UNIVERSIDAD DE GRANADA

DEPARTAMENTO DE INGENIERÍA CIVIL

ESTUDIO CINÉTICO DE BIORREACTORES DE MEMBRANA CON Y SIN LECHO MÓVIL APLICADOS AL TRATAMIENTO DE AGUAS RESIDUALES URBANAS

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KINETIC STUDY OF MOVING BED BIOFILM REACTOR-MEMBRANE BIOREACTOR SYSTEMS AND MEMBRANE BIOREACTORS FOR MUNICIPAL WASTEWATER TREATMENT

JUAN CARLOS LEYVA DÍAZ

TESIS DOCTORAL

PARA LA OBTENCIÓN DEL GRADO DE DOCTOR CON

MENCIÓN INTERNACIONAL POR LA UNIVERSIDAD DE GRANADA

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GRANADA, MARZO 2015

Editorial: Universidad de Granada. Tesis Doctorales
Autor: Juan Carlos Leyva Díaz
ISBN: 978-84-9125-079-1
URI: http://hdl.handle.net/10481/40050

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Memoria presentada por D. Juan Carlos Leyva Díaz para aspirar al Grado de Doctor por la Universidad de Granada.

Fdo.: D. Juan Carlos Leyva Díaz

Directores:

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GRANADA, MARZO 2015

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En Granada, a 6 de febrero de 2015,

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Este trabajo ha estado financiado por el Programa de Formación de Profesorado Universitario (FPU) del Ministerio de Educación, Cultura y Deporte en el marco del Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica 2008-2011, con referencia de la beca-contrato AP2010-1552, y por la empresa Abengoa Water en el marco del Proyecto Tecoagua, con referencia CEN-20091028, en colaboración con el Centro para el Desarrollo Tecnológico Industrial (CDTI), dependiente del Ministerio de Economía y Competitividad.

AGRADECIMIENTOS

En primer lugar, me gustaría agradecer a D. José Manuel Poyatos y a Dña. María del Mar Muñío, mis directores de tesis, la labor realizada hacia mi persona en la dirección de dicha investigación y todo lo aprendido a lo largo de los últimos años. En todo momento, han sabido orientar, de una manera excelente, el trabajo realizado, ayudándome a obtener siempre el máximo rendimiento, buscando el sentido práctico del estudio llevado a cabo y transmitiéndome el ánimo necesario en los momentos de adversidad.

Agradezco al profesor D. Ernesto Hontoria su buena disposición a la hora de aceptar la tutoría de mi beca-contrato FPU y todo lo aprendido en sus clases sobre depuración de aguas residuales. Mi más sincero agradecimiento al profesor D. Jesús González por estar siempre disponible para atenderme y ayudarme en todo lo concerniente a la investigación.

Doy las gracias a todos los miembros del grupo de investigación RNM-270 de Microbiología y Tecnologías Ambientales por facilitarme la integración en el mismo desde el primer momento. También, expresar mi gratitud hacia todos los miembros del Departamento de Ingeniería Civil por su acogida. Quiero recordar a todos los compañeros de laboratorio, tanto a los de la ETS de Ingeniería de Caminos, Canales y Puertos como a los del Instituto Universitario de Investigación del Agua, especialmente a mi compañero y amigo Juan Jesús Narváez que me ayudó en gran medida en mis inicios y del que he aprendido mucho. No podría dejar de mencionar a Bernabé, Ginés y Maribel, que siempre han estado dispuestos a ayudarme en todo lo que he necesitado.

Considero necesario expresar mi gratitud a la empresa Abengoa Water, especialmente a Dña. Inmaculada Salcedo y a D. Celestino Montero por su participación en el proyecto de investigación y su gran implicación a la hora de que el proyecto avanzara con éxito. Además, también agradezco al personal de la EDAR Puente de los Vados de Granada, en especial a Miguel Alonso, su amabilidad en el trato recibido a lo largo de estos años y su buena disposición para facilitarme el trabajo de recogida de muestras realizado.

Deseo manifestar mi agradecimiento a los profesores D. Massimiliano Fenice y D. Paolo Barghini, de la Universidad de la Tuscia, por su gran acogida y ayuda en todo lo

que necesité durante mi estancia de investigación en Viterbo (Italia), así como a mi compañera de laboratorio Susanna Gorrasi.

En este momento, deseo recordar a mis abuelos Ramón y Juan, a los que nunca olvidaré, que son para mí el modelo a seguir en cuanto a humildad, esfuerzo, sacrificio y amor hacia su familia, valores estos imprescindibles para conseguir los objetivos marcados a lo largo de la vida. No podría olvidarme de mis abuelas Paca y Julia, que siempre me manifiestan su cariño. Y gracias a mi padre Sebastián y a mi madre Carmen, a los que debo lo que soy, que siempre se han desvivido por mí para estar donde hoy estoy, me han brindado su apoyo en los momentos difíciles y han sabido darme, en todo momento, el consejo adecuado.

Muchas gracias, con letras mayúsculas, a la persona más especial de mi vida, María José, que es la alegría de mi corazón y a la que debo la energía, comprensión y ánimo que siempre me sabe transmitir para afrontar todas las situaciones que acontecen en nuestro peregrinar diario, desde el más profundo amor y cariño.

Finalmente, me quiero acordar de mi hermana María del Carmen, siempre dispuesta a ayudarme, mis tíos, en especial mi tío Antonio, mis primos Antonio Jesús y Cristina, Adela, José y Adela, por estar siempre pendientes de lo que en mi vida acontece y por el enorme cariño que, a diario, me profesan.

Finalmente, recuerdo a mi mejor amigo, Jesús, en el que siempre puedo confiar y que nunca me ha fallado.

A todos ellos les debo la realización de esta Tesis Doctoral.

Muchas gracias a todos.

A mis abuelos, Ramón y Juan,

a mis padres, Carmen y Sebastián,

y a María José.

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AAO	anaerobic/anoxic/oxic
AOB	ammonium-oxidizing bacteria bacterias oxidadoras de amonio
AOP(s)	advanced oxidation process(es)
ASM	activated sludge model modelo de fangos activos
BD	biofilm density
BNR	biological nutrient removal
BOD_5 DBO_5	five-day biochemical oxygen demand demanda bioquímica de oxígeno a los 5 días
COD DQO	chemical oxygen demand demanda química de oxígeno
COD_b	biodegradable fraction of COD
DBO	demanda bioquímica de oxígeno
DCA	detrended correspondence analysis
DeNB	denitrifying bacteria bacterias desnitrificantes
EBPR	enhanced biological phosphorus removal
EDAR	estación depuradora de aguas residuales
EPS	extracellular polymeric substances sustancias poliméricas extracelulares
f_{cv}	yield coefficient factor to convert to (mg VSS mg COD^{-1})
f_{cv}^{*}	yield coefficient factor to convert to (mg VSS mg N^{-1})
HRT(s) TRH(s)	hydraulic retention time(s) tiempo(s) de retención hidráulico
$k_{1,\ TOC}$	rate constant for TOC degradation
k_d	endogenous or decay coefficient for total biomass

K_M	half-saturation coefficient for organic matter
K_{NH}	half-saturation coefficient for ammonia-nitrogen
K_{NOB}	half-saturation coefficient for nitrite-nitrogen
K_S	substrate half-saturation coefficient
MBBR(s)	moving bed biofilm reactor(s) reactor(es) de biopelícula de lecho móvil
MBBR-MBR	moving bed biofilm reactor-membrane bioreactor biorreactor de membrana con lecho móvil
MBR(s)	membrane bioreactor(s) biorreactor(es) de membrana
MLSS	mixed liquor suspended solids sólidos en suspensión del licor mezcla
MLVSS	mixed liquor volatile suspended solids
NOB	nitrite-oxidizing bacteria bacterias oxidadoras de nitrito
NTK	nitrógeno total Kjeldahl
OC	oxygen consumption
OTU	operational taxonomic unit
OUR	oxygen uptake rate
OUR_{end}	endogenous oxygen uptake rate
PAO(s)	polyphosphate accumulative organism(s) bacteria(s) acumuladora(s) de polifosfatos
PHA(s)	polihidroxialcanoato(s)
PHB	polyhydroxybutyrate polihidroxibutirato
r_d	cellular decay rate
RDA	redundancy analysis
r_{su}	substrate degradation rate

r_x	cellular growth rate
r_x'	net cellular growth rate
R_s	dynamic oxygen uptake rate
S	substrate concentration
S_{NH}	ammonium concentration
S_S	organic matter concentration
SEM	scanning electron microscopy microscopía electrónica de barrido
SRT(s) TRC(s)	sludge retention time(s) tiempo(s) de retención celular
t	time
TGGE	temperature gradient gel electrophoresis electroforesis en gel con gradiente de temperatura
TN NT	total nitrogen nitrógeno total
TOC COT	total organic carbon carbono orgánico total
TP PT	total phosphorus fósforo total
TSS SST	total suspended solids sólidos en suspensión totales
VBD	volatile biofilm density
VFA(s)	volatile fatty acid(s)
VSS	volatile suspended solids
WWTP(s)	wastewater treatment plant(s) planta(s) de tratamiento de aguas residuales
X	biomass concentration
$X_{B, A}$	active autotrophic biomass concentration
$X_{B, H}$	active heterotrophic biomass concentration

X_T	total biomass concentration
Y	yield coefficient
Y_A	yield coefficient for autotrophic biomass
Y_H	yield coefficient for heterotrophic biomass
Y_{NOB}	yield coefficient for nitrite-oxidizing bacteria

Greek symbols

$\eta_{max,\ TOC}$	maximum rate of TOC degradation
$\eta_{\ TOC}$	rate of TOC removal of the pseudofirst-order model
μ	specific growth rate
μ_{emp}	empirical specific growth rate
μ_m	maximum specific growth rate
μ_A	specific growth rate for autotrophic biomass
$\mu_{m,\ A}$	maximum specific growth rate for autotrophic biomass
μ_H	specific growth rate for heterotrophic biomass
$\mu_{m,\ H}$	maximum specific growth rate for heterotrophic biomass
$\mu_{m,\ NOB}$	maximum specific growth rate for nitrite-oxidizing bacteria

RESUMEN

Se han desarrollado nuevas tecnologías respecto al tratamiento de aguas residuales. Entre ellas, el biorreactor de membrana con lecho móvil (MBBR-MBR) constituye una solución alternativa a los procesos convencionales. Siete sistemas diferentes aplicados al tratamiento de aguas residuales urbanas fueron estudiados en base a la eliminación de materia orgánica y nutrientes.

Las plantas de tratamiento de aguas residuales (WWTPs) que fueron diseñadas para la eliminación de materia orgánica y nitrógeno, consistían en un biorreactor de membrana (MBR), un MBBR-MBR híbrido que contenía material soporte en las zonas anóxica y aeróbica del biorreactor (MBBR-MBR híbrido$_a$), un MBBR-MBR híbrido que disponía de relleno solamente en la zona aeróbica del biorreactor (MBBR-MBR híbrido$_b$) y un MBBR-MBR puro que también contenía relleno sólo en la zona aeróbica del reactor biológico. Las WWTPs que fueron diseñadas para la eliminación de materia orgánica, nitrógeno y fósforo, consistían en un MBR$_p$, un MBBR-MBR híbrido que contenía material soporte en las zonas anaeróbica, anóxica y aeróbica del biorreactor (MBBR-MBR híbrido$_{ap}$) y un MBBR-MBR híbrido que tenía relleno solamente en las zonas anaeróbica y anóxica del biorreactor (MBBR-MBR híbrido$_{bp}$).

Las WWTPs operaron bajo diferentes tiempos de retención hidráulico (TRHs), 30.4 h, 26.5 h, 18 h, 9.5 h y 6 h, y diferentes concentraciones de biomasa, que se agruparon en concentraciones de biomasa bajas en torno a un valor medio de 2,700 mg L^{-1}, concentraciones de biomasa intermedias alrededor de un valor medio de 3,700 mg L^{-1} y concentraciones de biomasa altas entorno a un valor medio de 6,500 mg L^{-1}.

La cinética microbiana se estudió en relación a la biomasa heterótrofa y autótrofa, principalmente las bacterias oxidadoras de amonio (AOB) y las bacterias oxidadoras de nitrito (NOB), con el objetivo de explicar la eliminación de materia orgánica y nutrientes. Las comunidades microbianas de AOB, NOB y bacterias desnitrificantes (DeNB) de cada planta de tratamiento de aguas residuales (WWTP) se analizaron mediante métodos de pirosecuenciación 454 para detectar y cuantificar la contribución de las bacterias nitrificantes dentro de la comunidad bacteriana total. Además, se estudió la evolución de las actividades enzimáticas de α-glucosidasa, fosfatasa ácida y fosfatasa alcalina, se evaluó la diversidad bacteriana mediante electroforesis en gel con gradiente

de temperatura (TGGE) y se analizó la estructura de la comunidad bacteriana a través de microscopía electrónica de barrido (SEM).

El sistema MBBR-MBR híbrido$_b$ presentaba la mayor eficacia en relación a la eliminación de demanda química de oxígeno (DQO) para TRHs inferiores a 9.5 h, con valores de 87.39±6.01% y 84.10±2.25% para 9.5 h y 6 h, respectivamente. No había diferencias estadísticamente significativas respecto a la eliminación de DQO entre las diferentes configuraciones para TRHs mayores de 18 h. El rendimiento en eliminación de nitrógeno total (NT) era ligeramente superior en el sistema MBBR-MBR híbrido$_b$ para TRHs mayores de 9.5 h. El sistema MBBR-MBR puro presentaba los mayores porcentajes en eliminación de NT para TRHs inferiores a 9.5 h. Por lo tanto, una zona anóxica sin material soporte facilitaba el contacto entre el nitrato y los microorganismos. La eliminación de material soporte de la zona anóxica del biorreactor (MBBR-MBR híbrido$_b$) originó un aumento de las actividades enzimáticas estudiadas, así como de la capacidad para eliminar NT. Los resultados en relación a la eliminación de materia orgánica y nitrógeno estaban en consonancia con el estudio cinético de biomasa heterótrofa y autótrofa, respectivamente.

La introducción de una zona anaeróbica en el biorreactor mejoró la eliminación de fósforo total (PT), con un valor de 81.42±3.85% para el sistema MBBR-MBR híbrido$_{ap}$, que mostraba el mayor rendimiento.

El modelado cinético y el estudio microbiológico mejoraron el modelo ASM3 básico mediante la introducción del proceso de nitrificación en dos etapas. Los sistemas MBR y MBR$_p$ tenían, en general, el mejor comportamiento cinético respecto a la cinética de NOB. Los sistemas MBBR-MBR híbrido$_b$ y MBBR-MBR híbrido$_{bp}$ bajo un tiempo de retención hidráulico (TRH) de 18 h y el sistema MBBR-MBR puro con los TRHs de 9.5 h y 6 h podían tener un mejor comportamiento cinético en relación a las AOB porque, globalmente, la cinética de biomasa autótrofa era más eficaz en estos sistemas.

ABSTRACT

New technologies regarding wastewater treatment have been developed. Among these technologies, the moving bed biofilm reactor-membrane bioreactor (MBBR-MBR) is a recent alternative solution to conventional processes. Seven different systems for municipal wastewater treatment were studied regarding the removal of organic matter and nutrients.

The wastewater treatment plants (WWTPs), which were designed for organic matter and nitrogen removal, consisted of a membrane bioreactor (MBR), a hybrid MBBR-MBR system containing carriers both in the anoxic and aerobic zones of the bioreactor (hybrid MBBR-MBR_a), a hybrid MBBR-MBR which contained carriers only in the aerobic zone of the bioreactor (hybrid MBBR-MBR_b) and a pure MBBR-MBR which also contained carriers only in the aerobic zone of the biological reactor. The WWTPs, which were designed for organic matter, nitrogen and phosphorus removal, consisted of an MBR_p, a hybrid MBBR-MBR containing carriers in the anaerobic, anoxic and aerobic zones of the bioreactor (hybrid MBBR-MBR_{ap}) and a hybrid MBBR-MBR which contained carriers only in the anaerobic and anoxic zones of the bioreactor (hybrid MBBR-MBR_{bp}).

The WWTPs operated under different hydraulic retention times (HRTs), 30.4 h, 26.5 h, 18 h, 9.5 h and 6 h, and different biomass concentrations, which were grouped in low biomass concentrations around an average value of 2,700 mg L^{-1}, intermediate biomass concentrations around an average value of 3,700 mg L^{-1} and high biomass concentrations around an average value of 6,500 mg L^{-1}.

A study of the microbial kinetics concerning the heterotrophic and autotrophic biomass, mainly ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), was carried out to explain the removal of organic matter and nutrients. The microbial communities of AOB, NOB and denitrifying bacteria (DeNB) of each wastewater treatment plant (WWTP) were analyzed by 454 pyrosequencing methods to detect and quantify the contribution of nitrifying bacteria in the total bacterial community. Additionally, the evolution of the enzymatic activities of α-glucosidase and acid and alkaline phosphatase was studied, the bacterial diversity was evaluated by temperature gradient gel electrophoresis (TGGE) fingerprints and the bacterial

community structure was analyzed throughout the scanning electron microscopy (SEM).

The hybrid MBBR-MBR_b had the highest efficiency regarding the chemical oxygen demand (COD) removal for HRTs lower than 9.5 h, with values of 87.39±6.01% and 84.10±2.25% for 9.5 h and 6 h, respectively. There were not statistically significant differences regarding the COD removal between the different configurations for HRTs higher than 18 h. The efficiency concerning the total nitrogen (TN) removal was slightly higher in the hybrid MBBR-MBR_b for HRTs higher than 9.5 h. The pure MBBR-MBR had the highest percentages of TN removal for HRTs lower than 9.5 h. Therefore, an anoxic zone without carriers provided better contact between nitrate and the microorganisms. The removal of carrier from the anoxic zone of the bioreactor (hybrid MBBR-MBR_b) involved an increase of the enzymatic activities studied, as well as the capacity to remove TN. The results concerning the organic matter and nitrogen removal were in accordance with the kinetic study for heterotrophic and autotrophic biomass, respectively.

The introduction of an anaerobic zone in the bioreactor improved the total phosphorus (TP) removal, with a value of 81.42±3.85% for the hybrid MBBR-MBR_{ap}, which showed the highest performance.

Kinetic modeling and microbiological study enhanced the basic ASM3 model by introducing two-step nitrification. The MBR and MBR_p had usually the best kinetic behavior regarding the NOB kinetics. The hybrid MBBR-MBR_b and hybrid MBBR-MBR_{bp} under a hydraulic retention time (HRT) of 18 h and the pure MBBR-MBR with the HRTs of 9.5 h and 6 h could have a better kinetic behavior regarding the AOB because, as a whole, the kinetics of autotrophic biomass was more effective in these systems.

I. INTRODUCCIÓN GENERAL

1. Antecedentes y problemática de las aguas residuales

Aunque la captación y drenaje de aguas pluviales datan de tiempos antiguos, la recogida de aguas residuales no aparece hasta principios del siglo XIX, mientras que el tratamiento sistemático de las aguas residuales data de finales del siglo XIX y principios del siglo XX. El desarrollo de la teoría del germen a cargo de Koch y Pasteur en la segunda mitad del siglo XIX marcó el inicio de una nueva era en el campo del saneamiento. Hasta ese momento se había profundizado poco en la relación entre contaminación y enfermedades, y no se había aplicado al tratamiento de aguas residuales la bacteriología, disciplina entonces en sus inicios (Metcalf, 2003).

En el año 1849, Snow demostró la relación entre la transmisión del cólera y el consumo de agua contaminada por agua residual procedente de domicilios habitados por personas que padecían la enfermedad. A partir de estos descubrimientos, se empezó a tomar conciencia del peligro que las aguas residuales conllevan, al ser estas transmisoras de enfermedades de naturaleza feco-hídrica, principalmente de origen intestinal. Los patógenos aportados al agua, mayoritariamente desde las heces, pueden alcanzar las aguas limpias por un vertido directo de agua residual sin tratar, cerrándose el ciclo cuando el agua contaminada es consumida. Esto supone un grave problema que es necesario resolver mediante un tratamiento del agua residual antes de ser vertida para evitar la contaminación del medio y con ello la transmisión de enfermedades (Gómez-Nieto and Hontoria-García, 2003).

2. Definiciones y clasificación de las aguas residuales

La generación de aguas residuales es una consecuencia inevitable de las actividades humanas. El concepto de aguas residuales engloba las aguas residuales domésticas que son aquellas aguas recogidas en las aglomeraciones urbanas, procedentes de los vertidos de la actividad humana doméstica, o la mezcla de estas con las aguas que proceden de actividades comerciales, industriales y agrarias integradas en el núcleo urbano, así como las aguas de lluvia. En este sentido, las aguas pluviales o blancas proceden de drenajes o de escorrentía superficial y se caracterizan por grandes aportaciones intermitentes de caudal y escasa contaminación. Dicha contaminación se incorpora al agua al atravesar la lluvia la atmósfera y/o por el lavado de superficies y terrenos (escorrentía superficial). Por otro lado, las aguas procedentes de los vertidos de

la actividad humana, doméstica, comercial, industrial o agrícola reciben el nombre de aguas urbanas o negras y se caracterizan por presentar un caudal menor y más continuo, siendo la contaminación mucho mayor.

Por lo tanto, las aguas residuales urbanas se pueden definir como las aguas naturales contaminadas por las distintas sustancias (orgánicas, inorgánicas y microorganismos) aportadas por los vertidos de las aglomeraciones urbanas, junto con las aguas procedentes del drenaje pluvial en el caso de sistemas de saneamiento unitario (Trapote-Jaume, 2011). Dichas aguas se componen principalmente de aguas residuales domésticas, en las que predomina una contaminación orgánica, lo que hace que puedan ser depuradas mediante tratamientos biológicos al tratarse de aguas residuales urbanas biodegradables. Además, contienen un cierto porcentaje de aguas residuales industriales, siempre que estas no alteren sensiblemente las características de las aguas residuales domésticas, lo que ocurre en un gran porcentaje de los núcleos urbanos. Cuando no ocurre esto y la composición de la mezcla se ve alterada de manera significativa por las aguas residuales industriales, se denominan aguas residuales mixtas, que son asimilables a las aguas residuales industriales. Las aguas de escorrentía pluvial también pueden formar parte de las aguas residuales urbanas si la red de saneamiento es unitaria, es decir si las aguas de lluvia son recogidas por el mismo sistema de alcantarillado que se emplea para la recogida y conducción de las domésticas e industriales.

3. Composición de las aguas residuales urbanas

El conocimiento de la naturaleza del agua residual desde un punto de vista físico, químico y biológico es fundamental para proyectar y explotar las infraestructuras necesarias de recogida, tratamiento y evacuación de las aguas residuales, así como para la gestión de la calidad medioambiental.

La composición de las aguas residuales urbanas es muy variable y depende de diversos factores como el consumo de agua, aguas industriales que vierten a la red urbana, régimen alimenticio, costumbres de la población, etc.

La Tabla I.1 recoge la composición típica de un agua residual urbana, con tres grados posibles de contaminación (fuerte, media y débil). Generalmente, consumos de

agua bajos dan lugar a concentraciones elevadas de los parámetros y viceversa (Metcalf, 2003).

Tabla I.1. Composición típica de un agua residual urbana.

Parámetro	Concentración (mg L^{-1})		
	Fuerte	**Media**	**Débil**
Sólidos Totales (ST)	**1,200**	**700**	**350**
Fijos	600	350	175
Volátiles	600	350	175
Sólidos en Suspensión (SS)	350	200	100
(SS sedimentables+SS coloidales)			
Fijos (SSF)	75	50	30
Volátiles (SSV)	275	150	70
SS sedimentables (SSs)	20	10	5
SS coloidales (SSc)	330	190	95
Sólidos Disueltos (SD)	850	500	250
Fijos	525	300	145
Volátiles	325	200	105
Demanda Bioquímica de Oxígeno a 5 días (DBO_5)	**400**	**220**	**110**
Demanda Química de Oxígeno (DQO)	**1,000**	**500**	**250**
Carbono Orgánico Total (COT)	**290**	**160**	**80**
Nitrógeno Total (NT)	**85**	**40**	**20**
Nitrógeno Total Kjeldahl (NTK)	85	40	20
Nitrógeno Orgánico (N-NO)	35	15	8
Nitrógeno Amoniacal ($N\text{-}NH_3$)	50	25	12
Nitritos (NO_2^-)	0	0	0
Nitratos (NO_3^-)	0	0	0
Fósforo Total (PT)	**15**	**8**	**4**
Fósforo Orgánico (PO)	5	3	1
Fósforo Inorgánico (PI)	10	5	3
Cloruros	**100**	**50**	**30**
Alcalinidad (como $CaCO_3$)	**200**	**100**	**50**
Grasas	**150**	**100**	**50**

4. Legislación sobre aguas residuales

Actualmente, para controlar los problemas medioambientales que pueden originar las aguas residuales existe una amplia legislación al respecto. La legislación aplicable en España a la depuración de las aguas residuales urbanas se concreta en tres niveles normativos, como son el europeo, estatal y autonómico.

A nivel europeo, la Directiva 91/271/CEE (modificada parcialmente por la Directiva 98/15/CE), sobre el tratamiento de las aguas residuales urbanas, y la Directiva

2000/60/CE (Directiva Marco del Agua), sobre el establecimiento de un marco comunitario de actuación en el ámbito de la política de aguas, constituyen el referente fundamental.

A nivel nacional, las principales normas legales que incluye la legislación sobre esta materia son las siguientes:

- RDL 11/1995, por el que se transpone la Directiva 91/271/CEE al ordenamiento interno español.
- RD 509/1996, que desarrolla el RDL 11/1995, modificado por el RD 2116/1998.
- RDL 1/2001, por el que se aprueba el texto refundido de la Ley de Aguas, modificado por la Ley 62/2003.
- RD 849/1986, por el que se aprueba el Reglamento del Dominio Público Hidráulico, modificado por el RD 9/2008.
- RD 927/1988, por el que se aprueba el Reglamento de la Administración Pública del Agua y de la Planificación Hidrológica.
- Ley 10/2001, del Plan Hidrológico Nacional, parcialmente modificada por la Ley 11/2005.
- Ley 62/2003, por la que se transpone la Directiva 2000/60/CE al ordenamiento interno español.
- RD 1620/2007, por el que se establece el régimen jurídico de la reutilización de las aguas depuradas.

A nivel autonómico, las comunidades que disponen de competencias en materia de depuración de aguas han venido legislando en sus respectivos territorios. En este sentido, en la Comunidad Autónoma de Andalucía se promulgó la Ley 9/2010, de 30 de julio, de Aguas de Andalucía. Toda la regulación contenida en esta Ley, desde la normativa propia de la Administración Andaluza del Agua, planificación hidrológica y régimen de las obras hidráulicas, hasta la regulación del ciclo integral del agua de uso urbano y políticas de abastecimiento y saneamiento, aguas subterráneas, comunidades de usuarios, régimen de prevención de inundaciones y sequías, régimen económico financiero del agua y régimen de infracciones, se orienta en el sentido de dar cumplimiento a la Directiva 91/271/CEE y la Directiva Marco del Agua. Se trata de

construir, a partir del actual ordenamiento estatal, un régimen jurídico del agua adecuado a las concretas necesidades de Andalucía.

Como se ha comentado anteriormente, la Directiva 91/271/CEE es la referencia más importante en materia de depuración. Contiene estipulaciones relativas a la recogida, tratamiento y vertido de las aguas residuales urbanas y el tratamiento y vertido de las aguas residuales procedentes de determinados sectores industriales, estableciendo unos requisitos mínimos para el vertido de dichas aguas según el tamaño de la población, que el vertido afecte a aguas continentales o marinas y que vierta a zonas sensibles o menos sensibles.

Esta Directiva se trasladó al ordenamiento jurídico español mediante el RDL 11/1995 y el RD 509/1996 que desarrolla el anterior. Los criterios para fijar la obligación de tratar las aguas residuales urbanas son el número de "habitantes-equivalentes", concepto definido en función de la carga contaminante tanto de personas, como de animales e industrias, las "aglomeraciones urbanas", que son las zonas que presentan una concentración suficiente para la recogida y conducción de las aguas residuales a una instalación de tratamiento o a un punto de vertido final, y también se toma en consideración la mayor o menor sensibilidad de la zona en la que van a realizarse los vertidos, definiéndose "zona sensible" como el medio o zona de aguas declaradas expresamente con los criterios que se establecerán reglamentariamente.

Con carácter general, la legislación establece dos obligaciones claramente diferenciadas. En primer lugar, las "aglomeraciones urbanas" deberán disponer, según los casos, de sistemas colectores para la recogida y conducción de las aguas residuales; en segundo lugar, se prevén distintos tratamientos a los que deberán someterse dichas aguas antes de su vertido a las aguas continentales o marítimas. En la determinación de los tratamientos a que deberán ser sometidas las aguas residuales urbanas antes de su vertido, se tiene en cuenta si dichos vertidos se efectúan en "zonas sensibles" o "zonas menos sensibles", lo cual determinará un tratamiento más o menos riguroso. En la Tabla I.2 y en la Tabla I.3 se indican los parámetros cuyo control es obligatorio y los valores máximos permitidos si el vertido se realiza en zonas normales o zonas sensibles, según el RD 509/1996.

Tabla I.2. Requisitos para los efluentes de depuradoras urbanas para zonas normales y zonas sensibles eutóficas, según RD 509/1996. DBO_5 (demanda bioquímica de oxígeno de cinco días), DQO (demanda química de oxígeno), SS (sólidos en suspensión), h-e (habitantes equivalentes).

Parámetro	Concentración	Porcentaje mínimo de reducción (%) (1)
DBO_5	25 mg O_2 L^{-1}	70-90
DQO	125 mg O_2 L^{-1}	75
SS	35 mg L^{-1} (>10,000 h-e)	90
	60 mg L^{-1} (<10,000 h-e)	70

(1) Reducción relacionada con la carga del caudal de entrada.

Tabla I.3. Requisitos para los efluentes de depuradoras urbanas para zonas sensibles eutróficas, según RD 509/1996. P total (fósforo total), N total (nitrógeno total), h-e (habitantes equivalentes).

Parámetro	Concentración	Porcentaje mínimo de reducción (%) (1)
P total (2)	2 mg P L^{-1} (10,000-100,000 h-e) 1 mg P L^{-1} (>100,000 h-e)	80
N total (3)	15 mg N L^{-1} (10,000-100,000 h-e) 10 mg N L^{-1} (>100,000 h-e)	70-80

(1) Reducción relacionada con la carga del caudal de entrada.
(2) Fósforo total equivale a la suma de fósforo orgánico y fósforo inorgánico.
(3) Nitrógeno total equivale a la suma de nitrógeno Kjeldahl total (nitrógeno orgánico y amoniacal), nitrógeno en forma de nitrato y nitrógeno en forma de nitrito.

5. Eliminación de nutrientes

La depuración de las aguas residuales se centró inicialmente en reducir el contenido de materia orgánica antes de su vertido al medio acuático receptor, de forma que, hasta fechas relativamente recientes, el objetivo principal de las plantas de tratamiento de las aguas residuales venía siendo la eliminación de los compuestos de carbono orgánico. Sin embargo, actualmente se incluye la eliminación de nitrógeno y/o fósforo debido al establecimiento de leyes y normativas ambientales cada vez más restrictivas en cuanto a la calidad del agua residual tratada (Tabla I.3).

El vertido de agua residual con alto contenido en nutrientes, principalmente nitrógeno en forma de amonio, nitrito, nitrato o nitrógeno orgánico y fósforo en forma de ortofosfato, en ecosistemas acuáticos ha originado un problema concreto de contaminación de las aguas denominado eutrofización. Conforme aumenta la disponibilidad de nutrientes, se aumenta la producción fotosintética primaria, la cual se encuentra representada principalmente por la proliferación de microalgas. Este

fenómeno conlleva una secuencia de cambios en los ecosistemas acuáticos y causa un desequilibrio en el nivel de fertilidad acuática debido a que la velocidad de producción de los niveles tróficos inferiores es superior a la velocidad de consumo de los niveles tróficos superiores, ocasionando trastornos en el equilibrio entre la biodiversidad, los niveles tróficos y los ciclos de nutrientes en los ecosistemas acuáticos afectados. En este sentido, se produce un consumo de oxígeno que puede llegar a reducir la presencia de oxígeno disuelto en dichos ecosistemas por debajo de los valores necesarios para la vida acuática, afectando negativamente a los mismos y convirtiéndolos en la mayoría de los casos en hábitats inhóspitos para el desarrollo y la supervivencia de los organismos aerobios.

Por lo tanto, se hace necesaria la eliminación de estos nutrientes del agua residual. Esto contribuye a desarrollar e implementar tecnologías de tratamiento cada vez más especializadas, obligando a una mejora continua de los procesos de depuración de las aguas residuales.

5.1. Eliminación biológica de nitrógeno

Existe una variedad de tecnologías de eliminación de nitrógeno del agua residual que implican procesos físicos y químicos tales como el arrastre con aire del amoniaco, el intercambio iónico y la cloración al breakpoint. Sin embargo, debido a su elevado coste, funcionamiento irregular y problemas de explotación y mantenimiento, la aplicación de este tipo de tecnologías se ha centrado en situaciones concretas, principalmente sobre efluentes de agua residual de tipo industrial, en donde su baja biodegradabilidad y/o toxicidad hacen inviable el uso de procesos biológicos. La eliminación de nitrógeno de las aguas residuales en una estación depuradora de aguas residuales (EDAR) se lleva a cabo generalmente mediante procesos biológicos, entre los cuales, los procesos de nitrificación y desnitrificación vía nitrato están dentro de los más comúnmente utilizados.

5.1.1. Compuestos de nitrógeno en el agua residual

El origen principal del nitrógeno en el agua residual urbana son las proteínas ingeridas por las personas en su alimentación, llegando al agua fundamentalmente como urea, $CO(NH_2)_2$. Tanto la urea como los compuestos de nitrógeno orgánico son hidrolizados total o parcialmente en iones amonio (NH_4^+) y oxhidrilo (OH^-) en las redes

de alcantarillado de forma que la mayor parte del nitrógeno se encuentra en forma no oxidada a la entrada de una EDAR. La totalidad del nitrógeno no oxidado, resultante de la suma del nitrógeno orgánico y el nitrógeno amoniacal, es conocida como Nitrógeno Total Kjeldahl (NTK) (Trapote-Jaume, 2011). Normalmente, el nitrógeno orgánico (urea, aminoácidos, péptidos, proteínas, ácidos nucleicos y otros compuestos orgánicos sintéticos) se puede encontrar aproximadamente en un 40-50% y el nitrógeno amoniacal representaría casi el 50% restante. Eventualmente, puede ocurrir que haya parte de nitrógeno oxidado, en forma de nitrito o nitrato, en el influente de una EDAR como consecuencia de vertidos industriales o infiltraciones de la red. Esto obligaría a analizar la concentración de estos compuestos junto con el NTK para conocer la concentración total de nitrógeno en el medio.

5.1.2. Fundamentos de la eliminación biológica de nitrógeno

Los procesos biológicos que se pueden desencadenar en el agua residual como consecuencia de la alta carga bacteriana que contienen, daría lugar a diferentes transformaciones de los compuestos de nitrógeno, predominando inicialmente los procesos de proteolisis y amonificación mediante los cuales se incrementa el porcentaje de amonio y desciende el de nitrógeno orgánico. Dentro de estas transformaciones biológicas habría que destacar las acontecidas en los tratamientos aerobios aplicados al agua, en los cuales predominan los procesos indicados anteriormente y los contrarios (aminación y síntesis proteica) que permitirían nuevas síntesis celulares y con ello la proliferación bacteriana. Estos procesos permitirían una eliminación de nitrógeno del medio por biofloculación de la masa bacteriana formada pasando, junto con aquella materia orgánica sedimentable, al fango. Esto puede llegar a suponer una eliminación en torno al 15%. Junto con estos procesos destacan otros de oxidación que permiten transformar el nitrógeno amoniacal en formas más oxidadas como nitrato y nitrito debido a procesos biológicos como los de nitrificación. Estos procesos son aprovechados en aquellos sistemas de depuración en los que se pretende eliminar nitrógeno, el cual tras su transformación en las formas más oxidadas puede ser transformado mediante desnitrificación a nitrógeno molecular, tal y como queda reflejado en la Figura I.1 correspondiente al ciclo del nitrógeno (Gómez-Nieto and Hontoria-García, 2003).

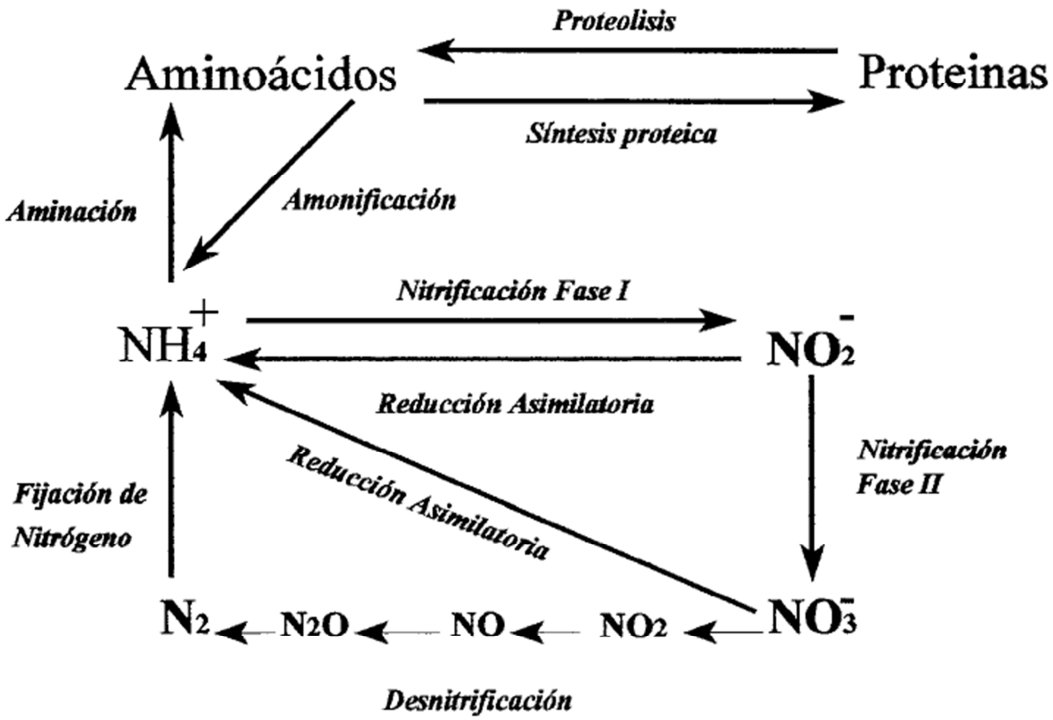

Figura I.1. Ciclo del nitrógeno (Gómez-Nieto and Hontoria-García, 2003).

Los avances en el conocimiento de los microorganismos involucrados en los procesos de eliminación de nitrógeno han llevado a la generación de una gran variedad de opciones de tratamiento en los últimos años. La tecnología aplicada en cada caso dependerá en gran medida de las características de la corriente de agua residual, los límites de vertido y el espacio disponible para su emplazamiento.

Entre los diferentes procesos de eliminación de nitrógeno se pueden destacar el proceso de nitrificación y desnitrificación vía nitrato, el proceso SHARON de nitrificación y desnitrificación vía nitrito, el proceso ANAMMOX de oxidación anaerobia de amonio, la nitritación parcial combinada con oxidación anaerobia de amonio, el proceso SND de nitrificación y desnitrificación simultánea y el proceso BABE de potenciación de organismos nitrificantes, entre otros.

Los procesos de nitrificación y desnitrificación vía nitrato son los más comúnmente utilizados para la eliminación del nitrógeno de las aguas residuales. La Figura I.2 muestra las transformaciones del nitrógeno en las aguas residuales a partir de los procesos de nitrificación y desnitrificación vía nitrato.

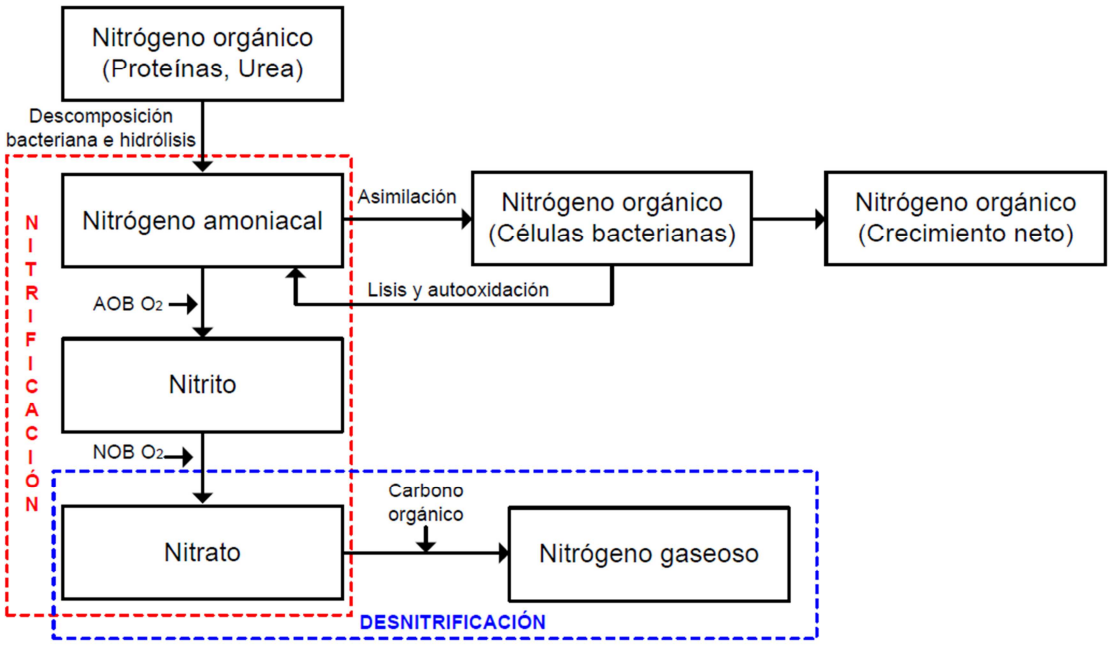

Figura I.2. Esquema del proceso de nitrificación-desnitrificación (Reyero-Cobo, 2010).

En la Figura I.2 se pueden observar los procesos de nitrificación y desnitrificación. Además, se encuentran representados los procesos de hidrólisis del nitrógeno orgánico, y los procesos de asimilación, crecimiento y lisis bacteriana a partir de nitrógeno inorgánico en forma amoniacal.

La nitrificación biológica es el proceso a través del cual el NTK presente en el agua residual se convierte a nitrato. Se trata de un proceso autotrófico y aerobio (es necesario un nivel de oxígeno alto del orden de 2 mg O_2 L^{-1}), en el que los microorganismos (autótrofos) obtienen la energía necesaria para el crecimiento bacteriano de la oxidación de compuestos de nitrógeno, principalmente del amoniaco. El proceso de nitrificación del nitrógeno amoniacal se lleva a cabo en dos etapas:

- 1ª Etapa (nitritación): el amonio (NH_4^+) es oxidado a nitrito (NO_2^-) por las bacterias oxidadoras de amonio (AOB), principalmente de los géneros *Nitrosomonas*, *Nitrosospira*, *Nitrosolobus* y *Nitrosovibrio*, de acuerdo a la ecuación:

$$NH_4^+ + \frac{3}{2}O_2 \xrightarrow{AOB} NO_2^- + 2H^+ + H_2O + \text{Energía}$$

- 2ª Etapa (nitratación): el nitrito es oxidado a nitrato (NO_3^-) por las bacterias oxidadoras de nitrito (NOB), principalmente pertenecientes a los géneros *Nitrobacter* y *Nitrospira*, según la ecuación:

$$NO_2^- + \frac{1}{2}O_2 \xrightarrow{NOB} NO_3^- + \text{Energía}$$

La energía liberada en estas reacciones es utilizada por los *Nitrosomonas* y los *Nitrobacter* para el crecimiento y mantenimiento celular. En la siguiente ecuación se representa la reacción energética global:

$$NH_4^+ + 2O_2 \xrightarrow{AOB+NOB} NO_3^- + 2H^+ + H_2O + \text{Energía}$$

La desnitrificación es el proceso mediante el cual los nitratos se reducen a nitrógeno gas (N_2) por medio de bacterias heterótrofas (*Pseudomonas*, *Achromobacter* y *Bacillus*), que pasa a la atmósfera y, de esta forma, se elimina del vertido. Se trata de un proceso llevado a cabo en condiciones anóxicas, es decir en ausencia de oxígeno disuelto (se estima que deben darse valores por debajo de 0.2 mg O_2 L^{-1}). Las bacterias heterótrofas utilizan los nitratos como fuente de oxígeno para las reacciones de síntesis y oxidación biológica, siempre y cuando haya carbono orgánico disponible para ser oxidado, constituyendo la fuente de energía de los microorganismos heterótrofos. Este proceso puede quedar resumido en la siguiente ecuación:

$$4H^+ + 5C + 4NO_3^- \xrightarrow{\text{Heterótrofas}} 5CO_2 + 2N_2 + 2H_2O$$

Un factor muy importante dentro de este proceso de eliminación de nitrógeno es el pH que debe estar comprendido entre 7 y 8.

5.2. Eliminación biológica de fósforo

La eliminación de fósforo se abordó inicialmente por precipitación química dada la gran sencillez de su aplicación práctica. Sin embargo, poco a poco se ha ido introduciendo la vía biológica para el fósforo que consiste en una eliminación por almacenamiento incrementado en la biomasa, ya que supone un ahorro de reactivos y una menor producción de fangos que además presentan un mayor contenido en fósforo,

lo que los hace apropiados para uso agrícola (Ferrer-Polo and Seco-Torrecillas, 2007). En los años 70 se realizaron numerosas investigaciones, sobre todo en Sudáfrica, para aclarar los fundamentos y el proceso de la retirada biológica incrementada de fósforo (Levin and Shapiro, 1965).

5.2.1. Compuestos de fósforo en el agua residual

En el caso de las aguas residuales urbanas, el fósforo procede de los residuos humanos (heces y orina) y de los detergentes. Dichas aguas contienen fósforo de origen orgánico que se encuentra en forma de fosfolípidos, ácidos nucleicos y numerosos compuestos fosforilados. Sin embargo, la mayor cantidad de fósforo en las aguas residuales urbanas está en forma inorgánica (Tabla I.1), bien como ortofosfatos (anión PO_4^{3-}) o como polifosfatos entre los que podemos destacar el hexametafosfato ($(PO_3)_6^{3-}$), el tripolifosfato ($P_3O_{10}^{3-}$) o el pirofosfato ($P_2O_7^{4-}$). Tanto la fracción orgánica como la inorgánica pueden aparecer en solución o bien asociadas a materia particulada (Gómez-Nieto and Hontoria-García, 2003). El fósforo total es el resultado de la suma del fósforo orgánico y el fósforo inorgánico.

5.2.2. Fundamentos de la eliminación biológica de fósforo

Los polifosfatos y otras combinaciones hidrolizables de fósforo son, normalmente, disociados de forma rápida por los microorganismos mediante exoenzimas, pasando a ortofosfatos. En el reactor biológico, las combinaciones disueltas de fósforo están predominantemente como ortofosfato. El problema de la eutrofización es producido básicamente por las formas solubles (ortofosfato) ya que el resto no es directamente asimilable. Sin embargo, la presencia de fósforo orgánico supone un posible incremento en la concentración de las formas asimilables debido a la mineralización realizada por diferentes microorganismos como hongos o bacterias. Igualmente, otras formas no asimilables, como los polifosfatos o el fósforo precipitado, pueden transformarse en ortofosfatos debido a fenómenos de hidrólisis o solubilización, en los cuales pueden jugar un papel importante los microorganismos o diversos factores fisicoquímicos. Al igual que se forma el ortofosfato, este también puede desaparecer debido a su asimilación o bien a la precipitación o acumulación, fenómenos en los cuales, junto con procesos fisicoquímicos, también pueden participar los

microorganismos. La Figura I.3 muestra el ciclo del fósforo donde quedan esquematizados cada uno de estos procesos (Gómez-Nieto and Hontoria-García, 2003).

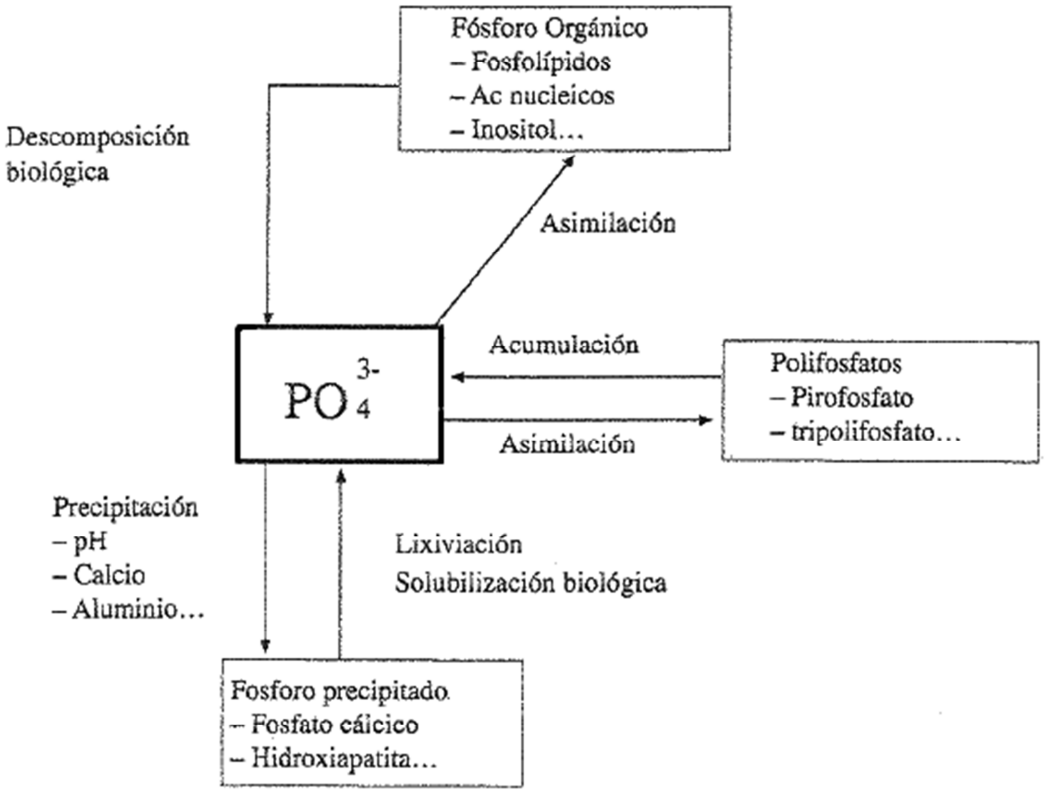

Figura I.3. Ciclo del fósforo (Gómez-Nieto and Hontoria-García, 2003).

Por lo tanto, además de la eliminación de los compuestos de carbono orgánico y nitrógeno, el objetivo de las plantas de tratamiento de aguas residuales también incluye la eliminación de fósforo. Las concentraciones permisibles de fósforo en las aguas tratadas dependen de las características del medio receptor, estableciéndose en la legislación valores máximos para zonas sensibles (Tabla I.3) debido a su incidencia en el proceso de eutrofización de las aguas receptoras, que provoca el deterioro del medio ambiente y afecta de forma negativa a la calidad de las aguas para los abastecimientos públicos.

En este sentido, se ha observado que determinados tipos de bacterias son capaces de almacenar fósforo intracelularmente en forma de gránulos de polifosfatos, bacterias acumuladoras de polifosfatos (PAOs), dando lugar a una eliminación neta de fósforo cuando son sometidas a una alternancia de condiciones anaerobias y aerobias. Se debe destacar el hecho de que el fósforo se incorpora a las células en forma de ortofosfato de

modo que en lugar de una retirada biológica de fósforo se puede hablar también de una retirada biológica de fosfatos. De esta forma, el fósforo o el fosfato pueden ser incorporados biológicamente a la biomasa por la normal asimilación de fósforo durante el crecimiento celular, por una toma incrementada en la célula en cantidad superior a la necesaria para el crecimiento y su consiguiente almacenamiento como polifosfato o bien indirectamente, por cambio de las condiciones exteriores (como por ejemplo el pH) que favorezcan una precipitación química de fosfatos en los flóculos de fango. En consecuencia, puede aumentarse la retirada de fósforo por medio de diversas formas de operación que produzcan un incremento de bacterias que tomen fosfatos en más alta cantidad y los almacenen como polifosfatos. Normalmente, la proporción de bacterias PAO en el fango activo es escasa. Su aumento y, como consecuencia, un incremento de la retirada de fósforo del agua residual se puede alcanzar en el momento en que el fango activo se vea sometido a condiciones anaerobias y aerobias alternativamente (Cortacans-Torre, 2004).

Bajo condiciones anaerobias, el fango activo descarga grandes cantidades de fosfatos al agua. Si el fango activo es aireado nuevamente, el fosfato es absorbido en cantidades mayores que la descarga, dando lugar a una eliminación neta del mismo (Figura I.4).

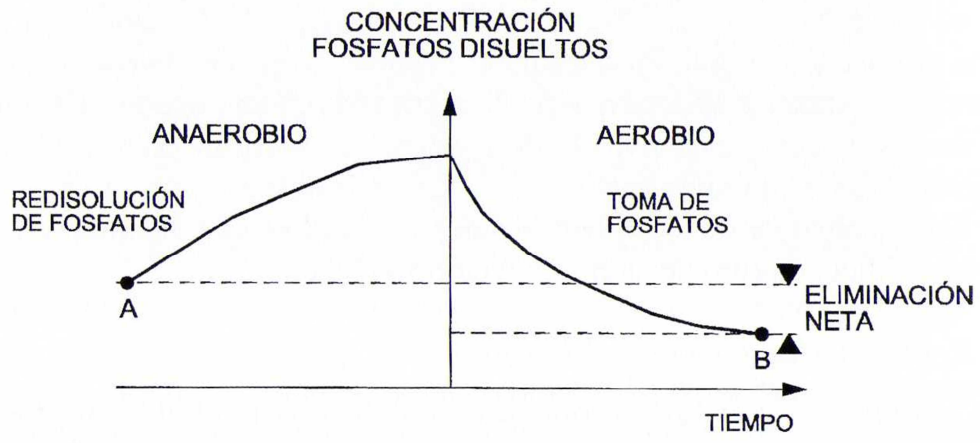

Figura I.4. Eliminación biológica del fósforo (Cortacans-Torre, 2004).

En condiciones anaerobias la materia orgánica fácilmente biodegradable es descompuesta por las bacterias acidogénicas (*Aeromonas*) a ácidos grasos de cadena corta. Los ácidos grasos de cadena corta (fundamentalmente ácido acético) son absorbidos por las bacterias PAO y almacenados como polihidroxibutirato (PHB) y

otros polihidroxialcanoatos (PHAs). Dado que las bacterias PAO no pueden ganar energía bajo condiciones anaerobias, la energía necesaria para el almacenamiento de los ácidos grasos, es obtenida de la descomposición de los polifosfatos. Durante este proceso se produce la liberación de ortofosfatos al medio y el consumo de materia orgánica. Esto hace que, en condiciones anaerobias, la concentración de ortofosfatos en el reactor aumente. Las bacterias PAO no son capaces de crecer en condiciones anaerobias, pero son capaces de almacenar sustrato intracelularmente en estas condiciones, lo que supone una ventaja competitiva frente a otras bacterias aerobias. El proceso que tiene lugar en condiciones anaerobias puede representarse de forma simplificada mediante la ecuación siguiente, en la que se supone que la materia orgánica utilizada es ácido acético, acumulado intracelularmente como PHB:

$$\underset{}{2C_2H_4O_2} + \underset{\text{Poli-P}}{(HPO_3)} + H_2O \rightarrow \underset{\text{M.O. almacenada}}{(C_2H_4O_2)_2} + PO_4^{3-} + 3H^+$$

Bajo condiciones aerobias, las bacterias PAO pueden utilizar el sustrato almacenado, polihidroxialcanoato (PHA), como fuente de carbono y energía para el crecimiento celular. Asimismo, utilizan parte de este sustrato almacenado para acumular fósforo intracelularmente en forma de polifosfatos, tomando el ortofosfato disponible en el licor mezcla, asegurando las reservas de energía necesarias para la etapa anaerobia. El proceso que tiene lugar en condiciones aerobias viene dado de forma simplificada por la siguiente ecuación (al igual que en la etapa anaerobia, se supone que la materia orgánica utilizada es ácido acético):

$$(C_2H_4O_2)_2 + 0.2PO_4^{3-} + 1.2O_2 + 0.16NH_4^+ \rightarrow$$

$$0.16C_5H_7NO_2 + 1.2CO_2 + \underset{\text{Poli-P}}{0.2(HPO_3)} + 0.44OH^- + 1.44\ H_2O$$

Esta reacción puede darse también en condiciones anóxicas, utilizando el nitrato como aceptor de electrones y produciendo nitrógeno gas.

La cantidad de fosfato incorporada por las bacterias PAO durante la fase aerobia supera la cantidad liberada durante la etapa anaerobia. Un esquema simplificado del proceso global aparece reflejado en la Figura I.5.

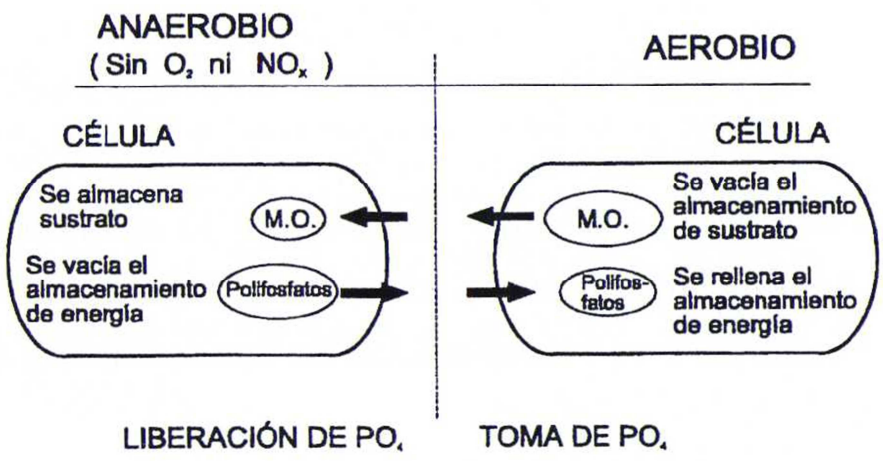

Figura I.5. Esquema de la liberación y toma de PO_4^{3-} en el proceso de eliminación de fósforo (Cortacans-Torre, 2004).

El fósforo sale del sistema con la purga de fango que se realiza tras la etapa aerobia (fango rico en polifosfatos). Este proceso permite un incremento, del orden de tres a cuatro veces, en la eliminación neta de fósforo respecto al producido por la sola síntesis celular de las bacterias heterótrofas no acumuladoras de polifosfatos. En esta línea, es necesario indicar que la presencia de nitratos (como aceptores de electrones) u oxígeno en la etapa anaerobia puede permitir que las bacterias no acumuladoras (bacterias heterótrofas desnitrificantes) metabolicen el sustrato fácilmente degradable reduciendo la cantidad de ácidos grasos de cadena corta disponibles para las bacterias acumuladoras de polifosfatos, de modo que estas bacterias no podrán ni crecer ni acumular polifosfato puesto que no disponen de sustrato y por tanto podrían llegar a desaparecer del sistema, hecho este que tiene como consecuencia una disminución en la eliminación de fósforo (Ferrer-Polo and Seco-Torrecillas, 2007).

5.3. Eliminación conjunta de nitrógeno y fósforo

Se han desarrollado diversos esquemas de proceso orientados a la eliminación biológica simultánea de materia orgánica y nutrientes. Los procesos de eliminación de nutrientes son más complejos que los de eliminación de materia orgánica, siendo necesaria la combinación de al menos dos etapas, aerobia y anóxica, en el caso del nitrógeno y otras dos, aerobia y anaerobia, en el caso del fósforo. Los procesos de eliminación simultánea de materia orgánica, nitrógeno y fósforo requieren la creación de una adecuada combinación de al menos tres etapas, anaerobia, anóxica y aerobia (Ferrer-Polo and Seco-Torrecillas, 2007).

Entre los sistemas de operación más empleados para la eliminación conjunta de materia orgánica, nitrógeno y fósforo están el proceso Bardenpho, proceso A^2/O, proceso UCT, proceso UCT modificado, proceso JHB, proceso EASC, proceso Biodenipho, proceso simultáneo y proceso SBR (Trapote-Jaume, 2011).

El esquema más sencillo para la eliminación conjunta de nitrógeno y fósforo es el A^2/O, que introduce un tanque anóxico entre el tanque anaerobio y el aerobio del proceso de eliminación de fósforo. Una variante de este sistema se empleó en **Chapter 7** para la eliminación conjunta de nitrógeno y fósforo. El esquema de este proceso se muestra en la Figura I.6. El fosfato se libera bajo condiciones anaeróbicas y posteriormente una acumulación del mismo tiene lugar en la zona aeróbica por parte de las bacterias PAO. En condiciones aeróbicas, tiene lugar el proceso de nitrificación, y la recirculación de nitratos desde el tanque de membranas hasta la zona anóxica permite la eliminación de nitrógeno ya que el nitrato es convertido a nitrógeno gaseoso que pasa a la atmósfera. Esta recirculación evita una posible inhibición en la liberación de fosfato en la zona anaeróbica como consecuencia de la recirculación que tiene lugar entre las cámaras anóxica y anaerobia, que permite completar la eliminación de materia orgánica del sistema.

Figura I.6. Esquema A^2/O modificado para la eliminación de nitrógeno y fósforo (modificado de Ferrer-Polo and Seco-Torrecillas, 2007).

6. Principios y procesos de depuración biológica

Como consecuencia de la legislación existente en materia de aguas residuales, es necesario realizar una serie de tratamientos a las aguas residuales para poder ser vertidas al medio. En el caso de las aguas residuales urbanas, los tratamientos biológicos constituyen el proceso fundamental dentro del tratamiento secundario de la línea de aguas dentro de la depuración de las aguas residuales urbanas, de ahí que al tratamiento

secundario se le conozca comúnmente como tratamiento biológico. Los objetivos de los tratamientos biológicos de las aguas residuales son, por un lado, la transformación o estabilización de la materia orgánica y, por otra parte, la coagulación y eliminación de los sólidos coloidales no sedimentables. Por lo tanto, la reducción del contenido orgánico y los nutrientes (nitrógeno y fósforo) constituye el principal objetivo cuando se trata de aguas residuales urbanas.

6.1. Metabolismo microbiano

Los procesos biológicos de depuración de las aguas residuales se basan en el metabolismo de los microorganismos presentes en los reactores biológicos. Entre los principales microorganismos que intervienen en los procesos biológicos de tratamiento se encuentran las bacterias, protozoos, hongos, algas, rotíferos y nematodos. Estos microorganismos necesitan principalmente una fuente de carbono para la síntesis de material celular nuevo y una fuente de energía para el desarrollo de sus funciones vitales. Además, necesitan elementos inorgánicos, denominados nutrientes, como el nitrógeno y fósforo, así como azufre, potasio, calcio y magnesio a nivel de trazas.

Las células de los microorganismos, por tanto, intercambian continuamente materia y energía con el medio. Introducen materia y la transforman con el objetivo de construir y renovar sus estructuras y conseguir la energía necesaria para sus funciones. Estas transformaciones que tienen lugar en la célula ocurren por medio de un conjunto de reacciones bioquímicas, catalizadas por enzimas, que se denominan genéricamente metabolismo.

En el metabolismo, se pueden distinguir dos fases, catabolismo o desasimilación y anabolismo o asimilación (Gòdia-Casablancas and López-Santín, 1998). El catabolismo consiste en una serie de reacciones bioquímicas que transforman la materia viva en deshechos o residuos. Las moléculas orgánicas complejas (polisacáridos, triglicéridos, proteínas, etc.) se transforman en otras más sencillas, orgánicas o inorgánicas (ácido pirúvico, ácido láctico, amoniaco, dióxido de carbono, etc.). En estas reacciones se libera la energía contenida en los enlaces de estas macromoléculas, es decir se pasa de moléculas con alto contenido energético (muy reducidas) a otras con escaso contenido (muy oxidadas). En el caso del anabolismo, se produce la síntesis de los componentes

orgánicos celulares necesarios para el crecimiento y la reproducción, consistentes en polímeros simples que necesitan energía y sustrato para su formación.

El metabolismo microbiano para aguas residuales se puede explicar dividiendo las reacciones bioquímicas en tres etapas (Ronzano and Dapena, 2002):

- Etapa I: consiste en la incorporación de la materia orgánica presente en las aguas residuales mediante reacciones de síntesis o asimilación, originando nuevo tejido celular, es decir un crecimiento de la masa de microorganismos. Para ello, la materia orgánica debe entrar en la célula a través de la membrana citoplasmática. Si la materia orgánica está en forma disuelta, demanda biológica de oxígeno (DBO) rápidamente biodegradable debida a compuestos solubles y constituidos por moléculas simples, pasa directamente a través de la membrana celular y se metaboliza a alta velocidad. Si la materia orgánica se encuentra en forma de materia en suspensión o coloidal, es decir en forma de partículas o grandes moléculas, que representa la mayor parte de la DBO lentamente biodegradable, es absorbida sobre las células con un efecto de almacenamiento sobre la membrana citoplasmática. Esta DBO debe ser transformada previamente en moléculas más simples para poder ser asimilada por la célula. Este proceso de hidrólisis origina moléculas más simples y se realiza en la pared celular, es llevado a cabo por enzimas extracelulares o exoenzimas producidas por la propia célula y con una velocidad relativamente lenta en comparación con la de la DBO rápidamente biodegradable; esta transformación en moléculas simples es el factor limitante en esta reacción de transformación bioquímica.
- Etapa II: consiste en la oxidación de una fracción de la materia orgánica mediante reacciones de oxidación o desasimilación, originando productos finales y liberando la energía necesaria para la síntesis de nuevo tejido celular.
- Etapa III: consiste en un proceso de respiración endógena o autooxidación que tiene lugar en ausencia de materia orgánica. El tejido celular será utilizado endógenamente produciendo compuestos gaseosos finales y energía para el mantenimiento de las células. De forma simultánea a la oxidación y producción de energía, hay una pérdida neta de masa activa llamada pérdida

de masa endógena. El 80% de la materia asimilada queda completamente oxidada como CO_2 y H_2O, y el 20% restante no es degradable y queda como residuo.

6.2. Cinética de crecimiento microbiano

Como se ha expuesto en el apartado anterior, los microorganismos presentes en los sistemas biológicos de depuración de aguas emplean los contaminantes orgánicos presentes en las aguas residuales como fuente de carbono y energía para el desarrollo y mantenimiento de sus funciones vitales. Dichos contaminantes son transformados en productos finales y nuevo material celular a partir de reacciones de oxidación biológica que tienen lugar durante el proceso de depuración de aguas residuales.

Los microorganismos que utilizan el carbono orgánico para la formación de tejido celular se denominan heterótrofos. Los organismos que obtienen carbono celular a partir de dióxido de carbono reciben el nombre de autótrofos. El proceso de conversión del dióxido de carbono a tejido celular orgánico es un proceso reductivo que precisa un suministro neto de energía. Por lo tanto, los microorganismos autótrofos deben emplear una parte mayor de su energía para la síntesis de tejido celular que los microorganismos heterótrofos, lo cual comporta unas tasas de crecimiento menores que las de estos. La energía necesaria para la síntesis celular se obtiene de la luz (organismos fotótrofos) o bien de las reacciones químicas de oxidación (organismos quimiótrofos). En el caso del tratamiento de las aguas residuales, estamos ante organismos quimiótrofos. Estos organismos pueden ser heterótrofos (protozoos, hongos y la mayoría de las bacterias) o autótrofos (bacterias nitrificantes). Los organismos quimioautótrofos consiguen la energía a partir de la oxidación de compuestos inorgánicos reducidos tales como el amoniaco, el nitrito y el sulfuro. Los organismos quimioheterótrofos suelen obtener la energía mediante la oxidación de compuestos orgánicos (Metcalf, 2003). En consecuencia, los estudios cinéticos respecto a las biomasas heterótrofa y autótrofa se basan en el análisis del comportamiento cinético de los organismos quimioheterótrofos y quimioautótrofos, respectivamente.

Por lo tanto, se puede indicar que uno de los aspectos más importantes en todos los sistemas de depuración mediante tratamiento biológico es la generación de biomasa, que está íntimamente relacionada con el metabolismo microbiano. Una manera de

explicar la generación de biomasa y la velocidad con que esta se genera es mediante la cinética de crecimiento microbiana. Un proceso de crecimiento celular implica el consumo de sustratos que suministren la energía y materia prima necesarias para la síntesis de nuevo material celular y demás productos del metabolismo. El crecimiento celular obedece a las leyes de conservación de la materia; los átomos de carbono, nitrógeno, oxígeno y demás elementos se reordenan en los procesos metabólicos de las células de manera que la cantidad total incorporada coincide con la que aparece en el entorno. Esto hace factible el planteamiento de balances de materia y de energía en los procesos de crecimiento celular, expresados de forma general en la Figura I.7:

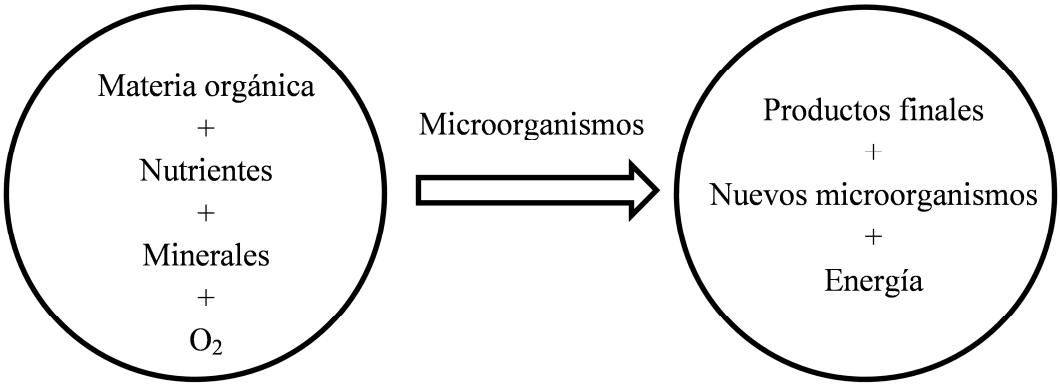

Figura I.7. Metabolismo de los microorganismos presentes en un sistema de depuración aerobio.

Se hace la consideración de célula promedio, que consiste en aceptar que todas las células de una población son iguales y se comportan de la misma forma (esta aproximación es la más comúnmente utilizada). Considerando un reactor discontinuo de mezcla perfecta, el crecimiento de las células tiene lugar dentro del reactor, y se detiene cuando hay algún tipo de limitación.

Se puede expresar la tasa o velocidad de crecimiento celular ($\mathbf{r_x}$) con la ecuación (1):

$$r_x = \frac{dX}{dt} = \mu X \qquad (1)$$

donde **μ** es la velocidad específica de crecimiento y **X** es la concentración de microorganismos.

A pesar de que el crecimiento celular es un fenómeno muy complejo, se puede obtener una descripción global razonablemente buena mediante el empleo del modelo de Monod, que es uno de los más comúnmente utilizados y que describe el crecimiento celular en función de la disponibilidad de un sustrato limitante (Monod, 1949). La reacción bioquímica se expresa de la siguiente manera:

$$\text{Sustrato (S)} + \text{Células (X)} \rightarrow \text{Más Células (X)} + \text{Producto (P)}$$

Según Monod (1949), la velocidad específica de crecimiento se puede expresar con la ecuación (2):

$$\mu = \mu_m \frac{S}{K_s + S} \qquad (2)$$

donde **S** es la concentración de sustrato, $\boldsymbol{\mu_m}$ es la velocidad específica máxima de crecimiento y $\mathbf{K_s}$ es la constante de Monod.

Por lo tanto, la expresión de la tasa de crecimiento celular queda de la forma indicada por la ecuación (3):

$$r_x = \frac{dX}{dt} = \frac{\mu_m X S}{K_s + S} \qquad (3)$$

Las ecuaciones anteriores muestran un crecimiento de los microorganismos (X) en función del reactivo limitante (S). En un proceso discontinuo, este sustrato irá disminuyendo con lo cual la velocidad de crecimiento de los microorganismos disminuirá.

A continuación, se introduce el concepto de coeficiente de producción (**Y**), que se define como la relación existente entre la masa de células producidas y la masa de sustrato consumido, como indica la ecuación (4):

$$Y = -\frac{dX}{dS} \qquad (4)$$

El signo negativo de la anterior expresión es debido a que existe una desaparición de sustrato. Si ahora se considera la expresión dada por la ecuación (5) de la tasa o velocidad de utilización de sustrato ($\mathbf{r_{su}}$), nos queda lo siguiente:

$$r_{su} = \frac{dS}{dt} \qquad (5)$$

De modo que el coeficiente de producción relaciona las velocidades de crecimiento celular y de consumo de sustrato, según indica la ecuación (6):

$$Y = -\frac{r_x}{r_{su}} \qquad (6)$$

Sustituyendo en la expresión anterior la velocidad de crecimiento celular por su expresión, se puede obtener la velocidad de consumo de sustrato de acuerdo con la ecuación (7):

$$r_{su} = -\frac{\mu_m X S}{Y (K_s + S)} \qquad (7)$$

Teniendo en cuenta que se está considerando un medio discontinuo, habrá un momento donde empezará a desaparecer el sustrato y la tasa media de crecimiento de microorganismos empezará a disminuir hasta hacerse constante y cuando empiecen a morirse los microorganismos presentes empezará a ser negativa. Esto se puede contemplar en las ecuaciones anteriores considerando la tasa o velocidad de muerte de los microorganismos ($\mathbf{r_d}$), que viene indicada por la ecuación (8):

$$r_d = -k_d X \qquad (8)$$

donde $\mathbf{k_d}$ es la constante de muerte de los microorganismos.

Por lo tanto, la tasa o velocidad neta de crecimiento de los microorganismos (r_x'), introduciendo la velocidad de muerte de los microorganismos, queda como expresa la ecuación (9):

$$r_x' = \frac{\mu_m X S}{K_s + S} - k_d X \qquad (9)$$

Como se observa en la expresión anterior, en un sistema en discontinuo las células no pueden reproducirse indefinidamente, y al final de una primera fase de crecimiento exponencial, la velocidad va disminuyendo a medida que aparecen limitaciones, dándose una fase estacionaria donde la concentración de microorganismos se mantiene constante y finalmente se llega a una fase de muerte celular donde desciende; esta fase, en realidad, se ve incrementada por la depredación entre los distintos microorganismos. Esto se puede ver reflejado en la Figura I.8, que muestra el modelo de crecimiento bacteriano (número de bacterias) en función del tiempo:

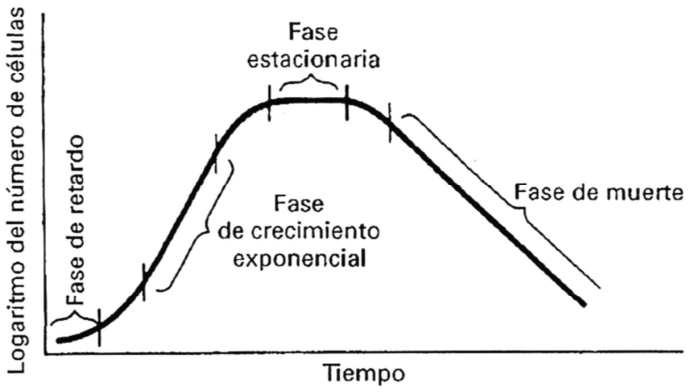

Figura I.8. Curva de crecimiento bacteriano (Metcalf, 2003).

En dicho modelo se pueden distinguir cuatro fases diferenciadas (Metcalf, 2003):

- Fase de retardo: representa el tiempo necesario para que los microorganismos se aclimaten a las nuevas condiciones ambientales y comiencen a dividirse, de tal forma que se produce un crecimiento lento.
- Fase de crecimiento exponencial: durante esta fase, la célula se divide a una velocidad determinada por su tiempo de generación y su capacidad de procesar alimento (tasa constante de crecimiento porcentual).
- Fase estacionaria: en esta fase, la población permanece constante. Esto puede ocurrir debido a que las células han agotado el sustrato o los nutrientes necesarios para el crecimiento, o bien como consecuencia de que la generación de células nuevas se compensa con la muerte de células viejas.
- Fase de muerte exponencial: durante esta fase, la tasa de mortalidad de bacterias excede la de generación de células nuevas. La tasa de mortalidad suele ser función de la población viable y de las características ambientales.

Se trata de una fase endógena en la que los microorganismos consumen sus propias reservas protoplasmáticas en primer lugar y posteriormente unos sirven de alimento a otros, denominándose este fenómeno como predación, por lo que se produce un decrecimiento. En algunos casos, la fase de muerte exponencial se corresponde con la inversa de la fase de crecimiento exponencial.

6.3. Diversidad bacteriana en los biorreactores de membrana con y sin lecho móvil

En las plantas de tratamiento de aguas residuales, las comunidades microbianas desarrolladas se caracterizan por presentar una amplia y compleja diversidad genética y metabólica (Erijman et al., 2011). Asimismo, estas comunidades son dinámicas, permitiendo una continua adaptación de las poblaciones microbianas a las nuevas condiciones ambientales u operacionales (Reboleiro-Rivas, 2014).

En este sentido, el conocimiento de las actividades enzimáticas que tienen lugar en un microcosmos, como es el caso de los biorreactores de membrana con y sin lecho móvil estudiados en este trabajo, es junto con el conocimiento de la concentración de biomasa (como cuantificación de la densidad de los distintos grupos bacterianos) de vital importancia en la caracterización biológica del sistema.

Durante el proceso de génesis de un fango activo y, en particular, de una biopelícula fijada al relleno del lecho móvil, complejas comunidades microbianas utilizan tanto enzimas extracelulares como intracelulares para hidrolizar y, en última instancia, mineralizar compuestos orgánicos. Estas actividades se pueden aplicar como indicadores de poblaciones específicas, como medida de biomasa activa y como indicadores de procesos específicos en un biorreactor como la eliminación de materia orgánica, nitrógeno y fósforo.

Algunas de las actividades enzimáticas más importantes aplicadas en los procesos biológicos de depuración son las fosfatasas y glucosidasas, debido en parte a que la composición química de un agua residual presenta una fracción orgánica mayoritariamente formada por carbohidratos y proteínas, en cuya hidrólisis juegan un importantísimo papel las actividades enzimáticas descritas.

Los análisis se realizarán sobre los licores mezcla de los reactores biológicos y en las biopelículas producidas sobre el relleno del lecho móvil.

Las fosfatasas son enzimas que hidrolizan ésteres de fosfatos, liberando grupos fosfato al medio. Existen dos tipos de fosfatasas que presentan diferencia en lo referente al valor de pH óptimo de acción y en cuanto a la preferencia frente a determinados sustratos. De esta forma, existen fosfatasas ácidas y alcalinas.

Las glucosidasas (a-glucosidasas y ß-glucosidasas) hidrolizan disacáridos procedentes de la degradación de polisacáridos. La primera de ellas hidroliza maltosa y sacarosa, mientras que la segunda hidroliza la celobiosa.

Los estudios sobre actividades enzimáticas proporcionarán datos importantes para el conocimiento de la actividad biológica desarrollada en los diferentes sistemas. Es por ello necesario completar esta información con la procedente de los estudios sobre la eliminación de materia orgánica y nutrientes. Las conclusiones obtenidas mediante esta transferencia de resultados permitirán conocer la influencia del relleno y la formación de biopelículas fijadas al mismo en las diferentes variables del proceso y en la actividad biológica responsable del proceso depurador. Estas conclusiones permitirán la realización de mejores diseños a la vez que facilitarán la fase de explotación. Además, las conclusiones alcanzadas en esta fase pueden ser también extensibles a otros sistemas convencionales de tratamiento.

Por otro lado, la diversidad bacteriana existente en cada uno de los sistemas de tratamiento de aguas residuales se evaluó a partir de la electroforesis en gel con gradiente de temperatura (TGGE). Esta técnica de biología molecular permite abordar la identificación de microorganismos y el estudio de la biodiversidad bacteriana a partir de una muestra de ADN proveniente de la biomasa suspendida y biopelícula adherida presente en los biorreactores de membrana con y sin lecho móvil objeto del presente estudio. Se trata de una de las técnicas más ampliamente utilizadas (Muyzer et al., 1993) y el empleo de la misma proporciona el perfil de la diversidad genética de una comunidad microbiana, permitiendo el estudio de la estructura y dinámica de la misma (Wittebolle et al., 2005). En un TGGE, el número de bandas, su posición precisa y la intensidad de las mismas ofrecen una estimación del número y la abundancia relativa de las poblaciones dominantes en la muestra (Boon et al., 2002). Con esta técnica se

pueden estudiar las variaciones poblacionales a lo largo del tiempo o en función de las condiciones ambientales y/o experimentales (Muyzer, 1999). Sin embargo, el estudio de las comunidades microbianas mediante TGGE presenta algunas limitaciones, entre las cuales cabe destacar que sólo son capaces de detectar las poblaciones mayoritarias que componen la comunidad estudiada. Además, puede ocurrir una co-migración de diferentes fragmentos de ADN que conlleva la agrupación de diferentes secuencias en una misma banda, impidiendo la identificación filogenética de las poblaciones microbianas. Finalmente, es necesario enfatizar que esta técnica no proporciona datos cuantitativos absolutos de las poblaciones (Reboleiro-Rivas, 2014).

En esta línea, las comunidades microbianas de bacterias nitrificantes y desnitrificantes de los diferentes sistemas objeto de estudio fueron analizadas mediante pirosecuenciación para detectar y cuantificar la contribución de dichas bacterias a la comunidad bacteriana total. Esto permitía complementar el estudio cinético en relación a la biomasa heterótrofa y autótrofa.

7. Tratamientos biológicos de las aguas residuales

7.1. Introducción

Como se ha indicado anteriormente, los objetivos del tratamiento biológico de las aguas residuales son la coagulación de la materia orgánica disuelta convirtiéndola en materia orgánica coloidal y eliminación de los sólidos coloidales no sedimentables, así como la estabilización de la materia orgánica. En el caso de las aguas residuales urbanas, los tratamientos biológicos tuvieron en un principio como objeto la reducción de la materia orgánica presente aunque posteriormente se les han ido dando otros usos como la eliminación de nitrógeno y/o la eliminación de fósforo de dichas aguas. A menudo, la eliminación de compuestos a nivel de traza que puedan resultar tóxicos, también constituye un objetivo de tratamiento importante.

Los principales procesos biológicos utilizados en el tratamiento de las aguas residuales se pueden dividir en cuatro grupos principales, aerobios, anóxicos, anaerobios y combinación de aerobios con anóxicos o anaerobios. Estos procesos se dividen a su vez en función de que el tratamiento se lleve a cabo en sistemas de cultivo en suspensión, cultivo fijo o una combinación de ambos (Trapote-Jaume, 2011). A continuación, se presenta la Tabla I.4 donde se puede observar dicha clasificación.

Tabla I.4. Procesos biológicos utilizados en el tratamiento de las aguas residuales (modificado de Metcalf, 2003).

Procesos biológicos		
Tipo	**Subtipo**	**Denominación común del proceso**
Procesos aerobios	**Cultivo en suspensión**	Fangos activos
		Nitrificación de cultivos en suspensión
		Lagunas aerobias
		Digestión aerobia de fangos
		Estanques aerobios de alta carga
	Cultivo fijo	Lechos bacterianos
		Filtros de pretratamiento
		Contactores biológicos rotativos
		Reactores de lecho compacto
	Procesos combinados	Lechos bacterianos-Fangos activos
		Fangos activos-Lechos bacterianos
Procesos anóxicos	**Cultivo en suspensión**	Desnitrificación con cultivo en suspensión
	Cultivo fijo	Desnitrificación con cultivo fijo
Procesos anaerobios	**Cultivo en suspensión**	Digestión anaerobia
		Proceso anaerobio de contacto
	Cultivo fijo	Filtro anaerobio
		Lagunas anaerobias
Procesos combinados aerobios-anaerobios	**Cultivo en suspensión**	Fase única nitrificación-desnitrificación
	Procesos combinados de cultivo fijo	Nitrificación-desnitrificación
		Lagunas facultativas
		Lagunas de maduración
		Lagunas anaerobias-facultativas
		Lagunas anaerobias-facultativas-aerobias

En todos estos procesos es necesario retener en el sistema la biomasa creada con el objetivo de que se produzca el proceso. En los de cultivo en suspensión se suele recurrir a una decantación y recirculación de la biomasa, mientras que en los de cultivo fijo la retención de la misma queda asegurada por las características del propio proceso (Ferrer-Polo and Seco-Torrecillas, 2007).

En el presente trabajo, se ha abordado el estudio de los reactores de biopelícula de lecho móvil (MBBR), a los que se les ha integrado un módulo de membranas, constituyendo lo que se denomina como biorreactor de membrana con lecho móvil (MBBR-MBR).

Los sistemas MBBR constituyen una tecnología intermedia entre los procesos de cultivo en suspensión y los procesos de cultivo fijo, o de biopelícula. El principio básico de este proceso es el crecimiento de la biomasa en soportes plásticos, formando una biopelícula, que se encuentran en suspensión en el reactor biológico (Trapote-Jaume, 2011). El medio soporte puede encontrarse fijo en una columna, y el agua fluye formando una fina película, o puede encontrarse en movimiento dentro del fluido (Ferrer-Polo and Seco-Torrecillas, 2007).

El espesor de la película de biomasa (biopelícula) oscila entre 0.1 y 2 mm y consta de una capa superficial donde el proceso que se realiza es idéntico al de los fangos activos (la materia llega al sistema por transporte convectivo), y una interna donde el transporte de sustrato, aceptor de electrones y nutrientes se produce por transporte molecular (difusión). Por ello, los modelos que se han desarrollado para representar el comportamiento de la biopelícula consideran tanto la reacción bioquímica como los procesos de transferencia de materia. Esta capa biológica es un sistema muy complejo y su composición no es homogénea. La proporción de biomasa activa es mayor en la superficie que en el interior donde se acumula una mayor cantidad de residuo orgánico inerte. En todo caso, se produce una migración continua de productos desde el interior hasta el exterior, donde son arrastrados del sistema por los esfuerzos cortantes superficiales, lo cual permite, así mismo, mantener constante el espesor total de la capa. Si no fuera así, cuando el espesor aumentara excesivamente, el sustrato no podría alcanzar la capa interna y los microorganismos situados en ella se desprenderían del soporte, siendo arrastrados por el agua. En la Figura I.9 se muestra una representación esquemática de la biopelícula (Ferrer-Polo and Seco-Torrecillas, 2007).

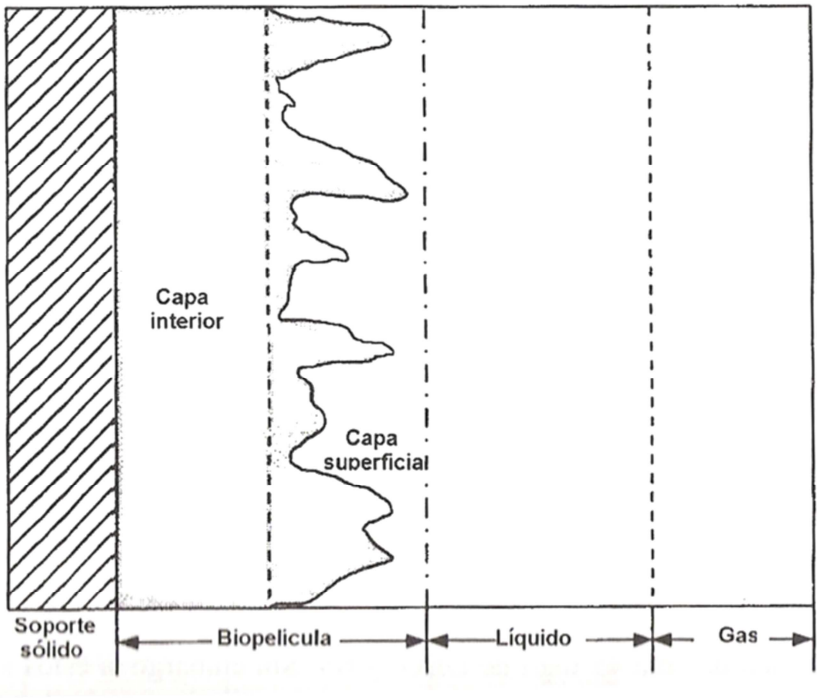

Figura I.9. Representación esquemática de la biopelícula (Ferrer-Polo and Seco-Torrecillas, 2007).

7.2. Reactores de biopelícula de lecho móvil (MBBRs)

7.2.1. Introducción

Debido a la exigencia cada vez mayor en el tratamiento de aguas residuales, tanto urbanas como industriales, y de las necesidades de reutilización, los trabajos de investigación en este campo han ido dirigidos a nuevos sistemas de tratamiento biológico que aumenten la capacidad de tratamiento de los reactores convencionales, incrementando la cantidad de microorganismos presentes en el sistema, sin tener que aumentar el volumen o el número de reactores. Dentro de las nuevas tecnologías para el tratamiento biológico se encuentran los procesos de biomasa fija sobre lecho móvil.

Como se ha indicado anteriormente, el principio básico del proceso de lecho móvil es el crecimiento de la biomasa en soportes plásticos que se mueven en el reactor biológico mediante la agitación generada por sistemas de aireación (reactores aerobios) o por sistemas mecánicos (en reactores anóxicos o anaerobios). Los soportes son de material plástico con densidad próxima a 1 g cm^{-3}, lo cual les permite moverse fácilmente en el reactor, incluso con porcentajes de relleno del 70%. En la Figura I.10 se

presenta un esquema con el movimiento del relleno en un reactor aerobio y en un reactor anóxico o anaerobio.

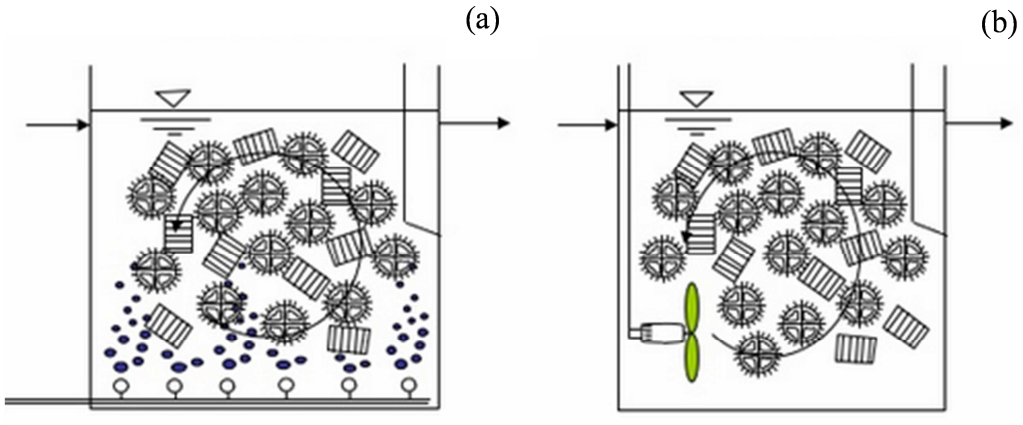

Figura I.10. Esquema del movimiento del relleno en un reactor aerobio (a) y en un reactor anóxico o anaerobio (b) mediante el empleo de un sistema de aireación en el fondo del reactor o un sistema mecánico de agitación, respectivamente (modificado de Zalakain and Manterola, 2011).

Inicialmente, las investigaciones se centraron en el uso de procesos de lecho fijo, sin embargo, se ha observado que este tipo de procesos presenta una serie de inconvenientes operacionales como es el atascamiento del lecho por crecimiento excesivo de biomasa, que obliga a la limpieza periódica del mismo. Estos inconvenientes han llevado a la necesidad de crear simples procesos de biopelícula o "biofilm" que los eliminen y que faciliten su operación tales como los procesos de lecho móvil (Zalakain and Manterola, 2011).

En estos procesos, la biopelícula que se forma en las paredes del relleno se caracteriza por una mayor efectividad respecto a los flóculos biológicos. A su vez, los soportes plásticos empleados contienen una elevada superficie específica por unidad de volumen que los convierte en elementos ideales para el desarrollo de la biopelícula. Estas dos particularidades hacen que los reactores de lecho móvil sean de volumen mucho menor que los de fangos activos (Trapote-Jaume, 2011). Por otra parte, el crecimiento de la biopelícula en el soporte hace que las capas más internas entren en anaerobiosis haciendo que se desprenda parte de la misma de forma automática; este hecho hace que la formación de biopelícula necesaria según la carga, tenga lugar de forma automática. A su vez estos sólidos desprendidos del soporte vienen a ser parte del exceso de fangos que hay que extraer del sistema (purga de fangos) y, por tanto, no es imprescindible la recirculación de los mismos al reactor. La operación de la planta

queda muy simplificada ya que la extracción de los fangos en exceso del reactor es automática y no se requiere de una recirculación.

Respecto a la ingeniería del proceso, el sistema de aireación está formado por una parrilla de tubos perforados de acero inoxidable que evita problemas de pérdida de eficiencia, cambio de difusores, etc. En cuanto al proceso de separación de la biomasa procedente del reactor biológico, hay varias alternativas. Se pueden emplear decantadores que se diseñan como decantadores secundarios considerando velocidades ascensionales. Por otra parte, y aunque inicialmente comenzaron a emplearse en aguas residuales industriales, también se pueden utilizar sistemas de flotación para la separación en el tratamiento de aguas residuales urbanas. Además, suele ser cada vez más habitual utilizar tecnología de membranas a continuación del reactor biológico (Zalakain and Manterola, 2011).

Los requerimientos de oxígeno, nutrientes (para el caso de vertidos industriales) y producción de fangos son similares a otros procesos biológicos de biomasa en suspensión, con lo que los costes de explotación de un proceso de lecho móvil vienen a ser similares a los convencionales de fangos activos. El ahorro en la reducción de volumen, tanto del reactor como del sistema de separación de sólidos, y en el sistema de aireación se compensan con el gasto en el soporte plástico haciendo que los costes de inversión sean también similares. Los costes de personal se ven reducidos debido a que el funcionamiento de la instalación es automático (Zalakain and Manterola, 2011).

Este tipo de procesos puede aplicarse tanto a plantas de tratamiento para la biodegradación de materia orgánica como para instalaciones con eliminación de nutrientes, en aguas residuales urbanas e industriales, permitiendo alcanzar los objetivos de los tratamientos biológicos, indicados al inicio de este apartado. Otra aplicación es el empleo de esta tecnología en la rehabilitación de plantas de fangos activos que tratan únicamente materia orgánica para su ampliación a la eliminación de nutrientes de forma sencilla y sin la necesidad de construir nuevos reactores biológicos.

7.2.2. Clasificación

Dentro de los sistemas de biomasa adherida sobre soporte móvil, se pueden distinguir dos grupos, los sistemas híbridos con recirculación de fangos y los sistemas puros sin recirculación de fangos.

Los sistemas híbridos contienen biomasa en suspensión y biomasa adherida en el mismo reactor. En estos sistemas, para conseguir una concentración adecuada de biomasa en suspensión en el reactor, se necesita recircular parte de los fangos sedimentados, en el caso de un decantador secundario posterior al reactor biológico, o parte de los fangos retenidos en la membrana, en el caso de un tanque con un módulo de membranas posterior al biorreactor, hasta el propio reactor (Figure III.1b y Figure III.1c de la sección **Materials and Methods**).

En los sistemas puros, el crecimiento bacteriano se da exclusivamente en los soportes plásticos, no existiendo recirculación de fangos, por lo que la concentración de sólidos en suspensión del licor mezcla (MLSS) en el reactor biológico es similar a la concentración en el agua residual influente, más los sólidos que se van desprendiendo de la biopelícula (Figure III.1d de la sección **Materials and Methods**).

7.2.3. Concepto, características y formación de la biopelícula

En la actualidad está bien establecido el hecho de que el estilo de vida bacteriano más común en los ambientes naturales es aquel en que las bacterias se adhieren a una superficie formando una estructura conocida como biopelícula (Decho, 2000; Watnick and Kolter, 2000) donde encuentran las necesidades fundamentales para su desarrollo (Gómez et al., 2000).

Se puede hablar de biopelícula o “biofilm” como una estructura compleja formada por agregados celulares (grupos de células densamente empaquetados) y huecos intersticiales, adherida a un material o interfase que puede ser de naturaleza abiótica (rocas, metales, vidrios, plásticos, etc.) o biótica (mucosa intestinal, plantas, etc.) (Lewandowski et al., 1995). Su estructura es morfológica y fisiológicamente distinta a la de bacterias libres, utilizándose incluso mediadores químicos intercelulares para desarrollar la biopelícula (Geesey, 2001).

Según Characklis and Wilderer (1989), las biopelículas son conjuntos de microorganismos que están dispuestos en forma de capas en los que sus polímeros extracelulares hacen la unión. Hay procesos biológicos donde los microorganismos se fijan en la superficie de un material, medio o soporte, y crean una capa con alto contenido en agua y una gran concentración de biomasa que recubren el soporte y que se denomina "biopelícula fija".

Zhang and Bishop (1994), consideran las biopelículas como agrupaciones de células simples o microcolonias embebidas en una matriz polimérica de origen microbiano, formada sobre un sustrato, las cuales les permiten realizar sus funciones vitales de forma más selectiva y permitiéndoles a la vez captar una mayor concentración de nutrientes.

La característica principal de esta asociación de células consiste en que estos microorganismos están unidos a la superficie de un sólido que actúa de soporte.

Las películas biológicas, que son células inmovilizadas, tienen un interés cada vez más importante en procesos utilizados en el control de la contaminación, tales como lechos móviles, filtros percoladores, lechos inundados, contactores biológicos rotativos, etc. Estos procesos de biopelícula son simples, fiables y estables debido a que esa inmovilización natural permite una retención y acumulación de biomasa excelente, sin necesidad de otros sistemas de separación de sólidos (Rittmann and McCarty, 2001).

7.2.3.1. Características fisicoquímicas y microbiológicas

Existen varias hipótesis que intentan explicar la estructura de las biopelículas. Por un lado, Bishop (1996) considera las biopelículas como sistemas estratificados que crecen de forma perpendicular al soporte y en los que habría una transferencia de masa desde la capa superficial a la capa interna. Por su parte, Lewandowski et al. (1995) las consideran sistemas tridimensionales donde existen estructuras heterogéneas, con canales que están llenos de agua y por donde circulan los nutrientes. Sin embargo, Wimpenny and Colasanti (1997) añaden que además de los otros modelos existe otro que es el de las biopelículas densas y que especifica que en función de la concentración de nutrientes, la estructura de la biopelícula será más o menos densa.

El crecimiento de toda la biopelícula será el resultado de la transferencia de masa y su posterior transformación por parte de los componentes de la biopelícula. Los nutrientes circularán a través de ella de dos modos, uno sobre los canales o las capas superficiales mediante difusión o convección y otro en las capas interiores o celulares mediante fenómenos de transporte de masa (Beer and Stoodley, 1995).

Respecto a la composición química de las biopelículas, en primer lugar, hay que destacar que son sistemas muy hidratados que facilitarán la transferencia de nutrientes. La biopelícula está compuesta por microorganismos, sustancias poliméricas extracelulares (EPS), cationes multivalentes, partículas orgánicas, inorgánicas en estado coloidal o disuelto. El principal responsable de la integridad funcional y estructural de la biopelícula son las EPS, que están constituidas por biopolímeros, polisacáridos, proteínas y otras macromoléculas como ADN, lípidos y sustancias húmicas (Nielsen et al., 1993). La composición de las EPS determina la mayor parte de las propiedades más importantes de la biopelícula, como densidad, porosidad, difusividad, resistencia a la fricción, conductividad térmica y actividad metabólica (Zhang et al., 1999).

En cuanto a la composición bacteriana, esta va a responder a la capacidad de los grupos bacterianos de adaptarse a las condiciones del medio donde se desarrollan las biopelículas. Independientemente de lo comentado anteriormente, las biopelículas no solamente van a estar compuestas por bacterias, sino que microorganismos como protozoos, hongos, rotíferos, nematodos, anélidos e insectos pueden formar parte de ellas (Bitton, 1994).

7.2.3.2. Formación de la biopelícula

Se ha demostrado como la colonización bacteriana de una superficie y la estructura de la biopelícula que va a formar están controladas por varios factores como la hidrodinámica, el genotipo de las células que la forman y la química de la superficie (Geesey, 2001). Las fases que determinan la formación de una biopelícula madura son diversas y complejas.

En el proceso de colonización y formación de una biopelícula se pueden establecer las siguientes etapas (Allison et al., 2000; Stephens, 2002):

- Etapa 1: se produce un acondicionamiento del medio soporte al adsorberse moléculas orgánicas sobre su superficie.
- Etapa 2: las bacterias perciben la proximidad de la superficie. En el caso de las células planctónicas esta percepción está mediada por la emisión al medio de señales moleculares que difunden a través del medio y generan un gradiente de concentración, el cual indica, cuando aumenta, la proximidad de una superficie (Costerton, 1999).
- Etapa 3: las células pasan desde el líquido hasta el medio soporte acondicionado, estando controlado este paso, principalmente, por interacciones electrostáticas. Una vez detectada la superficie, los microorganismos se han de transportar hacia la misma y este fenómeno puede tener lugar mediante transporte difusivo (movimiento Browniano), transporte convectivo de las células o mediante transporte activo donde las células bacterianas se mueven cerca de la superficie del soporte pudiendo haber un choque casual con la superficie o quimiotaxis en respuesta a un gradiente de concentración en la región interfase (Costerton, 1999).

  Hay que tener en cuenta que las bacterias se unen a una superficie u otra en función de que el medio donde se encuentren sea rico o no en nutrientes, es decir cuando el medio es rico en nutrientes se unen a cualquier superficie pero cuando ocurre lo contrario se fijan sobre una superficie rica en estos (Watnick and Kolter, 2000), de tal forma que la bacteria tiene acceso a los nutrientes en ambientes tanto ricos como pobres en estos.

- Etapa 4: se lleva a cabo la adhesión de las células al medio soporte. La fase de adhesión comienza con un proceso reversible en el que las células llegan a la superficie, se adhieren a ella por un tiempo limitado y se separan después. La desorción (desprendimiento) se puede producir debido a factores físicos, químicos y/o biológicos. En este proceso hay un intercambio continuo entre células libres y fijadas, siendo difícil establecer distinción entre la actividad de las células adheridas y las libres. En el momento en el que hay bacterias que se unen de forma irreversible, para un tiempo de adsorción suficiente, a la superficie tiene lugar el acondicionamiento físico de la misma, depositando

sustancias nutritivas (macromoléculas orgánicas) que atraen a las bacterias y permiten su adherencia y crecimiento.

La adhesión microbiana varía en función de las características de la superficie (Geesey, 2001), dependiendo de su carácter hidrofóbico-hidrofílico, del área de superficie disponible, así como del tipo de microorganismos colonizadores y del pH del medio.

En esta fase, las bacterias emplean flagelos y pilis para moverse a lo largo de la superficie hasta que contactan con otras bacterias, formando una microcolonia o aumentando la ya existente (Costerton, 1999; Watnick and Kolter, 2000).

Para la adhesión irreversible las bacterias cuentan con fimbrias, que favorecen adhesiones de tipo específico, y con EPS, que favorecen adhesiones de tipo inespecífico (Costerton, 1999). Respecto a las EPS, las células las producen, formando una matriz polisacárida que se extiende desde la superficie de las bacterias adhiriendo a estas en la superficie soporte. Las células adsorbidas crecen a expensas del sustrato y del agua incrementando el número de células en la biopelícula. A su vez, también se pueden producir cantidades significativas de productos excretando algunos de ellos y constituyendo parte de la biopelícula. De esta forma, se produce la adhesión de las células microbianas y otros organismos, así como material particulado, a la biopelícula.

Por lo tanto, para que las bacterias puedan ser miembros de una biopelícula han de reprimir la síntesis de flagelos que desestabilizarían la estructura, y activar la síntesis de exopolisacáridos que la refuerzan (Watnick and Kolter, 2000).

- Etapa 5: tiene lugar el crecimiento de las células adheridas al medio soporte. Como se ha indicado en la etapa anterior, tras la adsorción irreversible se produce un incremento en el número de células de la biopelícula a expensas del sustrato.

Cuando las biopelículas alcanzan su madurez y máximo grosor, se puede considerar que el crecimiento neto de la microbiota es cero. En estas circunstancias, las bacterias no se dividen pero son viables y cultivables. De esta forma, en las biopelículas maduras la división celular es infrecuente, utilizando esa energía en exceso para producir exopolisacárido que podría ser digerido por las células en momentos de carencia (Watnick and Kolter, 2000). A partir de este momento, la biopelícula funciona como un conjunto de microorganismos a la vez independientes y relacionados entre sí, captando células libres y presentes en el medio acuoso (Decho, 2000). La captura de macromoléculas orgánicas y células microbianas podría considerarse como un proceso normal de floculación influyendo el tamaño de la partícula, así como la fuerza y la composición iónica.

- Etapa 6: se produce un desprendimiento o separación de parte de la biopelícula formada. En el transcurso de la formación y estabilización de la biopelícula, parte de la misma se separa y vuelve al agua, estas células vuelven a su estado libre quizás para formar otros agregados (Costerton, 1999). Esta separación puede ser debida al esfuerzo cortante producido por el movimiento del agua (erosión), a la acción mecánica de otras partículas que chocan contra la biopelícula (abrasión), a la pérdida de adherencia de la biopelícula y al aumento en espesor por el crecimiento de esta.

  Además, durante el crecimiento de la biopelícula, se crea una estratificación en los grupos fisiológicos debido, fundamentalmente, a la limitación de la transferencia de oxígeno. Conforme crece la biopelícula, el oxígeno desaparece del interior produciéndose fenómenos anaerobios en los que se forman gases en el interior de la biopelícula como el CH_4, H_2S y otros, de manera que provocan desprendimientos masivos de biopelícula, especialmente aquella que está inactiva, produciéndose de esta forma una regeneración continua de la misma.

Como conclusión, se puede indicar que el crecimiento neto de las biopelículas es el resultado de un balance entre colonización, multiplicación y desorción, de modo que unas condiciones óptimas en el crecimiento determinarán el desarrollo óptimo de una biopelícula (Lewandowski et al., 1995).

Todo el proceso de formación de una biopelícula queda reflejado en la Figura I.11.

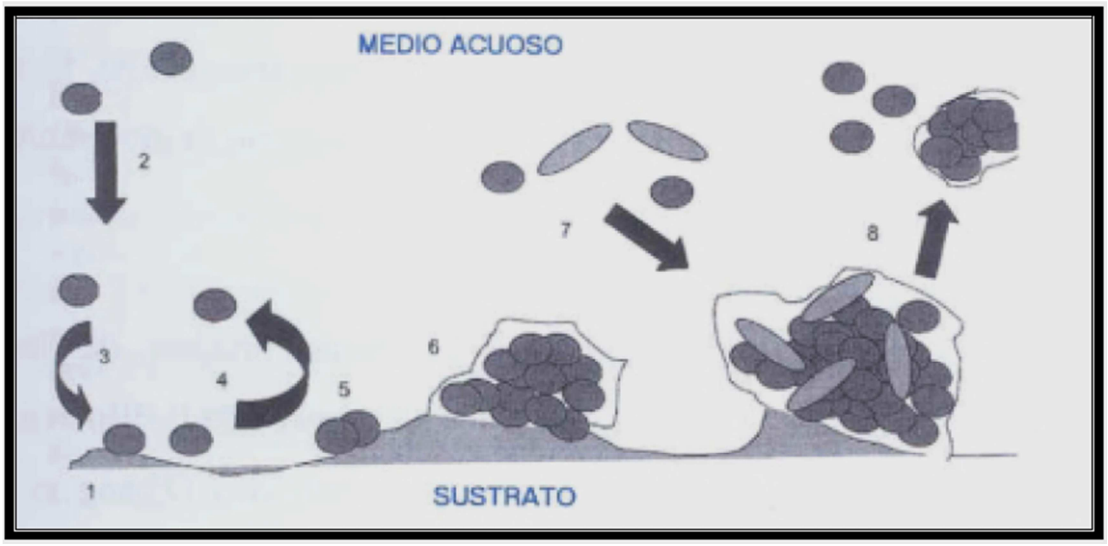

Figura I.11. Etapas en la formación de una biopelícula. (1) Acondicionamiento del soporte (Etapa 1). (2) Percepción de la superficie por parte de las células y transporte de las mismas desde el líquido hasta el soporte (Etapas 2 y 3). (3), (4) y (5) Adhesión de las células al soporte (Etapa 4). (6) y (7) Crecimiento de las células (Etapa 5). (8) Desprendimiento de parte de la biopelícula formada (Etapa 6) (Phillips et al., 2011).

7.2.4. Fundamentos de operación

La biomasa adherida en los procesos basados en la formación de biopelícula origina la capacidad de operar a altas concentraciones de biomasa activa lo cual aumenta la velocidad de eliminación biológica y hace a estos procesos más resistentes a sobrecargas y compuestos tóxicos (Lee et al., 2006). En los procesos de biopelícula, la biomasa puede especializarse para objetivos de tratamiento específicos (Ødegaard, 2006). Por ejemplo, la nitrificación y desnitrificación se pueden llevar a cabo con éxito en procesos de biopelícula ya que las bacterias nitrificantes, que son microorganismos de crecimiento más lento, son retenidos por la biopelícula (Wang et al., 2006; Aygun et al., 2008).

Mientras que los sistemas de biopelícula han sido desarrollados para aprovechar estas características, también tienen sus retos. Por ejemplo, los filtros percoladores requieren grandes volúmenes, los contactores biológicos rotativos están sujetos a fallos mecánicos, los biofiltros sumergidos de cultivo fijo presentan problemas de mantenimiento con la distribución de flujo sobre la superficie del medio y los biofiltros granulares requieren contralavados y, por consiguiente, no pueden operar de forma

continua. Los sistemas MBBR se han desarrollado para superar estos inconvenientes, aprovechando las ventajas de dichas tecnologías (Ødegaard, 2006). Las ventajas de los MBBR sobre el resto de reactores de biopelícula incluyen la ausencia de contralavados, no tienen tendencia a obstruirse, pueden funcionar de manera continua, proporcionan una alta zona superficial para el crecimiento microbiológico, y tienen una baja pérdida de carga (Rusten et al., 1998). Desde su introducción en el tratamiento de aguas residuales al final de la década de los ochenta, los MBBR han sido utilizados con éxito en el tratamiento de aguas residuales urbanas e industriales tales como el tratamiento de aguas residuales procedentes del procesado de productos lácteos (Aygun et al., 2008) y de la producción de papel y patatas fritas (Ødegaard et al., 1994).

Las ventajas de los MBBR se alcanzan usando pequeños soportes en suspensión que se mueven libremente en la fase líquida del reactor. Dichos soportes se mantienen en el reactor mediante el empleo de un tamiz en la salida. Los soportes normalmente son pequeños cilindros de polietileno diseñados para conseguir una alta superficie específica para el crecimiento de la biopelícula. Como resultado, el reactor no requiere recirculación de fangos para alcanzar las altas concentraciones de biomasa requeridas. Además, se puede lograr un alto tiempo de retención celular (edad del fango) y, por lo tanto, la generación de fango es más baja que la de los sistemas de fangos activos convencionales. Esta es una importante ventaja debido a los costes crecientes de tratamiento de los fangos. El movimiento de los soportes está originado por aireación de burbuja gruesa en aplicaciones aeróbicas y por mezcladoras en procesos MBBR anaeróbicos.

El parámetro más importante en el diseño de un MBBR es la región de la biopelícula y, por consiguiente, la zona superficial efectiva del soporte. La superficie específica en los sistemas MBBR se establece en base al propósito del tratamiento y se alcanza estableciendo la proporción de relleno adecuada. Los sistemas MBBR pueden funcionar con cargas orgánicas e hidráulicas más altas si se dispone de suficiente superficie. Se recomienda mantener la fracción de relleno por debajo del 70% para permitir que los soportes se muevan libremente dentro del reactor (Ødegaard, 2006). El rendimiento de los sistemas MBBR se puede incrementar aumentando el tiempo de retención hidráulico (TRH), o a través del empleo de varios compartimentos en el sistema MBBR (Leiknes and Ødegaard, 2007).

Di Trapani et al. (2008) estudiaron diferentes fracciones de relleno para los MBBR. Concluyeron que había una fracción de relleno óptima por encima de la cual el rendimiento de eliminación del reactor disminuía. Esto se atribuyó a la competencia entre la biomasa en suspensión y la biomasa adherida y a la importancia de los sólidos en suspensión en el MBBR. Con un aumento en la fracción de relleno, la concentración de biomasa en suspensión que crece disminuye y concentraciones de biomasa en suspensión bajas pueden reducir la eficiencia de eliminación de los sistemas MBBR ya que tienen un papel muy importante en la hidrólisis enzimática y en la biofloculación que tiene lugar en el reactor. Se observó que una fracción de relleno del 35% presentaba un rendimiento de eliminación de demanda química de oxígeno (DQO) mayor que una fracción de relleno del 66%. Por otro lado, una fracción de relleno del 66% tenía un rendimiento en el proceso de nitrificación ligeramente mejor debido a la existencia de concentraciones mayores de microorganismos nitrificantes de crecimiento lento que podían ser retenidos en el reactor. Estos resultados indican que la fracción de relleno es un parámetro importante en el diseño de los sistemas MBBR y debe ser elegida en base a los objetivos del tratamiento.

En cuanto a la cantidad de oxígeno necesaria, Wang et al. (2006) recomendaron que el oxígeno disuelto en el reactor se mantuviera por encima de 2 mg L^{-1} para una eliminación eficiente de DQO. En sus conclusiones, la disminución del oxígeno disuelto desde 2 a 1 mg L^{-1} reducía el rendimiento de eliminación de DQO en un 13% indicando que el oxígeno disuelto es un factor limitante. Por otro lado, el aumento del oxígeno disuelto desde 2 a 6 mg L^{-1} aumentaba la eficiencia de eliminación de DQO solamente en un 5.8%. Sus resultados también mostraron que en un único reactor MBBR se podían alcanzar la nitrificación y desnitrificación simultáneas con un TRH de 6 h debido a la limitación del proceso de difusión de oxígeno dentro de la biopelícula. El rendimiento de eliminación de nitrógeno más alto (89.1% de media) se obtuvo cuando el oxígeno disuelto se mantuvo en 2 mg L^{-1}. A concentraciones de oxígeno disuelto más bajas, se generaban condiciones anóxicas y la conversión de amonio a nitrito o nitrato era limitada, y a concentraciones de oxígeno disuelto mayores, las condiciones anóxicas y, por lo tanto, el proceso de desnitrificación en las capas más profundas de la biopelícula no tenía lugar.

Se ha mostrado en varios estudios que la concentración de la biomasa, tanto en la forma adherida como en suspensión, en el volumen del reactor MBBR es

aproximadamente la misma que en los procesos de fangos activos (2-5 kg m^{-3} de volumen del reactor). Sin embargo, el rendimiento de eliminación del reactor MBBR es varias veces mayor, lo que indica que los procesos MBBR son más viables. Rusten et al. (1998) descubrieron que a mayores velocidades de carga orgánica, los reactores MBBR presentan mayores concentraciones de sólidos en suspensión en el efluente. Sin embargo, se debería observar que la concentración de sólidos en suspensión final puede verse afectada de manera diferente dependiendo del tipo de velocidad de carga considerado.

En la bibliografía se ha informado sobre la producción de fangos en los reactores MBBR. Tanto Orantes and González-Martínez (2004) como Aygun et al. (2008) concluyeron que la producción de fango seguía una relación lineal con la velocidad de carga de DQO y era menor que en los procesos de fangos activos convencionales. Los resultados de Orantes and González-Martínez (2004) mostraron que los coeficientes de producción aumentaban desde 0.12 a 0.40 kg SST kg DQO $total^{-1}$ cuando la velocidad de carga orgánica se aumentaba desde 5.7 hasta 17.8 g DQO total m^{-2} $día^{-1}$. Sin embargo, el aumento de la velocidad de carga más allá de estos valores hasta 35.7 g DQO total m^{-2} $día^{-1}$ disminuía el coeficiente de producción a 0.34 kg SST kg DQO $total^{-1}$. La producción de fangos era de 979 g SST $día^{-1}$ para la velocidad de carga orgánica más alta en este estudio (35.7 g DQO total m^{-2} $día^{-1}$). Helness et al. (2005) propusieron un coeficiente de producción de 0.5 g SST g DQO $soluble^{-1}$.

Además, Orantes and González-Martínez (2004) observaron que la biomasa adherida sobre los soportes aumentaba con la velocidad de carga hasta la carga límite (30 g DQO m^{-2} $día^{-1}$), por encima de la cual la cantidad de biomasa sobre los soportes no podía aumentar más. Como resultado, se lograba un rendimiento de eliminación máximo para la velocidad de carga límite ya que la eficiencia de eliminación del sistema MBBR se ve afectada por la concentración de biomasa en el reactor.

Xiao and Ganczarczyk (2006) estudiaron el efecto de los caudales de influente sobre el sistema MBBR y apreciaron un cambio hacia partículas más grandes con el aumento del caudal. Atribuyeron esta observación a mayores colisiones de partículas de tal forma que se vencían las fuerzas de repulsión y al ser la frecuencia de colisión mayor, esto originaba una mayor agregación y la formación de flóculos más grandes. Otra conclusión de estos resultados fue que eso afectaría al rendimiento del proceso de

separación de sólidos posterior ya que el tamaño de los flóculos bajo diferentes condiciones de operación puede variar. Por ejemplo, cuando se usa una membrana, el mecanismo de ensuciamiento puede ser distinto y, en consecuencia, la velocidad de ensuciamiento variará en función del tamaño de los flóculos de los MLSS.

Si los reactores MBBR operan con velocidades de carga orgánica altas, el TRH sería bastante bajo para la eliminación completa del carbono orgánico. En estos sistemas, la DQO soluble es consumida rápidamente mientras que la mayor parte de la DQO particulada pasa por el reactor sin ningún tipo de cambio (Ødegaard, 2006). Una parte de la DQO particulada puede ser hidrolizada (lo cual complica el análisis del rendimiento de eliminación de DQO soluble en el reactor MBBR). Por ende, los sólidos en suspensión totales (SST) en el efluente del reactor MBBR que opera a altas velocidades de carga orgánica se pueden calcular como la suma de los SST presentes en el influente y la producción de fango biológico. Esto explica que la presencia de SST en el efluente sea mayor que en el influente en diferentes estudios de sistemas MBBR tales como los de Orantes and González-Martínez (2004) y Helness et al. (2005).

Un reto asociado con los reactores MBBR que trabajan con velocidades de carga orgánica altas es que la sedimentabilidad del fango disminuye (Ødegaard, 2006). La menor sedimentabilidad en reactores MBBR muy cargados se puede deber a la existencia de una fracción mayor de biomasa no floculada a la salida del reactor (Rusten et al., 1998). Si se incluyera un proceso de separación de sólidos mejorado, tal como una membrana, los reactores MBBR probablemente podrían operar a velocidades de carga significativamente mayores o a tiempos de retención hidráulicos (TRHs) bajos.

Varios estudios han evaluado el rendimiento del sistema que combina el reactor MBBR con un proceso de separación por filtración con membranas (Melin et al., 2005; Ahl et al., 2006; Leiknes and Ødegaard, 2007) para el tratamiento de aguas residuales urbanas y dieron resultados prometedores.

7.3. Biorreactores de membrana con lecho móvil (sistemas MBBR-MBR)

Como se ha comentado anteriormente, los sistemas MBBR combinan tecnologías de cultivo en suspensión y de biopelícula. Este efecto combinatorio hace que la tecnología sea capaz de asimilar mayor cantidad de materia orgánica, supuestamente, con un volumen inferior que un sistema de fangos activos convencional. Sin embargo,

los fangos producidos en los reactores MBBR presentan peores características de decantabilidad (especialmente cuando operan a altas tasas de carga) comparados con el proceso de fangos activos (Lee et al., 2006). Por lo tanto, su eficiencia está limitada por el rendimiento del decantador posterior, requiriendo una superficie de decantación mayor.

Por otro lado, la aplicación de biorreactores de membrana (MBRs) como sistemas compactos y fiables para el tratamiento de las aguas residuales ha aumentado en la pasada década debido a avances en su diseño y operación. En comparación con los sistemas de fangos activos convencionales, los MBRs tienen unos tiempos de retención celulares (TRCs) mayores, una separación total de sólidos, una desinfección del permeado y una producción de fango menor sin problemas de bulking (Defrance et al., 2000; Juang et al., 2007). Sin embargo, el ensuciamiento de las membranas supone la barrera más importante para la aplicación de los MBRs, ya que los costes de esta tecnología aumentan debido a la necesidad de mayores frecuencias de limpieza, reduciendo esto la vida útil de la membrana. Todos los constituyentes del licor mezcla contribuyen al ensuciamiento de la membrana en un biorreactor de membrana (MBR) y el alcance de su efecto depende de su aportación relativa en el fango y de las condiciones de operación del proceso. Tak and Bae (2005) encontraron que los sólidos en suspensión eran los que más contribuían al ensuciamiento o fouling de la membrana (72-83%) y la formación de una torta representaba el 90% del ensuciamiento total. Defrance et al. (2000) estudiaron un MBR que se alimentaba con un agua residual urbana sin tratar procedente de una planta de tratamiento y también encontraron que los sólidos en suspensión constituían el agente más significativo (65%) de ensuciamiento de las membranas.

La combinación de los sistemas MBBR y MBR resuelve, en gran medida, las limitaciones que presentan ambos procesos por separado. De este modo, surgen los sistemas MBBR-MBR como una integración de los reactores MBBR con una tecnología de membranas.

La aplicación de un sistema de separación de partículas mejorado, como es el caso de una membrana, elimina la limitación de los reactores MBBR en cuanto a la sedimentabilidad del fango, mejorando su aplicabilidad. Además, en estos sistemas, los reactores MBBR originan una producción de sólidos en suspensión significativamente

menor y, por lo tanto, la membrana estará expuesta a concentraciones de sólidos inferiores, lo cual conllevará una reducción del ensuciamiento de las mismas, permitiendo aumentar el flujo de operación, resolviendo en parte el problema de fouling que presentan los MBR. Además, el componente rápidamente biodegradable de la DQO será eliminado en el MBBR y esto ocasionará que la actividad biológica sobre la superficie de la membrana se reduzca. Estos reactores son más compactos que los correspondientes a los procesos de fangos activos convencionales y debido al desarrollo de biomasa adherida sobre los soportes o carriers, operan con concentraciones altas de biomasa activa (Lee et al., 2006). Los sistemas MBBR-MBR pueden operar con TRHs relativamente bajos o velocidades de carga orgánica altas (Ivanovic et al., 2008) ya que la materia rápidamente biodegradable (materia biodegradable soluble) es eliminada en el MBBR y la materia particulada (procedente del influente y de la biomasa producida en el reactor) es separada por la membrana. Por consiguiente, se debería mantener el TRH suficientemente bajo para minimizar la hidrólisis y la biodegradación de la materia particulada pero suficientemente alto para permitir la máxima eliminación de la materia orgánica soluble (Helness et al., 2005). De este modo, el sistema MBBR-MBR tiene el potencial de ser compacto, pudiendo tratar una tasa de carga alta y tener un elevado rendimiento en eliminación de materia orgánica, produciendo un efluente de alta calidad que puede ser reutilizado, lo cual hace de estos sistemas una opción interesante para el tratamiento de aguas residuales

Leiknes and Ødegaard (2007) demostraron que los procesos MBBR-MBR pueden funcionar con agua residual urbana con unas tasas de carga de DQO altas, comprendidas entre 2-8 kg m^{-3} $día^{-1}$, operando con TRHs incluso inferiores a 4 h, presentando un flujo sostenido relativamente alto de 50 L m^{-2} h^{-1} y, consecuentemente, alcanzando un rendimiento de eliminación de DQO alto. Los MBR, por comparación, operan típicamente a tasas de carga de DQO más bajas de 1-3 kg m^{-3} $día^{-1}$, requieren TRHs mayores de 4-10 h y producen flujos inferiores de 15-25 L m^{-2} h^{-1}.

A pesar de los beneficios potenciales de una tecnología que combina un MBBR con una membrana, ha habido muy pocos estudios de esta configuración. Se requiere más investigación para evaluar diferentes condiciones operacionales en estos sistemas con el objetivo de desarrollar los mismos y aumentar su aceptación en el tratamiento de las aguas residuales.

7.4. Ventajas frente a procesos biológicos convencionales

De forma general, las principales ventajas que presenta el proceso de lecho móvil frente a los procesos biológicos convencionales son (Zalakain and Manterola, 2011):

- Reducción de volumen del reactor biológico por el empleo de un soporte que proporciona una superficie específica elevada.
- Son procesos con gran flexibilidad ya que en función del porcentaje de soporte plástico empleado en el reactor, se recomienda no superior al 70%, se consigue modificar la superficie y, en consecuencia, la eficiencia del proceso.
- No requiere, en general, recirculación de biomasa al reactor. Esto da lugar a que la biomasa no dependa de la separación final del fango y en consecuencia de problemas habituales encontrados en procesos convencionales de fangos activos relacionados con la sedimentabilidad del fango (bulking filamentoso, foaming, etc.).
- La operación y control de este tipo de procesos son sencillos. Por una parte, el proceso evita los problemas de atascamiento y consecuentemente periodos de limpieza continuados, además, no es necesario un control de la purga de fangos ya que el sistema mantiene la biomasa en el reactor hasta que es desprendida del soporte.
- Permiten la generación de una biomasa característica de cada tipo de reactor (aerobio, anóxico o anaerobio) dando lugar a la obtención de un biofilm con una elevada actividad. Experimentalmente se ha constatado que las tasas de nitrificación y desnitrificación en este tipo de procesos son superiores a las obtenidas en los procesos convencionales.

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II. OBJETIVOS/OBJECTIVES

OBJETIVOS

Como **objetivo principal de esta investigación**, se plantea llevar a cabo un estudio cinético de biorreactores de membrana con y sin lecho móvil en relación con el análisis de su capacidad de eliminación de materia orgánica, nitrógeno y fósforo en el tratamiento de aguas residuales urbanas.

Para alcanzar el objetivo global de la investigación, se han desarrollado los siguientes **objetivos secundarios**:

1. Análisis de la influencia de diferentes configuraciones de relleno, tiempos de retención hidráulico (TRHs) y concentraciones de biomasa suspendida y/o adherida sobre la eliminación de materia orgánica y nutrientes en biorreactores de membrana con y sin lecho móvil, así como el estudio del proceso de eliminación de fósforo mediante un esquema de tratamiento que combina etapas anaerobia, anóxica y aerobia.

2. Evaluación de la cinética heterótrofa y autótrofa, estudio de la nitrificación en dos etapas a través del análisis cinético de las bacterias oxidadoras de amonio (AOB) y bacterias oxidadoras de nitrito (NOB), y su relación con la eliminación de materia orgánica y nutrientes y con las concentraciones de nitrito y nitrato en los diferentes efluentes de biorreactores de membrana con y sin lecho móvil en el tratamiento de aguas residuales urbanas.

3. Estudios microbiológicos de la influencia de las actividades enzimáticas de α-glucosidasa, fosfatasa ácida y fosfatasa alcalina de la biomasa suspendida y adherida en la eliminación de materia orgánica y nutrientes, diversidad bacteriana mediante electroforesis en gel con gradiente de temperatura (TGGE), estructura de las comunidades bacterianas de la biopelícula a través de microscopía electrónica de barrido (SEM), y poblaciones microbianas nitrificantes (AOB y NOB) y desnitrificantes desarrolladas en la biomasa suspendida y adherida mediante técnicas de pirosecuenciación 454 en biorreactores de membrana con y sin lecho móvil.

OBJECTIVES

The **main objective of this research** is a kinetic study of moving bed biofilm reactor-membrane bioreactor (MBBR-MBR) systems and membrane bioreactors (MBRs) regarding the analysis of the organic matter, nitrogen and phosphorus removal, which were carried out for municipal wastewater treatment.

The following **supporting objectives** have been developed to accomplish the main aim of the study:

1. Analysis of the influence of different carrier configurations, hydraulic retention times (HRTs) and concentrations of suspended and/or attached biomass on the organic matter and nutrient removal in MBBR-MBR systems and MBRs, as well as the study of the phosphorus removal with a treatment process which combines anaerobic, anoxic and aerobic zones.

2. Evaluation of the heterotrophic and autotrophic kinetics, study of two-step nitrification through the kinetic analysis of the ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), and their relation to the organic matter and nutrient removal and concentrations of nitrite and nitrate in the different effluents of MBBR-MBR systems and MBRs in the municipal wastewater treatment.

3. Microbiological studies of the influence of the enzymatic activities of α-glucosidase, acid phosphatase and alkaline phosphatase of the suspended and attached biomass on the organic matter and nutrient removal, bacterial diversity by the use of temperature gradient gel electrophoresis (TGGE), bacterial community structure of the biofilm via scanning electron microscopy (SEM), and nitrifying (AOB and NOB) and denitrifying microbial populations which grow in the suspended and attached biomass through 454 pyrosequencing in MBBR-MBR systems and MBRs.

1. General description of the wastewater treatment plants

Seven different systems for wastewater treatment were studied regarding the removal of organic matter and nutrients. Figure III.1 shows the four processes for organic matter and nitrogen removal, and Figure III.2 indicates the three systems for organic matter, nitrogen and phosphorus removal in municipal wastewater treatment.

1.1. Configurations for organic matter and nitrogen removal

The wastewater treatment plants (WWTPs), which were designed for organic matter and nitrogen removal, consisted of an MBR (Figure III.1a), a hybrid moving bed biofilm reactor-membrane bioreactor (hybrid MBBR-MBR_a) containing carriers both in the anoxic and aerobic zones of the bioreactor (Figure III.1b), a hybrid MBBR-MBR_b which contained carriers only in the aerobic zone of the bioreactor (Figure III.1c) and a pure MBBR-MBR which also contained carriers only in the aerobic zone of the biological reactor (Figure III.1d).

The bioreactors of these WWTPs were divided into four zones (C1, C2, C3 and C4), i.e. one anoxic zone (C2) and three aerobic ones (C1, C3 and C4), as well as the membrane tank (C5). The working volumes of the bioreactor and the membrane tank were 24 L and 4.32 L, respectively.

Furthermore, three different advanced oxidation process (AOP) technologies, an H_2O_2/UV system, a photo-Fenton (Fe^{2+}/H_2O_2/UV) process and a TiO_2/H_2O_2/UV system at two different H_2O_2 concentrations, treated in batch the effluents of two MBR systems and the hybrid MBBR-MBR_b under a hydraulic retention time (HRT) of 18 h (**Chapter 5**).

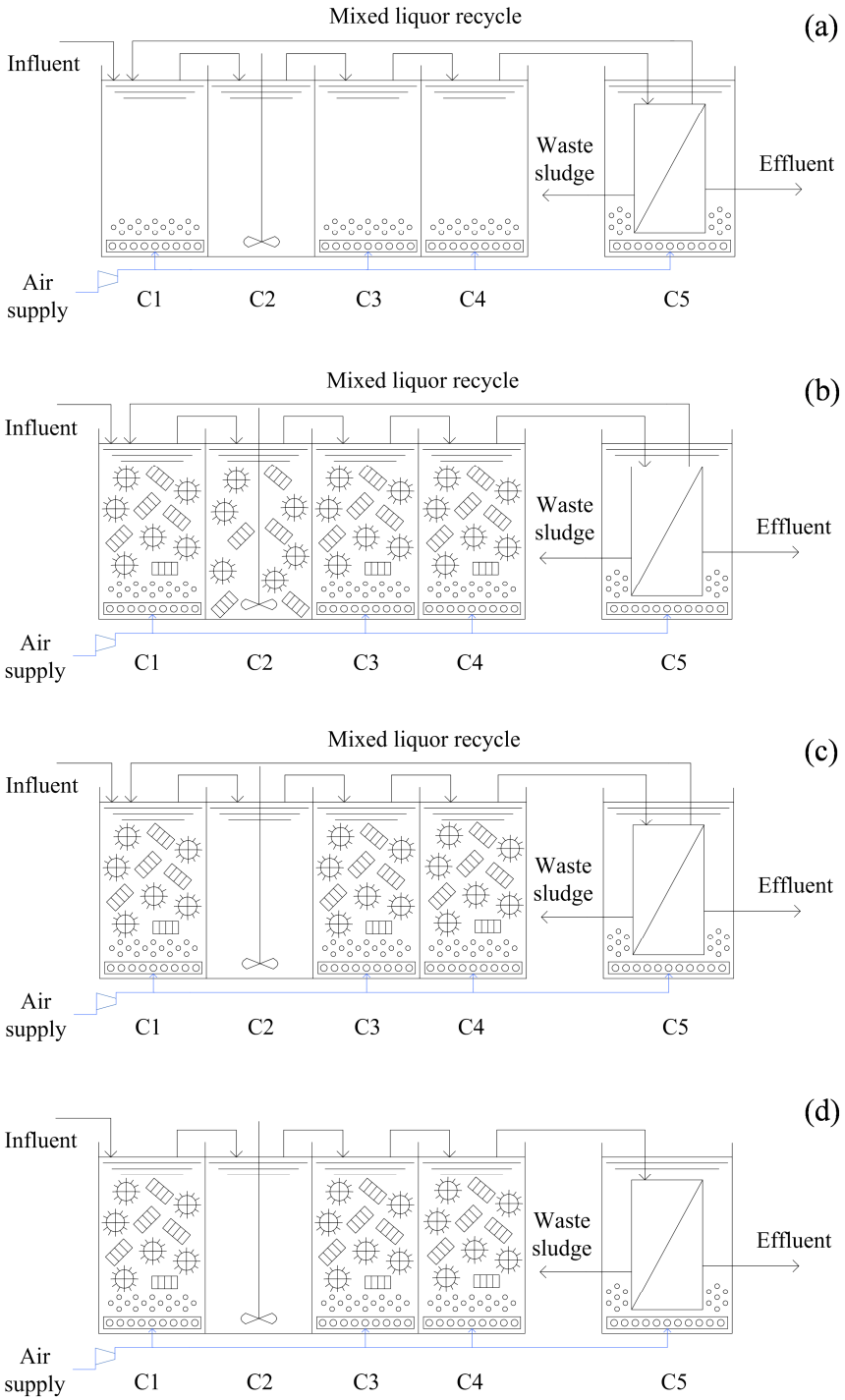

Figure III.1. Schematic diagram of the four systems for organic matter and nitrogen removals in municipal wastewater treatment. (a) Membrane bioreactor (MBR). (b) Hybrid moving bed biofilm reactor-membrane bioreactor containing carriers in the aerobic and anoxic zone of the bioreactor (Hybrid MBBR-MBR$_a$). (c) Hybrid moving bed biofilm reactor-membrane bioreactor containing carriers only in the aerobic zone of the bioreactor (Hybrid MBBR-MBR$_b$). (d) Pure moving bed biofilm reactor-membrane bioreactor (Pure MBBR-MBR).

1.2. Configurations for organic matter, nitrogen and phosphorus removal

The WWTPs, which were designed for organic matter, nitrogen and phosphorus removals, consisted of an MBR_p (Figure III.2a), a hybrid MBBR-MBR system containing carriers in the anaerobic, anoxic and aerobic zones of the bioreactor (hybrid MBBR-MBR_{ap}) (Figure III.2b) and a hybrid MBBR-MBR system which contained carriers only in the anaerobic and anoxic zones of the bioreactor (hybrid MBBR-MBR_{bp}) (Figure III.2c).

The bioreactors of the WWTPs were divided into four zones (C1, C2, C3 and C4), i.e. one anaerobic zone (C1), one anoxic zone (C2) and two aerobic zones (C3 and C4), as well as the membrane tank (C5). The working volumes of the bioreactor and the membrane tank were 24 L and 4.32 L, respectively

These systems were studied in **Chapter 7**.

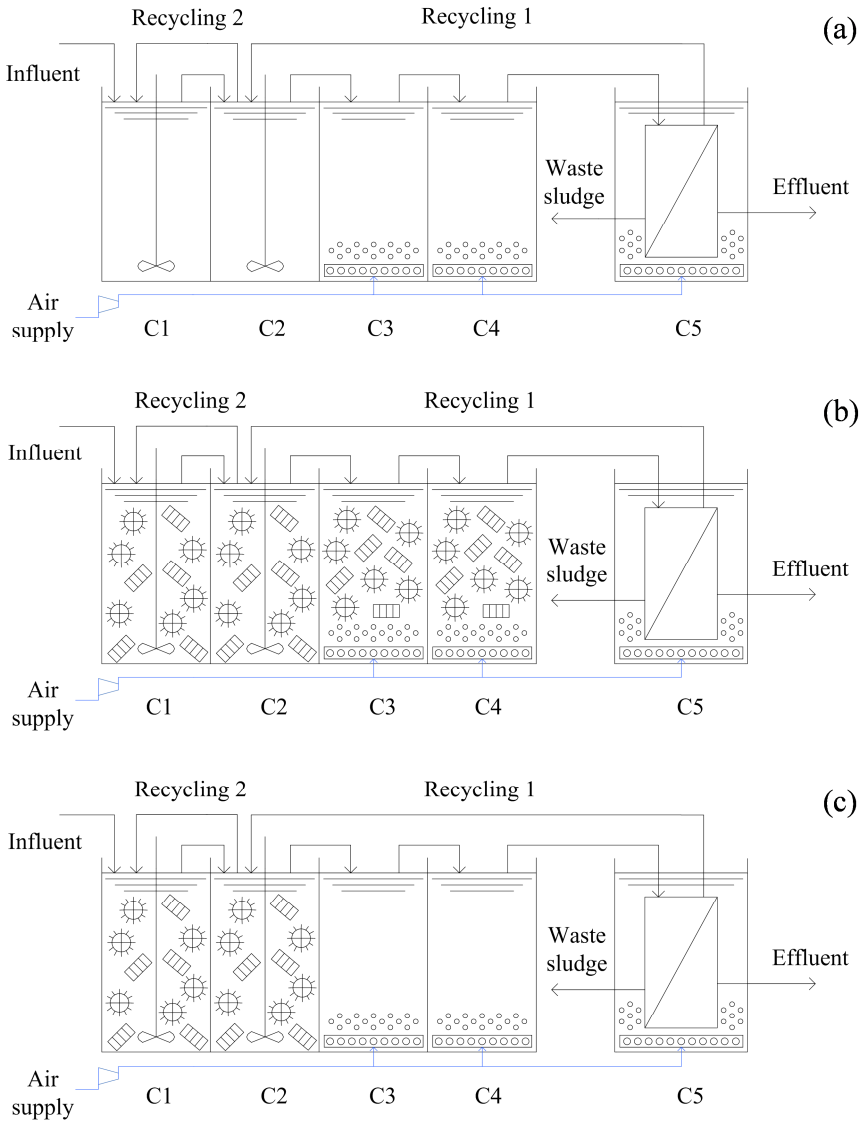

Figure III.2. Schematic diagram of the three systems for organic matter, nitrogen and phosphorus removal in municipal wastewater treatment. (a) Membrane bioreactor (MBR_p). (b) Hybrid moving bed biofilm reactor-membrane bioreactor containing carriers in the anaerobic, anoxic and aerobic zones of the bioreactor (Hybrid MBBR-MBR_{ap}) (c) Hybrid moving bed biofilm reactor-membrane bioreactor containing carriers in the anaerobic and anoxic zones of the bioreactor (Hybrid MBBR-MBR_{bp}).

2. Work plan and general operation conditions

The work plan was divided into seven research phases, as shown in Table III.1. Table III.1 includes the duration of each experimental phase, as well as the different systems, hydraulic retention times (HRTs) and biomass concentrations studied.

Table III.1. Work plan of the different experimental phases. HRT (hydraulic retention time), MLSS (mixed liquor suspended solids), BD (biofilm density).

Research phase	Start date	End date	Systems studied	HRT (h)	Biomass concentration	
					MLSS (mg L^{-1})	BD (mg L^{-1})
IV. Chapter 1	09/05/2011	17/12/2011	MBR	30.4	2,691.30±114.99	-
			Hybrid MBBR-MBR_a		1,569.87±82.01	1,228.18±75.89
			Hybrid MBBR-MBR_b		1,823.99±51.11	880.00±43.01
V. Chapter 2	19/12/2011	29/03/2012	MBR	26.5	4,383.86±316.01	-
			Hybrid MBBR-MBR_a		2,553.75±293.42	1,000.35±345.26
			Hybrid MBBR-MBR_b		2,999.14±400.18	675.00±175.39
VI. Chapter 3	10/04/2012	16/08/2012	MBR	18	3,574.34±175.26	-
			Hybrid MBBR-MBR_a		2,028.93±155.52	1,610.83±73.60
			Hybrid MBBR-MBR_b		2,306.66±112.93	1,207.50±76.61
VII. Chapter 4	03/09/2012	20/12/2012	MBR	9.5	3,326.83±233.95	-
			Hybrid MBBR-MBR_a		2,498.25±138.40	1,270.19±81.55
			Hybrid MBBR-MBR_b		2,457.58±156.90	1,250.00±66.51
VIII. Chapter 5	07/01/2013	30/04/2013	MBR_a	18	6,405.56±365.36	-
			MBR_b		2,739.68±211.75	-
			Hybrid MBBR-MBR_b		4,369.84±232.79	2,008.93±171.15
IX. Chapter 6	02/05/2013	29/11/2013	MBR_a	9.5 / 6	2,820.59±243.87 (9.5 h) / 2,777.78±282.27 (6 h)	-
			MBR_b		6,656.67±445.02 (9.5 h) / 6,566.67±255.73 (6 h)	-
			Hybrid MBBR-MBR_b		2,041.90±258.37 (9.5 h) / 2,243.75±216.95 (6 h)	997.73±124.62 (9.5 h) / 748.53±111.97 (6 h)
			Pure MBBR-MBR		208.00±61.30 (9.5 h) / 258.75±79.99 (6 h)	1,920.45±127.16 (9.5 h) / 2,070.00±202.97 (6 h)
X. Chapter 7	02/12/2013	28/03/2014	MBR_p	18	6,431.67±256.94	-
			Hybrid MBBR-MBR_{ap}		4,419.30±254.42	2,028.95±149.13
			Hybrid MBBR-MBR_{bp}		4,485.00±336.39	1,991.25±154.17

The WWTPs operated under different hydraulic retention times (HRTs), 30.4 h, 26.5 h, 18 h, 9.5 h and 6 h, and different biomass concentrations, which are grouped in low biomass concentrations around an average value of 2,700 mg L^{-1}, intermediate biomass concentrations around an average value of 3,700 mg L^{-1} and high biomass concentrations around an average value of 6,500 mg L^{-1}. These operational conditions are shown in Table III.2.

Table III.2. Operational conditions regarding HRT and biomass concentration, as MLSS, BD and total biomass, of the experimental plants. HRT (hydraulic retention time), MLSS (mixed liquor suspended solids), BD (biofilm density).

HRT (h)	Biomass concentration									
	MBR	Hybrid MBBR-MBR$_a$			Hybrid MBBR-MBR$_b$			Pure MBBR-MBR		
	MLSS (mg L^{-1})	MLSS (mg L^{-1})	BD (mg L^{-1})	Total biomass (mg L^{-1})	MLSS (mg L^{-1})	BD (mg L^{-1})	Total biomass (mg L^{-1})	MLSS (mg L^{-1})	BD (mg L^{-1})	Total biomass (mg L^{-1})
30.4	**2,691.30±114.99**[a]	1,569.87±82.01	1,228.18±75.89	**2,798.05±157.90**[a]	1,823.99±51.11	880.00±43.01	**2,703.99±94.12**[a]			
26.5	**4,383.86±316.01**[b]	2,553.75±293.42	1,000.35±345.26	**3,554.10±238.68**[b]	2,999.14±400.18	675.00±175.39	**3,674.14±275.57**[b]			
18	**3,574.34±175.26**[b]	2,028.93±155.52	1,610.83±73.60	**3,639.76±229.12**[b]	2,306.66±112.93	1,207.50±76.61	**3,514.16±189.54**[b]			
	6,405.56±365.36[c]				4,369.84±232.79	2,008.93±171.15	**6,378.77±403.94**[c]			
	2,739.68±211.75[a]									
9.5	**3,326.83±233.95**[b]	2,498.25±138.40	1,270.19±81.55	**3,768.44±219.95**[b]	2,457.58±156.90	1,250.00±66.51	**3,707.58±223.41**[b]	208.00±61.30	1,920.45±127.16	**2,128.45±188.46**[a]
	2,820.59±243.87[a]				2,041.90±258.37	997.73±124.62	**3,039.63±282.99**[a]			
	6,656.67±445.02[c]									
6	**2,777.78±282.27**[a]				2,243.75±216.95	748.53±111.97	**2,992.28±228.92**[a]	258.75±79.99	2,070.00±202.97	**2,328.75±182.96**[a]
	6,566.67±255.73[c]									

(a) Low biomass concentrations around an average value of 2,700 mg L^{-1}
(b) Intermediate biomass concentrations around an average value of 3,700 mg L^{-1}
(c) High biomass concentrations around an average value of 6,500 mg L^{-1}

Furthermore, the biomass concentration in the MBR_p was established at 6,431.67±256.94 mg L^{-1}. The concentrations of mixed liquor suspended solids (MLSS) in the hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp} were 4,419.30±254.42 mg L^{-1} and 4,485.00±336.39 mg L^{-1}, respectively, and the attached biofilm density (BD) on the carriers contained in the hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp} had values of 2,028.95±149.13 mg L^{-1} and 1,991.25±154.17 mg L^{-1}, respectively.

3. Physical and chemical determinations

A multifunctional meter (PCE-PHD 1, PCE Ibérica, SL, Spain) was used to measure the pH, conductivity and temperature in the influent, effluents and the different zones of each bioreactor and the dissolved oxygen concentration in each chamber of the different bioreactors.

Chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), volatile suspended solids (VSS) and total phosphorus (TP) were measured in accordance with standard methods (APHA, 2012). Total organic carbon (TOC) was determined using a Formarcs^{HT} TOC/TN analyzer by oxidative combustion at 950°C. Total nitrogen (TN) was determined through the concentrations of ammonium (NH_4-N), nitrite (NO_2-N) and nitrate (NO_3-N), which were determined by ion chromatography using a conductivity detector (Metrohm®, Metrohm AG, Switzerland). Dilution and separation of anions were carried out through an anion column (Metrosep A Supp 5, Metrohm AG, Switzerland) using a solution of carbonate/bicarbonate as the eluent and sulphuric acid as the regenerant. A cation column (Metrosep C 4, Metrohm AG, Switzerland) was used for the dilution and separation of cations, for which a solution of dipicolinic acid was employed as the eluent and distilled water as the regenerant.

Biofilm carriers were tested in order to assess the amount of biomass attached to the carriers. The assessment of TSS on the fixed biomass carriers, BD, was executed as follows: four representative plastic elements were extracted from each hybrid MBBR-MBR system. Subsequently, they were diluted in Tween 80 to solubilize the organic components of the biofilm attached to the carriers. Then, the sample of the carriers was sonicated for three minutes to ensure the separation of these components. Afterwards, the sample was centrifuged and washed off to separate the biomass from the carrier.

Finally, the sample was filtered (0.45 µm), dewatered at 105ºC and weighed; the obtained value was referred to the four elements. The TSS concentration was assessed through the total number of carriers in a liter of reactor (Martín-Pascual et al., 2012; Zhang et al., 2014).

4. Kinetic study

4.1. Respirometry

Respirometric tests were carried out using a "flow-gas/static-liquid" type batch respirometer (Spanjers et al., 1998). The respirometric analyzer used in this research is called BM-Advance. The operation principle of this respirometer is based on the consumption of oxygen by the microorganisms contained in the mixed liquor of the biological reactor of a WWTP. The dissolved oxygen concentration of the mixed liquor was measured in a continuous regimen since dissolved oxygen is the result of microorganism respiration during the metabolism of organic matter and ammonium as well as the own consumption of oxygen of the microorganisms. The main measurements which can be carried out with this analyzer are: dynamic oxygen uptake rate (R_S, mg O_2 L^{-1} h^{-1}), oxygen uptake rate (OUR, mg O_2 L^{-1} h^{-1}), oxygen consumption (OC, mg O_2 L^{-1}), biodegradable fraction of COD (COD_b, mg O_2 L^{-1}), temperature, pH and others.

The respirometric tests allowed for assessing the kinetic parameters depending on the R_S, OUR, OC, substrate concentration (S, mg O_2 L^{-1} for heterotrophic bacteria and mg N L^{-1} for autotrophic and nitrite-oxidizing bacteria) and biomass concentration (X, mg VSS L^{-1}).

Respirometric experiments were conducted on biomass samples taken from the WWTPs to analyze the influence of the different conditions on the behavior of the biomass present in each bioreactor; these are called "exogenous respiration tests". For this purpose, one liter of mixed liquor (wastewater, suspended solids and biofilm, in the case of the plants with carriers in the bioreactor), containing 35% carrier elements for the hybrid MBBR-MBR and pure MBBR-MBR systems, was withdrawn from the bioreactor of each pilot plant and transferred to the reactor of the respirometer. Before starting the experiment, the mixed liquor was aerated using an air pump and a porous sparger for 18 h to reach endogenous conditions in which any kind of substrate

contained in the sample was consumed. The reactor was placed in a Peltier-type thermostatic device (Dinko Instruments, Spain) and the temperature was kept at 20.0±0.1ºC during the experiment. Agitation was provided by a mechanical stirrer (Dinko Instruments, Spain) and a recycling peristaltic pump continuously pumped the mixed liquor from the bottom to the top of the reactor to homogenize the contents of the reactor. Therefore, the content of the reactor was assumed to be completely mixed. The sample was aerated using an air pump and an aeration stone. The air flow was set to 0.906±0.001 L min^{-1}. Three stock solutions of sodium acetate (500 mg L^{-1}), ammonium chloride (150 mg L^{-1}) and sodium nitrite (200 mg L^{-1}) were prepared. Three dilutions (35, 70 and 100%) from each solution were added to the respirometer to characterize the heterotrophic, autotrophic and nitrite-oxidizing bacteria, respectively. The concentrations of NH_4^+ and NO_2^- were determined by ion chromatography and the sodium acetate concentration was expressed as COD. The values of these concentrations were used as substrate concentration (S) for the kinetic study of the heterotrophic, autotrophic and nitrite-oxidizing bacteria.

At the end of these respirometric experiments, a specific test (endogenous respiration experiment) was carried out to evaluate the decay coefficient by leaving the mixed liquor without aeration so that the dissolved oxygen concentration decreased to zero. The pH in the respirometer was maintained at 7.25±0.75 through the addition of hydrochloric acid and sodium hydroxide. The dissolved oxygen concentration was measured with an oxygen sensor (Hamilton Company, United States). The temperature, aeration, pH and mixed liquor recycling were automatically controlled using the BM-Advance program.

4.2. Kinetic parameter estimation for heterotrophic and autotrophic biomass

Data acquisition and visualization took place using the BM-Advance software, which generated respirograms corresponding to the different experiments. The assessment of the kinetic parameters of the process was carried out through two types of tests, as explained previously. The addition of the three substrates made the dissolved oxygen concentration decrease to a minimum value due to cellular metabolism. Subsequently, this concentration began to increase until the initial value, which was reached when the substrate had been totally metabolized. These experiments enabled the estimation of the maximum specific growth rate (μ_m), the substrate half-saturation

coefficient (K_S) and the yield coefficient (Y) for the heterotrophic, autotrophic and nitrite-oxidizing bacteria (suspended and attached biomass), as well as the endogenous or decay coefficient for the global biomass, k_d, according to the Monod model (Monod, 1949).

One supposition was considered. The biomass concentration remained constant during the test (the time of the experiments was not too long) and it was recalculated due to the different additions of substrate which diluted the content of the reactor. The concentrations for heterotrophic, autotrophic and nitrite-oxidizing bacteria, $X_{H,}$ X_A and X_{NOB}, respectively, were determined by supposing the percentages of heterotrophic, autotrophic and nitrite-oxidizing bacteria which were determined by Leyva-Díaz et al. (2015) for an MBR and two hybrid MBBR-MBR systems, as well as the percentages of heterotrophic, autotrophic and nitrite-oxidizing bacteria belonging to the pure MBBR-MBR system (**Chapter 6**). These percentages were applied to the different values of mixed liquor volatile suspended solids (MLVSS) and volatile biofilm density (VBD) existing in each system.

4.2.1. Kinetic parameters for heterotrophic bacteria

The estimation of these parameters is carried out in five steps:

- Step 1: the oxygen consumption (OC) was determined from the numerical integration of the dynamic oxygen uptake rate (R_S) for each one of the three additions of organic substrate, as shown in Eq. (1):

$$OC = \int_{t_0}^{t} R_s \, dt \qquad (1)$$

  Figure III.3 shows the typical evolution of the R_S in a respirometric experiment. Three values of OC (OC_1, OC_2 and OC_3) are calculated from three values of substrate concentration (S_1, S_2 and S_3).

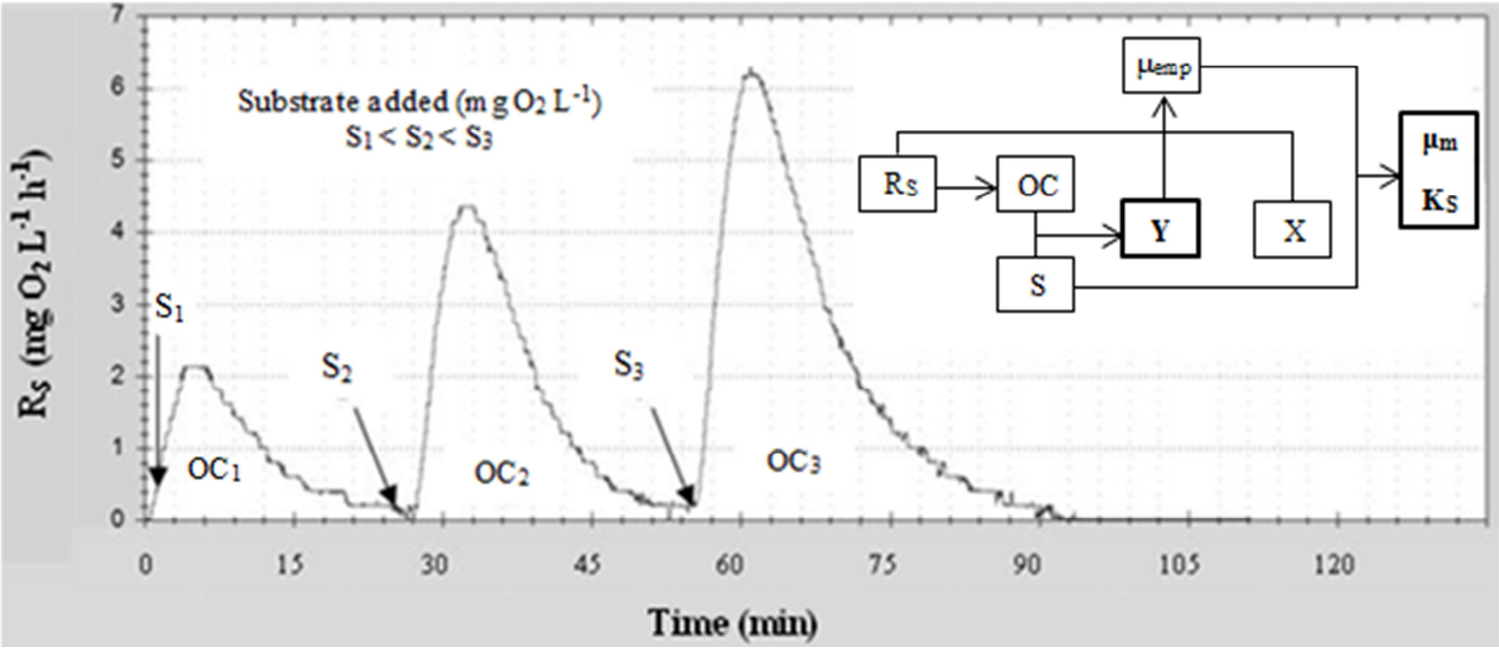

Figure III.3. Evolution of the dynamic oxygen uptake rate (R_S) in a respirometric experiment and schematic diagram of the assessment of the kinetic parameters.

- Step 2: the yield coefficient for heterotrophic biomass referred to the oxygen ($Y_{H, O2}$) was calculated according to Eq. (2) described by Helle (1999):

$$Y_{H,\ O_2} = \frac{S - OC}{S} \qquad (2)$$

where S (mg O_2 L^{-1}) is the substrate concentration and represented the organic matter concentration (S_S) for heterotrophic biomass.

The yield coefficient for heterotrophic biomass referred to the VSS ($Y_{H, VSS}$) can be evaluated by considering the conversion factor f_{cv}, with a value of 1.48 mg COD mg VSS^{-1}, according to Eq. (3):

$$Y_{H,\ VSS} = \frac{Y_{H, O_2}}{f_{cv}}\ (\text{mg VSS mg COD}^{-1}) \qquad (3)$$

- Step 3: the substrate degradation rate (r_{su}) can be calculated from the derivation of Eq. (2), as shown in Eq. (4):

$$r_{su} = \frac{dS}{dt} = \frac{1}{1 - Y_{H, O_2}} \frac{d(OC)}{dt} = \frac{1}{1 - Y_{H, O_2}} R_s\ (\text{mg } O_2\ L^{-1}\ h^{-1}) \qquad (4)$$

R_S is a measurement obtained from the respirometric analyzer.

- Step 4: the empirical specific growth rate for heterotrophic biomass ($\mu_{emp, H}$) was determined by considering the relation between the cellular growth rate (r_x) and the r_{su}, as shown in Eq. (5):

$$\mu_{emp,\ H} = \frac{r_x}{X_H} = \frac{Y_{H, VSS}\ r_{su}}{X_H} = \frac{Y_{H, VSS}\ R_s}{(1 - Y_{H, O_2})\ X_H}\ (h^{-1}) \qquad (5)$$

where X_H (mg VSS L^{-1}) is the concentration for heterotrophic bacteria.

- Step 5: Eq. (6) shows the linearization of the Monod model (Eq. (2) of the section **Introducción General**), which allowed for assessing $\mu_{m,\ H}$ (h^{-1}) and K_M (mg O_2 L^{-1}) from the linear regression of the inverse of the $\mu_{emp,\ H}$ depending on the inverse of S:

$$\frac{1}{\mu_{emp,\ H}} = \frac{K_M}{\mu_{m,\ H}} \frac{1}{S} + \frac{1}{\mu_{m,\ H}} \qquad (6)$$

4.2.2. Kinetic parameters for autotrophic and nitrite-oxidizing bacteria

The estimation of these parameters is carried out in five steps:

- Step 1: the oxygen consumption (OC) was determined according to Eq. (1).
- Step 2: the yield coefficient for autotrophic biomass referred to the oxygen ($Y_{A,\ O2}$) was calculated according to Eq. (7):

$$Y_{A,\ O_2} = \frac{S - OC}{S} \qquad (7)$$

where S (mg O_2 L^{-1}) is the ammonium concentration expressed as oxygen.

The yield coefficient for autotrophic biomass referred to the nitrogen ($Y_{A,\ N}$) can be evaluated, as shown in Eq. (8):

$$Y_{A,\ N} = \frac{S - OC}{S_{NH}} \ (\text{mg } O_2 \text{ mg } N^{-1}) \qquad (8)$$

where S_{NH} (mg N L^{-1}) is the ammonium concentration expressed as total nitrogen.

The yield coefficient for autotrophic biomass referred to the VSS is determined by considering the conversion factor f_{cv}^*, 1.42 mg O_2 mg VSS^{-1}, according to Eq. (9):

$$Y_{A,\ VSS} = \frac{Y_{A,\ N}}{f_{cv}^*} \ (\text{mg VSS mg } N^{-1}) \qquad (9)$$

- Step 3: the substrate degradation rate (r_{su}) can be calculated from the derivation of Eq. (7), as shown in Eq. (10):

$$r_{su} = \frac{dS}{dt} = \frac{1}{1 - Y_{A,O_2}} \frac{d(OC)}{dt} = \frac{1}{1 - Y_{A,O_2}} R_s \ (mg\ O_2\ L^{-1}\ h^{-1}) \qquad (10)$$

R_S is a measurement obtained from the respirometric analyzer.

- Step 4: the empirical specific growth rate for autotrophic biomass ($\mu_{emp,\ A}$) was determined by considering the relation between the r_x and the r_{su}, as shown in Eq. (11):

$$\mu_{emp,\ A} = \frac{r_x}{X_A} = \frac{\frac{1}{f_{cv}^*} r_{su}}{X_A} = \frac{\frac{1}{f_{cv}^*} R_s}{\left(1 - Y_{A,O_2}\right) X_A} \ (h^{-1}) \qquad (11)$$

where X_A (mg VSS L^{-1}) is the concentration for autotrophic bacteria.

- Step 5: Eq. (12) shows the linearization of the Monod model (Eq. (2) of the section **Introducción General**), which allowed for assessing $\mu_{m,\ A}$ (h^{-1}) y K_{NH} (mg N L^{-1}) from the linear regression of the inverse of the $\mu_{emp,\ A}$ depending on the inverse of S_{NH}:

$$\frac{1}{\mu_{emp,\ A}} = \frac{K_{NH}}{\mu_{m,\ A}} \frac{1}{S_{NH}} + \frac{1}{\mu_{m,\ A}} \qquad (12)$$

Figure III.3 includes a schematic diagram of the assessment of the kinetic parameters.

For the second type of experiments (endogenous respiration ones), the mixed liquor was left without aeration and the dissolved oxygen concentration decreased to zero. The OUR was calculated during the oxygen depletion by the BM-Advance software. The k_d was evaluated through the endogenous OUR (OUR_{end}) and the total biomass concentration (X_T), as described by Ramalho (1991) in Eq. (13):

$$k_d = \frac{OUR_{end}}{1.42\ X_T} \qquad (13)$$

The OUR_{end} was the value of the OUR in the interval in which it was constant.

These kinetic parameters allowed us to carry out kinetic modeling, which is an important tool for the design and operation of the biological processes in wastewater treatment (Hvala et al., 2002).

The r_{su} (Eq. (7) of the section **Introducción General)** was evaluated for each biological treatment in order to determine the WWTP which had the best kinetic behavior (Leyva-Díaz et al., 2014).

5. Microbiological analysis

5.1. Fixed biofilm recovery for microbiological analysis

The procedure detailed in Reboleiro-Rivas et al. (2013) was followed for the recovery of attached biomass from carriers. A total number of 50 carriers per chamber was collected from each bioreactor. These carriers were submerged in sterile saline solution (0.9 % NaCl) and then vortexed for 1 minute and sonicated for 3 minutes. Then, the recovered biomass was collected by centrifugation at 3000 x g for 5 minutes. The pellet was resuspended in 50 mL of sterile saline solution (0.9 % NaCl) for further analysis, such as the determination of enzymatic activities, the DNA extraction, the PCR 16S rRNA gene amplification and the TGGE fingerprint analysis

5.2. Determination of microbial enzymatic activities

The determination of the activities of α-glucosidase, acid phosphatase and alkaline phosphatase for attached and suspended biomass was carried out in accordance with Reboleiro-Rivas et al. (2013). The activity of α-glucosidase was measured following the colorimetric method protocol given by Goel et al. (1998), using Tris-HCl buffer (pH 7.6) and 1% p-nitrophenyl α-D-glucopyranoside as the substrate for the reaction. Acid and alkaline phosphatase activities were determined following Goel et al. (1998), using p-nitrophenyl phosphate (0.1 %) and different buffers for acid (acetate-acetic pH 4.8) and alkaline (carbonate-bicarbonate pH 9.6) phosphatase activities. Standard curves for α-glucosidase, acid and alkaline phosphatase activities were calculated through known concentrations of p-nitrophenol. All products were provided by Sigma-Aldrich (St. Louis, MO, USA).

5.3. DNA extraction and PCR 16S rRNA gene amplification

Biomass collected from each chamber for the different bioreactors was subjected to DNA extraction prior to the TGGE process. A total amount of 350 mg of the biomass pellet was taken for DNA extraction. DNA extraction was done using the Ultra Clean Soil Kit DNA (MoBio, USA) following the instructions given by the manufacturer. Amplification of the bacterial 16S rRNA gene was done in accordance with Molina-Muñoz et al. (2009). Then, a nested approach was used for polymerase chain reaction (PCR) amplification. A volume of 1 µL of extracted DNA was taken as the template for the nested PCR. Amplification of the bacterial 16S rRNA gene in almost its full length was accomplished with universal primers fD1 and rD1. After this PCR process, 1 µL of the product of the first amplification was subsequently amplified with universal primers targeting the V3 region of the bacterial 16S rRNA gene. PCR conditions were set following Molina-Muñoz et al. (2007).

5.4. TGGE fingerprint analysis

Amplicons of the V3 region of bacteria obtained from the nested PCR were subjected to TGGE procedure according to Molina-Muñoz et al. (2009). For this, 5 µL of nested PCR product were loaded in the wells of denaturing gels. The TGGE process used the TGGE Maxi system (Whatman-Biometra). The temperature gradient was 43-63°C. Electrophoresis was carried out at 125 V for 18 h. TGGE bands were then visualized using the Gel Code Silver Staining kit (Pierce). TGGE band patterns were normalized, compared and clustered using Gel Compar II software (Applied Maths, Belgium) for the estimation of phylogenetic profiles representing bacterial communities in chambers C1, C2, C3 and C4 of the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b. The dendrogram relating to the phylogenetic profiles of the band patterns was calculated with Dice coefficients and the unweighted pair groups method with arithmetic mean (UPGMA) algorithms. Cophenetic correlation coefficients were then calculated for the estimation of UPGMA clustering significance.

5.5. Scanning electron microscopy

Carriers with biofilm adhered were sampled from the bioreactors of the hybrid MBBR-MBR systems to observe and analyze the structure of the biofilm by scanning electron microscopy (SEM). The protocol for SEM was carried out in accordance with

Calderon et al. (2011). Pieces of carriers were fixed, post-fixed and dehydrated, transferred to ethanol, critical-point dried and coated with gold before examination with SEM. Photographs were taken using a Jeol JSM 5310LV microscope (Jeol Ltd., Tokyo, Japan) and analyzed with the software provided by the manufacturer.

5.6. DNA extraction and PCR Tag-pyrosequencing

Samples of 100 mL of mixed liquor were collected from each zone of the bioreactor for the MBR, hybrid MBBR-MBR_a, hybrid MBBR-MBR_b and pure MBBR-MBR. For fixed biofilm samples, 200 mL of carriers were obtained from each bioreactor. Samples from mixed liquor and fixed biofilm were then introduced into saline solution at 0.9% NaCl. Fixed biofilm samples were sonicated for detachment of biomass from the carriers. Samples were centrifuged at 3000 rpm for 10 min at room temperature to obtain the biofilm fraction in a pellet and the supernatant was discarded. Sample harvesting and pretreatment before DNA extraction were done in accordance with Ni et al. (2010). The remaining biomass was stored at -20ºC for future DNA extraction.

Previous to DNA extraction, biomass samples were defrosted and then four subsamples, one for each sampling point of each bioreactor, were treated as independent samples from DNA extraction. The different samples were centrifuged at 4000 rpm for 10 min at 4ºC. The pellet (300 mg) of each sample was collected for DNA extraction with the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. The four DNA extractions were then merged together for the following PCR Tag-pyrosequencing process (Zhang et al., 2012).

PCR analysis using primers 28F (5'-GAGTTTGATCNTGGCTCAG-3') and 519R (5'-GTNTTACNGCGGCKGCTG-3') was done according to Fan et al. (2012) and used for the collection of amplicons. These amplicons were then subjected to pyrosequencing. Samples for pyrosequencing were stored at -20°C and sent to the Research and Testing Laboratory (Lubbock, TX, USA), (http://www.researchandtesting.com/). The pyrosequencing process was done using a 454 FLX instrument and 454 pyrotag methods according to several authors (Elahi and Ronaghi, 2004; Dowd et al., 2008). Pre-processing of data including denoising and chimera checking was performed by the sequencing facility. Denoised data were

analyzed using the QIIME pipeline (http://qiime.sourceforge.net/) (Caporaso et al., 2010) as previously reported by Sutton et al. (2013). Low quality sequences that did not comply with the following default quality parameters were removed: (a) include a perfect match to the sequence tag and the 16S rRNA gene primer; (b) be at least 200 bp in length; (c) have no ambiguous bases; and (d) have no homopolymers longer than six nucleotides. Once trimmed and assigned to samples, data were processed using the QIIME´s UCLUST method. Sequences were clustered in operational taxonomic units (OTUs) at the 97% identity level. The most abundant sequences in each operational taxonomic unit (OTU), used as representative sequences, were aligned using PyNAST (DeSantis et al., 2006a) against the Greengenes core set (DeSantis et al., 2006b). Finally, sequences were clustered into operational taxonomic units (OTUs), and the abundance and diversity of them were checked by using proper statistical analysis.

In this way, heat maps showing the OTU community structures for ammonium-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and denitrifying bacteria (DeNB) were generated based on the relative abundance of these OTUs. Heat maps were generated for the exploration of the differences in the bacterial community structure between the different WWTPs and growth modes (planktonic growth and fixed biofilm growth associated with mixed liquor and carrier samples, respectively).

6. Statistical analysis

A statistical analysis using the software SPSS 20.0 for Windows was used to evaluate the existence of statistically significant differences between the results. Tukey's HSD post hoc procedure was used to analyze the data obtained concerning COD, BOD_5, TOC, TSS, TN, TP, concentrations of NH_4^+, NO_2^- and NO_3^-, and enzymatic activities (α-glucosidase, acid phosphatase and alkaline phosphatase) under the null hypotheses of independence and homogeneity with a significance level of 5% ($\alpha=0.05$).

A Bray-Curtis similarity analysis for the OTUs identified as AOB, NOB and DeNB was performed in the different systems using the package vegan 2.0 implemented in the statistical software R-Project v.2.15.1, with $p < 0.05$ (R Development Core Team 2008) (Ji et al., 2013; Posmanik et al., 2014). The relative abundance of each OTU was taken as weight for the similarity analysis.

A multivariable statistical analysis using the software Canoco for Windows version 4.5 was used to quantify the influence of the environmental variables, COD and TN of the influent, temperature (T), HRT, MLSS and BD, on the COD and TN removal and the kinetic parameters for heterotrophic and autotrophic biomass, and to obtain the variables with the highest influence on the behavior of the different systems studied. A Detrended Correspondence Analysis (DCA), the most appropriate ordination statistical analysis, was carried out in order to obtain the gradient lengths. DCA revealed that the longest ordination axis was lower than three, so the distribution of the model was linear. Redundancy Analysis (RDA) was used due to the fact that the distribution of the model was linear, as the statistical method recommended by Lepš and Šmilauer (1999). Statistical significance was tested using a Monte Carlo test with 499 permutations and a selected significance level of 0.05.

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IV. CHAPTER 1

Start-up of membrane bioreactor and hybrid moving bed biofilm reactor-membrane bioreactor (operational conditions of HRT=30.4 h and low biomass concentration).

Abstract

A hybrid moving bed biofilm reactor-membrane bioreactor (hybrid MBBR-MBR) system was studied as an alternative solution to conventional processes. This paper shows the results obtained from three wastewater treatment plants (WWTPs) working in parallel in the start-up and steady states. The first wastewater treatment plant was a membrane bioreactor (MBR), the second one was a hybrid MBBR-MBR system containing carriers both in anoxic and aerobic zones of the bioreactor (hybrid MBBR-MBR_a), and the last one was a hybrid MBBR-MBR system which contained carriers only in the aerobic zone (hybrid MBBR-MBR_b). The reactors operated with a hydraulic retention time (HRT) of 30.40 h. A kinetic study for characterizing heterotrophic biomass was carried out and organic matter and nutrients removals were evaluated. The evolution of the enzymatic activities of α-glucosidase and acid and alkaline phosphatase was analyzed and the bacterial diversity was studied by temperature gradient gel electrophoresis (TGGE). The hybrid MBBR-MBR_b showed the highest removal percentage of organic matter and total nitrogen with values of 91.71±2.59%, 98.21±0.85% and 64.07±8.69% for chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD_5) and total nitrogen (TN), respectively. These results were supported by the kinetic study for the heterotrophic biomass and the highest values of α-glucosidase, acid phosphatase and alkaline phosphatase in the hybrid MBBR-MBR_b (0.7347±0.0634 mM g VSS^{-1} min^{-1}, 7.2419±0.7428 mM g VSS^{-1} min^{-1} and 25.4047±0.3178 mM g VSS^{-1} min^{-1}, respectively). Furthermore, differences in the bioreactor configurations led to differences in the bacterial diversity in the different systems.

1. Introduction

Advanced technologies for wastewater treatment have been developed to control stricter effluent limits or upgrade existing overloaded activated sludge plants (Wang et al., 2006). A conventional membrane bioreactor (MBR) system uses suspended biomass and membrane filtration to treat wastewater and separate biomass (Zhou and Smith, 2002). Several advantages are attributed to MBR treatment according to Rodríguez et al. (2014), although maintaining membrane permeability and preventing fouling are the main problems of this technology (Judd, 2006). On the other hand, the moving bed biofilm reactor (MBBR) systems have been proved to be reliable for organic matter and nutrients removal without suffering the typical problems of suspended biomass processes (Ivanovic and Leiknes, 2008). In these systems, biomass grows as biofilm attached to small inert elements called carriers, usually made of plastic, working as support media for biomass immobilization. Carriers with a lighter density than water keep moving inside the bioreactor by aeration in an aerobic reactor or by a mechanical stirrer in an anaerobic or anoxic reactor. The settleability of biosolids is the largest challenge in MBBR design (Ødegaard, 2000).

The moving bed biofilm reactor-membrane bioreactor (MBBR-MBR) has emerged as a highly effective biological process which solves the problems of the MBR and MBBR systems (Leiknes and Ødegaard, 2007) regarding the fouling and settleability, respectively. These systems combine a biofilm reactor with a membrane bioreactor to separate the suspended solids. There are two ways of working in an MBBR-MBR system: hybrid MBBR-MBR or pure MBBR-MBR, depending on whether or not suspended biomass is present, respectively, as well as attached biomass. In this study, a hybrid MBBR-MBR was used, combining suspended and attached biomass inside the bioreactor since there was recycling between the MBR and the MBBR (Mannina and Viviani, 2009).

Kinetic modeling is an important tool to design, evaluate, control and predict the behavior of the biological processes which take part in the wastewater treatment (Hvala et al., 2002). However, there are still some uncertainties concerning the kinetic behavior of hybrid MBBR-MBR as the coexistence of suspended and attached biomass could lead to a modification in the kinetics of both biomasses, compared with processes involving pure suspended or attached biomass (Di Trapani et al., 2010).

It has been showed that hydrolysis of organic matter is the first step for the organic matter removal in a biological process for wastewater treatment (Burgess and Pletschke, 2008; Reboleiro-Rivas et al., 2013). Hydrolysis of organic matter is mediated by extracellular microbial enzymes (Cadoret et al., 2002; Gessesse et al., 2003). Some of the most important extracellular microbial enzymes are the phosphatase and the α-glucosidase enzymes. In this way, α-glucosidase and phosphatase activities have been proposed as useful tools for the characterization, monitoring and optimization of organic matter biodegradation in a biological process for wastewater treatment (Liwarska-Bizukojc and Ledakowicz, 2003; Anupama et al., 2008; Molina-Muñoz et al., 2010). The enzyme α-glucosidase breaks the α-1,4 glucosidic linkage and releases glucose from maltose, and phosphatase hydrolyzes phosphate esters and releases phosphate groups (Calderon et al., 2013). Therefore, α-glucosidase and phosphatase enzymes have a great importance in MBBR-MBR systems due to high organic loading rates of carbohydrates and phosphorus with the influent (Reboleiro-Rivas et al., 2013). Variations of these enzymatic activities are an excellent indicator of the physiology of the suspended or attached biomass present in an MBBR-MBR system. Moreover, the kinetic study can be complemented by the evaluation of the enzymatic activities.

Molecular biology techniques are useful tools for the investigation of microbial communities in natural and engineered environments, and offer several advantages over other identification methods (Molina-Muñoz et al., 2007). One of the most used molecular biology techniques is temperature gradient gel electrophoresis (TGGE), developed by Muyzer (1999). TGGE has been successfully used for the investigation of bacterial community structure of wastewater treatment systems (Wagner et al., 2002; Cortés-Lorenzo et al., 2006).

The aim of this investigation was to compare an MBR configuration and two hybrid MBBR-MBR systems with different carrier configurations regarding the organic matter removal through the determination of the kinetic parameters relating to the heterotrophic biomass and the enzymatic activities of α-glucosidase, acid phosphatase and alkaline phosphatase in the suspended and attached biomasses, as well as the nutrients removal, during the start-up and steady states. The differences regarding bacterial diversity were analyzed by TGGE. The three WWTPs operated under a hydraulic retention time (HRT) of 30.40 h.

2. Materials and methods

2.1. Description of the wastewater treatment plants

Three urban wastewater treatment plants (WWTPs) working in parallel were fed with municipal wastewater. The first wastewater treatment plant (WWTP) consisted of an MBR (Figure IV.1a), the second one was a hybrid MBBR-MBR system containing carriers in the anoxic and aerobic zones of the bioreactor (hybrid MBBR-MBR_a) (Figure IV.1b), and the last one consisted of a hybrid MBBR-MBR system which contained carriers only in the aerobic zone (hybrid MBBR-MBR_b) (Figure IV.1c).

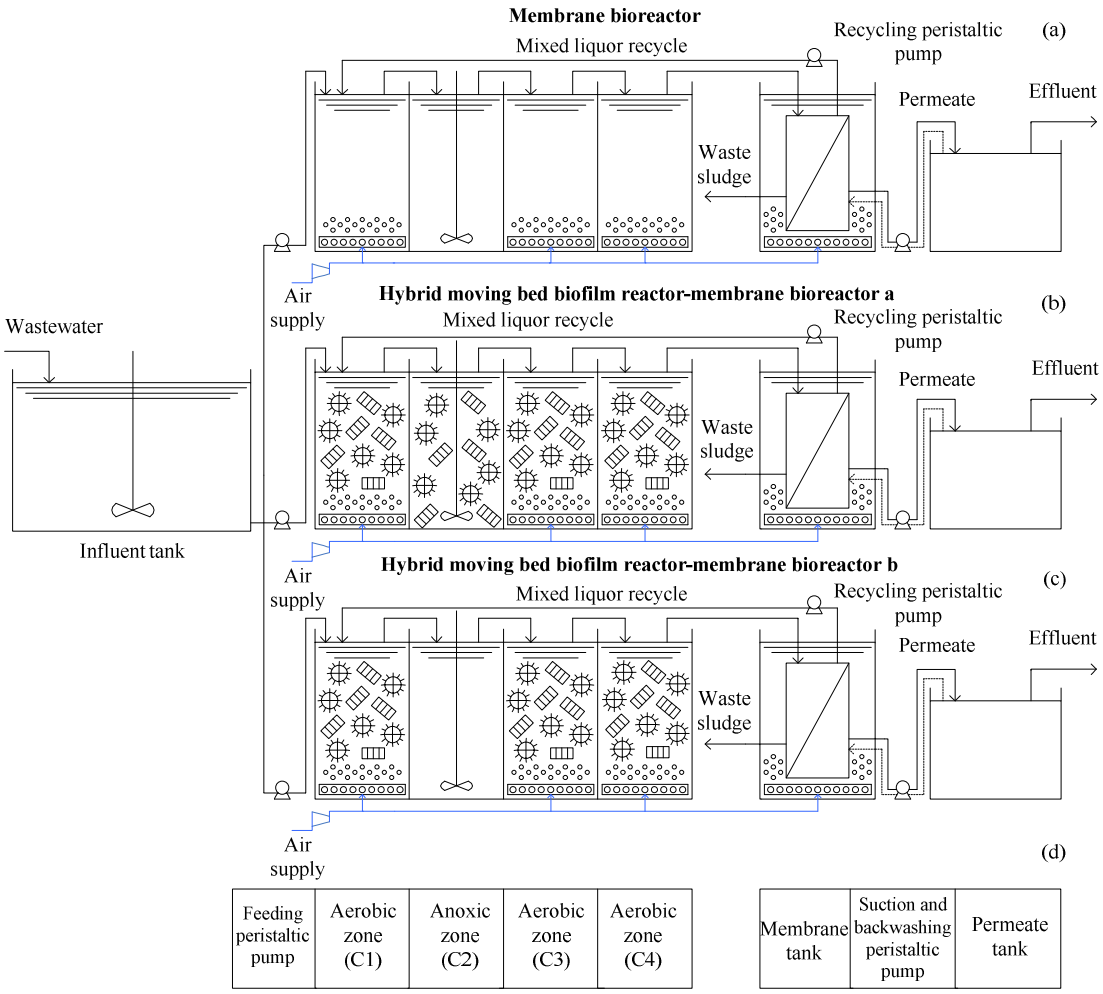

Figure IV.1. Diagram of the three pilot plants of municipal wastewater treatment. (a) Plant with an MBR. (b) Plant with a hybrid MBBR-MBR containing carriers both in the anoxic zone and in the aerobic zone (Hybrid MBBR-MBR_a). (c) Plant with a hybrid MBBR-MBR which contained carriers only in the aerobic zone (Hybrid MBBR-MBR_b). (d) Nomenclature concerning the reactor zones, membrane tank, permeate tank and some peristaltic pumps.

The bioreactors of the WWTPs were divided into four zones, i.e. one anoxic zone (C2) and three aerobic ones (C1, C3 and C4). Both the stirrers and the diffusers in the anoxic and aerobic zones, respectively, had the objectives of homogenizing the mixed liquor and keeping the carriers moving in the hybrid MBBR-MBR systems. Municipal wastewater was pumped into the first aerobic chamber of the bioreactor from the influent tank. It went through the anoxic zone and the rest of the aerobic compartments by a communicating vessel system. The anoxic chamber was in the second compartment to avoid recycling from the membrane tank to the first compartment could change the anoxic conditions as the mixed liquor of the membrane tank contained a higher concentration of dissolved oxygen to prevent membrane fouling. Recycling from the membrane tank to the first chamber of the bioreactor was necessary for maintaining the working mixed liquor suspended solids (MLSS) concentration inside the bioreactor and allowing the nitrogen removal. The recirculation rate was three times and a half the influent flow rate. Subsequently, the outlet of the bioreactor was led into the membrane tank and the permeate was extracted through the membrane by a suction-backwashing peristaltic pump (323U, Watson-Marlow Pumps Group, USA) to collect it into the permeate tank. A cyclic mode of operation was carried out by production and backwashing periods of 9 min and 1 min, respectively. The operational conditions of the WWTPs are shown in Table IV.1.

Table IV.1. Technical data, operational conditions and stabilization concentrations of MLSS, MLVSS, attached BD and VBD of the experimental plants. HRT (hydraulic retention time), MLSS (mixed liquor suspended solids), MLVSS (mixed liquor volatile suspended solids), BD (biofilm density), VBD (volatile biofilm density).

Parameter	MBR		Hybrid MBBR-MBR$_a$		Hybrid MBBR-MBR$_b$	
	Aerobic zone	Anoxic zone	Aerobic zone	Anoxic zone	Aerobic zone	Anoxic zone
Working volume of bioreactor (L)	18	6	18	6	18	6
Filling ratio with carriers (%)	0	0	35	35	35	0
Flow rate (L h^{-1})	0.93		0.93		0.93	
HRT (h)	30.40		30.40		30.40	
SRT (day)	91		91		91	
Working volume of membrane tank (L)	4.32		4.32		4.32	
Total membrane area (m^2)	0.10		0.10		0.10	
Nominal pore size (µm)	0.4		0.4		0.4	
Membrane flux (L m^{-2} h^{-1})	9.3		9.3		9.3	
MLSS (mg L^{-1})	2,691.30±114.99		1,569.87±82.01		1,823.99±51.11	
MLVSS (mg L^{-1})	2,232.14±95.37		1,321.50±69.03		1,552.67±43.50	
BD (mg L^{-1})	-		1,228.18±75.89		880.00±43.01	
VBD (mg L^{-1})	-		983.44±60.77		720.21±35.20	

2.2. Experimental procedure and analytical determinations

Samples were collected from the influent, the three effluents and the anoxic and aerobic zones of the bioreactors and the membrane tanks every day. Physical and chemical determinations were carried out regarding the pH, conductivity, temperature, dissolved oxygen, chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), volatile suspended solids (VSS), total phosphorus (TP) and total nitrogen (TN) according to section **Materials and Methods**.

Furthermore, the kinetic parameters for heterotrophic biomass were evaluated, the enzymatic activities of α-glucosidase, acid phosphatase and alkaline phosphatase were determined and a TGGE fingerprint analysis was carried out (**Materials and Methods**).

The evaluation of statistically significant differences between the results concerning COD, BOD_5, TSS, TN, TP and enzymatic activities was carried out according to section **Materials and Methods.**

3. Results and discussion

3.1. Evolution of the biomass and physical and chemical parameters

The evolutions of MLSS and attached biofilm density (BD) during the start-up and steady states are shown in Figure IV.2.

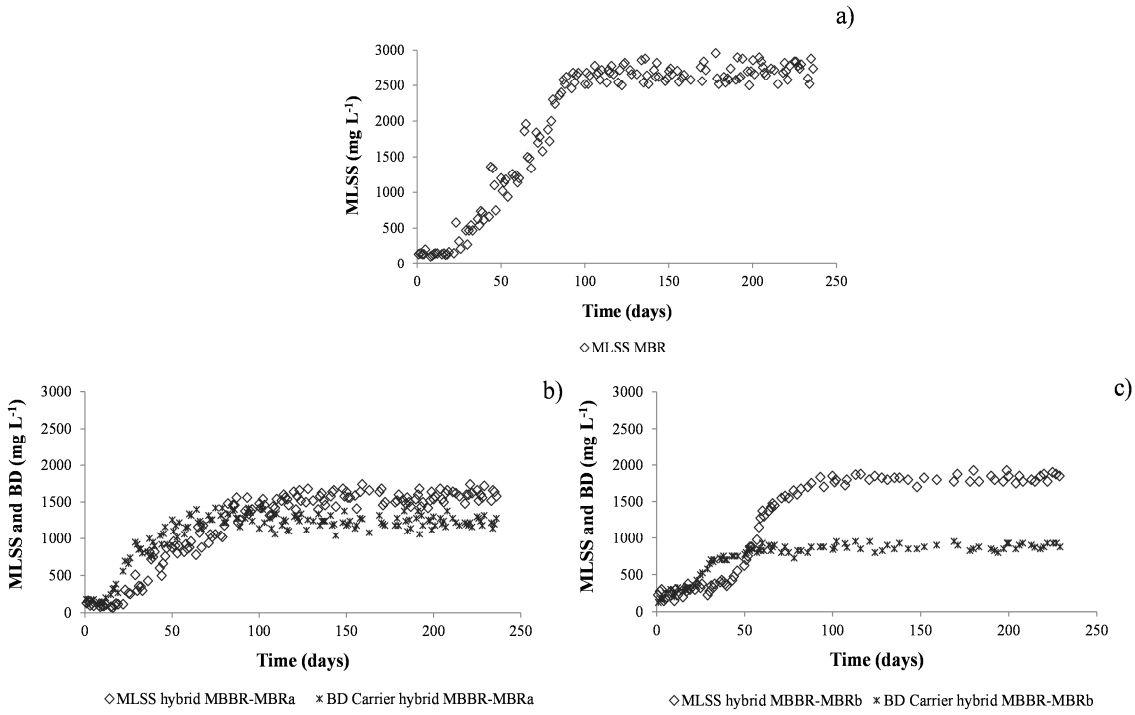

Figure IV.2. Evolution of the mixed liquor suspended solids (MLSS) and attached biofilm density (BD) during the start-up and steady states. (a) MLSS from the MBR. (b) MLSS and BD from the hybrid MBBR-MBR_a. (c) MLSS and BD from the hybrid MBBR-MBR_b.

The total time of the start-up and steady states was 110 and 127 days, respectively, although the steady state was reached before in the MBR. The biomass concentration in the three WWTPs was similar as the difference between the concentrations of MLSS in the WWTPs was compensated by the attached BD on the carriers contained in the hybrid MBBR-MBR systems. Sriwiriyarat and Randall (2005) conducted their research with similar values of MLSS and BD. Mixed liquor volatile suspended solids (MLVSS) and volatile biofilm density (VBD) were used for the estimation of kinetic parameters (Table IV.1).

Table IV.2 shows the average values of pH, conductivity, temperature and dissolved oxygen concentration of the influent, effluents and mixed liquors of each bioreactor in the start-up and steady states. The pH values of the mixed liquors and the effluents were more acidic in the steady state due to the nitrification process, which was more effective after the start-up state (Canziani et al., 2006). The variation of the temperature was higher in the steady state (21.1±4.1ºC) than that observed in the start-up state (22.2±1.9 ºC) as the start-up state was carried out between the months of May and July, and the steady state lasted until December. Wang et al. (2006) recommend a concentration of dissolved oxygen over 2.0±0.1 mg O_2 L^{-1} to obtain an efficient removal of COD and an effective nitrification process, as occurred in the aerobic zone of the different bioreactors.

Table IV.2. Average values of pH, conductivity, temperature and dissolved oxygen of the influent, effluents and mixed liquors of the bioreactors of the experimental plants in the start-up and steady states.

Parameter	Sampling zone									
	Influent	MBR			Hybrid MBBR-MBR_a			Hybrid MBBR-MBR_b		
		Effluent	Anoxic zone	Aerobic zone	Effluent	Anoxic zone	Aerobic zone	Effluent	Anoxic zone	Aerobic zone
Start-up state										
pH	7.99±0.20	7.92±0.56	8.12±0.55	7.68±0.49	7.75±0.65	8.06±0.40	7.50±0.50	7.90±0.68	8.13±0.58	7.56±0.52
Conductivity (µS cm^{-1})	1,261±134	990±151	1,140±185	1,042±153	964±145	1,083±138	993±140	980±148	1,076±129	998±155
Temperature (ºC)	22.7±2.0	22.8±1.9	21.6±1.7	21.8±1.8	22.8±1.9	21.8±1.8	21.8±1.8	22.8±1.9	21.8±1.8	21.9±1.9
Dissolved oxygen (mg O_2 L^{-1})	-	-	0.2±0.1	3.8±1.4	-	0.1±0.1	4.0±1.2	-	0.1±0.1	3.8±1.4
Steady state										
pH	7.83±0.29	6.31±0.98	6.65±0.98	6.47±0.90	5.88±0.94	6.22±0.93	6.06±0.90	6.00±0.95	6.58±0.93	6.24±0.91
Conductivity (µS cm^{-1})	1,200±139	960±76	1,009±103	969±83	980±84	977±96	961±102	968±81	995±87	945±102
Temperature (ºC)	21.0±4.0	21.2±4.0	21.0±4.2	21.1±4.1	21.3±4.0	21.1±4.1	21.1±4.1	21.2±4.1	21.1±4.2	21.1±4.2
Dissolved oxygen (mg O_2 L^{-1})	-	-	0.3±0.1	4.2±1.2	-	0.3±0.1	5.4±0.6	-	0.3±0.1	5.2±0.8

3.2. Organic matter and nutrient removal

The average values of COD, BOD_5, TSS, TN and TP of the influent of the experimental plants and the reduction percentages of these parameters in the start-up and steady states are shown in Table IV.3.

Table IV.3. Average values of COD, BOD_5, TSS, TN and TP of the influent and removal percentages of the experimental plants in the start-up and steady states. COD (chemical oxygen demand), BOD_5 (five-day biochemical oxygen demand), TSS (total suspended solids), TN (total nitrogen), TP (total phosphorus).

Parameter	Sampling zone	Removal percentage	Wastewater treatment plant		
	Influent		MBR	Hybrid MBBR-MBR_a	Hybrid MBBR-MBR_b
Start-up state					
COD (mg O_2 L^{-1})	386.01±136.64	**COD (%)**	85.10±9.14	84.11±11.05	86.60±10.35
BOD_5 (mg O_2 L^{-1})	240.00±88.85	**BOD_5 (%)**	95.00±3.10	93.66±5.35	95.92±2.37
TSS (mg L^{-1})	172.63±89.60	**TSS (%)**	95.82±4.76	93.04±8.79	96.07±3.79
TN (mg N L^{-1})	109.42±23.18	**TN (%)**	48.96±17.69	42.18±19.84	48.53±20.08
TP (mg P L^{-1})	12.68±6.20	**TP (%)**	39.86±26.20	43.15±20.93	37.46±29.30
Steady state					
COD (mg O_2 L^{-1})	336.08±104.48	**COD (%)**	90.75±3.30	90.83±3.53	91.71±2.59
BOD_5 (mg O_2 L^{-1})	262.78±80.78	**BOD_5 (%)**	98.18±1.01	98.18±0.84	98.21±0.85
TSS (mg L^{-1})	157.56±65.71	**TSS (%)**	95.62±4.67	94.82±6.33	94.28±8.27
TN (mg N L^{-1})	99.17±36.50	**TN (%)**	63.06±8.42	61.80±11.95	64.07±8.69
TP (mg P L^{-1})	10.15±4.50	**TP (%)**	36.16±18.31	38.74±16.57	41.30±14.07

The removal percentages of COD, BOD_5 and TN were lower in the start-up phase than those obtained in the steady state. There were not statistically significant differences between the WWTPs concerning these parameters in the start-up and steady states. However, the hybrid MBBR-MBR_b had the best performance regarding COD, BOD_5 and TN removal with values of 91.71±2.59%, 98.21±0.85% and 64.07±8.69%, respectively, in the steady state. These results indicated that the nitrification and denitrification processes in the hybrid MBBR-MBR were more effective than in the MBR, but an anoxic zone without carriers was necessary to provide better contact between nitrate and the microorganisms (Larrea et al., 2007). The removal percentages of BOD_5 and TN were higher with a hydraulic retention time (HRT) of 26.5 h than those obtained with an HRT of 30.40 h since biomass concentrations were higher

(Leyva-Díaz et al., 2013). The values relating to TSS were very similar in the start-up and steady states as the WWTPs contained a module including hollow-fiber microfiltration membranes in the membrane tank. The removal percentages of TP were low in the WWTPs as there was not a strict anaerobic zone to initialize the process of biological phosphorus removal (Kermani et al., 2009), although small anaerobic zones were created in the anoxic compartments of the bioreactor which made phosphorus removal possible together with the physical process of the membrane separation.

3.3. Biological kinetic modeling of MBR, hybrid MBBR-MBR$_a$ and hybrid MBBR-MBR$_b$

Kinetic parameters for the characterization of heterotrophic biomass in the start-up and steady states are shown in Table IV.4.

Table IV.4. Kinetic parameters for the characterization of heterotrophic biomass in the start-up and steady states of the experimental plants. Y_H (yield coefficient for heterotrophic biomass), $\mu_{m,H}$ (maximum specific growth rate for heterotrophic biomass), K_M (half-saturation coefficient for organic matter), k_d (decay coefficient for total biomass).

Parameter	Sampling zone		
	MBR	Hybrid MBBR-MBR$_a$	Hybrid MBBR-MBR$_b$
Start-up state			
Y_H (mg VSS mg COD^{-1})	0.4000	0.4294	0.4592
$\mu_{m,H}$ (h^{-1})	0.0701	0.0185	0.0173
K_M (mg O_2 L^{-1})	54.8786	23.0705	20.6506
k_d (d^{-1})	0.0350	0.1033	0.1032
Steady state			
Y_H (mg VSS mg COD^{-1})	0.2798	0.3453	0.3025
$\mu_{m,H}$ (h^{-1})	0.0028	0.0044	0.0031
K_M (mg O_2 L^{-1})	4.7464	10.8310	3.5491
k_d (d^{-1})	0.0333	0.0230	0.0207

The amount of heterotrophic biomass produced per substrate oxidized, measured by Y_H, in the bioreactors of the WWTPs were higher in the start-up phase because of the biomass growth during this period of time. The heterotrophic biomass of MBR had a better kinetic performance in the start-up phase when the substrate degradation rate (r_{su}) was evaluated through the different parameters of the kinetic model (Figure IV.3a) as

the biomass required more time to grow on the carriers in the hybrid MBBR-MBR systems (hybrid MBBR-MBR_a and hybrid MBBR-MBR_b). It involved that the steady state was reached in less time in MBR as observed in Figure IV.2. The hybrid MBBR-MBR_b showed the best kinetic behavior of heterotrophic biomass in the steady state when r_{su} was evaluated depending on the kinetic parameters, biomass concentration and substrate concentration (Figure IV.3b) under the operational conditions of this study. Thus, the heterotrophic biomass from the hybrid MBBR-MBR_b required less time for organic matter oxidation and the maximum specific growth rate was achieved with less available substrate.

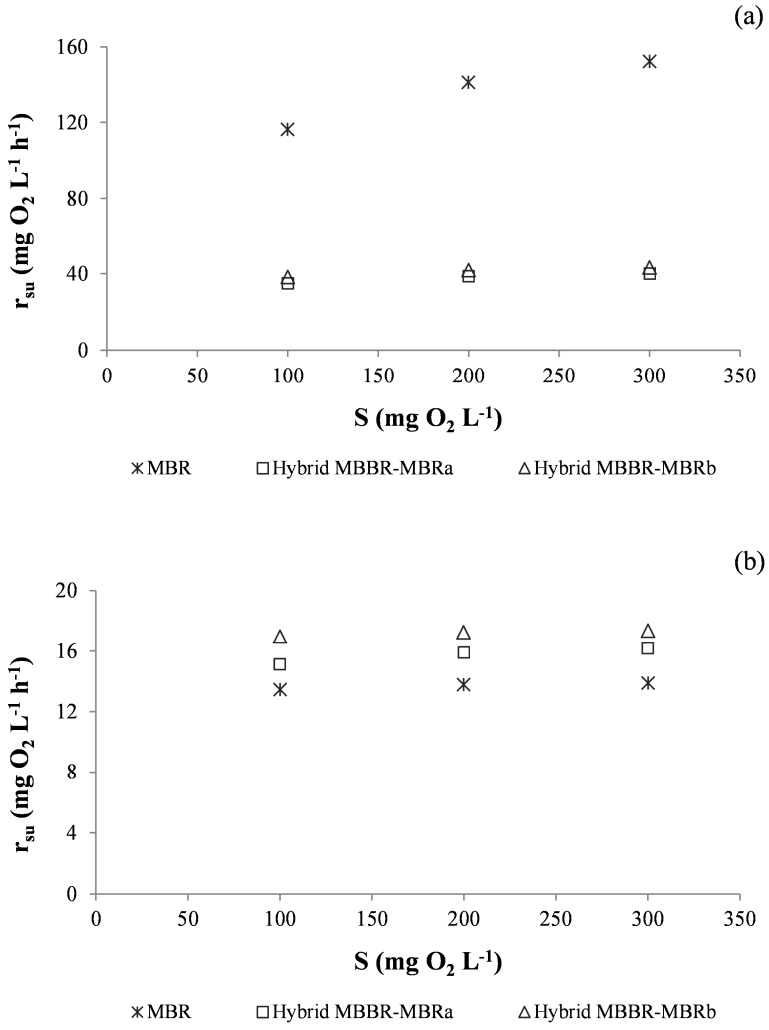

Figure IV.3. Substrate degradation rate (r_{su}) obtained in the heterotrophic kinetic study depending on the substrate concentration for the different bioreactors from the wastewater treatment plants. (a) Start-up phase. (b) Steady state.

These results were in accordance with the percentages of organic matter removal as the hybrid MBBR-MBR_b was the pilot plant with higher COD and BOD_5 removal

percentages in the steady state, as indicated in Table IV.3. Similar values of these kinetic parameters were obtained in other studies (Ferrai et al., 2010; Seifi and Fazaelipoor, 2012). The decay coefficient for the biomass contained in the bioreactors was lower in the steady state of the three WWTPs as the systems were stabilized. Therefore, the total quantity of biomass oxidized per day was higher in the start-up phase.

3.4. Enzymatic activities

The values of α-glucosidase, acid phosphatase and alkaline phosphatase enzymatic activities of suspended and attached biomass of the microbial communities in the four chambers of the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b are shown in Figure IV.4, Figure IV.5 and Figure IV.6, respectively.

It can be observed that the mean α-glucosidase enzymatic activity was higher within the attached biomass than in the suspended biomass in the hybrid MBBR-MBR_a (0.1585±0.0129 mM g VSS^{-1} min^{-1} and 0.1412±0.0162 mM g VSS^{-1} min^{-1}, respectively) and hybrid MBBR-MBR_b (0.5150±0.0381 mM g VSS^{-1} min^{-1} and 0.2197±0.0253 mM g VSS^{-1} min^{-1}, respectively). There is no a clear pattern in the α-glucosidase enzymatic activity with respect to the different chambers for each WWTP observed. The average values of α-glucosidase enzymatic activity relating to days 120, 140 and 160 of the steady state were calculated by considering the mean of the values corresponding to the four chambers. The evaluation of the contribution of suspended and attached biomasses in hybrid MBBR-MBR_a and hybrid MBBR-MBR_b was carried out according to Reboleiro-Rivas et al. (2013). The results show that the α-glucosidase enzymatic activities in the hybrid MBBR-MBR_b and hybrid MBBR-MBR_a, i.e. 0.7347±0.0634 mM g VSS^{-1} min^{-1} and 0.2997±0.0291 mM g VSS^{-1} min^{-1}, respectively, were higher than in MBR with a value of 0.1481±0.0068 mM g VSS^{-1} min^{-1}.

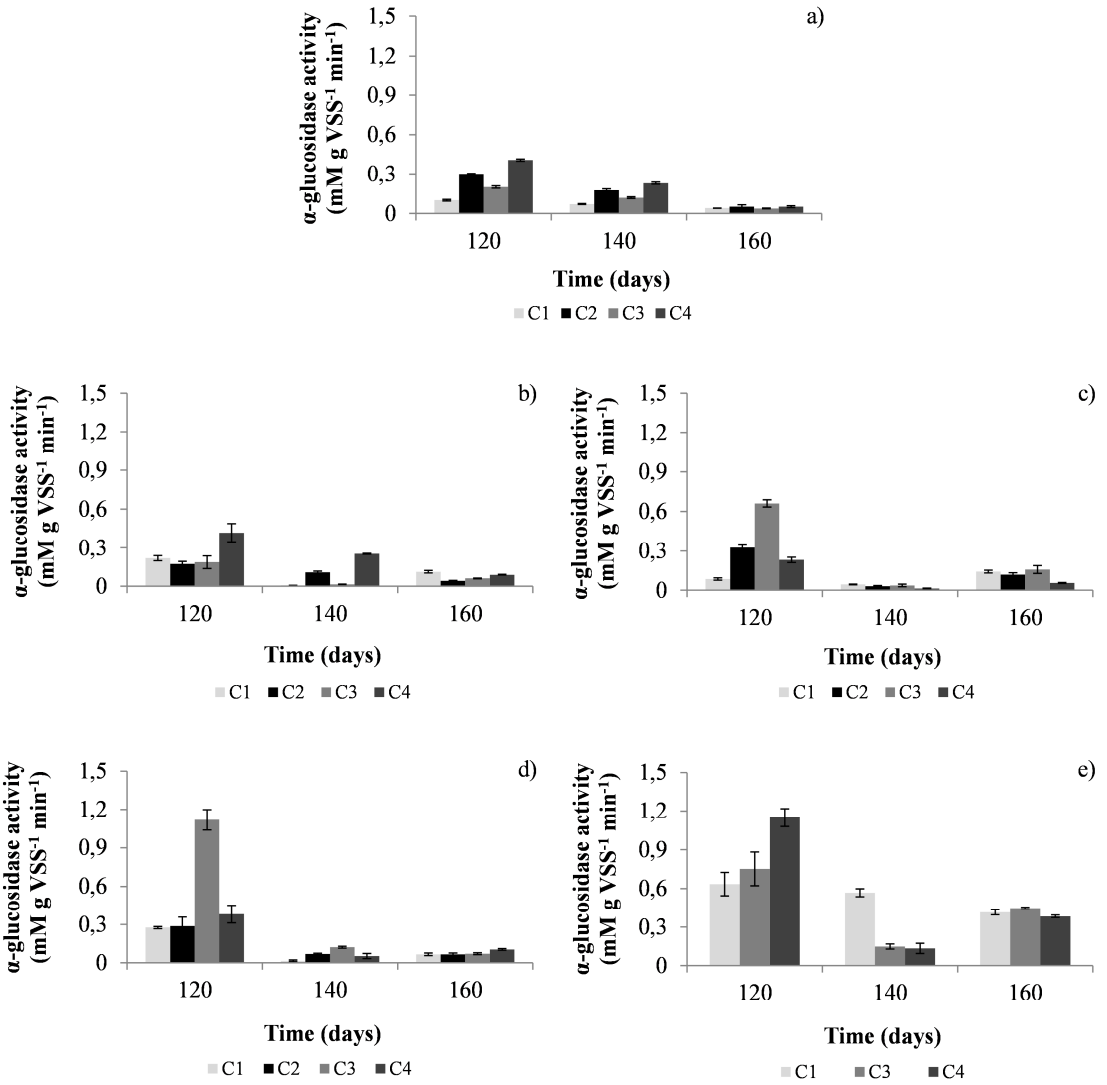

Figure IV.4. Enzymatic activity of α-glucosidase in the chambers C1, C2, C3 and C4 of the different bioreactors. (a) Suspended biomass in the MBR. (b) Suspended biomass in hybrid MBBR-MBR_a. (c) Attached biomass in hybrid MBBR-MBR_a. (d) Suspended biomass in hybrid MBBR-MBR_b. (e) Attached biomass in hybrid MBBR-MBR_b.

The mean acid phosphatase enzymatic activity was higher in the fixed biofilm communities than in the suspended biomass in the hybrid MBBR-MBR_b (4.4769±0.3373 mM g VSS^{-1} min^{-1} and 2.7650±0.4054 mM g VSS^{-1} min^{-1}, respectively); it was lower in the attached biomass than in the suspended biomass in the hybrid MBBR-MBR_a (1.3069±0.1393 mM g VSS^{-1} min^{-1} and 2.6429±0.3950 mM g VSS^{-1} min^{-1}, respectively). Differences between the different chambers were not clear regarding acid phosphatase enzymatic activity. The results show that the acid phosphatase enzymatic activities in the hybrid MBBR-MBR_b and hybrid MBBR-MBR_a, i.e. 7.2419±0.7427 mM g VSS^{-1} min^{-1} and 3.9498±0.5343 mM g VSS^{-1} min^{-1},

respectively, were the highest, followed by MBR with a value of 2.6692±0.3991 mM g VSS^{-1} min^{-1}.

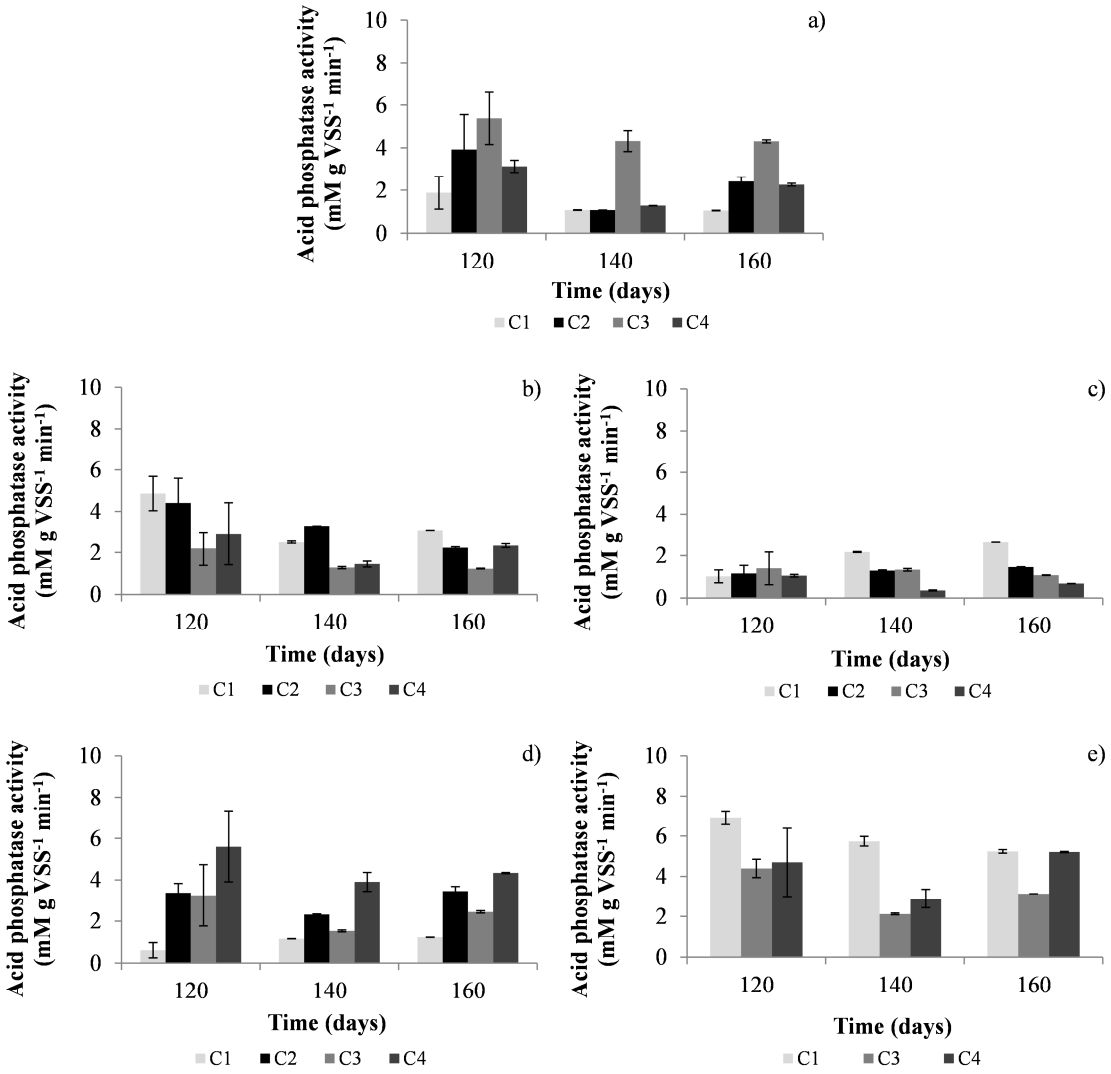

Figure IV.5. Enzymatic activity of acid phosphatase in the chambers C1, C2, C3 and C4 of the different bioreactors. (a) Suspended biomass in the MBR. (b) Suspended biomass in hybrid MBBR-MBR_a. (c) Attached biomass in hybrid MBBR-MBR_a. (d) Suspended biomass in hybrid MBBR-MBR_b. (e) Attached biomass in hybrid MBBR-MBR_b.

The values of alkaline phosphatase enzymatic activity within the fixed biofilm communities were also higher than within the suspended biomass communities in the hybrid MBBR-MBR_b (16.0685±0.1474 mM g VSS^{-1} min^{-1} and 9.3361±0.1704 mM g VSS^{-1} min^{-1}, respectively), but these values were lower in the attached biomass than in the suspended biomass in the hybrid MBBR-MBR_a (2.9025±0.2911 mM g VSS^{-1} min^{-1} and 7.9465±0.6918 mM g VSS^{-1} min^{-1}, respectively). Once again, differences between the different chambers were not related to the different conditions of the systems. The results show that the alkaline phosphatase enzymatic activity in the hybrid MBBR-

MBR_b and hybrid MBBR-MBR_a, i.e. 25.4046±0.3178 mM g VSS^{-1} min^{-1} and 10.8490±0.9829 mM g VSS^{-1} min^{-1}, respectively, were the highest, followed by MBR with a value of 10.0904±0.7878 mM g VSS^{-1} min^{-1}.

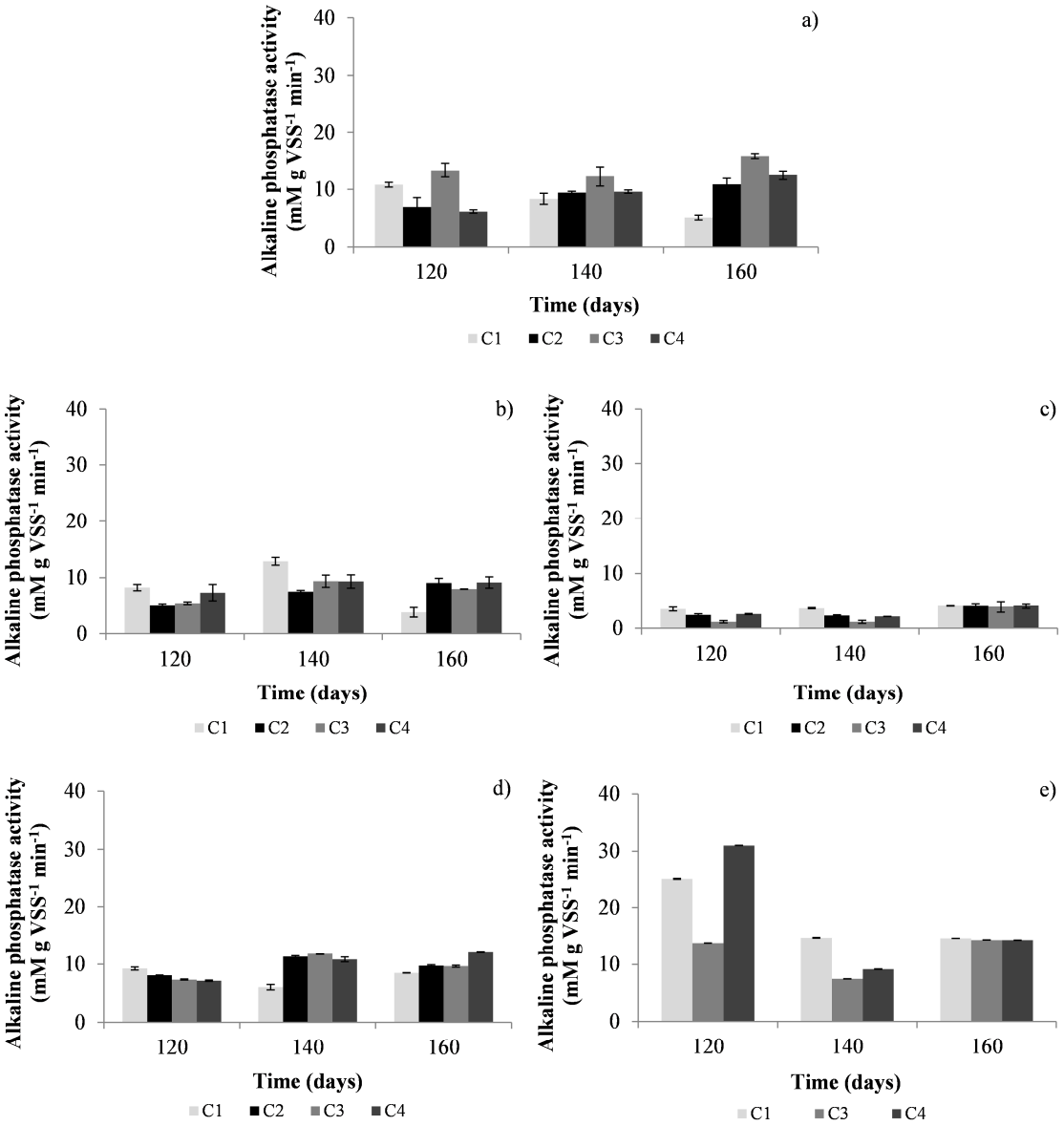

Figure IV.6. Enzymatic activity of alkaline phosphatase in the chambers C1, C2, C3 and C4 of the different bioreactors. (a) Suspended biomass in the MBR. (b) Suspended biomass in hybrid MBBR-MBR_a. (c) Attached biomass in hybrid MBBR-MBR_a. (d) Suspended biomass in hybrid MBBR-MBR_b. (e) Attached biomass in hybrid MBBR-MBR_b.

The hybrid MBBR-MBR_b had the highest values of α-glucosidase, acid and alkaline phosphatase enzymatic activities. Furthermore, the enzymatic activities within the attached biomass were higher than in the suspended biomass in the hybrid MBBR-MBR_b. This might be caused by differences in the structure of the biofilm which was developed on the carriers, or differences in the microbial community as a consequence

of the existence of an anoxic zone without carriers (Reboleiro-Rivas et al., 2013). Guelli and Souza (2012) reported that biofilms are less affected by environmental changes such as nutrient concentration, temperature, pH, metabolic products and toxic substances, than suspended cultures due to the greater stability of the extracellular polymeric substances of the attached biofilm, since the biofilm matrix has been described as a "protective barrier" against adverse environmental conditions (Flemming and Wingender, 2010). The differences regarding the enzymatic activities were statistically significant between the hybrid MBBR-MBR_b and the other two systems with an HRT of 30.40 h as the p-values obtained were lower than α=0.05. The hybrid MBBR-MBR_b system achieved higher removal percentages of COD, BOD_5, TN and TP in the steady state (Table IV.3) as the enzymatic activities were higher; the bacterial activity is closely related to the enzymatic activity within an ecosystem (Nybroe et al., 1992). In this sense, the evaluation of the enzymatic activities also supported the results obtained from the kinetic study, as the hybrid MBBR-MBR_b showed the highest values of the r_{su} for heterotrophic biomass in the steady state (Figure IV.3).

Differences among the different chambers for the same bioreactor configuration were not clear for α-glucosidase and phosphatase enzymatic activities. Similarly, it has been reported that enzymatic activity is not influenced by the dissolved oxygen concentration in the aerobic, anaerobic and anoxic zones of a bench scale activated sludge process (Goel et al., 1998).

3.5. TGGE fingerprint analysis

TGGE fingerprint band analysis regarding suspended and attached biomass communities for chambers C1, C2, C3 and C4 of the bioreactors of the three WWTPs is offered in Figure IV.7. Samples could be divided into five different groups, attending to band patterns. The first group was represented by suspended biomass from the chambers C1, C2 and C3 belonging to the MBR. The second group included the suspended biomass from chambers C1 and C2 and the attached biomass from chamber C1 of the hybrid MBBR-MBR_a, and the suspended biomass from chamber C4 of the MBR. Chambers C3 and C4 of the hybrid MBBR-MBR_a, both fixed biofilm and suspended biomass, as well as the fixed biomass of chamber C2 of the hybrid MBBR-MBR_a, conform another group. Suspended biomass from chambers C1, C2 and C3 and attached biomass from chamber C1 of the hybrid MBBR-MBR_b were grouped in the

fourth group. The remaining samples, i.e. the attached biomass from chambers C3 and C4 and the suspended biomass from chamber C4 of the hybrid MBBR-MBR_b, were clustered into the last one. Fingerprints belonging to the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b were clearly differentiated, with the exception of chamber C4 corresponding to the MBR. In this sense, it can be said that differences in the configuration of the bioreactors were shown in the TGGE fingerprints of their bacterial communities. However, differences in the disposition of bacterial communities, which can be seen in the clustering of TGGE fingerprints, seem to not be remarkable in the different chambers of the three bioreactors, as indicated in the study of the enzymatic activities.

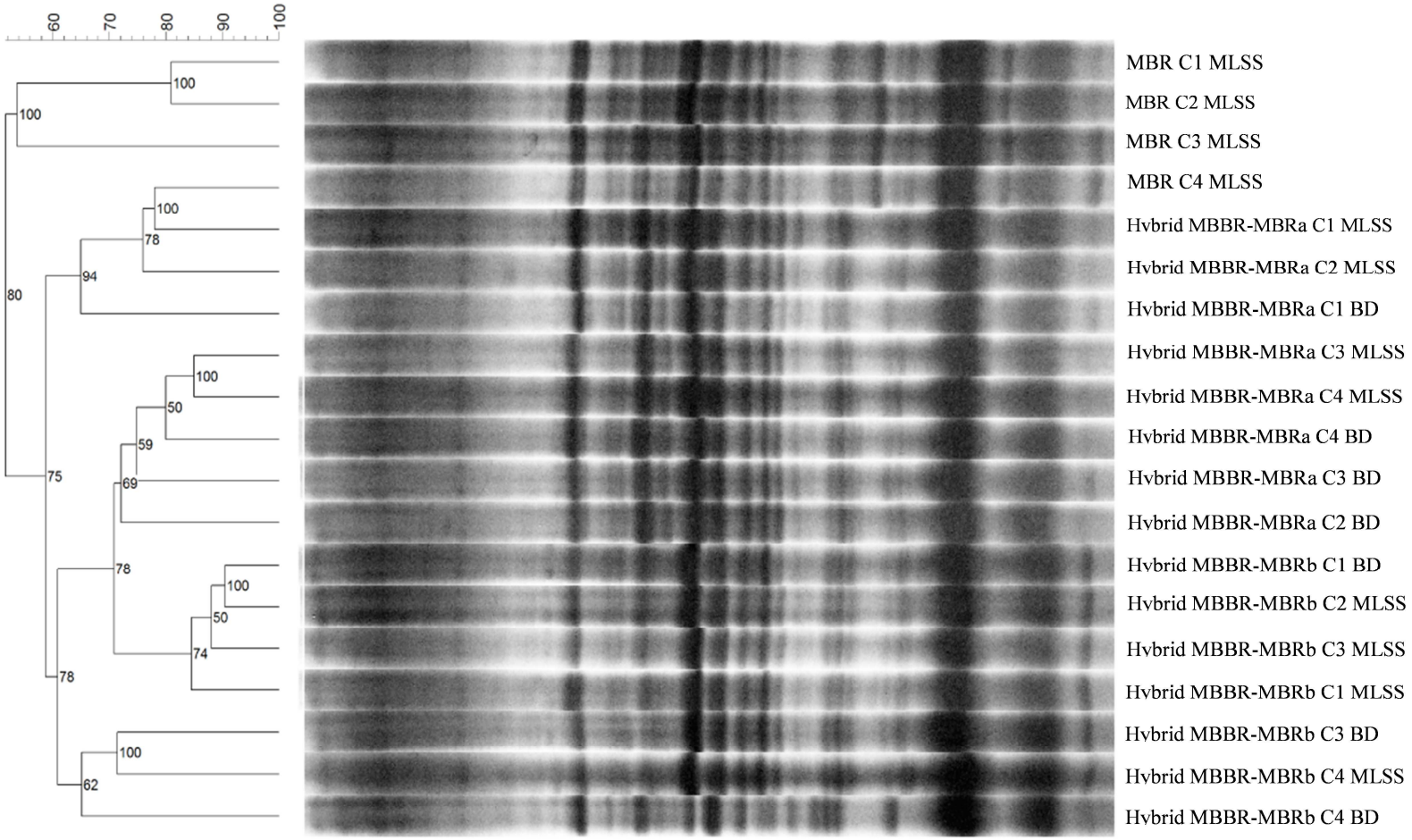

Figure IV.7. TGGE fingerprints of bacterial communities of suspended biomass (MLSS) and attached biomass (BD) in chambers C1, C2, C3 and C4 of the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b.

4. Conclusions

The following conclusions were drawn:

1. The attached biomass provided an extra contribution to the organic matter and nutrient removal as the reduction percentages of COD, BOD_5, TN and TP were higher in the hybrid MBBR-MBR_b which contained carriers only in the aerobic zone of the bioreactor in the steady state. The results regarding organic matter removal were supported by the kinetic study of heterotrophic biomass as the hybrid MBBR-MBR_b showed a better kinetic behavior than the MBR and the hybrid MBBR-MBR_a, with values of Y_H=0.3025 mg VSS mg COD^{-1}, $\mu_{m,\,H}$=0.0031 h^{-1} and K_M=3.5491 mg O_2 L^{-1} in the steady state. The results regarding organic matter and nutrient removal were also supported by the highest values of α-glucosidase, acid phosphatase and alkaline phosphatase in the hybrid MBBR-MBR_b (0.7347±0.0634 mM g VSS^{-1} min^{-1}, 7.2419±0.7428 mM g VSS^{-1} min^{-1} and 25.4047±0.3178 mM g VSS^{-1} min^{-1}, respectively), which implied higher bacterial activities in the hybrid MBBR-MBR_b. Therefore, an anoxic zone without carriers was necessary to provide better contact between nitrate and the microorganisms.

2. The enzymatic activities of α-glucosidase, acid and alkaline phosphatase showed different values in relation to the biomass configuration, with higher values for attached biomass than for suspended biomass in the hybrid MBBR-MBR_b. The improvement in the enzymatic activities of the hybrid MBBR-MBR_b by the presence of attached biomass could have been caused by differences in the structure of the biofilm which was developed on the carriers, or differences in the microbial community as a consequence of the existence of an anoxic zone without carriers.

3. Differences in the bioreactor configuration led to differences in the bacterial diversity in the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b systems according to the TGGE fingerprints of amplicons of the V3 region of the bacterial 16S rRNA gene.

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V. CHAPTER 2

Comparative kinetic study between hybrid moving bed biofilm reactor-membrane bioreactor and membrane bioreactor systems and their influence on organic matter and nutrient removal (operational conditions of HRT=26.5 h and intermediate biomass concentrations).

Abstract

New technologies regarding wastewater treatment have been developed. Among these technologies, the moving bed biofilm reactor combined with membrane bioreactor (MBBR-MBR) is a recent alternative solution to conventional processes. Three wastewater treatment plants (WWTPs), working in parallel, were studied under a hydraulic retention time of 26.5 h. The WWTPs consisted of a membrane bioreactor (MBR), a hybrid MBBR-MBR_a containing carriers in the anoxic and aerobic compartments and a hybrid MBBR-MBR_b containing carriers only in the aerobic zone. The microbial kinetics for heterotrophic and autotrophic biomass was analyzed, as well as the evolution of the enzymatic activities of α-glucosidase and acid and alkaline phosphatase, the bacterial diversity and bacterial community structure. During the study, the difference between the experimental plants was not statistically significant concerning organic matter and nutrients removal. However, different tendencies regarding nutrients removal were shown by the three wastewater treatment plants. In this sense, the performances in terms of nitrogen and phosphorus removal of the hybrid MBBR-MBR_b (67.34±11.22% and 50.65±11.13%, respectively) were slightly better than those obtained from the other experimental plants. As a whole, the MBR showed a better kinetic performance for the heterotrophic and autotrophic biomass, with values of $\mu_{m,\,H}$=0.0086 h^{-1}, $\mu_{m,\,A}$=0.0765 h^{-1}, K_M=2.3659 mg O_2 L^{-1} and K_{NH} =1.3070 mg N L^{-1}. The results regarding the enzymatic activities showed higher values for suspended biomass than for attached biomass in the hybrid MBBR-MBR systems. Temperature gradient gel electrophoresis (TGGE) fingerprints and scanning electron microscopy (SEM) analysis demonstrated the existence of differences in the bacterial diversity and bacterial community structure.

1. Introduction

In the course of the last few decades, industrial development, increase in urbanization and changes in farming practices, among other factors, have caused an outstanding rise in the consumption of water resources as well as deterioration in their quality. As a consequence of this, and due to the more restrictive limits imposed to the effluent of municipal wastewater treatment plants (WWTPs) by the Water Framework Directive (Chave, 2001), it has become necessary to improve existing municipal WWTPs. To achieve this, the development of more advanced technologies is necessary in order to comply with currently established effluent limits and water quality guideline as well as those that could be imposed in the future.

Water quality is influenced by several factors. Some of the most important are the organic matter content and the enrichment of nutrients in water bodies, like phosphorus and nitrogen (Mulkerrins et al., 2004). Wastewater with high levels of organic matter, phosphorus and nitrogen can be the main reason for several problems when released into the environment, such as oxygen consumption, eutrophication and toxicity (Luostarinen et al., 2006). Accordingly, it is necessary to remove these contaminants from wastewater in order to reduce the damage caused to the environment (Wang et al., 2006).

Secondary treatment is the main process in a municipal wastewater treatment plant (WWTP), with the aim of removing these contaminants. It is accomplished by biological processes classified into two different types: suspended biomass or biofilm processes. Suspended biomass processes are effective for the elimination of organic matter and nutrients in municipal WWTPs. The activated sludge process is the most commonly used suspended biomass process. However, these processes can have some drawbacks when exposed to high hydraulic and organic loads. To improve the performance of this process, the amount of biomass inside the reactor would have to be increased to the limitation imposed by the clarifier. Furthermore, sludge settleability, the large reactors and settling tanks required in these processes can be problematic (Pastorelli et al., 1999).

Membrane bioreactors can be highlighted as they solve most of the problems of conventional activated sludge systems. They combine membrane filtration and

biological treatment using activated sludge, thus providing several advantages (Stephenson et al., 2000). Firstly, the membrane replaces the clarifier in the wastewater treatment system (Gunder and Krauth, 1998; van der Roest et al., 2002). Apart from this, these are compact systems with practically complete solids removal which permit effluent disinfection. Moreover, they can operate at higher suspended biomass concentrations, resulting in long sludge retention times as well as low sludge production and avoidance of problems regarding sludge bulking. However, fouling is a common problem of this kind of system, which is caused by the accumulation of substances on the surface of the membrane with a consequent reduction in membrane permeability (Defrance et al., 2000).

On the other hand, biofilm processes have been proved to be reliable for organic matter and nutrients removal without suffering the typical problems of suspended biomass processes (Ødegaard et al., 1994). There are many different biofilm systems, such as trickling filters, rotating biological contactors, fixed media submerged biofilters, granular media biofilters, fluidized bed reactors, etc. They all have advantages and disadvantages (Rusten et al., 2006; Leiknes and Ødegaard, 2007). For these reasons, the moving bed biofilm reactor (MBBR) process was developed in Norway in the late 1980s and early 1990s. In this study, a hybrid technology between a MBBR and a membrane bioreactor (MBR), which combines suspended and attached biomass, was analysed. This system combines suspended biomass and biofilm processes through the addition of carrier media inside the biological reactor for biofilm growth (Ødegaard, 2006). This process has been proved to be a very simple and efficient technology in municipal wastewater treatment (Hem et al., 1994; Rusten et al., 1995). It was developed on the basis of conventional activated sludge and biofilter processes. In these systems, biomass grows as suspended flocs and biofilm. In the case of biofilm, it adheres and grows attached to small inert elements, usually made of plastic, working as support media for biomass immobilization. These elements have a lighter density than water and they keep moving inside the reactor. This movement can be driven by aeration in an aerobic reactor or by a mechanical stirrer in an anaerobic or anoxic reactor.

Moving bed biofilm reactors have several advantages when compared to suspended biomass processes: higher biomass concentration, high chemical oxygen demand loading, strong tolerance to loading impact, higher sludge age, lower hydraulic

residence times, higher volumetric removal rates, relatively small area requirements and no sludge bulking problem (Chen et al., 2008). Moreover, the combination of biofilm reactors with a membrane separation of suspended solids may reduce the effect of membrane fouling caused by high biomass concentrations inside membrane bioreactors (Leiknes and Ødegaard, 2001; Leiknes and Ødegaard, 2002). When they are compared to other kinds of biofilm processes, moving bed biofilm reactors present very good mixing conditions, resulting in efficient mass transfer and the elimination of the risks of clogging of the media with biomass or other solids. In this way, they have the capacity to handle high loads of particulate matter (Welander et al., 1998). Additionally, the increase of the sludge age in the system leads to the creation of a favorable environment for the growth of nitrifying bacteria (Randall and Sen, 1996), which is also supported by biomass immobilization as a biofilm allowing for maintaining slow-growing organisms in the system. Nitrification and denitrification are two of the most important processes used in wastewater treatment and they have been used successfully in biofilm reactors (Wang et al., 2006). In general, *Nitrosomonas* and *Nitrobacter* are assumed to be responsible for nitrification in wastewater, while denitrification is achieved by denitrifying organisms, although an organic carbon source is required (Barnes and Bliss, 1983; Wiesmann, 1994; Wang et al., 2006).

In the last few years, many studies have been carried out on these systems, with the goals of assessing process performance and the interaction between suspended and biofilm growth, comparing different biofilm carriers. In this way, interesting results have been obtained showing the effectiveness of these systems for organic matter and nitrogen removal. In this sense, operational results showed both a purification improvement of organic matter and ammonia and the existence of simultaneous denitrification (Müller, 1998; Di Trapani et al., 2008).

However, MBBR processes are relatively novel from the point of view of the kinetics and there are some uncertainties regarding the kinetic performance of these systems. Activated sludge plants have been widely modelled using the ASM model family (Henze et al., 2000). On the other hand, the modelling of MBBR systems remains very challenging to process engineers. The coexistence of two kinds of biomass, suspended and attached, could lead to a modification in the kinetic parameters of both biomasses, compared to those of a pure suspended biomass process. Modelling

and dynamic simulation can also be an important tool for the design and operation of MBBR plants (Hvala et al., 2002).

In this study, the Monod model was used to estimate the kinetic parameters which characterize autotrophic and heterotrophic biomass. This model describes cellular growth according to the availability of a limiting substrate (Monod, 1949). Moreover, the death of microorganisms is included in the model by adding the cellular decay rate (r_d). Therefore, the net cellular growth rate (r'_x) can be calculated according to Eq. (1):

$$r'_X = \frac{\mu_m S\, X}{K_S + S} - k_d X \qquad (1)$$

The relevant parameters which describe the model for autotrophic (A) and heterotrophic (H) biomass are the respective yield coefficients (Y_A and Y_H), the maximum specific growth rates ($\mu_{m,\,A}$ and $\mu_{m,\,H}$), the amounts of active biomass ($X_{B,\,A}$ and $X_{B,\,H}$) and the half-saturation coefficients for ammonia nitrogen (K_{NH}) and organic matter (K_M). The substrate concentration can be referred to ammonium concentration (S_{NH}) or organic matter concentration (S_S), depending on the kind of biomass studied. These parameters could be obtained by respirometric experiments which allow characterizing autotrophic and heterotrophic biomass according to the Monod model.

The study of the enzymatic activities of α-glucosidase, acid phosphatase and alkaline phosphatase can complement the kinetic analysis and support the results regarding organic matter and nutrient removal of the different WWTPs (Molina-Muñoz et al., 2010). These enzymatic activities have a great importance in moving bed biofilm reactor-membrane bioreactor (MBBR-MBR) systems since the biodegradation of organic matter (carbohydrates) and nutrients (nitrogen and phosphorus) contained in the influent is mediated by extracellular microbial enzymes such as the α-glucosidase and the phosphatase enzymes in a biological process for wastewater treatment (Cadoret et al., 2002; Liwarska-Bizukojc and Ledakowicz, 2003). Variations of these enzymatic activities indicate the different physiology of the suspended and attached biomass present in an MBBR-MBR system. It allows for characterizing, monitoring and optimizing the biological process which take place in the WWTPs (Anupama et al., 2008).

Molecular biology techniques are useful tools for the investigation of microbial communities in natural and engineered environments, and offer several advantages over other identification methods (Molina-Muñoz et al., 2007). One of the most used molecular biology techniques is temperature gradient gel electrophoresis (TGGE), developed by Muyzer (1999). TGGE has been successfully used for the investigation of bacterial community structure of wastewater treatment systems (Cortés-Lorenzo et al., 2006). Furthermore, scanning electron microscopy (SEM) allows for analyzing the community structure of bacterial biofilms developed in an MBBR-MBR system.

The principal aim of this investigation was to quantify organic matter removal, solids reduction as well as denitrification and nitrification capacities through the application of two hybrid MBBR-MBR systems with continuous operation under a hydraulic retention time (HRT) of 26.5 h. In addition, a comparison of the results obtained from the previously mentioned system with those of a MBR configuration was also performed. The secondary objective was to determine the kinetic parameters relating to the autotrophic and heterotrophic biomasses and the enzymatic activities of α-glucosidase, acid phosphatase and alkaline phosphatase which characterize the hybrid MBBR-MBR and MBR processes and then compare them. The bacterial diversity and the structure of the biomass present were evaluated by TGGE and SEM, respectively.

2. Materials and methods

2.1. Description of the experimental pilot plants

The experiments were conducted using three urban wastewater treatment systems working in parallel. There was a sewage storage tank which was filled with real wastewater coming from the WWTP of Puente de los Vados, located in Granada (Spain). The three pilot plants were fed by a feeding peristaltic pump (323S, Watson-Marlow Pumps Group, USA) with municipal wastewater from this tank. Each plant included a biological reactor divided into four zones, one anoxic zone and three aerobic ones, followed by a membrane tank with a submerged hollow-fiber module. The outlet of the bioreactor was subsequently led into the membrane tank from where the permeate was extracted through the membrane using a suction-backwashing peristaltic pump (323U, Watson-Marlow Pumps Group, USA). A small volume of the concentrate (retentate) was removed as excess sludge (Figure V.1).

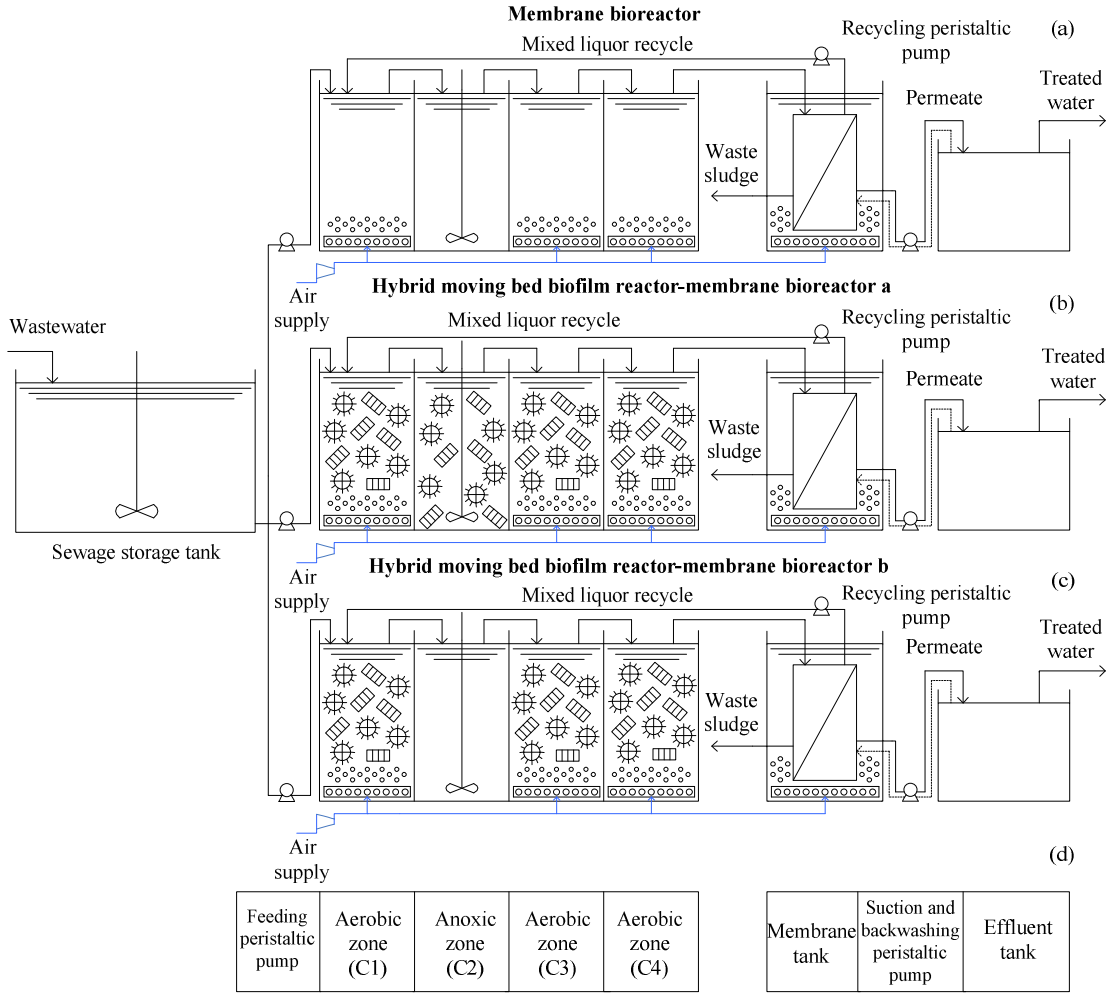

Figure V.1. Schematic diagram of the three pilot plants of municipal wastewater treatment used in the study. (a) Plant with an MBR. (b) Plant with a hybrid MBBR-MBR containing carriers both in the anoxic zone and in the aerobic zone (hybrid MBBR-MBR$_a$). (c) Plant with a hybrid MBBR-MBR which contained carriers only in the aerobic zone (hybrid MBBR-MBR$_b$). (d) Nomenclature concerning the reactor zones, membrane tank, effluent tank and some peristaltic pumps.

The first WWTP consisted of an MBR (Figure V.1a), the second one was a hybrid MBBR-MBR system containing carriers both in anoxic and aerobic zones (hybrid MBBR-MBR$_a$) (Figure V.1b), and the last one consisted of a hybrid MBBR-MBR system which contained carriers only in the aerobic zone (hybrid MBBR-MBR$_b$) (Figure V.1c). Figure V.1d shows the reactor zones, membrane tank, effluent tank and some peristaltic pumps.

The MBR (Figure V.1a) included a biological reactor divided into four zones, one anoxic zone and three aerobic ones, followed by a membrane tank with a submerged hollow-fiber module. The dimensions of the biological reactor were 50 cm long, 12 cm wide and 60 cm high. Each of the four compartments was 12 cm long, 12 cm wide and

60 cm high. The total volume of the reactor was 36 L; each compartment of the reactor had a volume of 9 L. The reactor had a security percentage with a value of 33% in relation to the total volume. Therefore, the operation volume was 24 L and each compartment of the reactor had a usable volume of 6 L (Table V.1).

Table V.1. Technical data, operation conditions and stabilization concentrations of MLSS and attached BD of the experimental plants. HRT (hydraulic retention time), MLSS (mixed liquor suspended solids), BD (biofilm density).

Parameter	MBR		Hybrid MBBR-MBR$_a$		Hybrid MBBR-MBR$_b$	
	Aerobic zone	Anoxic zone	Aerobic zone	Anoxic zone	Aerobic zone	Anoxic zone
Volume (L)	18	6	18	6	18	6
Filling ratio with carriers (%)	0	0	35	35	35	0
Flow rate (L h^{-1})	1.07		1.07		1.07	
HRT (h)	26.5		26.5		26.5	
SRT (day)	42		42		42	
MLSS (mg L^{-1})	4,383.86±316.01		2,553.75±293.42		2,999.14±400.18	
BD (mg L^{-1})	-		1,000.35±345.26		675.00±175.39	

Municipal wastewater, which came from the sewage storage tank, was pumped into the first aerobic chamber of each reactor. Then, it passed through the anoxic chamber and, subsequently, it reached the second and third aerobic chambers by a communicating vessel system. Finally, wastewater went into the membrane tank. The anoxic zone was in the second compartment in order to avoid that the recycling from the membrane tank, which contained a higher dissolved oxygen concentration to prevent the membrane fouling, could change the anoxic conditions. Therefore, the anoxic zone was set between the first and the third aerobic zones with dissolved oxygen concentrations which could be adjusted to values that were not too high. Eventually, a recycling was carried out from the membrane tank to pump out the aerobic mixed liquor, which contained oxidized organic matter and nitrate, into the anoxic chamber through a recycling peristaltic pump (323S, Watson-Marlow Pumps Group, USA). The anoxic chamber received the recycling flow from the membrane tank after passing through the first aerobic chamber to convert the nitrate into gaseous nitrogen (N_2) in the presence of organic matter acting as electron donor. This process is called denitrification and it allowed the elimination of nitrogen as a gaseous substance which was expelled to the atmosphere. The recycling rate was three times the influent flow rate.

The membrane tank was designed as an external submerged unit where the dimensions of the tank were adjusted for particle separation only. The membrane tank was cylindrical. It had a diameter of 10 cm and its height was 65 cm. The total volume of this tank was 6.7 L, but the effective volume was 4.32 L. The membrane tank consisted of a vertically oriented submerged module of hollow-fiber microfiltration membranes (Micronet Porous Fiber, SL, Spain). The membrane was flowing from the outside to the inner side by sucking. The total membrane area was 0.10 m^2. The hollow fibers were made of polyvinylidene fluoride and they had an inside braid-reinforcement made of polyester. The fibers had an outer diameter of 2.45 mm, an inner diameter of 1.10 mm and a pore size of 0.4 μm. Aeration was applied at the base of the module by a coarse bubble disk diffuser (CAP 3, ECOTEC, SA, Spain). The membranes were continuously aerated with a tangential air current to prevent any organic or inorganic solids from being settled on their surface. Air was supplied by an air compressor (ACO-500, Hailea, China). The airflow to the MBR was measured by a rotameter (2100 Model, Tecfluid, SA, Spain) and regulated by a manual valve. The flow rate of air had a value of 500 L h^{-1} and the air was supplied at a constant pressure and temperature of 0.5 bar and 20ºC.

The permeate was extracted by a suction-backwashing peristaltic pump to collect it into the permeate tank. The cyclic mode of operation consisted of production and backwashing periods of 9 min and 1 min, respectively. The MBR was operated at a constant flux with a value of 10.7 L m^{-2} h^{-1} (LMH) and the transmembrane pressures (TMP) varied between 0.1 and 0.5 bar. The operating parameters such as permeate flow, permeation and backwashing times could be adjusted by a control panel.

The systems which combined a MBBR with a MBR (Figure V.1b and Figure V.1c) had the same dimensions as the MBR (Table V.1). The membrane tanks of the hybrid MBBR-MBR systems were also the same as those used in the MBR. The operation of the hybrid MBBR-MBR systems was identical to that described for the MBR.

Biomass grew as suspended flocs and as a biofilm in the hybrid MBBR-MBR systems. The biomass which grew as a biofilm was developed on carriers which moved freely in the water volume by aeration in the aerobic zone and by a mechanical stirrer in the anoxic zone. This kind of carrier is called K1 and it was developed and supplied by

AnoxKaldnes AS (Norway). This carrier has been widely studied in similar experiments (Melin et al., 2005; Leiknes and Ødegaard, 2007; Di Trapani et al., 2008; Martín-Pascual et al., 2012). The K1 media filling-fraction (percentage of the reactor volume occupied with carriers in an empty tank) and the effective reactor volumes in the MBBR are shown in Table V.1. The filling ratio of 35% had an effective specific surface area of 175 $m^2 m^{-3}$ tank volume.

Sampling ports were provided in each reactor for sample collection. All anoxic zones had variable speed stirrers (Multi Mixer MM-1000, Biosan Laboratories, Inc., USA) which kept in movement the biofilm media in the anoxic zone. The sewage storage tank also had a variable speed propeller to homogenize municipal wastewater. This stirrer was identical to the previous ones. Normal propeller speed was 320 rpm both in the anoxic zone and in the feeding tank. Aerobic zones were equipped with a fine bubble disk diffuser (AFD 270, ECOTEC, SA, Spain) at the bottom of the reactor. Air to the aerobic zone was supplied by an air compressor (ACO-500, Hailea, China). The airflow to the reactor was measured by a rotameter (2100 Model, Tecfluid, SA, Spain) and regulated by a manual valve. The flow rate of air in each of the biological reactors had a value of 30 L h^{-1} and the air was supplied at a constant pressure and temperature of 0.5 bar and 20ºC. Both the stirrer in the anoxic zone and the diffuser in the aerobic one had the function of keeping the carriers moving inside the reactor and homogenising the mixed liquor.

2.2. Experimental procedure and analytical determinations

Reactors operated at a constant flow rate of 1.07 L h^{-1} and an HRT of 26.5 h. The recycling rate was five times the influent flow rate although it was reduced until a value of three times the influent flow rate in order to the concentrations of mixed liquor suspended solids (MLSS) and the attached biofilm density (BD) reached the values corresponding to the stabilization phase, which are indicated in Table V.1. The inf uent in each bioreactor was controlled by a level indicator connected to the feeding pump, ensuring that the level in the bioreactor was suitable and the membranes of the membrane tank were at all times covered by the mixed liquor. Samples were collected every 24 h from the influent, the three effluents, as well as the anoxic and aerobic zones of the different reactors and the membrane tank.

Physical and chemical determinations were carried out regarding the pH, conductivity, temperature, dissolved oxygen, chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), volatile suspended solids (VSS), total phosphorus (TP) and total nitrogen (TN) according to section **Materials and Methods**.

Furthermore, the kinetic parameters for heterotrophic and autotrophic biomass were evaluated, the enzymatic activities of α-glucosidase, acid phosphatase and alkaline phosphatase were determined, a TGGE fingerprint analysis was carried out and the structure of the attached biofilm was analyzed by SEM (**Materials and Methods**).

The evaluation of statistically significant differences between the results concerning COD, BOD_5, TSS, TN, TP and enzymatic activities was carried out according to section **Materials and Methods.**

3. Results and discussion

3.1. Biofilm formation and MLSS

The three pilot plants were started up with urban wastewater taken from the WWTP of Puente de los Vados located in Granada (Spain). The concentration of MLSS and the biofilm formation were developed by the feeding and recycling of municipal wastewater from the membrane tank until the first aerobic compartment at a flow rate of 5 L h^{-1}. During the start-up phase, lower mixing velocity of the mechanical stirrer (around 80 rpm) was applied in the anoxic zones in order to allow better attachment of microorganisms. Later it was gradually increased to 320 rpm to avoid clogging up the carriers. The total time of the start-up phase was 47 days. Subsequently, the stabilization phase started. This phase had a duration of 59 days.

The value relating to the concentration of MLSS from the MBR is shown in Figure V.2a. The values of the concentration of MLSS and attached BD from the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b are shown in Figure V.2b and Figure V.2c, respectively.

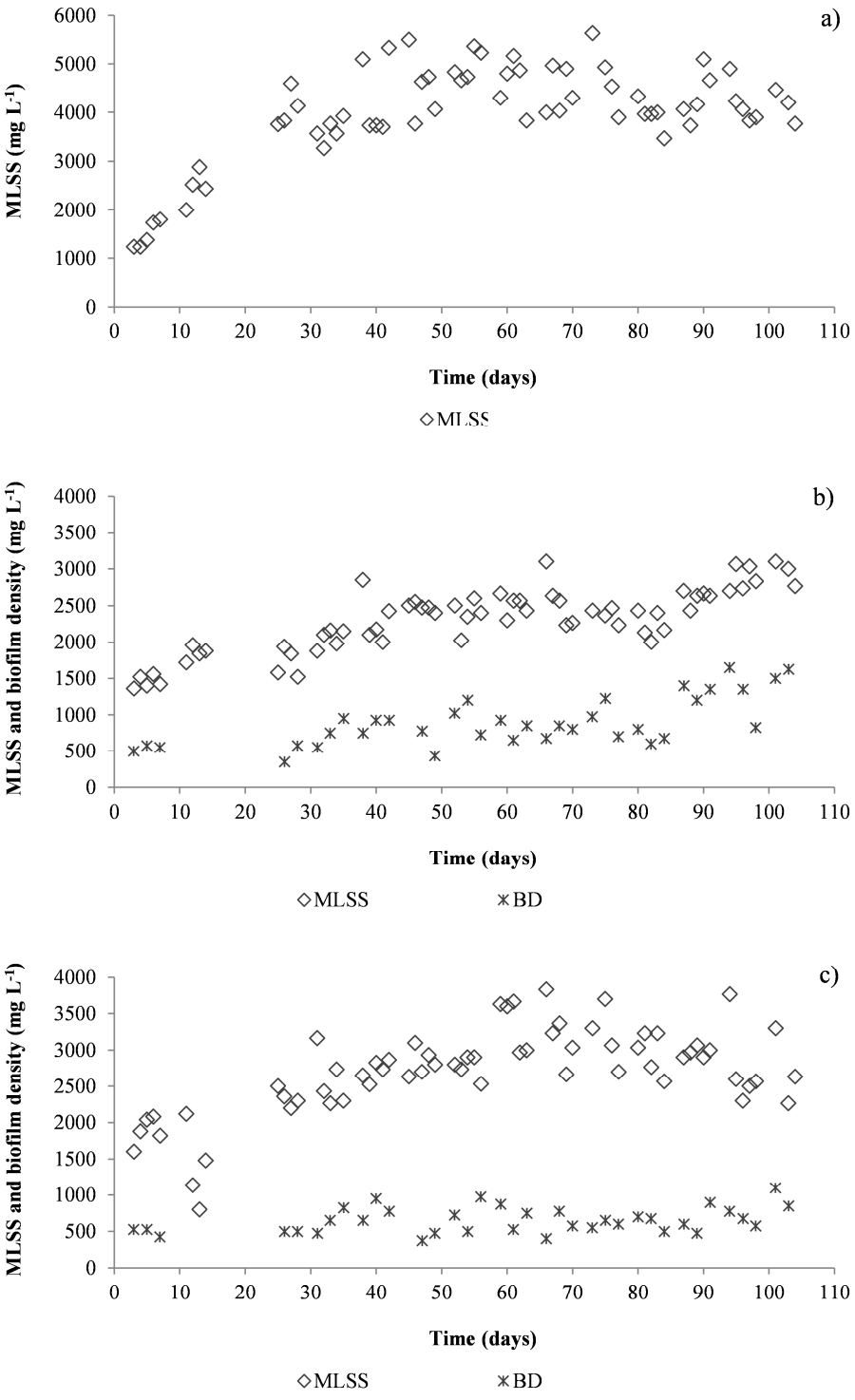

Figure V.2. Evolution of the mixed liquor suspended solids (MLSS) and attached biofilm density (BD) during the start-up and stabilization phases. (a) MLSS of the MBR. (b) MLSS and BD attached to the carrier of the hybrid MBBR-MBR_a. (c) MLSS and BD attached to the carrier of the hybrid MBBR-MBR_b.

Figure V.2a, Figure V.2b and Figure V.2c show the increase in MLSS and BD for the experimental plants until the day 47, when the start-up phase ended. MLSS and BD were kept at a constant value from that day to the end of this study (stabilization phase).

However, the last days relating to the stabilization phase from the hybrid MBBR-MBR_a (Figure V.2b) showed a slight increase in MLSS and biofilm density compared to the hybrid MBBR-MBR_b (Figure V.2c). It could be caused by a slight detachment of biofilm from the carriers contained in the hybrid MBBR-MBR_a which had a higher number of carriers than the hybrid MBBR-MBR_b, so this effect was not observed in the hybrid MBBR-MBR_b. Rutt et al. (2006) worked with similar concentrations of MLSS and attached BD to those used in this research.

The concentrations of MLSS in the plants with MBBR (hybrid MBBR-MBR_a and hybrid MBBR-MBR_b) were similar, as can be seen in Table V.1. These values were lower than the concentration of MLSS in the MBR (4,383.86±316.01 mg L^{-1}). However, this difference was compensated by the attached biofilm on the carriers contained in the hybrid MBBR-MBR systems, with values of BD of 1,000.35±345.26 mg L^{-1} for the hybrid MBBR-MBR_a (Figure V.2b) and 675.00±175.39 mg L^{-1} for the hybrid MBBR-MBR_b (Figure V.2c). These values of the concentration of MLSS and BD were similar to those employed by Kim et al. (2010) in their research. Sriwiriyarat and Randall (2005) also carried out their study with similar values of MLSS and BD. These attached biofilm densities were lower than those reported in other studies, such as the one carried out by Marques et al. (2008). The values of BD were relatively low. This was probably due to the sludge retention time, organic loading rate, aeration in the aerobic zone of the reactors and the mechanical agitation in the anoxic zone. Aeration and agitation made the biofilm detachment be higher than the expected value. Furthermore, there was not a direct relation between the organic matter contained in the influent and the biofilm, which was approximately constant during the study.

3.2. Physical and chemical parameters

The average values of pH, conductivity, temperature and dissolved oxygen concentration of the influent, effluents and mixed liquors of the biological reactors of the pilot plants of municipal wastewater treatment are indicated in Table V.2.

Table V.2. Average values of pH, conductivity, temperature and dissolved oxygen of the influent, effluents and mixed liquors of the biological reactors of the experimental plants.

Parameter	Sampling zone									
	Influent	MBR			Hybrid MBBR-MBR_a			Hybrid MBBR-MBR_b		
		Effluent	Anoxic zone	Aerobic zone	Effluent	Anoxic zone	Aerobic zone	Effluent	Anoxic zone	Aerobic zone
pH	8.01±0.16	4.82±0.75	6.13±0.52	5.66±0.56	4.49±0.19	5.60±0.48	4.98±0.42	4.63±0.32	5.41±0.54	5.09±0.46
Conductivity (µS cm^{-1})	1234±84	1021±72	1002±77	1003±76	1044±59	1030±62	1032±64	1035±54	1033±56	1021±63
Temperature (ºC)	14.8±1.1	14.9±1.1	14.9±1.1	15.0±1.1	15.0±1.2	15.0±1.1	15.0±1.2	14.9±1.2	15.0±1.2	15.0±1.2
Dissolved oxygen (mg O_2 L^{-1})	-	-	0.9±0.1	2.2±0.8	-	0.9±0.2	5.3±0.8	-	0.8±0.1	4.4±1.0

The values of pH in the biological reactors and the effluents were relatively acid due to the nitrification process, which released protons into the mixed liquor (Canziani et al., 2006). The aerobic zones had pH values lower than those in the anoxic zones due to the acid nature of the oxygen when it was spread throughout the mixed liquor. If the pH values had been higher than these obtained in this study, the percentages of TN removal would probably have been higher too because the nitrification process is more effective in a pH range around 7. The value of conductivity of the influent was slightly higher than those of the biological reactors and the effluents due to its composition. The temperature was 14.9±1.3ºC in the three experimental plants because the research was carried out in winter, during the months of January, February and March. Other studies about MBBR were carried out in winter with similar temperature values (Rutt et al., 2006). The dissolved oxygen concentration in the aerobic zone of the three biological reactors was over 2.0 mg O_2 L^{-1}, which is recommended in order to get an efficient removal of COD and an effective nitrification process, according to Wang et al. (2006). The dissolved oxygen concentration in the anoxic zone of the three biological reactors was slightly lower than 1.0 mg O_2 L^{-1} to help the denitrification process.

3.3. Organic matter and nutrient removal

In the three municipal WWTPs, the biological reactors usually biodegraded the organic matter from the wastewater while the membrane unit separated the biomass and particulate and colloidal matter from the bioreactor effluent. The removal of organic matter was studied by obtaining the COD and the BOD_5 in the influent and the effluent of each experimental plant. These parameters indicate the concentration of total organic matter and the concentration of biodegradable organic matter, respectively. Figure V.3a shows the values of COD and BOD_5 obtained from the influent, whereas Figure V.3b, Figure V.3c and Figure V.3d indicate the values of COD and BOD_5 obtained from the effluent relating to the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, respectively, during the stabilization phase of this research.

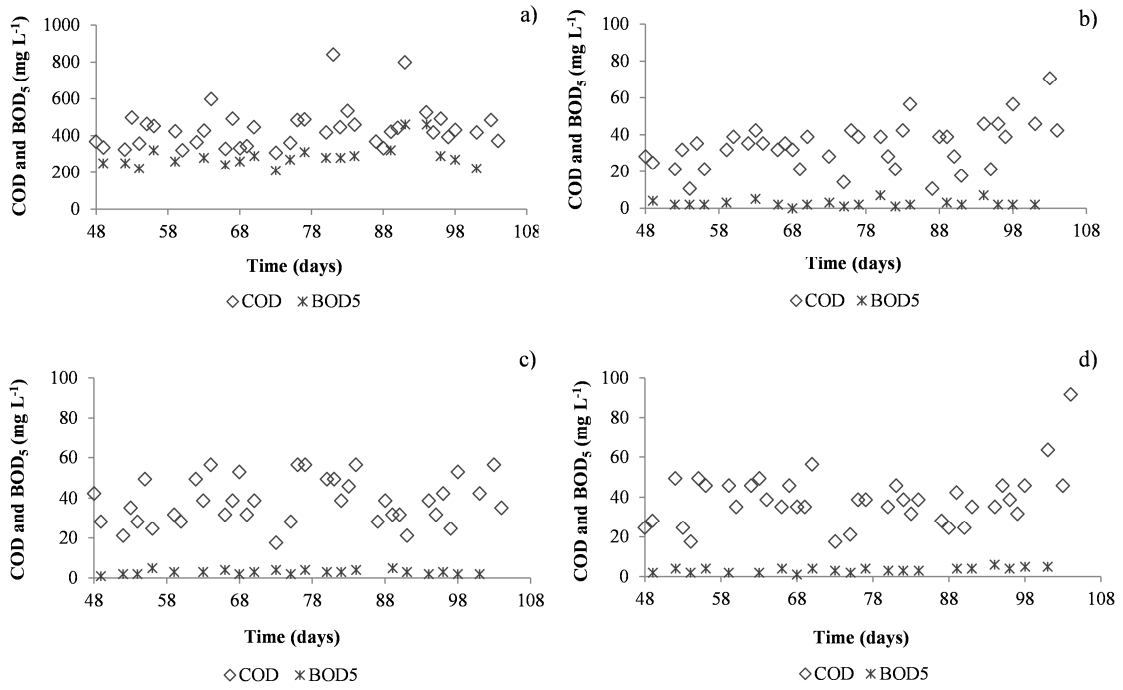

Figure V.3. Evolution of the chemical oxygen demand (COD) and five-day biochemical oxygen demand (BOD_5) of the influent and the three effluents of the experimental plants during the stabilization phase. (a) COD and BOD_5 of the influent. (b) COD and BOD_5 of the effluent in the MBR. (c) COD and BOD_5 of the effluent in the hybrid MBBR-MBR_a. (d) COD and BOD_5 of the effluent in the hybrid MBBR-MBR_b.

The organic matter was almost totally removed. This removal was very similar in the three experimental plants studied, as can be observed in Table V.3 through the parameters COD and BOD_5.

Table V.3. Average values and reduction percentages of COD, BOD_5, TSS, TN and TP of the influent and effluents of the experimental plants. COD (chemical oxygen demand), BOD_5 (five-day biochemical oxygen demand), TSS (total suspended solids), TN (total nitrogen), TP (total phosphorus).

Parameter	Sampling zone				Removal percentage	Wastewater treatment plant		
	Influent	Effluent MBR	Effluent Hybrid MBBR-MBR_a	Effluent Hybrid MBBR-MBR_b		MBR	Hybrid MBBR-MBR_a	Hybrid MBBR-MBR_b
COD (mg O_2 L^{-1})	437.73±112.90	34.01±12.60	38.53±11.28	39.08±13.45	**COD (%)**	91.97±2.96	90.97±2.55	90.74±3.69
BOD_5 (mg O_2 L^{-1})	287.14±65.05	2.67±1.77	2.95±1.07	3.38±1.24	**BOD_5 (%)**	99.07±0.57	98.94±0.40	98.81±0.44
TSS (mg L^{-1})	232.75±58.00	8.92±7.89	11.01±8.18	11.55±8.48	**TSS (%)**	95.89±4.39	95.00±4.00	94.82±3.84
TN (mg N L^{-1})	147.76±68.43	50.01±21.74	50.62±23.23	46.61±21.84	**TN (%)**	65.17±7.41	63.84±15.81	67.34±11.22
TP (mg P L^{-1})	13.83±4.47	6.93±1.99	7.24±2.44	6.51±1.31	**TP (%)**	48.31±10.77	45.97±15.05	50.65±11.13

The removal percentages of COD had values of 91.97±2.96%, 90.97±2.55% and 90.74±3.69% for the MBR (Figure V.1a), hybrid MBBR-MBR_a (Figure V.1b) and hybrid MBBR-MBR_b (Figure V.1c), respectively. Jonoud et al. (2003) obtained similar percentages of COD removal, which were higher than 85% with an HRT of 20 h, a flow rate of 0.45 L h^{-1} and concentrations of MLSS of 3,400 mg L^{-1} in the anaerobic MBBR and 1,810 mg L^{-1} in the aerobic MBBR. These operation conditions were similar to those used in this research as shown in Table V.1. If the HRT had a value lower than 26.5 h, the COD removal efficiencies would also be lower than those obtained in this study and similar studies (Melin et al., 2005).

The concentrations of COD in the effluents, excluding the first days of the start-up phase, were lower than 125 mg O_2 L^{-1} in such a way that the Spanish standard limit stated by the legislation about wastewater treatment and European Union recommendation was obeyed. The concentration of COD of the influent was 437.73±112.90 mg O_2 L^{-1} and it ranged between a minimum value of 300 mg O_2 L^{-1} and a maximum value of 840 mg O_2 L^{-1}, approximately. This occurred due to the time of sampling and the season of the year.

The tendency of BOD_5 was similar to the performance observed for the parameter COD. The removal of biodegradable organic matter had values of 99.07±0.57%, 98.94±0.40% and 98.81±0.44% for the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, respectively. The concentrations of BOD_5 of the effluents were always lower than the imposed Spanish standard limit and European Union recommendation of 25 mg O_2 L^{-1}, as indicated in the Spanish standard limit stated by the legislation regarding wastewater treatment. The average concentration of BOD_5 in the influent was 290.91 mg O_2 L^{-1} and ranged between 210 and 460 mg O_2 L^{-1}. Di Trapani et al. (2010) obtained BOD_5 removal efficiencies higher than 94%. These percentages were lower than those obtained in this research as the HRT (7.4 h) was lower than that employed in this study.

The difference between the experimental plants, regarding the parameters COD and BOD_5, was not statistically significant with an HRT of 26.5 h as the p-values were higher than α=0.05. The performance regarding organic matter removal was slightly better in the MBR. This was probably due to the higher concentration of MLSS in this experimental plant. Andreottola et al. (2000) carried out an experimental comparison between a MBBR and an activated sludge system. The COD removal efficiencies in the

activated sludge system (84%) were higher than those obtained in the MBBR (76%) due to the different biomass concentration. The biomass concentration ranged from 1.3 to 3.4 kg TSS m^{-3} (average value: 2.1 kg TSS m^{-3}) in the activated sludge system and from 0.8 to 1.5 kg TSS m^{-3} (average value: 1.0 kg TSS m^{-3}) in the MBBR system (Andreottola et al., 2000).

Di Trapani et al. (2010) carried out a comparison between a MBBR and an activated sludge system with values of HRT lower than that used in this study. In general, the performances of the two systems were almost comparable, in terms of COD, suggesting that the attached biomass did not provide an extra contribution to the removal process.

The biodegradability factor could be determined from the values of BOD_5 and COD over time. This parameter is defined as the quotient between BOD_5 and COD. This factor indicates the biodegradability of the organic matter contained in the influent and effluents of the experimental plants (Vollertsen and Hvitved-Jacobsen, 2002). Its value ranges between 0 and 1. The influent had a biodegradability factor of 0.68±0.08 in this research, so the biological treatment was suitable for this kind of municipal wastewater. The biodegradability factor had values of 0.09±0.05, 0.09±0.05 and 0.09±0.03 for the effluents from the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, respectively.

The process of microfiltration (a physical process) must naturally have a minimum flow of suspended solids through the membrane, as occurred in this study. The values of total suspended solids for the effluents of the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b were 8.92±7.89 mg L^{-1}, 11.01±8.18 mg L^{-1} and 11.55±8.48 mg L^{-1}, respectively. Garcia-Mesa et al. (2012) obtained similar concentrations of TSS in the effluent of an MBR with an efficiency of 97.45% in a full scale plant. The performances of the three plants were excellent with respect to the removal of total suspended solids, with values higher than 94%, as shown in Table V.3. The difference between the pilot plants, regarding the TSS, was not statistically significant with an HRT of 26.5 h as the p-values were higher than α=0.05. In accordance with the legislation requirements, the concentrations of TSS of the three effluents were lower than 35 mg L^{-1}. The high removal of TSS together with the high reduction percentages of COD and BOD_5 indicate that the membrane units had a period of useful life superior

to the period specified by the manufacturer, so the membrane units were able to accept, with the same volume, a higher organic load in the influent.

The concentrations of TN and TP in the influent and the effluents of the municipal wastewater treatment pilot plants are indicated in Table V.3. There, the respective removal percentages of these nutrients can also be seen. Both TN and TP concentrations in the effluent of each experimental plant exceeded the Spanish standard limits stated by the legislation regarding wastewater treatment (10 and 15 mg N L^{-1} for the TN concentration and 1 and 2 mg P L^{-1} for the TP concentration). Therefore, the different effluents could not be released into sensitive zones, so release would have to take place in normal zones.

Figure V.4a shows the values of TN concentration obtained from the influent whereas Figure V.4b, Figure V.4c and Figure V.4d indicate the values of TN concentration obtained from the effluent relating to the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, respectively, during the stabilization phase of this study.

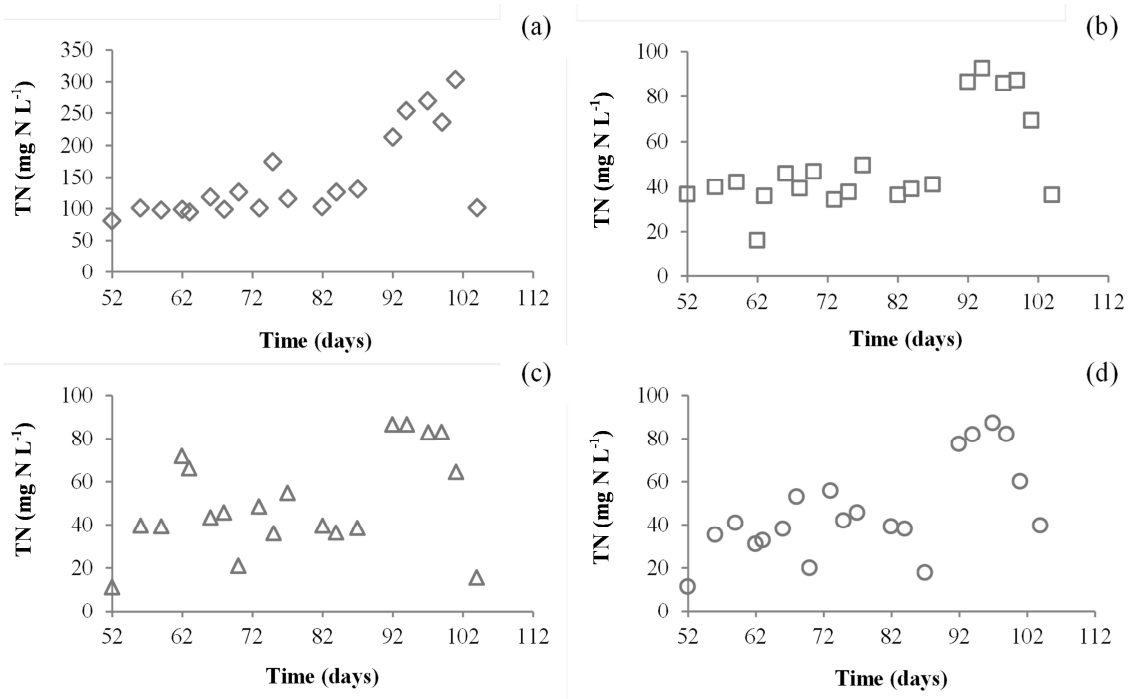

Figure V.4. Evolution of the total nitrogen (TN) concentration of the influent and the three effluents of the experimental plants during the stabilization phase. (a) TN of the influent. (b) TN of the effluent in the MBR. (c) TN of the effluent in the hybrid MBBR-MBR_a. (d) TN of the effluent in the hybrid MBBR-MBR_b.

The hybrid MBBR-MBR_b had a percentage of TN removal of 67.34±11.22%. This value was higher than the percentages obtained for the MBR and hybrid MBBR-

MBR_a, which had a value of 65.17±7.41% and 63.84±15.81%, respectively. The hybrid MBBR-MBR_b contained carriers in the aerobic zone but it did not contain any in the anoxic zone. These results indicate that the nitrification and denitrification processes in the hybrid MBBR-MBR systems were more effective than in the MBR, but an anoxic zone without carriers was necessary to provide better contact between nitrate and the microorganisms (Rusten et al., 1995; Rusten et al., 2000; Larrea et al., 2007). The TN removal efficiencies could be improved if the dissolved oxygen concentration in the anoxic zone of each biological reactor was decreased to help the denitrification process (Wang et al., 2006). Jonoud et al. (2003) obtained similar percentages of TN removal, which were higher than 50% with an HRT of 20 h, a flow rate of 0.45 L h^{-1} and concentrations of MLSS of 3,400 mg L^{-1} in the anaerobic MBBR and 1,810 mg L^{-1} in the aerobic MBBR. These operation conditions were similar to those used in this research as shown in Table V.1.

Di Trapani et al. (2010) generally obtained similar performances in a MBBR and an activated sludge system with respect to the TN removal, with values of HRT lower than that used in this study. Dong et al. (2011) carried out their research with similar hydraulic retention times (36 and 18 h) to those used in this study, using a ceramic biocarrier. They obtained COD removal efficiencies lower than those achieved in this study. However, the TN removal performances were higher than those obtained in this research.

The experimental plants also removed TP in spite of the fact that these systems had not a strict anaerobic zone to initialize the process of biological phosphorus removal (Kermani et al., 2009). The creation of some dead zones in the anoxic compartments of each reactor made the TP removal possible through the formation of small anaerobic zones as well as the physical process of microfiltration through the membrane. As occurred with TN, the hybrid MBBR-MBR_b had the highest percentage of TP removal with a value of 50.65±11.13%. The MBR and hybrid MBBR-MBR_a had percentages of TP removal lower than that of the hybrid MBBR-MBR_b, with values of 48.31±10.77% and 45.97±15.05%, respectively. The tendency for TP removal was similar to that for TN one. As a result of this, a hybrid MBBR-MBR system with an anoxic zone without carriers (hybrid MBBR-MBR_b) also facilitated biological TP removal.

The difference between the experimental plants, regarding the TN and TP concentrations, was not statistically significant with an HRT of 26.5 h as the p-values obtained from the three post hoc procedures used were higher than α=0.05. In spite of this, the performance of the hybrid MBBR-MBR_b, regarding these parameters, was higher than those of the MBR and hybrid MBBR-MBR_a.

3.4. Kinetic parameters for autotrophic and heterotrophic biomass

Respirometry was carried out on the mixed liquor, with carriers in the case of the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, in order to compare the kinetic behavior of the microorganisms contained in a hybrid MBBR-MBR system with the performance of a conventional system (MBR).

3.4.1. Kinetic parameters for heterotrophic biomass

The amount of heterotrophic biomass produced per substrate oxidized in the biological reactor of the hybrid MBBR-MBR_b (Y_H = 0.3967 mg VSS mg COD^{-1}) was lower than the biomass produced in the biological reactors of the MBR and hybrid MBBR-MBR_a, with values of 0.5040 mg VSS mg COD^{-1} and 0.5041 mg VSS mg COD^{-1}, respectively, as shown in Table V.4.

Table V.4. Kinetic parameters for the characterization of heterotrophic and autotrophic biomass. Y_H (yield coefficient for heterotrophic biomass), $\mu_{m,\,H}$ (maximum specific growth rate for heterotrophic biomass), K_M (half-saturation coefficient for organic matter), Y_A (yield coefficient for autotrophic biomass), $\mu_{m,\,A}$ (maximum specific growth rate for autotrophic biomass), K_{NH} (half-saturation coefficient for ammonia nitrogen), k_d (decay coefficient for autotrophic and heterotrophic biomass).

Parameter	Sampling zone		
	MBR	Hybrid MBBR-MBR_a	Hybrid MBBR-MBR_b
Heterotrophic biomass			
Y_H (mg VSS mg COD^{-1})	0.5040	0.5041	0.3967
$\mu_{m,\,H}$ (h^{-1})	0.0086	0.0048	0.0012
K_M (mg O_2 L^{-1})	2.3659	0.9597	1.2417
Autotrophic biomass			
Y_A (mg O_2 mg N^{-1})	0.9714	0.7772	0.6595
$\mu_{m,\,A}$ (h^{-1})	0.0765	0.0263	0.0331
K_{NH} (mg N L^{-1})	1.3070	0.7617	0.5327
Total biomass			
k_d (d^{-1})	0.0484	0.0314	0.0326

Consequently, sludge production in the hybrid MBBR-MBR_b should be lower than in the MBR and hybrid MBBR-MBR_a. Therefore, the amount of waste sludge would also be lower and it could involve an economic saving with respect to treatment of the waste products of the WWTP.

The rest of the parameters which fit the Monod model for the heterotrophic biomass contained in each of the biological reactors, $\mu_{m,\,H}$ and K_M, are also indicated in Table V.4. According to these kinetic parameters, the biomass of the MBR and hybrid MBBR-MBR_a showed a similar performance and this performance was better than that corresponding to the hybrid MBBR-MBR_b. This meant that the heterotrophic biomass of the MBR and hybrid MBBR-MBR_a required less time for organic matter oxidation under the experimental conditions of this research. Moreover, the obtaining of the maximum specific growth rate was carried out with less available substrate in the MBR and hybrid MBBR-MBR_a. Therefore, less time would be required to accomplish a steady state under the experimental conditions of this study. Furthermore, the stress conditions experienced by the biofilm could be an explanation for the low values of $\mu_{m,\,H}$ compared to the default value (2 d^{-1} at 20ºC) proposed in ASM3 by Gujer et al. (1999).

In general, these results were in accordance with the percentages of organic matter removal of the experimental pilot plants. The MBR and hybrid MBBR-MBR_a were the pilot plants with higher performances of COD and BOD_5, as indicated in Table V.3.

Ferrai et al. (2010) had similar values to those obtained in this research, regarding Y_H and $\mu_{m,\,H}$. Seifi and Fazaelipoor (2012) had similar values to those obtained in this study, with respect to Y_H and K_M. This was also indicated by Insel et al. (2009).

3.4.2. Kinetic parameters for autotrophic biomass

The autotrophic biomass contained in the biological reactor of the hybrid MBBR-MBR_b required the lowest quantity of oxygen to oxidize the same amount of substrate (Y_A=0.6595 mg O_2 mg N^{-1}), followed by the hybrid MBBR-MBR_a and MBR, with values of 0.7772 mg O_2 mg N^{-1} and 0.9714 mg O_2 mg N^{-1}, respectively, as shown in Table V.4. This led to an energy saving regarding the oxygen supply.

The rest of the parameters which fit the Monod model for the autotrophic biomass contained in each of the biological reactors, $\mu_{m,\,A}$ and K_{NH}, are also indicated in Table V.4. According to these kinetic parameters, the biomass of the MBR and hybrid MBBR-MBR_b showed a similar performance and this performance was better than that corresponding to the hybrid MBBR-MBR_a. This meant that the autotrophic biomass of the MBR and hybrid MBBR-MBR_b required less time for the oxidation of nitrogen contained in the influent under the experimental conditions of this research. Moreover, the obtaining of the maximum specific growth rate was carried out with less available substrate in the MBR and hybrid MBBR-MBR_b. Therefore, less time would be required to achieve a steady state under the experimental conditions of this study. In general, these results were in accordance with the percentages of TN removal of the experimental pilot plants. The MBR and hybrid MBBR-MBR_b were the pilot plants with higher performances of TN removal, as indicated in Table V.3, as they had the best kinetic behavior when the r_{su} is evaluated according to the kinetic parameters Y_A, $\mu_{m,\,A}$ and K_{NH}.

Seifi and Fazaelipoor (2012) had similar values to those obtained in this study, with respect to $\mu_{m,\,A}$ and K_{NH}. This was also indicated by Insel et al. (2009). The values of Y_A reported by Seifi and Fazaelipoor (2012) were slightly lower than those obtained

in this study. Ferrai et al. (2010) had similar values to those obtained in this research, regarding K_{NH}.

Moreover, the half-saturation coefficients are particularly interesting in view of the diffusional mass transport limitations in the MBBR (Plattes et al., 2007). It was found that the half-saturation coefficients for ammonia nitrogen (K_{NH}) and organic matter (K_M) were generally lower than the typical values for activated sludge systems proposed as default values in ASM1 (Henze et al., 1987). This indicated that mass transport limitations for ammonia nitrogen and organic matter were not more important in the MBBR system than in typical activated sludge systems. This supports the MBBR modelling concept proposed before and applied in this study.

3.4.3. Decay coefficient for autotrophic and heterotrophic biomass

Finally, the values of k_d are also reported in Table V.4. The values obtained for the experimental plants with a MBBR (hybrid MBBR-MBR_a and hybrid MBBR-MBR_b) were similar and they were lower than the value obtained for the MBR. This meant that 3.14% and 3.26% (for the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, respectively) of the total quantity of biomass contained in the hybrid MBBR-MBR systems represented the quantity of biomass oxidized per day. These values were lower than the percentage existing in the MBR (4.84% for the MBR).

Canziani et al. (2006) obtained similar values of k_d (0.03 d^{-1}) to those obtained in this study. These values resulted well below the lower limit of the range reported in the literature (0.06-0.2 d^{-1}) (Metcalf, 2003).

3.5. Enzymatic activities

The values of α-glucosidase, acid phosphatase and alkaline phosphatase enzymatic activities of suspended and attached biomass in the four chambers of the bioreactor of the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b are shown in Figure V.5, Figure V.6 and Figure V.7, respectively. The evaluation of the contribution of suspended and attached biomasses in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b was carried out according to Reboleiro-Rivas et al. (2013).

The average values of α-glucosidase enzymatic activity relating to days 50, 60, 70, 80, 90 and 100 of the steady state were calculated by considering the mean of the

values corresponding to the four chambers. The results show that the α-glucosidase enzymatic activities in the hybrid MBBR-MBR_b and hybrid MBBR-MBR_a, i.e. 0.5086±0.0478 mM g VSS^{-1} min^{-1} and 0.2704±0.0371 mM g VSS^{-1} min^{-1}, respectively, were higher than in MBR with a value of 0.2064±0.0210 mM g VSS^{-1} min^{-1}.

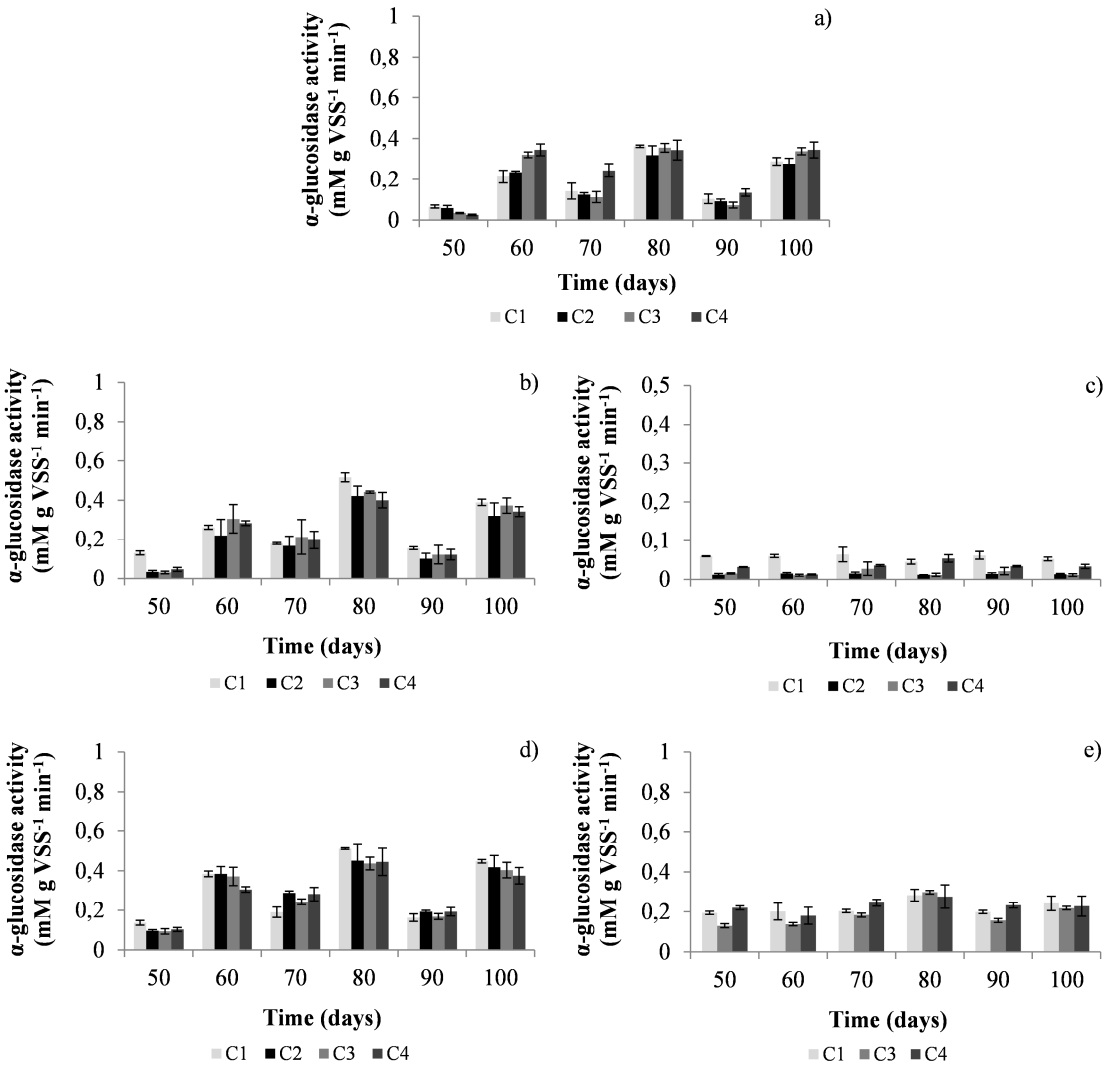

Figure V.5. Enzymatic activity of α-glucosidase in the chambers C1, C2, C3 and C4 of the different bioreactors. (a) Suspended biomass in the MBR. (b) Suspended biomass in hybrid MBBR-MBR_a. (c) Attached biomass in hybrid MBBR-MBR_a. (d) Suspended biomass in hybrid MBBR-MBR_b. (e) Attached biomass in hybrid MBBR-MBR_b.

The results show that the acid phosphatase enzymatic activities in the hybrid MBBR-MBR_b and hybrid MBBR-MBR_a, i.e. 7.6881±0.6510 mM g VSS^{-1} min^{-1} and 6.2920±0.4052 mM g VSS^{-1} min^{-1}, respectively, were the highest, followed by MBR with a value of 4.2315±0.2828 mM g VSS^{-1} min^{-1}.

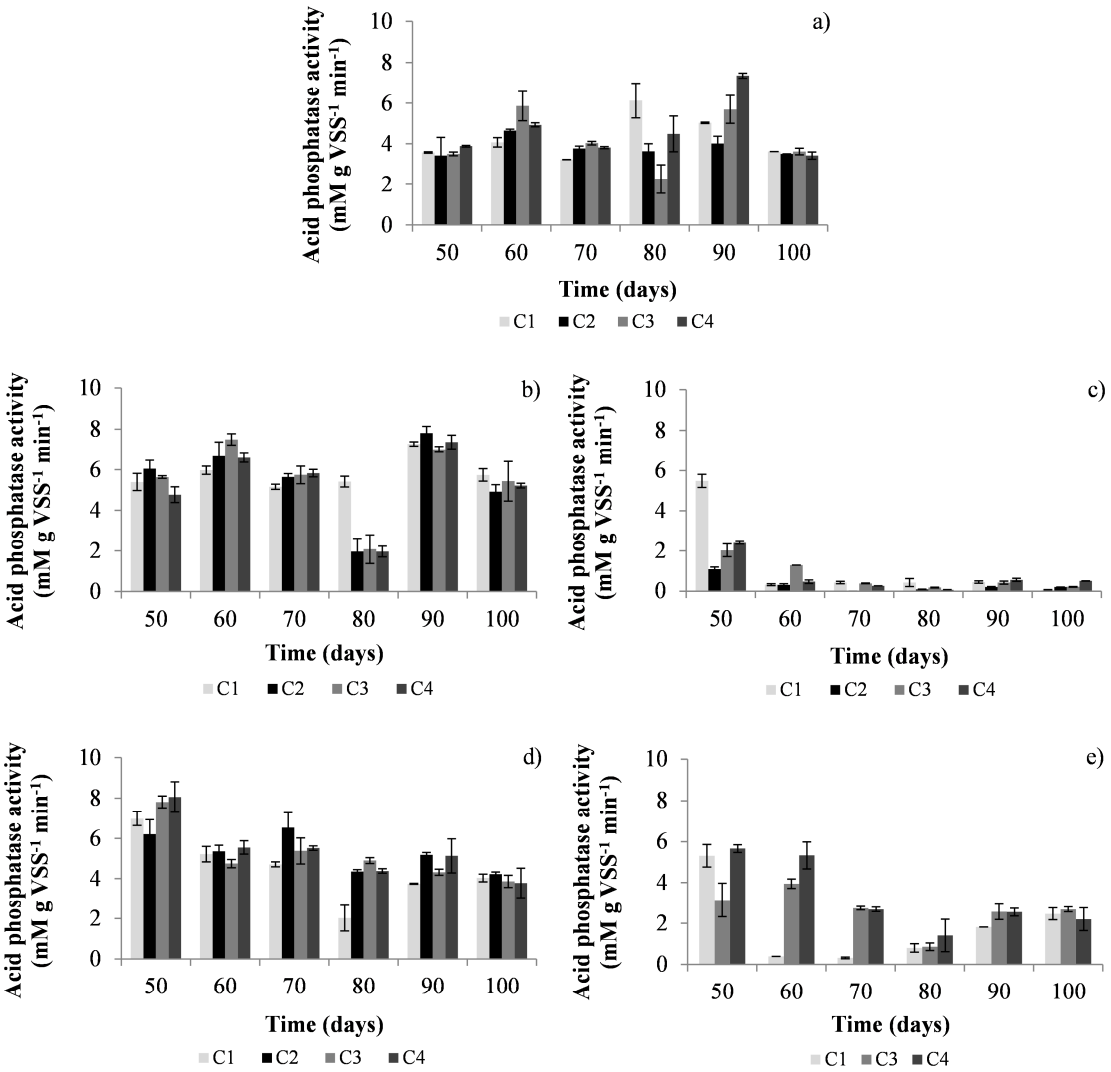

Figure V.6. Enzymatic activity of acid phosphatase in the chambers C1, C2, C3 and C4 of the different bioreactors. (a) Suspended biomass in the MBR. (b) Suspended biomass in hybrid MBBR-MBR_a. (c) Attached biomass in hybrid MBBR-MBR_a. (d) Suspended biomass in hybrid MBBR-MBR_b. (e) Attached biomass in hybrid MBBR-MBR_b.

On the other hand, the results show that the alkaline phosphatase enzymatic activity in the hybrid MBBR-MBR_b and MBR, i.e. 5.4326±0.4354 mM g VSS^{-1} min^{-1} and 5.0313±0.3468 mM g VSS^{-1} min^{-1}, respectively, were the highest, followed by the hybrid MBBR-MBR_a with a value of 3.3427±0.3825 mM g VSS^{-1} min^{-1}.

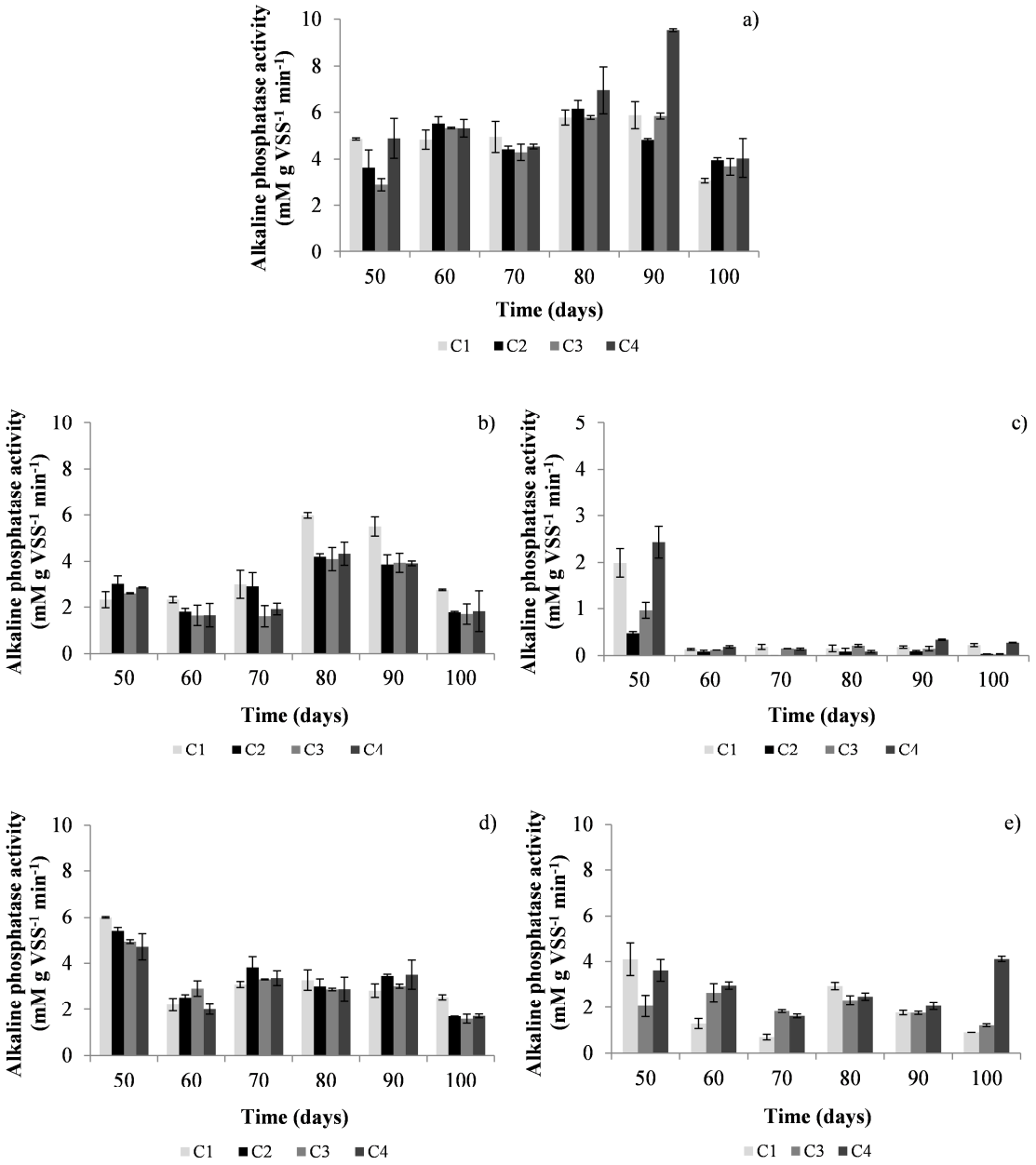

Figure V.7. Enzymatic activity of alkaline phosphatase in the chambers C1, C2, C3 and C4 of the different bioreactors. (a) Suspended biomass in the MBR. (b) Suspended biomass in hybrid MBBR-MBR_a. (c) Attached biomass in hybrid MBBR-MBR_a. (d) Suspended biomass in hybrid MBBR-MBR_b. (e) Attached biomass in hybrid MBBR-MBR_b.

The hybrid MBBR-MBR_b had the highest values of the enzymatic activities of α-glucosidase, acid and alkaline phosphatase. Furthermore, the enzymatic activities within the attached biomass were lower than in the suspended biomass in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b. This might be caused by the low diffusivity that extracellular polymeric substances (EPS) layers provide to the attached biomass, which impedes the release of extracellular microbial enzymes so the enzymatic activity is reduced. It implies that the contribution of the attached biomass in the improvement of

the enzymatic activities was not significant in the hybrid MBBR-MBR systems. As a consequence of this, the differences regarding the enzymatic activities were not statistically significant between the hybrid MBBR-MBR_b and the other two systems with an HRT of 26.5 h as the p-values obtained were higher than α=0.05. However, the hybrid MBBR-MBR_b achieved higher removal percentages of TN and TP in the steady state (Table V.3) as the enzymatic activities of acid phosphatase and alkaline phosphatase were higher and the bacterial activity is closely related to the enzymatic activity within an ecosystem (Nybroe et al., 1992). In this sense, the evaluation of the phosphatase enzymatic activities supported the results obtained from the kinetic study for autotrophic biomass, as the hybrid MBBR-MBR_b showed the best kinetic performance for this kind of biomass in the steady state (Table V.4).

There is no a clear pattern in the α-glucosidase, acid phosphatase and alkaline phosphatase enzymatic activities with respect to the different chambers for each WWTP observed. Similarly, it has been reported that enzymatic activity is not influenced by the dissolved oxygen concentration in the aerobic, anaerobic and anoxic zones of a bench scale activated sludge process (Goel et al., 1998).

3.6. TGGE fingerprint analysis

Band pattern analysis for chambers C1, C2, C3 and C4 of the bioreactors of the three WWTPs regarding suspended and attached biomass communities can be seen in Figure V.8. In terms of bacterial diversity, five different groups can be distinguished after clustering of samples. Two groups are formed by samples from the hybrid MBBR-MBR_b. Other group contains six samples from the hybrid MBBR-MBR_a, while another has three samples from the MBR. The rest of samples are contained in the last group. Based on bacterial diversity of the samples, it can be said that significant differences exist between the three WWTPs studied. Therefore, the different configurations of the bioreactors led to differences in the bacterial communities developed in each of the WWTPs.

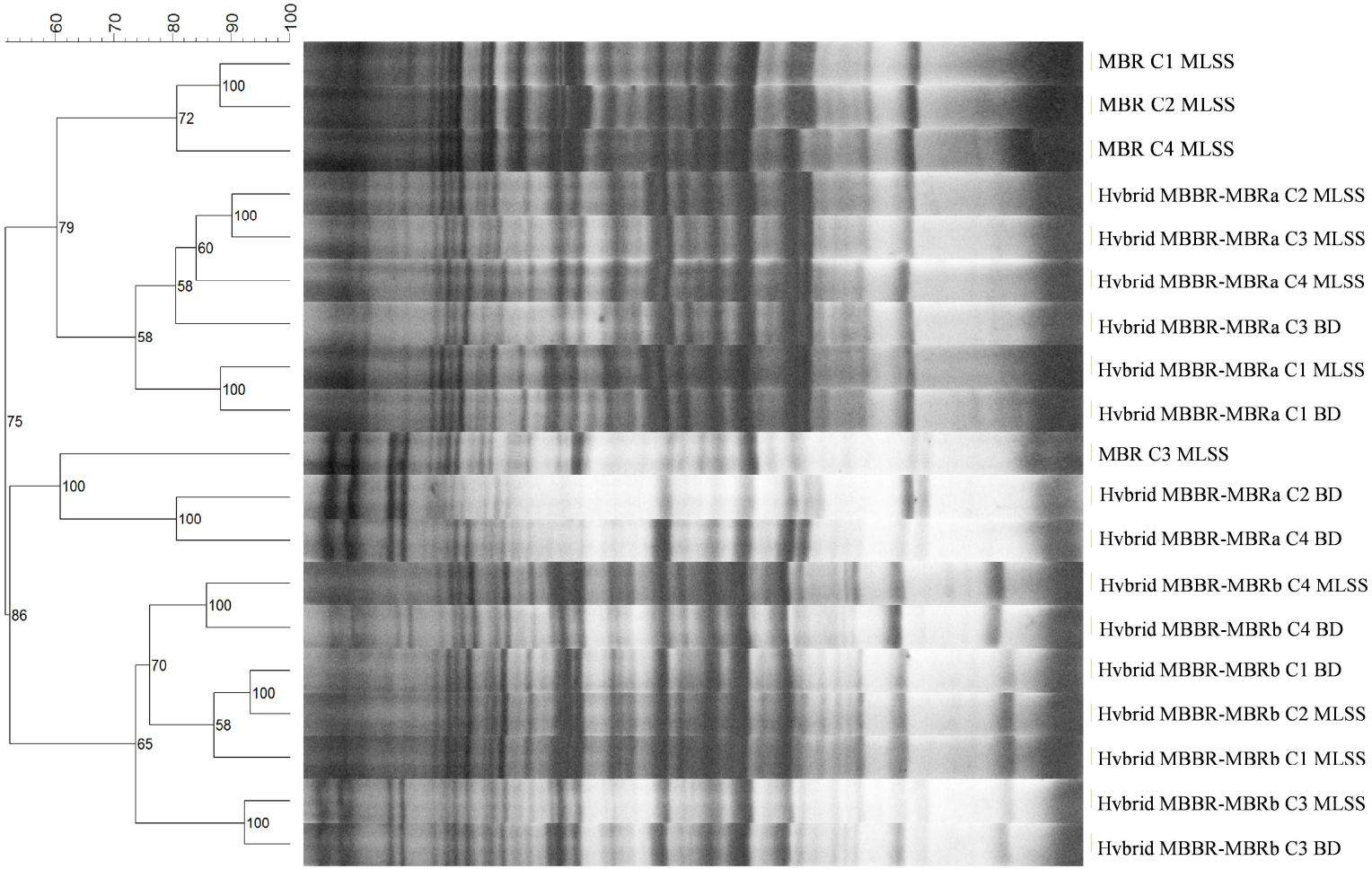

Figure V.8. TGGE fingerprints of bacterial communities of suspended biomass (MLSS) and attached biomass (BD) in chambers C1, C2, C3 and C4 of the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b.

3.7. Analysis of biofilm communities by SEM

The fixed biomass from the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b was observed by SEM. The SEM photographs are shown in Figure V.9 and Figure V.10 for the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, respectively. The SEM images show phylotypes with different morphologies such as cocci and bacilli, as well as filamentous bacteria and EPS layers, which are the basis for the formation of attached biomass (Calderon et al., 2011; Calderon et al., 2012).

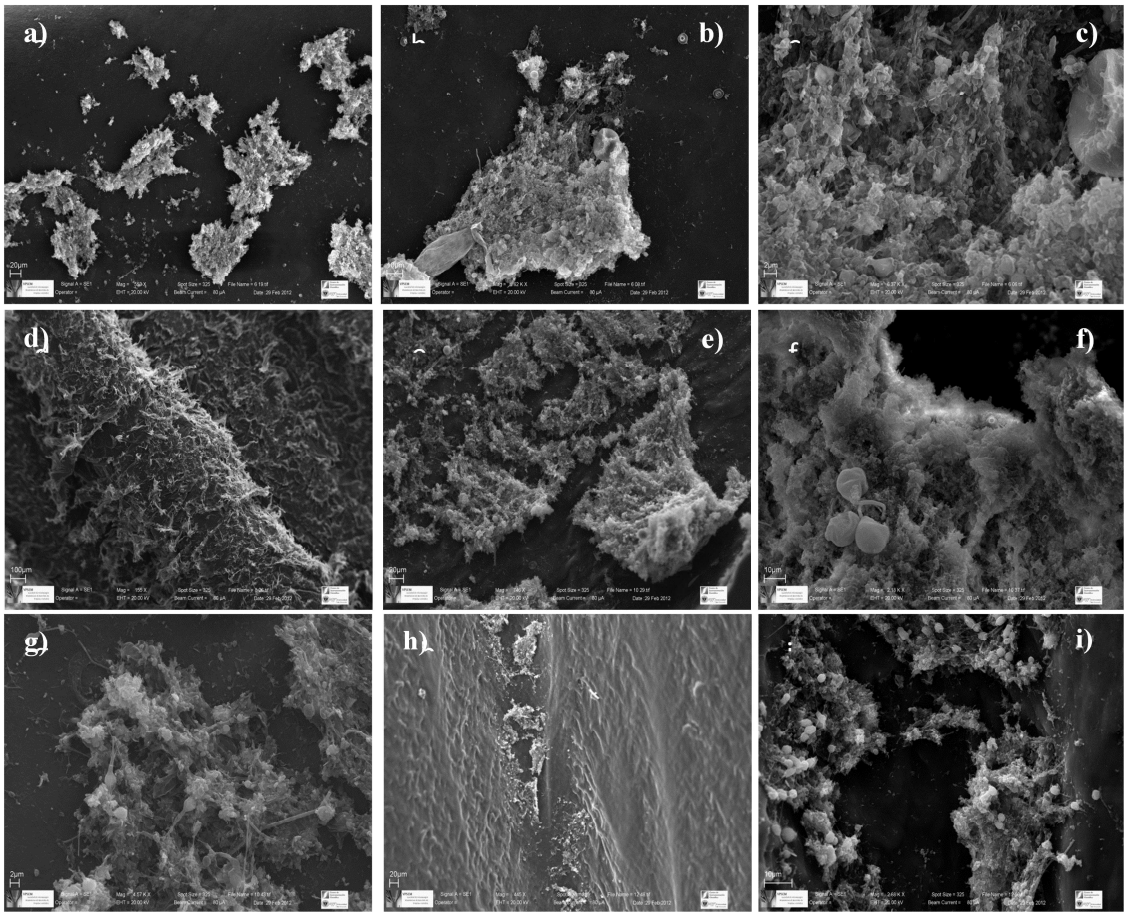

Figure V.9. Scanning electron microscopy (SEM) of attached biomass collected from the hybrid MBBR-MBR_a. (a), (b), (c) Chamber C1. (d) Chamber C2. (e), (f), (g) Chamber C3. (h), (i) Chamber C4.

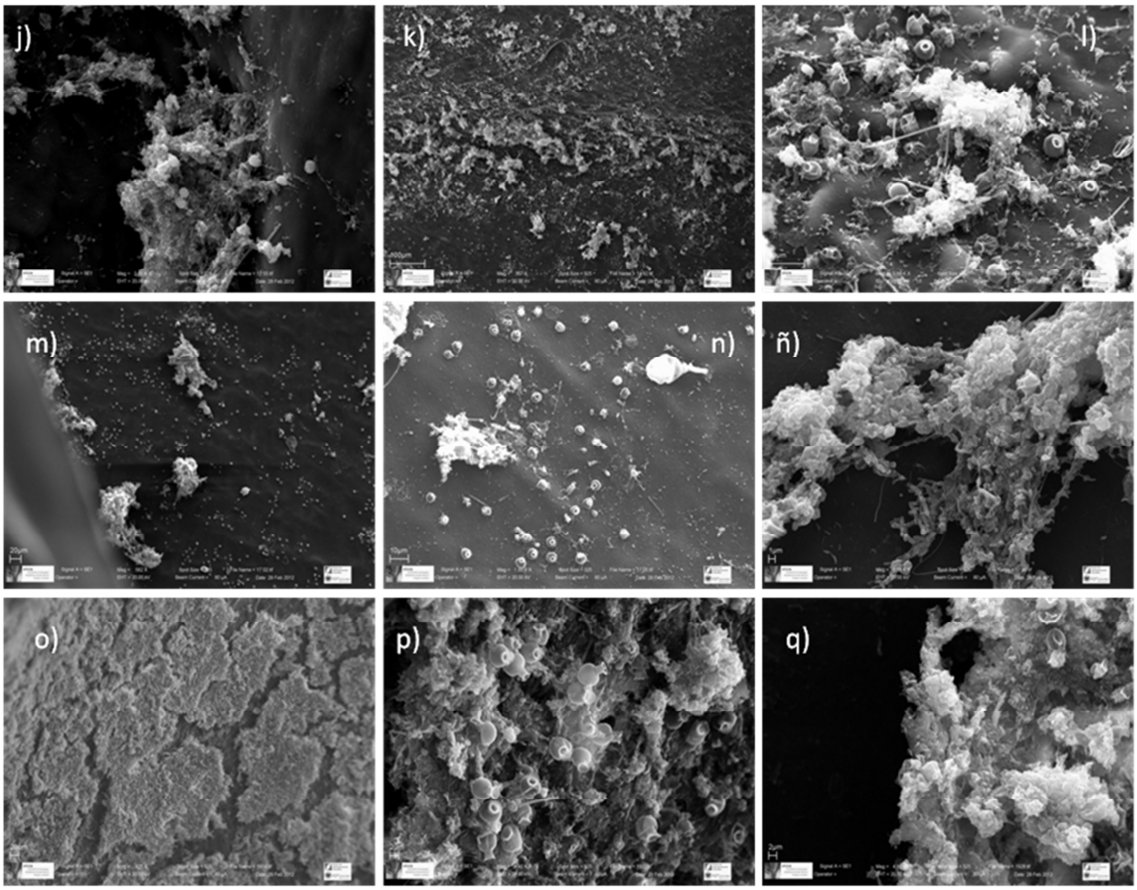

Figure V.10. Scanning electron microscopy (SEM) of attached biomass collected from the hybrid MBBR-MBR$_b$. (j), (k), (l) Chamber C1. (m), (n), (ñ) Chamber C3. (o), (p), (q) Chamber C4.

4. Conclusions

A comparison of the results obtained from the systems which combined a moving bed biofilm reactor with a membrane bioreactor (hybrid MBBR-MBR systems) with those of a membrane bioreactor configuration (MBR) was carried out. The following conclusions were drawn:

1. Concerning the organic matter and nutrient removal, the differences between the experimental plants were not statistically significant with an HRT of 26.5 h, suggesting that the attached biomass did not provide an extra contribution to the organic matter and nutrients removal process.

2. In spite of this, the hybrid MBBR-MBR$_b$ had a percentage of TN removal of 67.34±11.22%. This value was higher than the percentages obtained for the MBR and hybrid MBBR-MBR$_a$, which had values of 65.17±7.41% and 63.84±15.81%, respectively. It was supported by the study of the enzymatic

activities as the hybrid MBBR-MBR_b had the highest values of acid phosphatase (7.6882±0.6510 mM g VSS^{-1} min^{-1}) and alkaline phosphatase (5.4326±0.4354 mM g VSS^{-1} min^{-1}). These results indicate that the nitrification and denitrification processes in the hybrid MBBR-MBR systems were slightly more effective than in the MBR, but an anoxic zone without carriers was necessary to provide better contact between nitrate and the microorganisms. The tendency for TP removal was similar to that for TN one.

3. From the point of view of the kinetics of the heterotrophic biomass, the hybrid MBBR-MBR_b showed a sludge production lower than the other experimental plants with a value of Y_H=0.3967 mg VSS mg COD^{-1}. The heterotrophic biomass of the MBR and hybrid MBBR-MBR_a required less time for organic matter oxidation under the experimental conditions of this research and this biomass also required less time to accomplish a steady state with a value of $\mu_{m,\,H}$=0.0086 h^{-1} and K_M=2.3659 mg O_2 L^{-1}, corresponding to the MBR. As a result of this, the MBR and hybrid MBBR-MBR_a showed better performance than the hybrid MBBR-MBR_b. These results were in accordance with the percentages of organic matter removal of the experimental pilot plants.

4. From the point of view of the kinetics of the autotrophic biomass, the hybrid MBBR-MBR_b required the lowest quantity of oxygen to oxidize the same amount of substrate (Y_A=0.6595 mg O_2 mg N^{-1}). The autotrophic biomass of the MBR and hybrid MBBR-MBR_b required less time for the oxidation of nitrogen contained in the influent under the experimental conditions of this research and this biomass also required less time to accomplish a steady state with a value of $\mu_{m,\,A}$=0.0765 h^{-1} and K_{NH}=1.3070 mg N L^{-1}, corresponding to the MBR. As a result of this, the MBR and hybrid MBBR-MBR_b showed better efficiency than the hybrid MBBR-MBR_a. These results were in accordance with the percentages of TN removal of the experimental pilot plants.

5. The enzymatic activities of α-glucosidase, acid phosphatase and alkaline phosphatase showed different values in relation to the biomass configuration, with higher values for suspended biomass than for attached biomass. This

could have been caused by the low diffusivity of extracellular microbial enzymes caused by the EPS layer when the attached biomass is formed.

6. Differences in the bioreactor configuration led to differences in the bacterial diversity, bacterial community structure and the appearance of EPS in the hybrid MBBR-MBR systems according to the TGGE fingerprints of amplicons of the V3 region of the bacterial 16S rRNA gene and the SEM analysis.

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VI. CHAPTER 3

Analysis of microbial kinetics, enzymatic activities and bacterial community structure in membrane bioreactor and hybrid moving bed biofilm reactor-membrane bioreactor systems for wastewater treatment (operational conditions of HRT=18 h and intermediate biomass concentrations).

Abstract

A membrane bioreactor (MBR), a hybrid moving bed biofilm reactor-membrane bioreactor (hybrid MBBR-MBR_a) containing carriers in the anoxic and aerobic compartments and a hybrid MBBR-MBR_b containing carriers only in the aerobic zone were used in parallel and compared for treating municipal wastewater. The hydraulic retention time (HRT) was 18 h. A study of the microbial kinetics was carried out to explain the removal of organic matter and nutrients. The evolution of the enzymatic activities of α-glucosidase and acid and alkaline phosphatase as well as the bacterial diversity and bacterial community structure were studied. The MBR and the hybrid MBBR-MBR_b showed the highest reduction percentages of chemical oxygen demand (COD), 90.29±2.05% and 90.24±2.87%, respectively. Moreover, the hybrid MBBR-MBR_b had the highest removal performance of total nitrogen (TN) with a value of 63.96±7.00%. The MBR and the hybrid MBBR-MBR_b had the highest values of α-glucosidase, acid phosphatase and alkaline phosphatase enzymatic activities (0.4110±0.0414, 8.5154±0.8202 and 2.2052±0.1660 mM $gVSS^{-1}$ min^{-1}, respectively, for the hybrid MBBR-MBR_b). Temperature gradient gel electrophoresis (TGGE) fingerprints and scanning electron microscopy (SEM) analysis demonstrated the existence of differences in the bacterial diversity, bacterial community structure and the appearance of extracellular polymeric substances (EPS) in the biofilm systems.

1. Introduction

Recently there has been an increase of urbanization together with the even stricter environmental legislation regarding organic pollutant and nutrient concentrations imposed at the outlet of municipal wastewater treatment plants (WWTPs) (Onnis-Hayden et al., 2011; Pal et al., 2012). In addition, there has also been an increasingly limited area for the construction of new plants. These factors have often led to the necessity of upgrading existing WWTPs (Di Trapani et al., 2010); new technologies have been developed to obtain a better quality of effluent or to upgrade overloaded activated sludge plants (Wang et al., 2006).

The conventional activated sludge process has been used for removing pollutants from wastewater. However, technical development requires new technologies that are better adapted to the elimination of contaminants, enabling the effluent to reach an acceptable quality for the reuse of the treated wastewater (Molina-Muñoz et al., 2010). Among these technologies, a conventional membrane bioreactor (MBR) combines an activated sludge process with solid-liquid separation by membrane (ultra- or micro-) filtration replacing the usual sedimentation step to treat wastewater and separate biomass (Rodríguez-Hernández et al., 2014). These systems are characterized by excellent effluent quality, low sludge production, small size and flexibility for future expansion and upgrade, according to Rodríguez et al. (2012). Nevertheless, membrane fouling is the main problem with this technology, as it reduces filtration performance, shortens the life of the membrane and leads to higher operating costs (Drews, 2010).

The combination of a conventional MBR with a moving bed biofilm reactor (MBBR) constitutes a solution to the problems regarding membrane fouling (Liu et al., 2010). This technology is called moving bed biofilm reactor-membrane bioreactor (MBBR-MBR) and is very efficient in the removal of organic carbon, ammonium, nitrites and nitrates (Di Trapani et al., 2010). There are two ways of working in an MBBR-MBR system: hybrid MBBR-MBR or pure MBBR-MBR, depending on whether or not suspended biomass is present, respectively, as well as attached biomass to carry out the biodegradation process (Ivanovic and Leiknes, 2012). Attached biomass grows on small carrier elements suspended in constant motion throughout the entire volume of the reactor. This system becomes economically attractive when compact technology is required to accommodate space constraints or stringent effluent quality

requirements are mandatory (Yang et al., 2006). However, there are some uncertainties regarding the kinetic performance of MBBR-MBR systems, particularly the kinetic behavior of nitrite-oxidizing bacteria (NOB) which are involved in the nitrification process (Rongsayamanont et al., 2010), due to the coexistence of two kinds of biomass, i.e. suspended and attached, which could modify the kinetics of the system compared to processes involving pure suspended or attached biomass (Di Trapani et al., 2010). Several studies have been carried out to improve knowledge regarding the kinetic modeling of MBBR-MBR to facilitate the design, evaluation, control and prediction of the behavior of these systems (Leyva-Díaz et al., 2014; Leyva-Díaz et al., 2015).

The measurement of enzymatic activities is essential to characterize a complex microbial microcosm present in the suspended and attached biomass of MBR and MBBR-MBR systems (Liwarska-Bizukojc and Ledakowicz, 2003). In any wastewater treatment plant (WWTP), an important fraction of the organic matter entering with the influent needs to be hydrolyzed before bacterial uptake. In this sense, the hydrolysis of organic matter has been identified as the rate-limiting step for organic matter removal, so enzymatic activities give an estimation of the active biomass in a WWTP (Chróst and Siuda, 2002). In nature, extracellular microbial enzymes are responsible for this process (Cadoret et al., 2002; Gessesse et al., 2003). Therefore, the biodegradation of organic matter is mediated by extracellular microbial enzymes in MBR or MBBR-MBR systems (Burgess and Pletschke, 2008; Reboleiro-Rivas et al., 2013). Glucosidase and phosphatase activities are some of the most important enzymatic activities occurring during the biological treatment of wastewater (Boczar et al., 2001). The enzyme α-glucosidase releases glucose from maltose by breaking the α-1,4 glucosidic linkage, and phosphatase releases phosphate groups from phosphate esters by hydrolysis (Calderon et al., 2013). Therefore, α-glucosidase is important because carbohydrates comprise a large fraction of the organic matter entering with the influent. Furthermore, phosphatase (acid and alkaline) is also important because an important fraction of the total phosphorus entering the bioreactor comes in the form of organic phosphate (Reboleiro-Rivas et al., 2013). In this way, α-glucosidase and phosphatase activities have been found to be useful tools for the characterization, optimization and monitoring of organic matter biodegradation and nutrient removal by a biological process (Molina-Muñoz et al., 2010). Variations of these enzymatic activities are thus an excellent indicator of the physiology of the suspended or attached biomass present in an MBBR-MBR system.

Therefore, enzymatic activities may be used for kinetic data evaluation so the study of α-glucosidase and phosphatase can complement the kinetic modeling carried out in this work.

The structure and composition of the suspended and attached biomass involved in MBR and MBBR-MBR processes and the mechanisms by which configuration variations may influence on them are regarded as crucially important for the optimization and control of these processes (Boltz et al., 2011). In this sense, molecular biology techniques are useful tools for the investigation of microbial communities in natural and engineering environments, and offer advantages over culture-dependent methods (Molina-Muñoz et al., 2007). One of the most used molecular biology techniques is temperature gradient gel electrophoresis (TGGE), developed by Muyzer (1999). TGGE has been widely used for the investigation of the bacterial community structure of wastewater treatment systems (Wagner et al., 2002; Cortés-Lorenzo et al., 2006). Furthermore, scanning electron microscopy (SEM) allows for analyzing the community structure of bacterial biofilms developed in an MBBR-MBR system.

The aim of this work was to evaluate the microbial kinetics and the enzymatic activities of α-glucosidase, acid phosphatase and alkaline phosphatase in the suspended and attached biomasses in an MBR system and two hybrid MBBR-MBR processes and to relate them to the removal of organic matter and nutrients. It allowed for determining the effect of the biofilm in the MBBR-MBR system. The three WWTPs operated under a hydraulic retention time (HRT) of 18 h. The structure and composition of the biomass present involved in these processes were evaluated by TGGE and SEM.

2. Materials and methods

2.1. Description of the wastewater treatment plants

Three municipal WWTPs, working in parallel, were fed with urban wastewater. The first WWTP consisted of an MBR (Figure VI.1a), the second one was a hybrid MBBR-MBR system containing carriers in the anoxic and aerobic zones of the bioreactor (hybrid MBBR-MBR_a) (Figure VI.1b), and the last one consisted of a hybrid MBBR-MBR system which contained carriers only in the aerobic zone (hybrid MBBR-MBR_b) (Figure VI.1c). The carrier used in the hybrid MBBR-MBR systems was called K1, and was developed and supplied by AnoxKaldnes AS (Norway); this carrier was

previously studied in similar experiments (Melin et al., 2005; Di Trapani et al., 2008). The bioreactors of the WWTPs were divided into four zones (C1, C2, C3 and C4), i.e. one anoxic zone (C2) and three aerobic ones (C1, C3 and C4). The dimensions of the bioreactor were 50 cm long, 12 cm wide and 60 cm high and the working volume was 24 L.

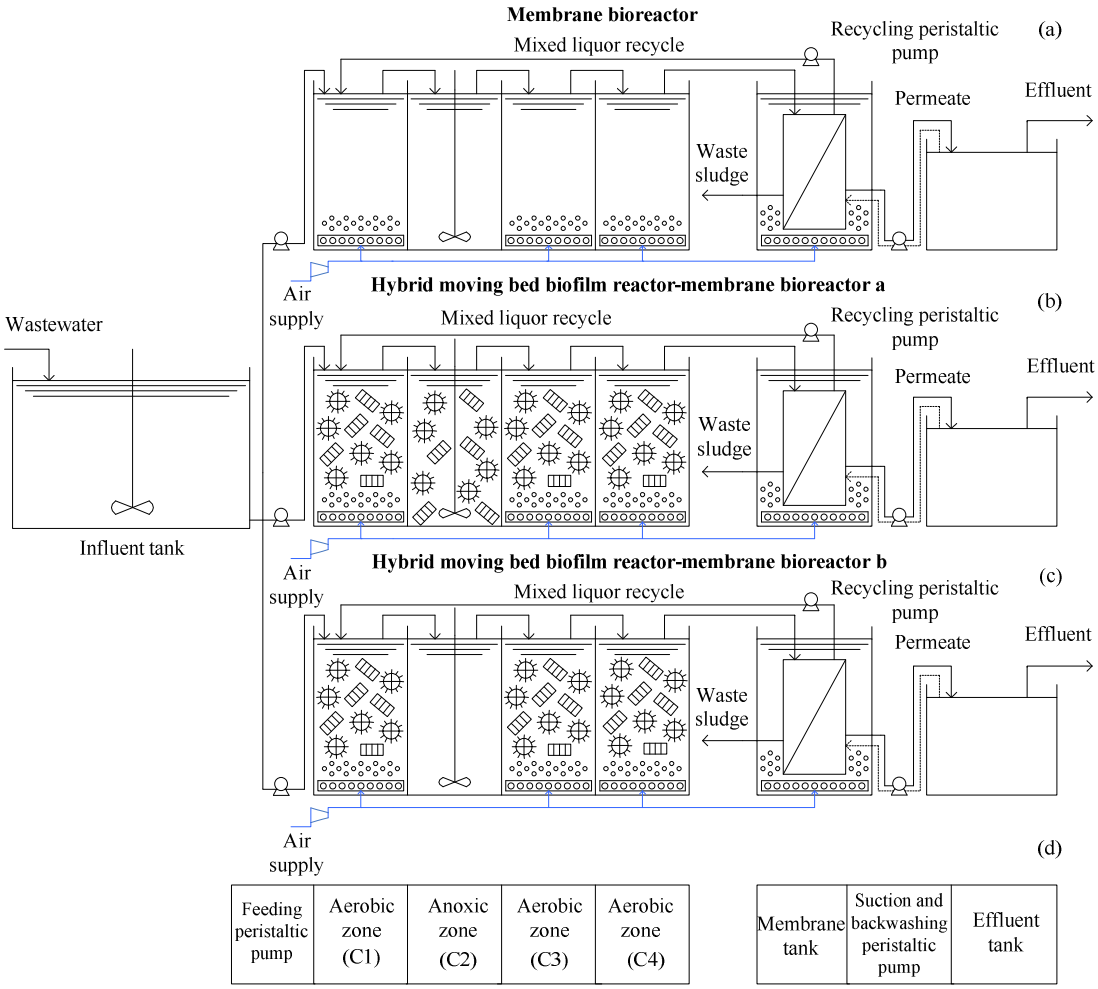

Figure VI.1. Diagram of the experimental pilot plants. (a) Membrane bioreactor (MBR). (b) Hybrid moving bed biofilm reactor-membrane bioreactor containing carriers in the aerobic and anoxic zones of the bioreactor (hybrid MBBR-MBR_a) (c) Hybrid moving bed biofilm reactor-membrane bioreactor containing carriers only in the aerobic zone of the bioreactor (hybrid MBBR-MBR_b).

Urban wastewater was pumped into the first aerobic compartment of each biological reactor from the influent tank. This tank had a stirrer (Multi Mixer MM-1000, Biosan Laboratories, Inc., USA) to homogenize the municipal wastewater. Then, it went through the anoxic zone which was situated in the second chamber to avoid recycling from the membrane tank to the first compartment could change the anoxic conditions since the mixed liquor had a higher concentration of dissolved oxygen in the membrane

tank to prevent membrane fouling. The recirculation rate was three times the influent flow rate. Subsequently, it went through the remaining aerobic chambers by a communicating vessel system. The diffusers (AFD 270, ECOTEC, SA, Spain) and stirrers (Multi Mixer MM-1000, Biosan Laboratories, Inc., USA) in the aerobic and anoxic compartments, respectively, had the functions of homogenizing the mixed liquor and keeping the carriers moving in the hybrid MBBR-MBR systems. An air flow rate of 30 L h^{-1} was supplied to the aerobic zones of the bioreactors by an air compressor (ACO-500, Hailea, China). The outlet of the bioreactor was led into the membrane tank, which was designed to be an external submerged unit. It was cylindrical, had a diameter of 10 cm, a height of 65 cm and a working volume of 4.32 L. The membrane module consisted of a vertically oriented submerged module of hollow-fiber ultrafiltration membranes (Micronet Porous Fiber, SL, Spain) with a total membrane area of 0.20 m^2 and a pore size of 0.04 μm. Another air compressor (ACO-500, Hailea, China) supplied aeration, which was applied to the base of the module by a coarse bubble disk diffuser (CAP 3, ECOTEC, SA, Spain) with an air flow rate of 100 L h^{-1}. Recycling from the membrane tank to the first compartment of the biological reactor was necessary for obtaining the working mixed liquor suspended solids (MLSS) concentration inside the bioreactor and allowing the nitrogen removal. The effluent was extracted through the membrane by a suction-backwashing peristaltic pump (323U, Watson-Marlow Pumps Group, USA) to collect it into the effluent tank. The pump worked under production and backwashing periods of 9 min and 1 min, respectively. A specific volume of waste sludge was removed from the membrane tank.

The reactor zones, the membrane tank, the effluent tank and some peristaltic pumps are shown in Figure VI.1d. Table VI.1 shows the operating conditions of the WWTPs.

Table VI.1. Technical data, operating conditions and stabilization concentrations of MLSS, MLVSS, attached BD and VBD of the experimental plants. HRT (hydraulic retention time), SRT (sludge retention time), MLSS (mixed liquor suspended solids), MLVSS (mixed liquor volatile suspended solids), BD (biofilm density), VBD (volatile biofilm density).

Parameter	MBR		Hybrid MBBR-MBR_a		Hybrid MBBR-MBR_b	
	Aerobic zone	Anoxic zone	Aerobic zone	Anoxic zone	Aerobic zone	Anoxic zone
Working volume (L)	18	6	18	6	18	6
Filling ratio with carriers (%)	0	0	35	35	35	0
Flow rate (L h^{-1})	1.6		1.6		1.6	
HRT (h)	18		18		18	
SRT (day)	32		32		32	
Membrane flux (L m^{-2} h^{-1})	8		8		8	
MLSS (mg L^{-1})	3,574.34±175.26		2,028.93±155.52		2,306.66±112.93	
MLVSS (mg L^{-1})	3,067.11±150.39		1,831.20±140.36		1,964.17±96.16	
BD (mg L^{-1})	-		1,610.83±73.60		1,207.50±76.61	
VBD (mg L^{-1})	-		1,462.73±66.83		1,025.21±65.04	

2.2. Experimental procedure and analytical determinations

Samples were collected from the influent, the three effluents and the anoxic and aerobic zones of the bioreactors and the membrane tanks every day. Physical and chemical determinations were carried out in relation to the pH, conductivity, temperature, dissolved oxygen, chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), volatile suspended solids (VSS), total phosphorus (TP), total nitrogen (TN) and the concentrations of ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) according to section **Materials and Methods**.

Furthermore, the kinetic parameters for heterotrophic, autotrophic and nitrite-oxidizing bacteria were evaluated, the enzymatic activities of α-glucosidase, acid phosphatase and alkaline phosphatase were determined, a TGGE fingerprint analysis was carried out and the structure of the attached biofilm was analyzed by SEM (**Materials and Methods**).

The evaluation of statistically significant differences between the results concerning COD, BOD_5, TSS, TN, TP, concentrations of NH_4^+, NO_2^- and NO_3^-, and enzymatic activities was carried out according to section **Materials and Methods.**

3. Results and discussion

3.1. Evolution of the biomass and physical and chemical parameters

The concentrations of MLSS and attached biofilm density (BD) increased during the start-up phase until the steady state started as the working concentrations were achieved in the day 50 of the study. The total duration of the study was 128 days. Figure VI.2a, Figure VI.2b and Figure VI.2c show the evolution of MLSS and attached BD for the experimental plants.

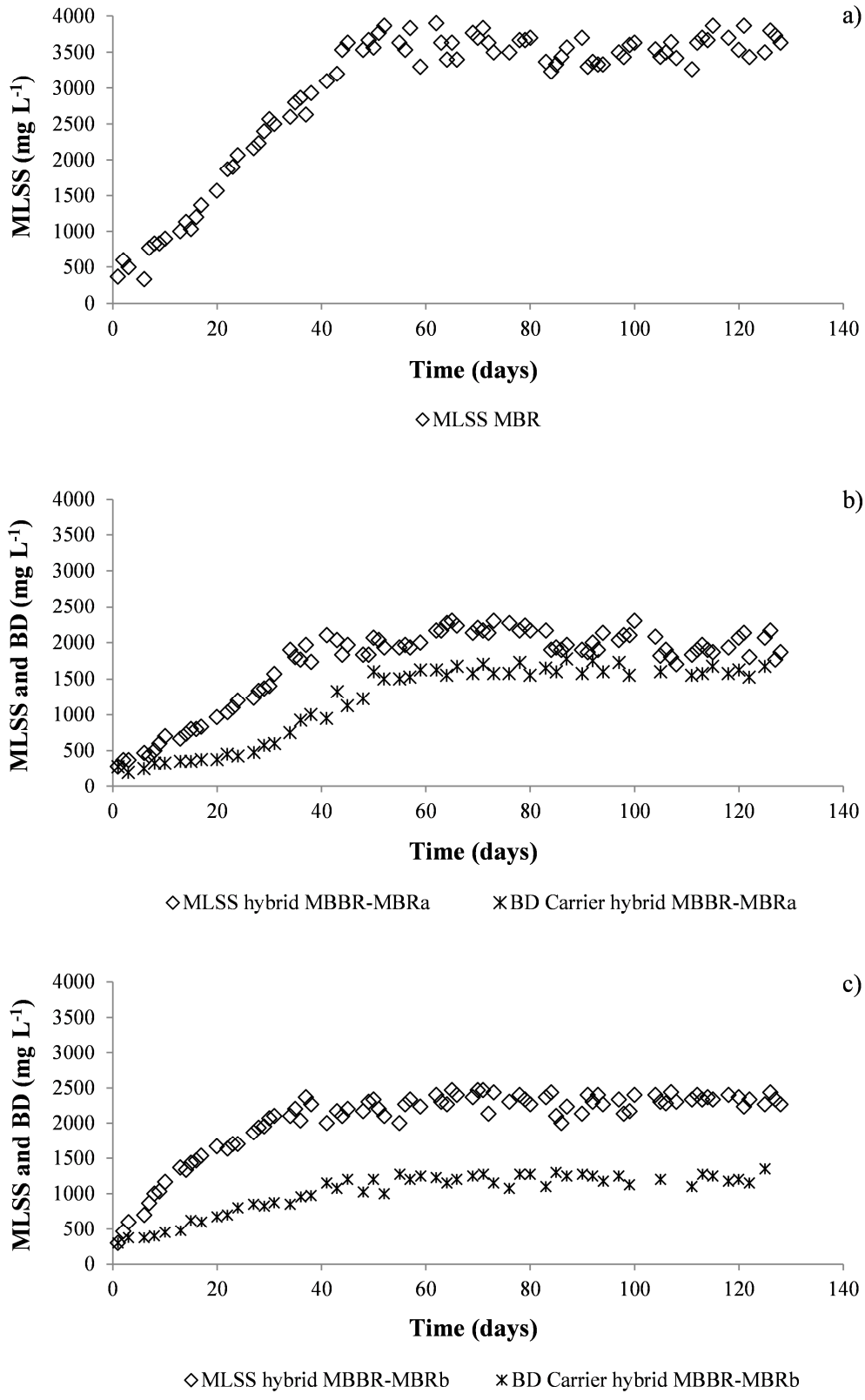

Figure VI.2. Evolution of the suspended and attached biomasses as mixed liquor suspended solids (MLSS) and biofilm density (BD), respectively, in the bioreactors of the WWTPs. (a) MLSS of the MBR. (b) MLSS and BD of the hybrid MBBR-MBR_a. (c) MLSS and BD of the hybrid MBBR-MBR_b.

The steady state values of the concentration of MLSS and attached BD are shown in Table VI.1. Moreover, mixed liquor volatile suspended solids (MLVSS) and volatile biofilm density (VBD) from Table VI.1 were used for the estimation of the kinetic parameters. The total biomass concentrations were similar in the three WWTPs since the concentration of MLSS in MBR (3,574.34±175.26 mg L^{-1}) was compensated for by the addition of the concentration of MLSS and attached BD in hybrid MBBR-MBR_a (2,028.93±155.52 mg L^{-1} and 1,610.83±73.60 mg L^{-1}, respectively) and hybrid MBBR-MBR_b (2,306.66±112.93 mg L^{-1} and 1,207.50±76.61 mg L^{-1}, respectively). It allowed for studying the differences regarding the microbial kinetics, enzymatic activities and bacterial diversity between the three WWTPs. Similar concentrations of MLSS and BD in hybrid MBBR systems were used by Falletti and Conte (2007).

The nitrification process caused a slight drop in the pH values in the mixed liquors of the bioreactors and the effluents (Canziani et al., 2006), as shown in Table VI.2. The study was carried out between the months of April and August and this is the reason why the temperature was high (23.3±1.5ºC). The concentrations of dissolved oxygen in the aerobic chambers of the different bioreactors were higher than 2.0±0.1 mg O_2 L^{-1} (2.7±1.0 mg O_2 L^{-1}, 3.0±0.7 mg O_2 L^{-1} and 3.2±0.9 mg O_2 L^{-1} for the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, respectively) according to the suggestion of Wang et al. (2006) to obtain efficient processes of organic matter oxidation and nitrification. The concentrations of dissolved oxygen in the anoxic zone of the bioreactors were 0.2±0.1 mg O_2 L^{-1}, 0.4±0.2 mg O_2 L^{-1} and 0.3±0.2 mg O_2 L^{-1} for the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, respectively (Table VI.2).

Table VI.2. Average values of pH, conductivity, temperature and dissolved oxygen of the influent, effluents and mixed liquors of the bioreactors of the experimental plants.

Parameter	Sampling zone									
	Influent	MBR			Hybrid MBBR-MBR_a			Hybrid MBBR-MBR_b		
		Effluent	Anoxic zone	Aerobic zone	Effluent	Anoxic zone	Aerobic zone	Effluent	Anoxic zone	Aerobic zone
pH	8.02±0.17	6.61±0.58	7.04±0.63	6.18±0.74	6.15±0.27	6.50±0.50	5.61±0.65	6.25±0.41	6.18±0.76	5.74±0.84
Conductivity (µS cm^{-1})	1,243±84	1,048±200	963±63	954±65	1,033±76	951±68	953±86	1,040±77	968±75	955±97
Temperature (ºC)	23.3±1.5	23.3±1.5	23.0±1.5	23.2±1.5	23.4±1.6	23,2±1.5	23.3±1.6	23.4±1.6	23.3±1.5	23.3±1.5
Dissolved oxygen (mg O_2 L^{-1})	-	-	0.2±0.1	2.7±1.0	-	0.4±0.2	3.0±0.7	-	0.3±0.2	3.2±0.9

3.2. Organic matter and nutrient removal

The removal percentages of organic matter and nutrients were very similar in the WWTPs studied, as can be observed in Table VI.3 through the parameters COD, BOD_5, TN and TP.

Table VI.3. Average values of COD, BOD_5, TSS, TP, TN, NH_4^+, NO_2^- and NO_3^- of the influent and effluents of the experimental plants and removal percentages of COD, BOD_5, TSS, TP and TN during the steady state. COD (chemical oxygen demand), BOD_5 (five-day biochemical oxygen demand), TSS (total suspended solids), TP (total phosphorus), TN (total nitrogen), NH_4^+ (concentration of ammonium), NO_2^- (concentration of nitrite), NO_3^- (concentration of nitrate).

Parameter	Sampling zone				Removal percentage	Wastewater treatment plant		
	Influent	Effluent MBR	Effluent hybrid MBBR-MBR_a	Effluent hybrid MBBR-MBR_b		MBR	Hybrid MBBR-MBR_a	Hybrid MBBR-MBR_b
COD (mg O_2 L^{-1})	320.16±63.63	31.09±9.41	32.66±6.40	31.25±9.35	**COD (%)**	90.29±2.05	89.80±2.25	90.24±2.87
BOD_5 (mg O_2 L^{-1})	242.14±88.16	5.76±1.16	5.93±1.09	5.93±0.98	**BOD_5 (%)**	97.62±1.56	97.55±1.32	97.55±1.49
TSS (mg L^{-1})	155.49±63.44	4.98±3.80	5.35±3.61	6.78±4.67	**TSS (%)**	96.80±2.40	96.56±2.67	95.64±3.13
TP (mg P L^{-1})	10.88±2.24	5.88±0.72	5.99±0.74	5.89±1.15	**TP (%)**	45.95±7.80	44.94±6.79	45.89±7.27
TN (mg N L^{-1})	95.48±46.01	34.63±15.95	35.59±15.33	34.41±14.65	**TN (%)**	63.73±8.05	62.72±8.22	63.96±7.00
NH_4^+ (mg NH_4^+ L^{-1})	115.86±36.56	ND	ND	ND				
NO_2^- (mg NO_2^- L^{-1})	4.16±0.11	17.16±11.55	33.02±14.02	39.53±17.50				
NO_3^- (mg NO_3^- L^{-1})	18.17±9.18	130.24±50.29	113.13±31.09	99.11±29.63				

ND: Not Detected

The differences between the three systems were not statistically significant regarding these parameters with an HRT of 18 h as the p-values obtained from the post hoc procedure, Tukey´s HSD, were higher than α=0.05. However, the MBR and the hybrid MBBR-MBR_b showed similar removal performances regarding COD, TN and TP, which were slightly higher than those obtained in the hybrid MBBR-MBR_a. The MBR had the best COD and TP removals with values of 90.29±2.05% and 45.95±7.80%, respectively, and the hybrid MBBR-MBR_b showed the best TN removal with a value of 63.96±7.00%. Therefore, a hybrid MBBR-MBR system is suitable to remove TN with an anoxic zone without carriers, which provides better contact between nitrate and the microorganisms, according to Larrea et al. (2007). Moreover, Jonoud et al. (2003) obtained similar percentages of COD and TN removal, i.e. higher than 85% and 50%, respectively, under an HRT of 20 h. The values of TP removal were low in all systems because a strict anaerobic zone was necessary to initialize the process of biological phosphorus removal, as indicated by Kermani et al. (2009). The concentrations of dissolved oxygen in the anoxic and aerobic zones of the different bioreactors were 0.2±0.1 mg O_2 L^{-1} and 2.7±1.0 mg O_2 L^{-1}, 0.4±0.2 mg O_2 L^{-1} and 3.0±0.7 mg O_2 L^{-1}, and 0.3±0.2 mg O_2 L^{-1} and 3.2±0.9 mg O_2 L^{-1} for the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, respectively.

The reduction percentages of TSS are also indicated in Table VI.3. There were no statistically significant differences regarding this parameter between the three systems studied as they contained a module including hollow-fiber ultrafiltration membranes in the membrane tank.

3.3. Biological kinetic modeling of MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b

Table VI.4 shows the kinetic parameters which fit the Monod model for the heterotrophic, autotrophic and nitrite-oxidizing bacteria from the different bioreactors.

Table VI.4. Kinetic parameters for the characterization of heterotrophic, autotrophic and nitrite-oxidizing bacteria. Y_H (yield coefficient for heterotrophic bacteria), $\mu_{m,\ H}$ (maximum specific growth rate for heterotrophic bacteria), K_M (half-saturation coefficient for organic matter), Y_A (yield coefficient for autotrophic bacteria), $\mu_{m,\ A}$ (maximum specific growth rate for autotrophic bacteria), K_{NH} (half-saturation coefficient for ammonia-nitrogen), Y_{NOB} (yield coefficient for nitrite-oxidizing bacteria), $\mu_{m,\ NOB}$ (maximum specific growth rate for nitrite-oxidizing bacteria), K_{NOB} (half-saturation coefficient for nitrite-nitrogen), k_d (decay coefficient for total bacteria).

Parameter	Sampling zone		
	MBR	Hybrid MBBR-MBR_a	Hybrid MBBR-MBR_b
Heterotrophic bacteria			
Y_H (mg VSS mg COD^{-1})	0.4235	0.3960	0.4338
$\mu_{m,\ H}$ (h^{-1})	0.0068	0.0065	0.0110
K_M (mg O_2 L^{-1})	8.6103	6.6382	9.0178
Autotrophic bacteria			
Y_A (mg O_2 mg N^{-1})	0.9852	1.8887	2.1970
$\mu_{m,\ A}$ (h^{-1})	0.0530	0.0840	0.0861
K_{NH} (mg N L^{-1})	15.2472	5.7525	3.1287
Nitrite-oxidizing bacteria			
Y_{NOB} (mg O_2 mg N^{-1})	0.5682	0.5078	0.4713
$\mu_{m,\ NOB}$ (h^{-1})	0.2032	0.1671	0.3311
K_{NOB} (mg N L^{-1})	0.9370	1.4769	1.6232
Total bacteria			
k_d (d^{-1})	0.0165	0.0311	0.0307

The hybrid MBBR-MBR_b showed the highest values of the maximum specific growth rate for heterotrophic ($\mu_{m,\ H}$) and autotrophic biomass ($\mu_{m,\ A}$) with 0.0110 h^{-1} and 0.0861 h^{-1}, respectively. Similar values concerning $\mu_{m,\ H}$ and $\mu_{m,\ A}$ were obtained by Canziani et al. (2006) and Plattes et al. (2007), respectively. The hybrid MBBR-MBR_b showed the best kinetic performance from the point of view of the heterotrophic and autotrophic biomass when the r_{su} was evaluated depending on the kinetic parameters, substrate concentration and biomass concentration (Figure VI.3a and Figure VI.3b). The r_{su} was higher for the MBR and hybrid MBBR-MBR_b concerning the heterotrophic biomass. It supported the highest removal percentages of COD for these systems, as can be observed in Table VI.3. The hybrid MBBR-MBR_b showed the highest values of r_{su} regarding autotrophic biomass which is the reason why this system had the best removal performance of TN (63.96±7.00%) under the operating conditions used in this study. Therefore, the required time for substrate oxidation was lower in the heterotrophic and

autotrophic biomasses from the hybrid MBBR-MBR_b; the μ_m was obtained with less available substrate and the steady state was reached in less time.

However, the MBR had the best kinetic behavior regarding the NOB kinetics with values of Y_{NOB} = 0.5682 mg O_2 mg N^{-1}, $\mu_{m,\ NOB}$ = 0.2032 h^{-1} and K_{NOB} = 0.9370 mg N L^{-1} (Pambrun et al., 2006), as shown in Figure VI.3c. This supported the fact that the nitrate concentration in the effluent from the MBR was higher than that from the hybrid MBBR-MBR_b (Table VI.3). Therefore, the hybrid MBBR-MBR_b could have a better kinetic behavior regarding the ammonium-oxidizing bacteria (AOB) because, as a whole, the kinetics of autotrophic bacteria was better, as previously mentioned, and the hybrid MBBR-MBR_b had the highest nitrite concentration in its effluent, as indicated in Table VI.3. In this sense, there were statistically significant differences regarding nitrite and nitrate formations between the MBR and hybrid MBBR-MBR_b with an HRT of 18 h as the p-values obtained were less than α=0.05, p-value $_{\text{MBR-Hybrid MBBR-MBRb}}$ (NO_2^-) = 0.01959 and p-value $_{\text{MBR-Hybrid MBBR-MBRb}}$ (NO_3^-) = 0.03435. Leyva-Díaz et al. (2015) obtained similar conclusions in a study carried out with the same WWTPs, similar values of MLSS and BD and an HRT of 9.5 h, although the MBR had the best kinetic behavior in relation to the autotrophic biomass and the hybrid MBBR-MBR_b showed the best kinetic performance regarding the NOB.

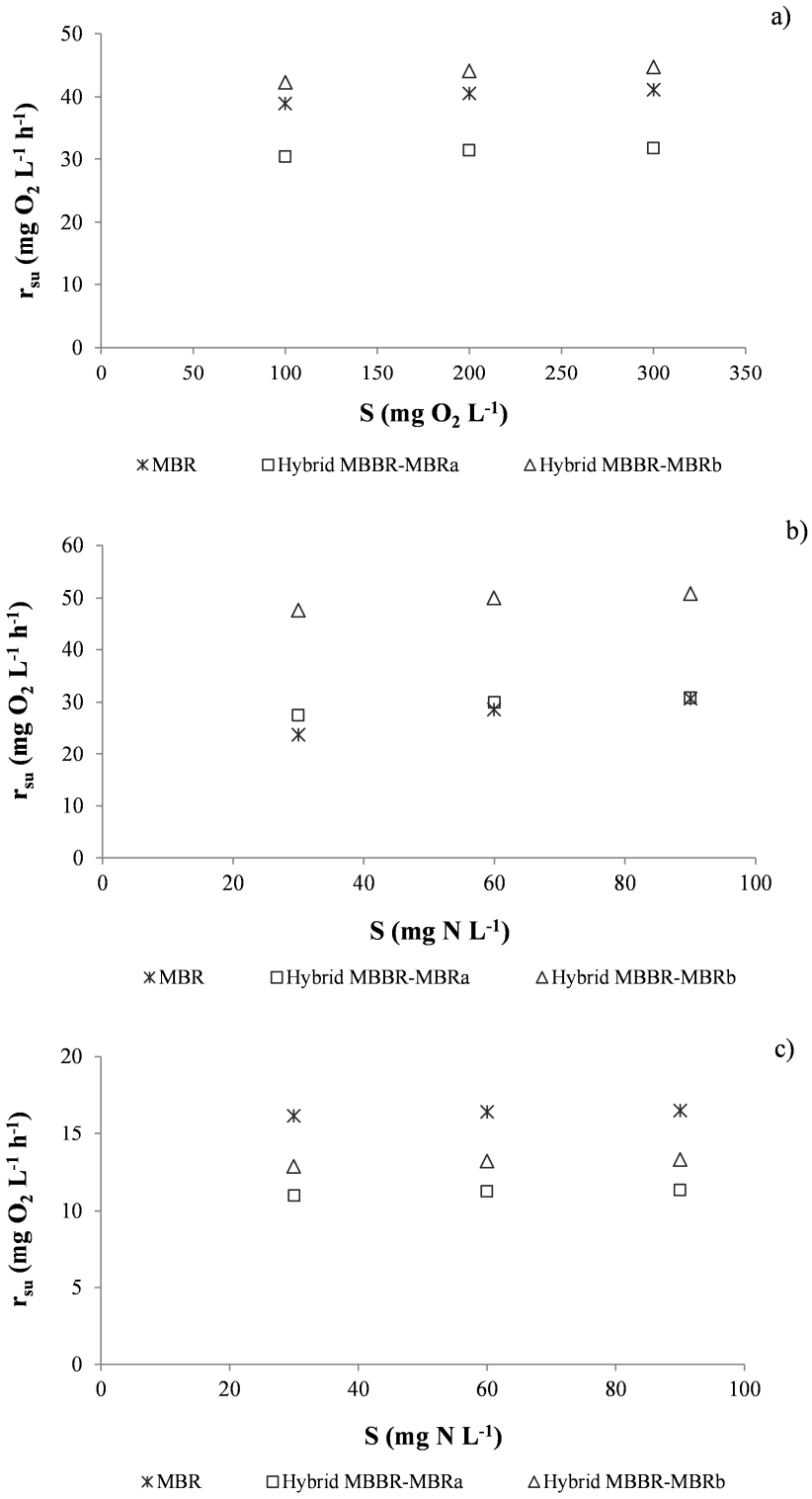

Figure VI.3. Evolution of the substrate degradation rate (r_{su}) in the kinetic study depending on the substrate concentration for the different bioreactors from the WWTPs. (a) Heterotrophic bacteria. (b) Autotrophic bacteria. (c) Nitrite-oxidizing bacteria.

3.4. Enzymatic activities

The values of α-glucosidase, acid phosphatase and alkaline phosphatase enzymatic activities of suspended and attached biomass of the microbial communities in the four chambers of the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b are shown in Figure VI.4, Figure VI.5 and Figure VI.6, respectively. It can be observed that the mean α-glucosidase enzymatic activity was lower within the attached biomass than in the suspended biomass. Differences of one order of magnitude were found between the different biomass configurations in hybrid MBBR-MBR_a. However, differences were much smaller in hybrid MBBR-MBR_b. On the other hand, α-glucosidase enzymatic activity was not affected by different conditions in different chambers. Bearing in mind the fact that the steady state started on day 50, the average values of α-glucosidase enzymatic activity relating to days 60, 80, 100 and 120 were calculated by considering the mean of the values corresponding to the four chambers. The evaluation of the percentage of contribution of suspended and attached biomasses in hybrid MBBR-MBR_a and hybrid MBBR-MBR_b was carried out according to Reboleiro-Rivas et al. (2013). The results show that the α-glucosidase enzymatic activities in the MBR and hybrid MBBR-MBR_b, i.e. 0.4650±0.0430 mM g VSS^{-1} min^{-1} and 0.4110±0.0414 mM g VSS^{-1} min^{-1}, respectively, were higher than in hybrid MBBR-MBR_a with a value of 0.2933±0.0316 mM g VSS^{-1} min^{-1}.

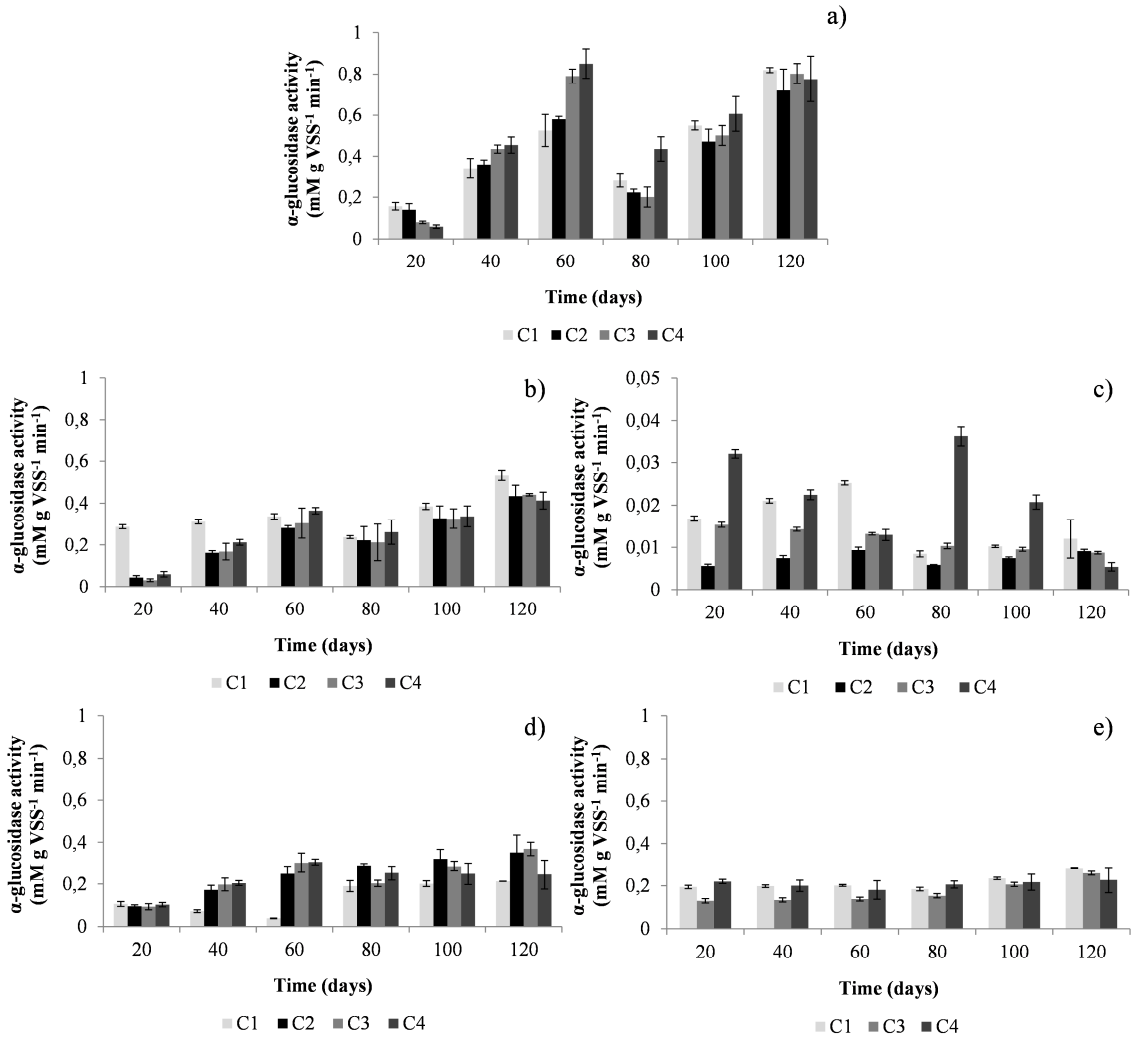

Figure VI.4. Enzymatic activity of α-glucosidase in the chambers C1, C2, C3 and C4 of the different bioreactors. (a) Suspended biomass in the MBR. (b) Suspended biomass in hybrid MBBR-MBR_a. (c) Attached biomass in hybrid MBBR-MBR_a. (d) Suspended biomass in hybrid MBBR-MBR_b. (e) Attached biomass in hybrid MBBR-MBR_b.

As with α-glucosidase, the mean acid phosphatase enzymatic activity was higher in the suspended biomass than in the fixed biofilm communities. Differences between the different chambers were not clear regarding acid phosphatase enzymatic activity. The results show that the acid phosphatase enzymatic activities in the MBR and hybrid MBBR-MBR_b, i.e. 8.6876±0.6129 mM g VSS^{-1} min^{-1} and 8.5155±0.8202 mM g VSS^{-1} min^{-1}, respectively, were the highest, followed by hybrid MBBR-MBR_a with a value of 6.1473±0.7642 mM g VSS^{-1} min^{-1}.

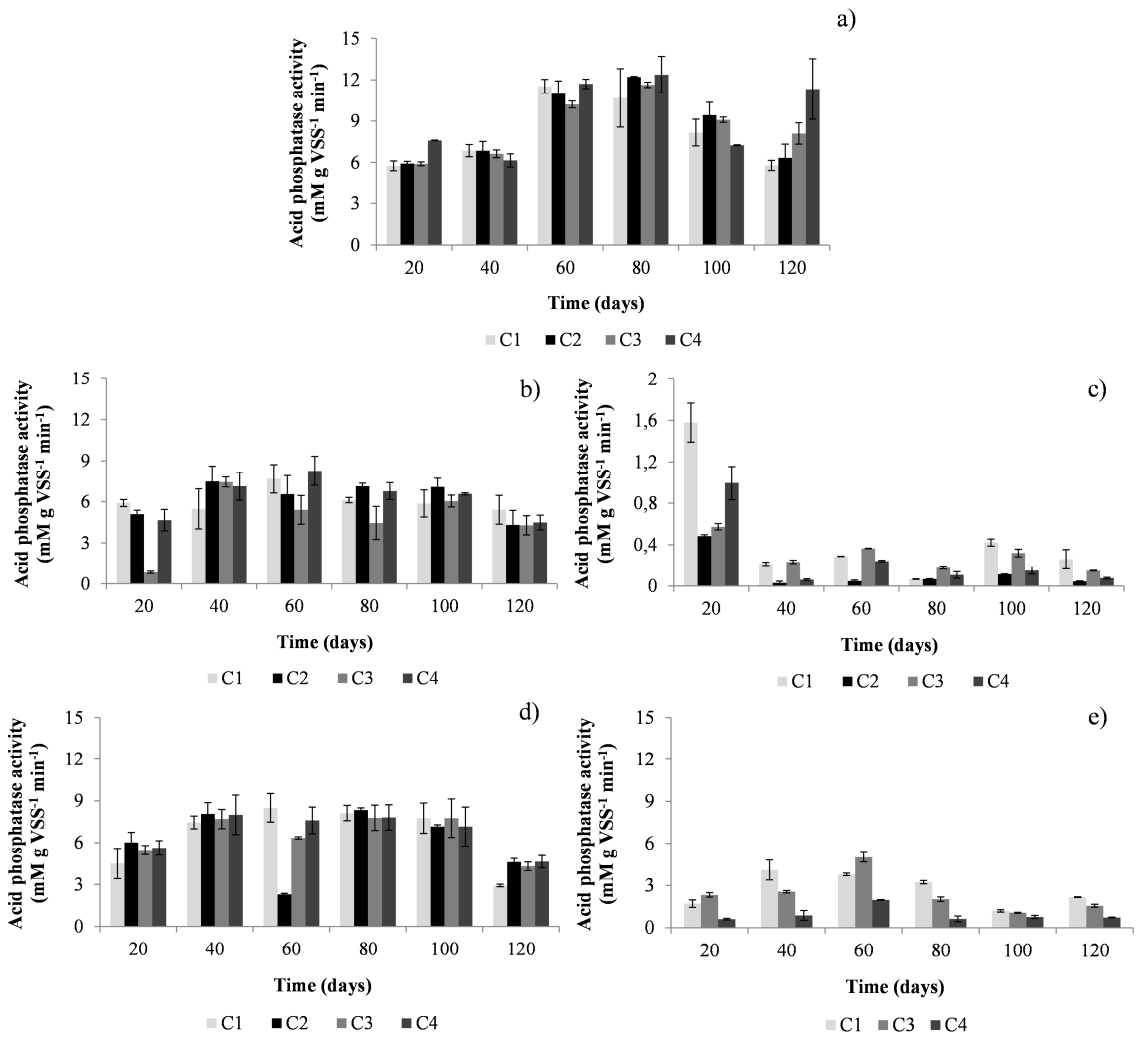

Figure VI.5. Enzymatic activity of acid phosphatase in the chambers C1, C2, C3 and C4 of the different bioreactors. (a) Suspended biomass in the MBR. (b) Suspended biomass in hybrid MBBR-MBR_a. (c) Attached biomass in hybrid MBBR-MBR_a. (d) Suspended biomass in hybrid MBBR-MBR_b. (e) Attached biomass in hybrid MBBR-MBR_b.

The values of alkaline phosphatase enzymatic activity within the suspended biomass communities were also higher than within the fixed biofilm communities. Once again, differences between the different chambers were not related to the different conditions of the systems. The results show that the alkaline phosphatase enzymatic activity in the MBR, 4.3872±0.2845 mM g VSS^{-1} min^{-1}, was the highest, followed by hybrid MBBR-MBR_b and hybrid MBBR-MBR_a with values of 2.2053±0.1660 mM g VSS^{-1} min^{-1} and 1.9624±0.1877 mM g VSS^{-1} min^{-1}, respectively.

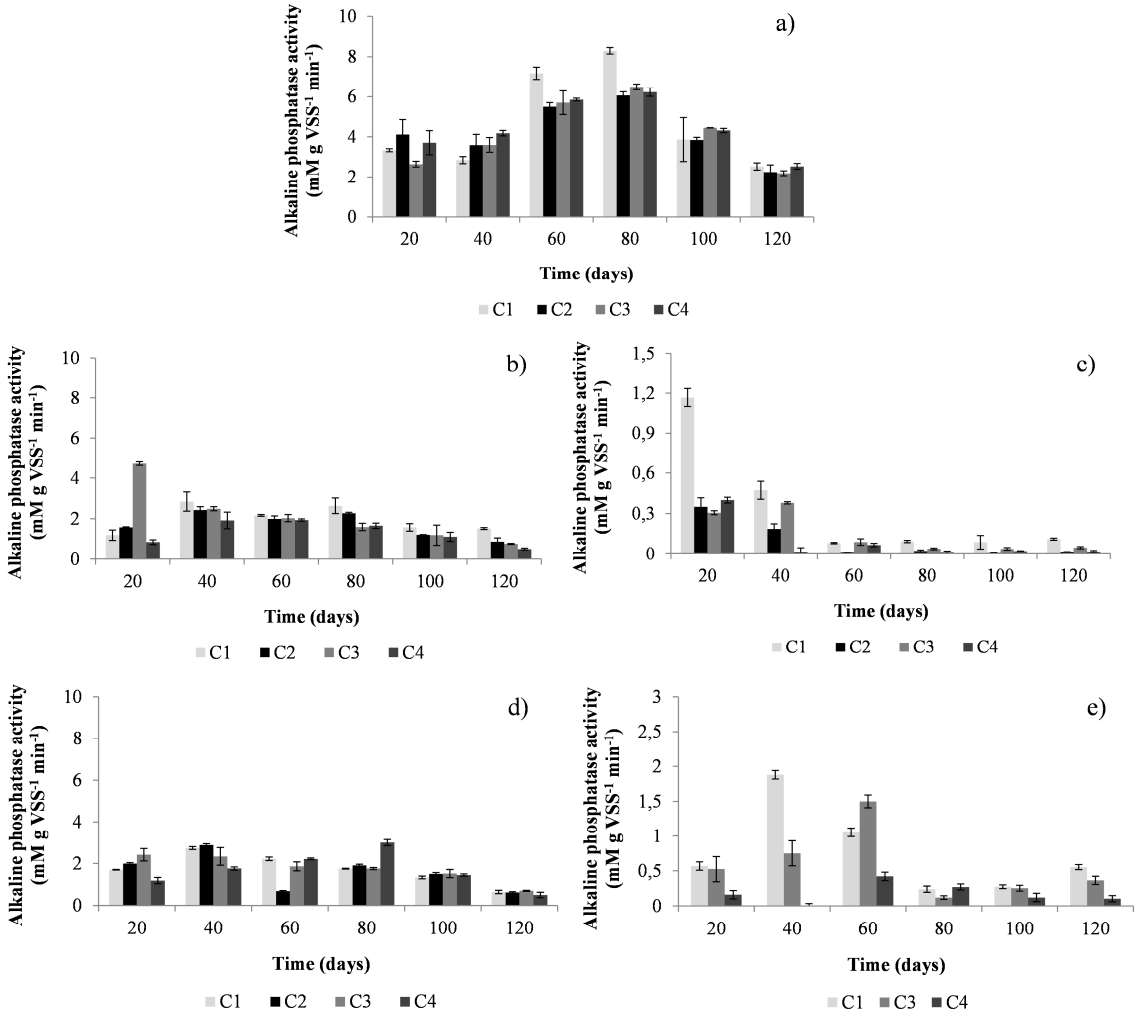

Figure VI.6. Enzymatic activity of alkaline phosphatase in the chambers C1, C2, C3 and C4 of the different bioreactors. (a) Suspended biomass in the MBR. (b) Suspended biomass in hybrid MBBR-MBR_a. (c) Attached biomass in hybrid MBBR-MBR_a. (d) Suspended biomass in hybrid MBBR-MBR_b. (e) Attached biomass in hybrid MBBR-MBR_b.

Therefore, acid phosphatase activity had the highest values, closely followed by alkaline phosphatase activity. This indicates that α-glucosidase activity was less important than acid phosphatase and alkaline phosphatase activities in all three bioreactors. Some authors have investigated the influence of temperature on phosphatase and α-glucosidase activities in this kind of wastewater treatment process (Calderon et al., 2012; Reboleiro-Rivas et al., 2013). It was found that summer temperatures increase phosphatase activity while winter temperatures increase α-glucosidase activity (Molina-Muñoz et al., 2007; Molina-Muñoz et al., 2010). The present results are in accordance with these authors, as the experiment was conducted between the months of April and August and the temperature was 23.3±1.5ºC. Furthermore, different behaviors of phosphatase and α-glucosidase enzymatic activities

have been identified regarding the VSS concentration in wastewater treatment processes (Burgess and Pletschke, 2008; Calderon et al., 2013). Molina-Muñoz et al. (2010) found that the optimum activity for α-glucosidase occurs at VSS concentrations around 8,533.3 mg L^{-1}, while optimum activity for phosphatase occurs at VSS concentrations of 5,766.6 mg L^{-1} and lower. In this sense, these results are in accordance as the VSS concentrations in the bioreactors of the three WWTPs were lower than those obtained by Molina-Muñoz et al. (2010) (Table VI.1).

Differences in the enzymatic activities of attached and suspended biomasses have been found, with higher values for the suspended biomass (Reboleiro-Rivas et al., 2013). A plausible explanation for these differences resides in the low diffusivity that extracellular polymeric substances (EPS) layers provide to the attached biomass, which impedes the release of extracellular microbial enzymes so the enzymatic activity is reduced. In spite of this, the differences regarding the enzymatic activities were not statistically significant between the WWTPs with an HRT of 18 h as the p-values obtained were higher than α=0.05. Furthermore, α-glucosidase and acid phosphatase enzymatic activities were very similar in the MBR and hybrid MBBR-MBR_b under an HRT of 18 h; differences might be higher at lower values of HRT, which would indicate the existence of an improvement in the enzymatic activities by the presence of attached biomass (Reboleiro-Rivas et al., 2013). Therefore, the MBR and hybrid MBBR-MBR_b systems achieved higher removal percentages of COD, TN and TP (Table VI.3) as the enzymatic activities were higher; the bacterial activity is closely related to the enzymatic activity within an ecosystem (Nybroe et al., 1992). In this sense, the study of the enzymatic activities also supported the results obtained from the kinetic analysis, as the hybrid MBBR-MBR_b showed the highest values of the r_{su} for heterotrophic and autotrophic biomasses. This system had the highest values of α-glucosidase, acid phosphatase and alkaline phosphatase, together with the MBR system.

Differences among the different chambers for the same bioreactor configuration were not clear for α-glucosidase and phosphatase enzymatic activities. Similarly, it has been reported that enzymatic activity is not influenced by the dissolved oxygen concentration in the aerobic, anaerobic and anoxic zones of a bench scale activated sludge process (Goel et al., 1998).

3.5. TGGE fingerprint analysis

TGGE fingerprints for the amplicons of V3 region of the bacterial 16S rRNA gene generated through nested PCR regarding suspended and attached biomass communities for chambers C1, C2, C3 and C4 of the bioreactors of the three WWTPs can be seen in Figure VI.7. The fingerprints of the microbial communities of the three bioreactors were related with 75% similarity. Fingerprints belonging to the MBR were clearly differentiated from those corresponding to hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, with the exception of chamber C4. The differentiation of hybrid MBBR-MBR_a and hybrid MBBR-MBR_b appeared at 78% similarity, with the exception of chamber C4 from hybrid MBBR-MBR_b, which stands as an individual sample. In this sense, it can be said that differences in the configuration of the bioreactors were shown in the TGGE fingerprints of their bacterial communities. Moreover, differences in the disposition of bacterial communities can be seen in the clustering of TGGE fingerprints. Nevertheless, these differences seem to not be remarkable. In this sense, the TGGE fingerprints suggest similar diversity regarding the bacterial communities of the suspended and attached biomasses.

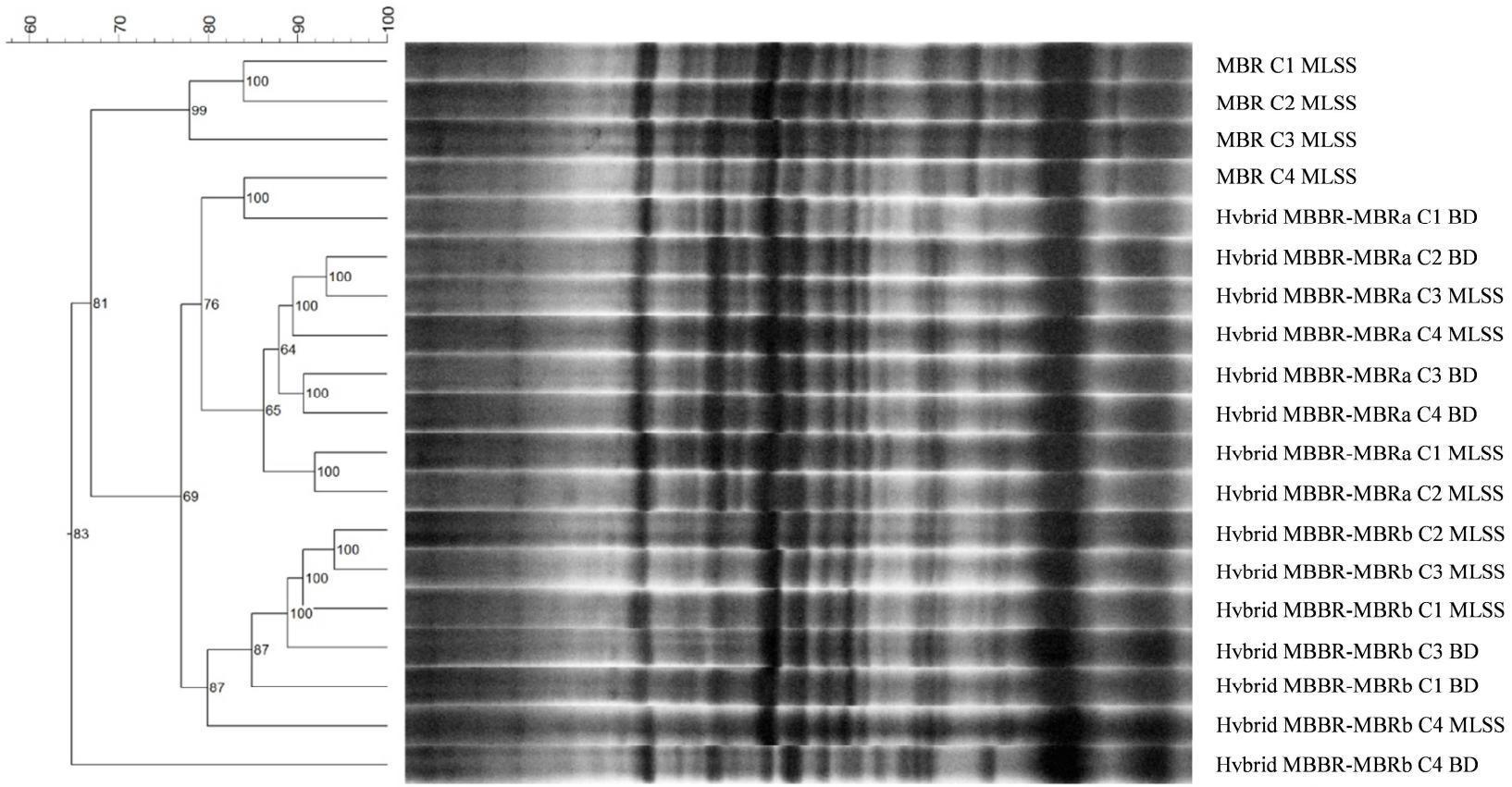

Figure VI.7. TGGE fingerprints of bacterial communities of suspended biomass (MLSS) and attached biomass (BD) in chambers C1, C2, C3 and C4 of the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b.

3.6. Analysis of biofilm communities by SEM

The SEM analysis was carried out to determine the morphology of the biofilm developed on the carriers of the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b. The SEM results demonstrate the presence of microorganisms with different structures such as bacilli (Figure VI.8c, Figure VI.8k and Figure VI.8o), cocci (Figure VI.8f, Figure VI.8l and Figure VI.8n and Figure VI.8q), filamentous bacteria (Figure VI.8g and Figure VI.8h) and also the appearance of EPS (Figure VI.8e, Figure VI.8f, Figure VI.8j, Figure VI.8ñ and Figure VI.8r), which are the basis for biofilm development (Calderon et al., 2011; Calderon et al., 2012).

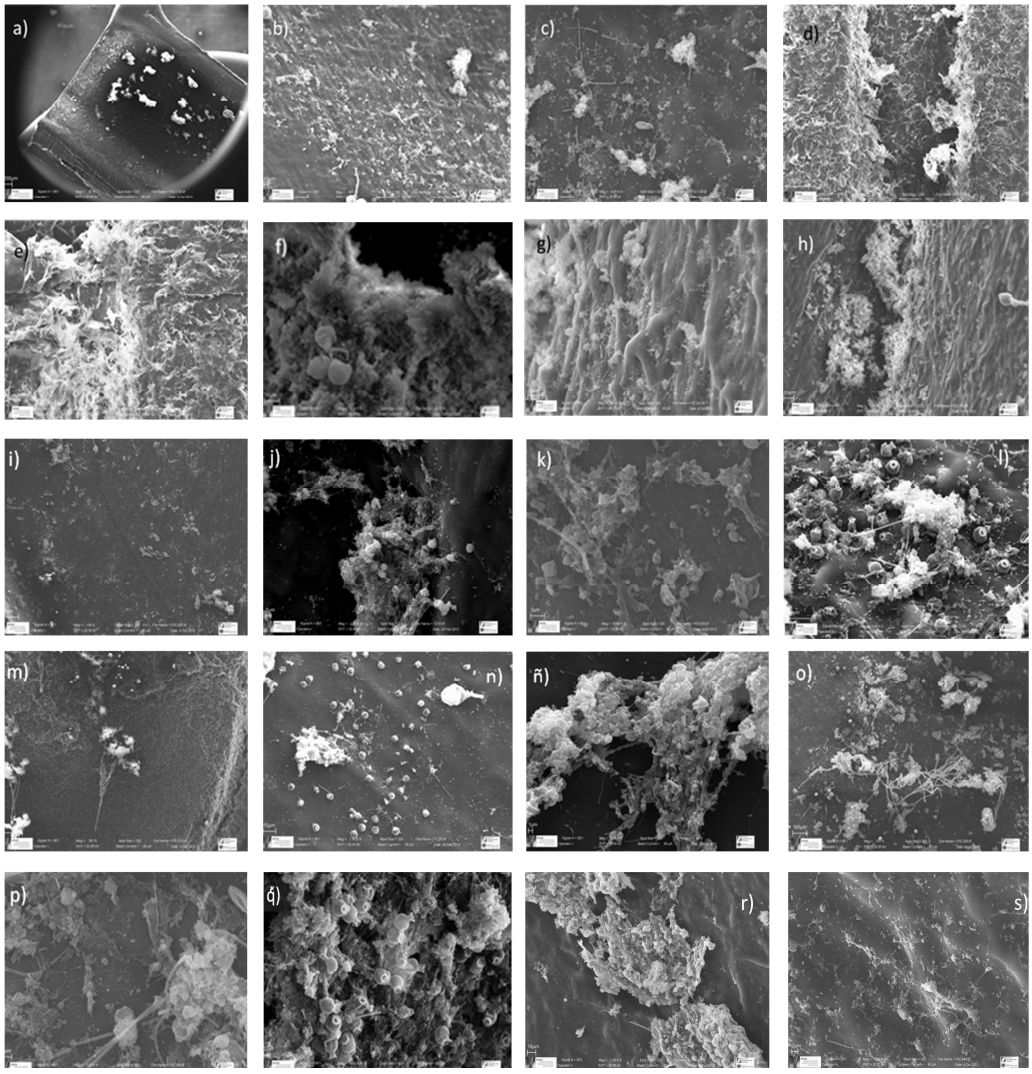

Figure VI.8. SEM of biomass collected from the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b. (a), (b), (c) Hybrid MBBR-MBR_a, C1. (d), (e) Hybrid MBBR-MBR_a, C2. (f), (g), (h) Hybrid MBBR-MBR_a, C3. (i), (j), (k) Hybrid MBBR-MBR_a, C4. (l), (m) Hybrid MBBR-MBR_b, C1. (n), (ñ), (o) Hybrid MBBR-MBR_b, C3. (p), (q), (r), (s) Hybrid MBBR-MBR_b, C4.

4. Conclusions

The following conclusions were drawn:

1. The MBR and hybrid MBBR-MBR_b (without carriers in the anoxic zone) showed the highest removal percentages of COD, which were supported by their best kinetic performance regarding the heterotrophic biomass with values of $\mu_{m,\,H}$ of 0.0068 h^{-1} and 0.0110 h^{-1}, respectively. The hybrid MBBR-MBR_b had the best kinetic behavior concerning the autotrophic biomass ($\mu_{m,\,A}$ = 0.0861 h^{-1}) with a reduction percentage of TN of 63.96±7.00%. The MBR showed the best performance from the point of view of the kinetics of nitrite-oxidizing bacteria, which supported the concentrations of nitrite and nitrate in the different effluents. Thus, an anoxic zone without carriers provides better contact between nitrate and the microorganisms. These results were also supported by higher values of α-glucosidase, acid phosphatase and alkaline phosphatase, which implied higher bacterial activities in the MBR and hybrid MBBR-MBR_b systems.

2. The enzymatic activities of α-glucosidase, acid phosphatase and alkaline phosphatase showed different values in relation to the biomass configuration, with higher values for suspended biomass than for attached biomass. This could have been caused by the low diffusivity of extracellular microbial enzymes caused by the EPS layer when the attached biomass is formed.

3. Acid and alkaline phosphatase enzymatic activities were higher than α-glucosidase enzymatic activity in the bioreactors of the three WWTPs. This fact can be explained by the temperature conditions and the VSS concentration inside the bioreactors, which favor phosphatase enzymatic activity over α-glucosidase enzymatic activity.

4. Differences in the bioreactor configuration led to differences in the bacterial diversity, bacterial community structure and the appearance of EPS in the hybrid MBBR-MBR systems according to the TGGE fingerprints of amplicons of the V3 region of the bacterial 16S rRNA gene and the SEM analysis.

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VII. CHAPTER 4

Kinetic modeling and microbiological study of two-step nitrification in a membrane bioreactor and hybrid moving bed biofilm reactor-membrane bioreactor for wastewater treatment (operational conditions of HRT=9.5 h and intermediate biomass concentrations).

Abstract

A membrane bioreactor (MBR), a hybrid moving bed biofilm reactor-membrane bioreactor (MBBR-MBR) containing carriers in the anoxic and aerobic zones of the bioreactor (hybrid MBBR-MBR_a) and a hybrid MBBR-MBR which contained carriers only in the aerobic zone (hybrid MBBR-MBR_b) were used in parallel with the same urban wastewater and compared. The reactors operated with a hydraulic retention time (HRT) of 9.5 h. Kinetic parameters for heterotrophic and autotrophic biomasses, mainly nitrite-oxidizing bacteria (NOB), were evaluated and related to organic matter and nutrients removals. The microbial communities of each wastewater treatment plant (WWTP) were analyzed by 454 pyrosequencing methods to detect and quantify the contribution of nitrifying bacteria in the total bacterial community. All three systems showed similar performance in terms of pollutant removal although the hybrid MBBR-MBR_b showed the best performance from the point of view of the kinetics of heterotrophic and nitrite-oxidizing bacteria, with values of $\mu_{m,\ H} = 0.0267\ h^{-1}$, $K_M = 8.8808\ mg\ O_2\ L^{-1}$, $\mu_{m,\ NOB} = 0.5369\ h^{-1}$ and $K_{NOB} = 2.1670\ mg\ N\ L^{-1}$. It supported the efficiencies of chemical oxygen demand (COD) and total nitrogen (TN) removals and the concentrations of nitrite and nitrate in the different effluents.

1. Introduction

The adverse environmental impacts of nitrogenous compounds in waters include increased eutrophication, toxicity to aquatic organisms, and the depletion of dissolved oxygen due to bacterial oxidation of ammonia to nitrate (Wang et al., 2006; Liu and Qiu, 2007).

Among the various methods for the removal of nitrogenous compounds from wastewater, biological removal is highly efficient compared to other methods. Biological removal of nitrogenous compounds involves the existence of nitrification and denitrification processes. The nitrification process is widely known (Barnes and Bliss, 1983; Wiesmann, 1994), in which ammonium is converted to nitrite and, subsequently, nitrite is converted to nitrate. Several groups of microorganisms, such as ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), are involved in the nitrification process. The conversion of ammonium into nitrite is carried out by AOB, according to reaction (1), and the conversion of nitrite into nitrate is carried out by NOB, as shown in reaction (2):

$$NH_4^+ + \frac{3}{2}O_2 \rightarrow NO_2^- + H_2O + 2H^+ \qquad (1)$$

$$NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^- \qquad (2)$$

Nitrate is converted into gaseous nitrogen and removed in the presence of an organic substrate through heterotrophic reduction during the denitrification process. Thus, the removal of nitrogenous compounds from wastewater is very important (He et al., 2009). Wastewater treatment techniques have become more sophisticated, shifting from sole chemical oxygen demand (COD) removal to simultaneous COD, nitrogen and phosphorus removal (Qiu et al., 2010).

The moving bed biofilm reactor-membrane bioreactor (MBBR-MBR), which was developed by Leiknes and Ødegaard (2007), represents a different spectrum in advanced wastewater treatment. This system is based on the addition of a freely moving carrier media inside the bioreactor (Ødegaard, 2006). There are two ways of working in an MBBR-MBR system. In a pure MBBR-MBR process, biofilm only grows attached to

carriers which are kept in constant motion throughout the entire volume of the reactor. The hybrid MBBR-MBR process combines suspended and attached biomass. The MBBR-MBR system aims to partially mitigate the fouling concerns regarding membrane bioreactor (MBR) systems and the settleability issues in relation to moving bed biofilm reactor (MBBR) systems. The immobilized microbial cells on the carriers offer an additional advantage of seamlessly integrated simultaneous nitrification and denitrification (Yang et al., 2009). It is based on the fact that there are dissolved oxygen concentration gradients within microbiological flocs as a result of diffusion limitations from the aqueous phase into the immobilized biofilm. The aerobic liquid provides an oxidizing environment where soluble five-day biochemical oxygen demand (BOD_5) is removed and ammonia is nitrified. The nitrite and nitrate produced during nitrification diffuses into the inner parts of the biofilm where there is an anoxic micro-zone. This micro-zone harbors heterotrophic denitrifiers which produce nitrogen gas in the traditional manner (Yang et al., 2009).

This process becomes economically attractive when compact technology is required to accommodate space constraints or stringent effluent quality requirements are mandatory (Yang et al., 2006). Moreover, the requirement to improve the wastewater treatment plants (WWTPs) has led to the necessity of using different molecular biology techniques. Thus, knowledge of the microbial community's composition involved in biofilm processes and the influence of the operation conditions on their structure are regarded as being crucially important to optimize the nutrient removal rates and to implement control strategies in MBBR-MBR systems. In the last decade, knowledge on nitrifying bacteria in wastewater treatment technologies has greatly expanded due to the application of molecular biology techniques like polymerase chain reaction (PCR) and 454 pyrosequencing methods (Elahi and Ronaghi, 2004; Sun et al., 2012; González-Martínez et al., 2013). In this context, molecular fingerprinting tools (454 pyrosequencing) and statistical multivariate analysis (Bray-Curtis cluster analysis) were used in this study to provide a broader view of the nitrifying bacteria present in an MBR and two different hybrid MBBR-MBR systems under different working conditions. In this way, questions about community structure, activity and population kinetics are answered by means of molecular monitoring tools, which allow identifying and quantifying the microbial population present in these WWTPs. These data allow the

monitoring of variations in the community profiles due to different operation conditions (Calderón et al., 2012).

An important tool to design, evaluate, control and predict the behavior of the biological processes which take part in the wastewater treatment is kinetic modeling. However, hybrid MBBR-MBR processes are relatively novel from the point of view of kinetics, and there are some uncertainties regarding the kinetic performance of these systems, particularly the kinetic behavior of NOB which has not been extensively studied in the literature (Rongsayamanont et al., 2010). The coexistence of suspended and attached biomass could lead to a modification in the kinetic parameters of both biomasses, compared to those of a pure suspended biomass process (Di Trapani et al., 2010).

The aim of this research was to determine the kinetic parameters relating to the autotrophic and heterotrophic biomasses, especially the kinetic performance of NOB, in an MBR system and two different hybrid MBBR-MBR processes and to relate them to the removal of organic matter and total nitrogen (TN). Furthermore, populations of nitrifying bacteria were identified and quantified to support the previous results.

2. Materials and methods

2.1. Description of the experimental pilot plants

Three pilot WWTPs, working in parallel, were fed by a feeding peristaltic pump (323S, Watson-Marlow Pumps Group, USA) with municipal wastewater from a sewage storage tank. Real wastewater came from the outlet of the primary settler of a wastewater treatment plant (WWTP) in Granada, Spain. The WWTPs consisted of an MBR (Figure VII.1a), an MBBR combined with an MBR containing carriers both in the anoxic and aerobic zones of the bioreactor (hybrid MBBR-MBR_a) (Figure VII.1b) and an MBBR combined with an MBR containing carriers only in the aerobic zone of the bioreactor (hybrid MBBR-MBR_b) (Figure VII.1c). The reactor zones, the membrane tank, the effluent tank and some peristaltic pumps are shown in Figure VII.1d.

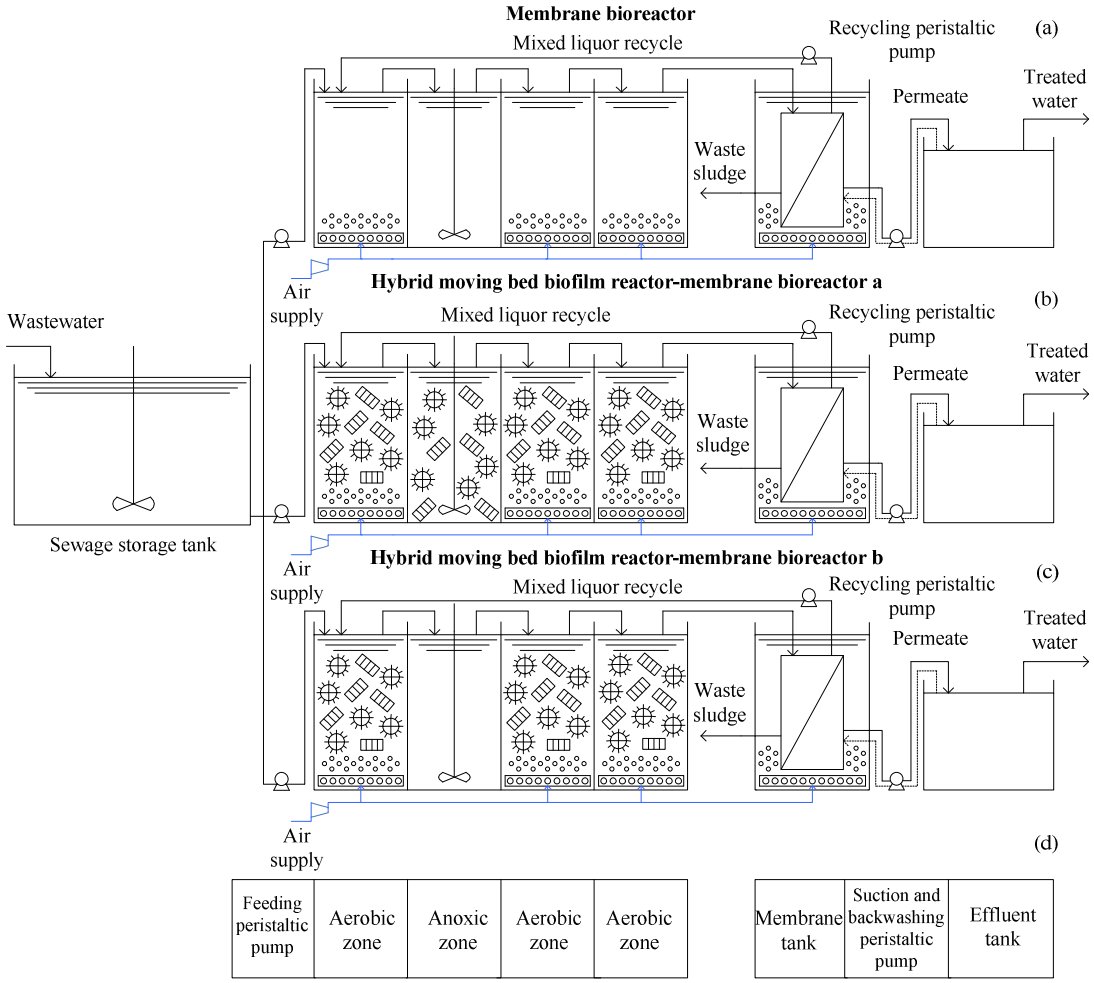

Figure VII.1. Schematic diagram of the three municipal wastewater treatment plants (WWTPs) used in the study. (a) Membrane bioreactor (MBR). (b) Hybrid MBBR-MBR containing carriers both in the anoxic and aerobic zones of the bioreactor (hybrid MBBR-MBR$_a$). (c) Hybrid MBBR-MBR containing carriers only in the aerobic zone of the bioreactor (hybrid MBBR-MBR$_b$). (d) Nomenclature concerning the reactor zones, membrane tank, effluent tank and some peristaltic pumps.

The MBR included a bioreactor divided into four zones, i.e. one anoxic zone and three aerobic ones (Figure VII.1a). The dimensions of the bioreactor were 50 cm long, 12 cm wide and 60 cm high and the total volume was 36 L. The working volume was 24 L because the reactor had a security percentage with a value of 33% in relation to the total volume (Table VII.1).

Table VII.1. Operation conditions and stabilization concentrations of MLSS and attached BD of the experimental plants. HRT (hydraulic retention time), SRT (sludge retention time), MLSS (mixed liquor suspended solids), BD (biofilm density).

Parameter	MBR		Hybrid MBBR-MBR_a		Hybrid MBBR-MBR_b	
	Aerobic zone	Anoxic zone	Aerobic zone	Anoxic zone	Aerobic zone	Anoxic zone
Volume (L)	18	6	18	6	18	6
Filling ratio with carriers (%)	0	0	35	35	35	0
Flow rate (L h^{-1})	3.00		3.00		3.00	
HRT (h)	9.5		9.5		9.5	
SRT (day)	11.7		11.7		11.7	
MLSS (mg L^{-1})	3,326.83±233.95		2,498.25±138.40		2,457.58±156.90	
MLVSS (mg L^{-1})	2,885.44±202.91		2,172.16±120.35		2,076.29±132.49	
BD (mg L^{-1})	-		1,270.19±81.55		1,250.00±66.51	
VBD (mg L^{-1})	-		1,129.66±72.53		1,044.41±55.57	

Municipal wastewater, coming from the sewage storage tank, was pumped into the first aerobic chamber of the bioreactor. Then, it went through the anoxic zone and, subsequently, it reached the second and third aerobic compartments through a communicating vessel system. The anoxic zone was in the second compartment to avoid recycling from the membrane tank, which contained a higher dissolved oxygen concentration to prevent membrane fouling; this could change the anoxic conditions. Therefore, the anoxic zone was set between the first and the third aerobic chambers with dissolved oxygen concentrations which could be adjusted to values that were not too high. The recycling rate was three times the influent flow rate.

The outlet of the bioreactor was led into a membrane tank which was designed to be an external submerged unit. It was cylindrical, had a diameter of 10 cm and was 65 cm high. The total volume of this tank was 6.7 L, whereas the working volume was 4.32 L. The membrane module consisted of a vertically oriented submerged module of hollow-fiber ultrafiltration membranes (Micronet Porous Fiber, SL, Spain). The membrane was fed from the outside to the inner side via a suction process. The total membrane area was 0.20 m^2. The hollow fibers were made of polyvinylidene fluoride and they had an inside braid-reinforcement made of polyester. The fibers had an outer diameter of 2.45 mm, an inner diameter of 1.10 mm and a pore size of 0.04 μm. Aeration was applied to the base of the module by a coarse bubble disk diffuser (CAP 3,

ECOTEC, SA, Spain). The membranes were continuously aerated with a tangential air current to prevent any organic or inorganic solids from settling onto the surface. Air was supplied by an air compressor (ACO-500, Hailea, China). The airflow to the MBR was measured by a rotameter (2100 Model, Tecfluid, SA, Spain) and regulated by a manual valve. The air flow rate had a value of 100 L h^{-1} and the air was supplied at a constant pressure and temperature of 0.5 bar and 20ºC. The permeate was extracted through the membrane using a suction-backwashing peristaltic pump (323U, Watson-Marlow Pumps Group, USA) to collect it into the permeate tank. The cyclic mode of operation consisted of production and backwashing periods of 9 min and 1 min, respectively. Transmembrane pressure (TMP) varied between 0.1 and 0.5 bar. The operating parameters such as permeate flow, permeation and backwashing times could be adjusted by a control panel.

A small volume of the retentate was removed from the membrane tank as excess sludge. Recycling was carried out from the membrane tank to pump out the aerobic mixed liquor into the anoxic chamber through a recycling peristaltic pump (323S, Watson-Marlow Pumps Group, USA). The anoxic chamber received the recycling flow from the membrane tank after passing through the first aerobic chamber. This allowed for maintaining the working mixed liquor suspended solids (MLSS) concentration inside the bioreactor and facilitated nitrogen removal.

The hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, which combined a MBBR with an MBR (Figure VII.1b and Figure VII.1c, respectively), had the same dimensions as the MBR (Table VII.1). The membrane tank of the hybrid MBBR-MBR systems was also the same as that used in the MBR. The operation was identical to that described for the MBR.

Biomass grew as suspended flocs and as a biofilm in the hybrid MBBR-MBR systems. Biofilm grew on carriers which moved freely in the water volume by aeration in the aerobic zone and by a mechanical stirrer in the anoxic one. The carrier used is called K1 and was developed and supplied by AnoxKaldnes AS (Norway). This carrier has been widely studied in similar experiments (Leiknes and Ødegaard, 2007; Di Trapani et al., 2008). The K1 media filling-fraction and the working reactor volumes are shown in Table VII.1.

Recycling was carried out from the membrane tank to the anoxic chamber to maintain the working MLSS concentration inside the bioreactor and to allow for nitrogen removal.

All anoxic zones had variable speed stirrers (Multi Mixer MM-1000, Biosan Laboratories, Inc., USA) which kept the biofilm media moving in the anoxic zone. The sewage storage tank also had a variable speed propeller to homogenize municipal wastewater. This stirrer was identical to the previous ones. The normal propeller speed was 320 rpm. Aerobic zones were equipped with a fine bubble disk diffuser (AFD 270, ECOTEC, SA, Spain) at the bottom of the reactor. Air to the aerobic zone was supplied by an air compressor (ACO-500, Hailea, China). The airflow to the reactor was measured by a rotameter (2100 Model, Tecfluid, SA, Spain) and regulated by a manual valve. The air flow rate in each of the bioreactors was 30 L h^{-1} and the air was supplied at a constant pressure and temperature of 0.5 bar and 20ºC. Both the stirrer in the anoxic zone and the diffuser in the aerobic one had the function of keeping the carriers moving inside the reactor and homogenizing the mixed liquor.

2.2. Experimental procedure and analytical determinations

Samples were collected every day from the influent, the three effluents and the anoxic and aerobic zones of the bioreactors and the membrane tank. Biomass samples were collected from the biofilm developed on the carriers and the mixed liquor. The three pilot WWTPs operated under a hydraulic retention time (HRT) of 9.50 h, a sludge retention time (SRT) of 11.75 days which involved a flow rate of waste sludge of 2.41 L day^{-1}, a flow rate of 3.00 L h^{-1} and a membrane flux of 15 L m^{-2} h^{-1} (Table VII.1). A level indicator connected to the feeding pump controlled the influent in each bioreactor to ensure that the level in the system was correct and the membranes were covered by the mixed liquor.

Physical and chemical determinations were carried out concerning the pH, conductivity, temperature, dissolved oxygen, chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), volatile suspended solids (VSS), total phosphorus (TP), total nitrogen (TN) and the concentrations of ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) according to section **Materials and Methods**.

The microbial communities of the three WWTPs were analyzed by 454 pyrosequencing methods in order to detect and quantify the contribution of nitrifying bacteria (AOB and NOB) and denitrifying bacteria in the total bacterial community. Furthermore, the kinetic parameters for heterotrophic, autotrophic and nitrite-oxidizing bacteria were evaluated (**Materials and Methods**).

The evaluation of statistically significant differences between the results concerning COD, BOD_5, TSS, TN, TP and concentrations of NH_4^+, NO_2^- and NO_3^- was carried out according to section **Materials and Methods.** Moreover, a Bray-Curtis cluster analysis was performed to quantify the compositional dissimilarity between the different samples, based on nitrifying bacteria (**Materials and Methods**).

3. Results and discussion

3.1. Evolution of the biomass and physical and chemical parameters

The start-up of the three pilot plants was carried out with urban wastewater taken from the WWTP at Puente Los Vados located in Granada, Spain. The concentration of MLSS and the attached biofilm density (BD) increased during the start-up phase until the steady state was reached. The total time of the start-up phase was 42 days. Subsequently, the stabilization phase started. This phase had a duration of 66 days. The evolutions of MLSS and BD in the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b are shown in Figure VII.2a, Figure VII.2b and Figure VII.2c, respectively.

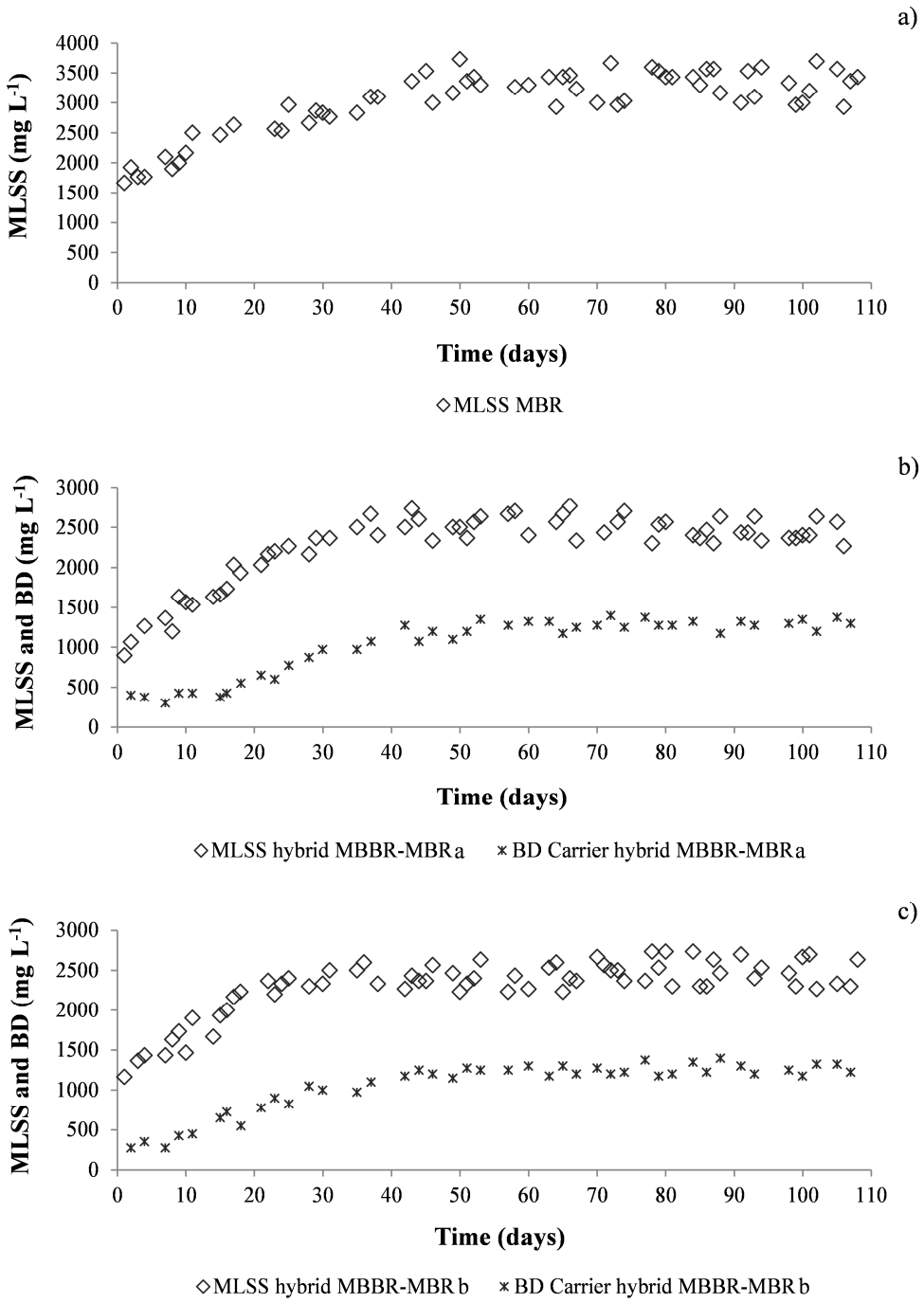

Figure VII.2. Evolution of the mixed liquor suspended solids (MLSS) and attached biofilm density (BD) during the start-up and stabilization phases. (a) MLSS of the MBR. (b) MLSS and BD attached to the carrier of the hybrid MBBR-MBR$_a$. (c) MLSS and BD attached to the carrier of the hybrid MBBR-MBR$_b$.

The values of the concentration of MLSS and attached BD from the WWTPs in the steady state are shown in Table VII.1. The concentrations of MLSS in the hybrid MBBR-MBR systems (hybrid MBBR-MBR$_a$ and hybrid MBBR-MBR$_b$) were similar and were lower than the one in the MBR, which had a value of 3,326.83±233.95 mg L^{-1}. This difference was compensated for by the attached BD on the carriers contained in the

hybrid MBBR-MBR_a and hybrid MBBR-MBR_b (1,270.19±81.55 mg L^{-1} and 1,250.00±66.51 mg L^{-1}, respectively). Falletti and Conte (2007) carried out a study with similar values of MLSS and BD in hybrid MBBR systems.

The pH values in the mixed liquors of the bioreactors and the effluents were slightly acid due to the nitrification process (Canziani et al., 2006). The temperature was 17.2±1.9ºC in the three WWTPs. The concentration of dissolved oxygen in the aerobic zone of the different bioreactors was over 2.0±0.1 mg O_2 L^{-1} (2.7 ± 1.7 mg O_2 L^{-1}, 2.9 ± 1.2 mg O_2 L^{-1} and 3.2 ± 1.4 mg O_2 L^{-1} for the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, respectively), which is recommended to obtain an efficient removal of COD and an effective nitrification process, according to Wang et al. (2006). The concentrations of dissolved oxygen in the anoxic zone of the bioreactors were 0.4 ± 0.1 mg O_2 L^{-1}, 0.3 ± 0.1 mg O_2 L^{-1} and 0.3 ± 0.1 mg O_2 L^{-1} for the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, respectively.

3.2. Organic matter and nutrient removal

The removal percentages and values of COD and BOD_5 obtained from the influent and effluents relating to the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b during the stabilization phase are shown in Table VII.2.

Table VII.2. Average values of COD, BOD_5, TSS, TP, TN, NH_4^+, NO_2^- and NO_3^- of the influent and effluents of the experimental plants and removal percentages of COD, BOD_5, TSS, TP and TN during the steady state. COD (chemical oxygen demand), BOD_5 (five-day biochemical oxygen demand), TSS (total suspended solids), TP (total phosphorus), TN (total nitrogen), NH_4^+ (concentration of ammonium), NO_2^- (concentration of nitrite), NO_3^- (concentration of nitrate).

Parameter	Sampling zone				Removal percentage	Wastewater treatment plant		
	Influent	Effluent MBR	Effluent hybrid MBBR-MBR_a	Effluent hybrid MBBR-MBR_b		MBR	Hybrid MBBR-MBR_a	Hybrid MBBR-MBR_b
COD (mg O_2 L^{-1})	257.47±73.57	34.05±12.40	33.35±12.75	31.88±9.64	**COD (%)**	86.77±3.24	87.05±4.60	87.62±2.82
BOD_5 (mg O_2 L^{-1})	176.36±90.27	2.18±1.43	2.09±1.48	2.50±2.32	**BOD_5 (%)**	98.76±1.00	98.81±1.42	98.58±1.63
TSS (mg L^{-1})	119.84±43.58	3.67±2.92	4.35±2.22	4.05±2.81	**TSS (%)**	96.94±2.33	96.37±2.28	96.62±2.76
TP (mg P L^{-1})	10.18±1.20	5.20±2.13	5.55±1.66	5.54±2.36	**TP (%)**	48.92±19.90	45.48±19.85	45.58±20.04
TN (mg N L^{-1})	100.37±27.60	42.12±23.26	45.33±19.60	43.58±17.65	**TN (%)**	58.03±16.87	54.84±11.61	56.58±11.51
NH_4^+ (mg NH_4^+ L^{-1})	118.36±37.35	ND	ND	ND				
NO_2^- (mg NO_2^- L^{-1})	36.21±0.99	21.89±14.73	16.46±6.99	11.97±5.30				
NO_3^- (mg NO_3^- L^{-1})	10.91±5.51	163.36±63.08	184.89±50.81	180.32±53.91				

ND: Not Detected

The pilot plants with carriers inside the bioreactor (hybrid MBBR-MBR_a and hybrid MBBR-MBR_b) showed a slightly better performance for COD removal. These values were similar to those reported by Ahl et al. (2006). Moreover, Di Trapani et al. (2010) obtained BOD_5 removal efficiencies similar to those shown in this study with approximately the same HRT and SRT. Organic matter removal was very similar in the three experimental plants studied, as can be observed in Table VII.2 through the parameters COD and BOD_5. Actually, the differences between the three WWTPs regarding the removal percentages of COD and BOD_5 were not statistically significant with an HRT of 9.5 h as the p-values obtained from the post hoc procedure, Tukey´s HSD, were higher than α=0.05.

The values of TSS for the effluents of the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b were 3.67±2.92 mg L^{-1}, 4.35±2.22 mg L^{-1} and 4.05±2.81 mg L^{-1}, respectively (Table VII.2). The difference between the pilot plants, regarding the removal percentage of TSS, was not statistically significant with an HRT of 9.5 h as the p-values obtained from the post hoc procedure, Tukey´s HSD, were higher than α=0.05. This occurred since the three pilot plants contained a module including hollow-fiber ultrafiltration membranes in the MBR.

Table VII.2 shows the concentrations of TN and TP in the influent and the effluents and the removal percentages of these nutrients in the municipal WWTPs. There were no statistically significant differences regarding the removal percentages of TN and TP with an HRT of 9.5 h as the p-values obtained were higher than α=0.05. In spite of this, the MBR had a removal percentage of TN slightly higher than the other pilot plants with a value of 58.03±16.87% as shown in Table VII.2. The hybrid MBBR-MBR_b showed better performance regarding TN removal than the hybrid MBBR-MBR_a; this also occurred in a study carried out by Leyva-Díaz et al. (2013) with an identical WWTP design, similar concentrations of MLSS and BD and an HRT of 26.5 h. Therefore, MBBR-MBR systems are suitable to remove TN, but an anoxic zone without carriers is necessary to provide better contact between nitrate and the microorganisms (Rusten et al., 1995; Rusten et al., 2000; Larrea et al., 2007). This is in agreement with the data provided in Table VII.3, as the total concentration of nitrifying populations were similar in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, but the

total concentration of the denitrifying population was higher in the hybrid MBBR-MBR_b.

Table VII.3. Total concentration of nitrifying bacteria (AOB and NOB), denitrifying bacteria (DeNB) and heterotrophic bacteria as MLVSS concentration and attached VBD in the experimental plants. AOB (ammonium-oxidizing bacteria), NOB (nitrite-oxidizing bacteria), MLVSS (mixed liquor volatile suspended solids), VBD (volatile biofilm density).

Microbial population	Total biomass concentration				
	MBR	Hybrid MBBR-MBR_a		Hybrid MBBR-MBR_b	
	MLVSS (mg L^{-1})	MLVSS (mg L^{-1})	VBD (mg L^{-1})	MLVSS (mg L^{-1})	VBD (mg L^{-1})
Nitrifying bacteria	86.56±6.09	195.49±10.83	203.34±13.06	166.10±10.59	187.99±10.01
Ammonium-oxidizing bacteria (AOB)	28.85±2.03	152.05±8.42	169.45±10.88	145.34±9.27	156.66±8.34
Nitrite-oxidizing bacteria (NOB)	57.71±4.06	43.44±2.41	33.89±2.18	20.76±1.32	31.33±1.67
Denitrifying bacteria (DeNB)	115.42±8.12	130.33±7.22	124.26±7.98	415.26±26.50	83.55±4.45
Heterotrophic bacteria	2,250.64±158.27 (78%)	1,716.01±95.08 (79%)	869.84±55.85 (77%)	1,598.74±102.02 (77%)	772.86±41.12 (74%)

Furthermore, the removal percentages of TN could be higher if the anoxic zone of the bioreactor from the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b was larger, as the total concentration of the denitrifying populations was higher in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b than in the MBR (Table VII.3). Di Trapani et al. (2010) generally obtained similar performance in an MBBR with respect to nitrogen removal, with similar values of the HRT.

The removal percentages of COD, BOD_5 and TN were lower than those obtained by Leyva-Díaz et al. (2013) with similar WWTPs, concentrations of MLSS and BD but a higher HRT of 26.5 h.

These systems did not have a strict anaerobic zone to initialize the process of biological phosphorus removal (Kermani et al., 2009), but the creation of small anaerobic zones in the anoxic compartments of each bioreactor as well as the physical process of ultrafiltration made TP removal possible.

3.3. Study of the nitrifying and denitrifying microbial populations

Differences in the structure of the bacterial population in each bioreactor were detected in this research (Figure VII.3 and Table VII.3).

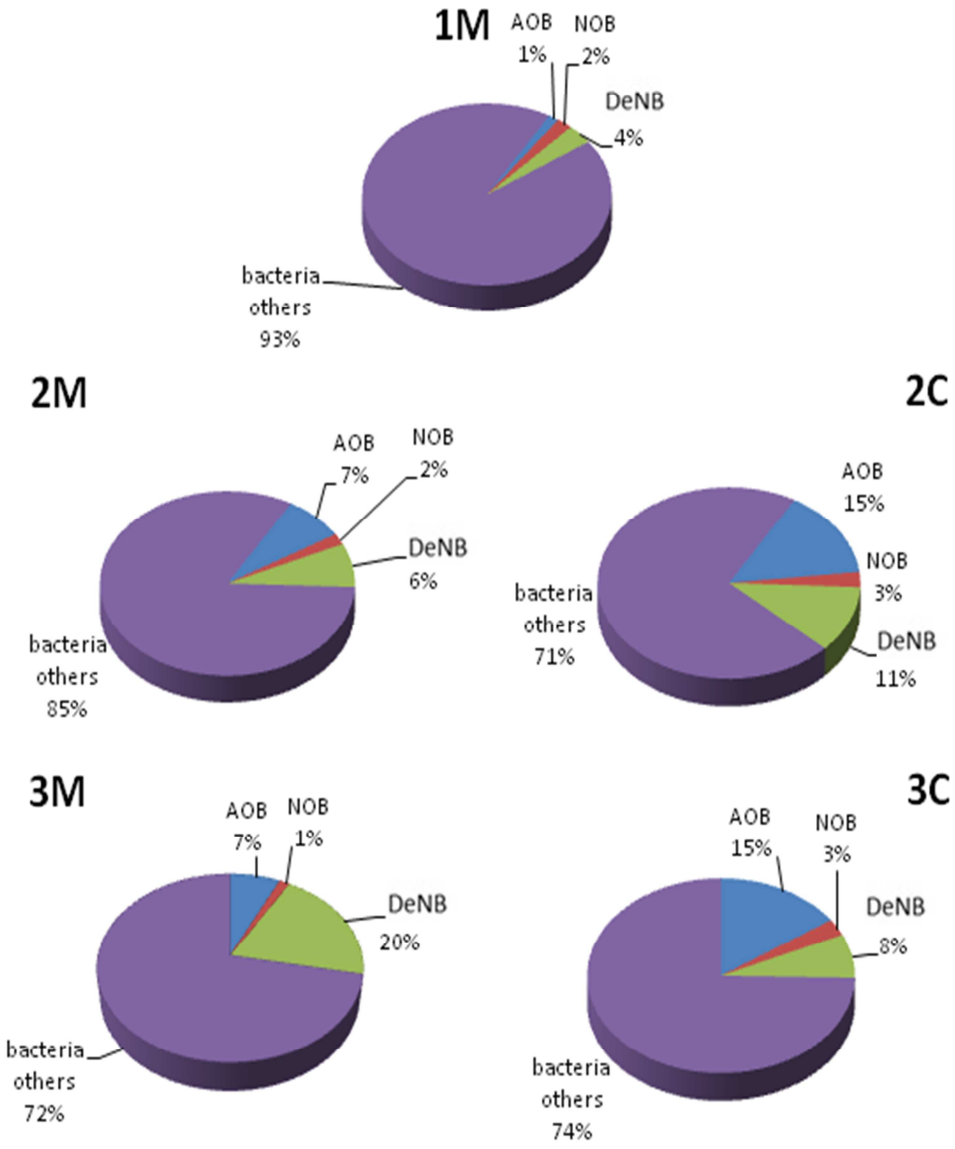

Figure VII.3. Percentage of AOB, NOB, DeNB and other bacteria in relation to the total bacteria in MLSS (M) and BD attached to carriers (C) in the MBR (1), hybrid MBBR-MBR_a (2) and hybrid MBBR-MBR_b (3). AOB (ammonium-oxidizing bacteria), NOB (nitrite-oxidizing bacteria), DeNB (denitrifying bacteria).

Table VII.2 shows that the nitrifying activities in the hybrid MBBR-MBR systems (hybrid MBBR-MBR_a and hybrid MBBR-MBR_b) were similar, maintaining their high capacity to transform all the ammonium into nitrite and nitrate. Moreover, these nitrifying activities were higher than that in the MBR, as the conversion of ammonium into nitrate was higher in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b (184.89±50.81 mg NO_3^- L^{-1} and 180.32±53.91 mg NO_3^- L^{-1}, respectively). There were statistically significant differences regarding nitrate formation between the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b concerning the MBR with an HRT of 9.5 h as

the p-values obtained were less than α=0.05, p-value $_{\text{MBR-Hybrid MBBR-MBRa}}$ (NO_3^-) = 0.04546 and p-value $_{\text{MBR-Hybrid MBBR-MBRb}}$ (NO_3^-) = 0.04089.

However, the microbial population changed in each WWTP, as can be observed in Figure VII.3. Table VII.3 is based on the mixed liquor volatile suspended solids (MLVSS), the volatile biofilm density (VBD) and the values in Figure VII.3; it shows that nitrifying population present in the MLSS from the MBR was significantly lower than in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, while the heterotrophic population was higher in the MBR. This supported the fact that the nitrifying activity was higher in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b. In this context, an inhibitory effect could be suggested for the heterotrophic microbiota on the AOB and NOB populations in the MBR, mainly caused by the higher yields and growth rates of the heterotrophic bacteria as well as localized competition between heterotrophic and nitrifying bacteria (Okabe et al., 1996).

Nitrifying populations were very similar in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b (Figure VII.3 and Table VII.3), regardless of their location (suspended or attached biomass). However, very different results were observed when denitrifying populations were studied in the suspended biomass. In particular, the high concentrations of denitrifying bacteria detected in the hybrid MBBR-MBR_b could be explained by the absence of carriers in the anoxic zone. Attached growth on the surfaces of supporting materials has certain advantages, such as the protection of microorganisms in a hostile environment (Simões et al., 2010), e.g. in the presence of antimicrobial agents, ultraviolet light, and oxygen (Lyon, 2008). Consequently, the absence of attached biomass can reduce the growth of some aerobic microorganisms in the anoxic compartment of the bioreactor, resulting in the enrichment of denitrifying bacteria under these environmental conditions. Therefore, the conversion of nitrate into molecular nitrogen (the denitrification process) would be higher if the anoxic zone was larger, as the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b had the potential capacity to remove a greater amount of TN, taking into account the higher total concentration of denitrifying bacteria (Table VII.3), although the MBR had a slightly higher removal percentage of TN with a volume of anoxic zone of 6 L.

This study showed that nitrifying populations were heterogeneous in all WWTPs, showing a large number of different species (Figure VII.4).

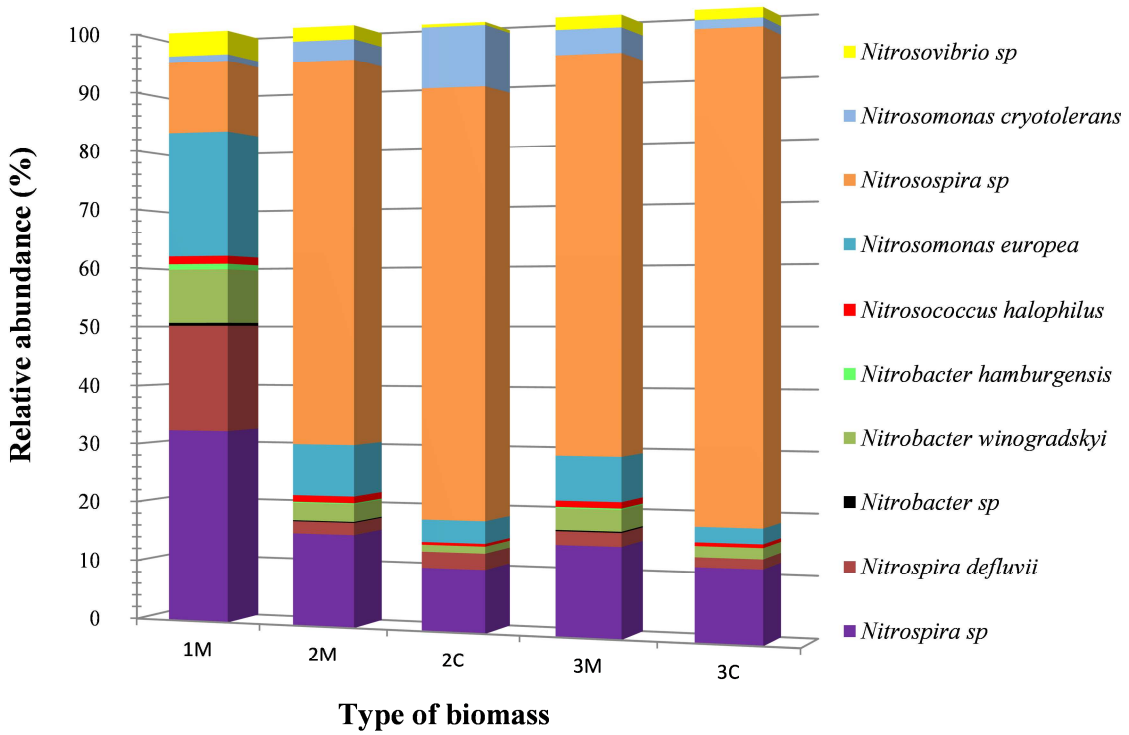

Figure VII.4. Relative abundance of the total nitrifying bacteria in MLSS (M) and BD attached to carriers (C) in the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b.

Furthermore, the *Nitrosomonas* sp. and *Nitrosospira* sp. OTUs were the most important AOB microorganisms identified in this research, independently of the operational conditions of the WWTP, as reported by various authors in conventional nitrification-denitrification processes (Dionisi et al., 2002; Limpiyakorn et al., 2007). However, other significant AOB OTUs were also detected, such as *Nitrosococcus halophilus* and *Nitrosovibrio* sp. These results are in agreement with those obtained in ammonium-rich systems like activated sludge (Pal et al., 2012).

The nitrifying bacteria were dominated by NOB with 61% of the total relative abundance in the MBR. All resulting sequences were related to the typical nitrite-oxidizing species of the genera *Nitrospira* and *Nitrobacter* (Figure VII.4). It is well-known that species of these genera are the key NOB in WWTPs (Schramm et al., 1998; Kim and Kim, 2006; Vanparys et al., 2007). The nitrifying population was very similar in the suspended and attached biomass in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b (Figure VII.4), with *Nitrosospira* as the predominant genus. Similar results have been previously reported by other authors (Prinčič et al., 1998; Vejmelkova et al., 2012). Therefore, the AOB population was predominant in comparison with the NOB

population with a percentage of relative abundance higher than 70% in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b (Figure VII.4).

The statistical Bray Curtis analysis showed the similarity between the different samples from each bioreactor (Figure VII.5) (Clarke et al., 2006).

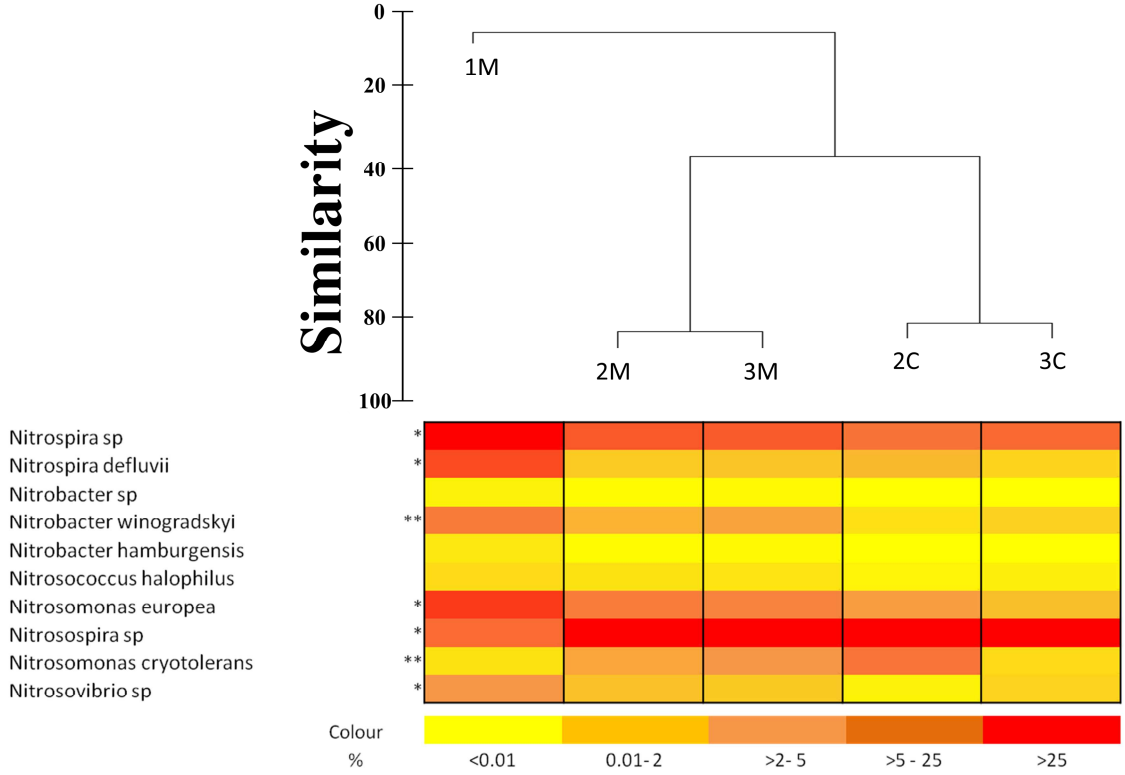

Figure VII.5. Heat map of nitrifying OTUs in the mixed liquor and carrier samples in all bioreactors. (*): OTU found in all bioreactor samples. (**): OTU found in all mixed liquor samples. OTUs were grouped by following taxonomic affiliation at the species level. Samples were clustered by similarities in dominant nitrifying OTUs distribution. The scale at the bottom represents the contribution of a particular OTU and is expressed as a percentage of the total. The closest bacterial relative is shown on the left side of the map.

Similarity values higher than 80% were reached for MLSS (90%) and BD attached to carriers (81%) between the hybrid MBBR-MBR systems (hybrid MBBR-MBR_a and hybrid MBBR-MBR_b). In this way, the absence of carriers in the anoxic zone produced important differences in the denitrifying bacteria (Figure VII.4), but did not lead to similar changes in the nitrifying bacteria. On the other hand, the non-existence of carriers in the MBR produced a very low similarity with the pilot plants which contained carriers (hybrid MBBR-MBR_a and hybrid MBBR-MBR_b). Even so, some AOB species such as *Nitrosomonas europaea*, *Nitrosospira* sp. and *Nitrosovibrio* sp., and some NOB such as *Nitrospira* sp. and *Nitrospira defluvii* appeared in all the

bioreactors (Figure VII.5). The presence of these nitrifiying bacteria are in agreement with several studies (Dionisi et al., 2002; Vejmelkova et al., 2012).

3.4. Kinetic parameters for heterotrophic, nitrifying and nitrite-oxidizing bacteria

The biological reactors in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b had the highest values of the yield coefficient for heterotrophic bacteria (Y_H), i.e. 0.5331 mg VSS mg COD^{-1} and 0.5498 mg VSS mg COD^{-1}, respectively, as shown in Table VII.4.

Table VII.4. Kinetic parameters for the characterization of heterotrophic and autotrophic biomass. Y_H (yield coefficient for heterotrophic bacteria), $\mu_{m,\ H}$ (maximum specific growth rate for heterotrophic bacteria), K_M (half-saturation coefficient for organic matter), Y_A (yield coefficient for nitrifying bacteria), $\mu_{m,\ A}$ (maximum specific growth rate for nitrifying bacteria), K_{NH} (half-saturation coefficient for ammonia-nitrogen), Y_{NOB} (yield coefficient for nitrite-oxidizing bacteria), $\mu_{m,\ NOB}$ (maximum specific growth rate for nitrite-oxidizing bacteria), K_{NOB} (half-saturation coefficient for nitrite-nitrogen), k_d (decay coefficient for total bacteria).

Parameter	Sampling zone		
	MBR	Hybrid MBBR-MBR_a	Hybrid MBBR-MBR_b
Heterotrophic bacteria			
Y_H (mg VSS mg COD^{-1})	0.4609	0.5331	0.5498
$\mu_{m,\ H}$ (h^{-1})	0.0192	0.0214	0.0267
K_M (mg O_2 L^{-1})	16.4736	9.8251	8.8808
Nitrifying bacteria			
Y_A (mg O_2 mg N^{-1})	1.0389	1.5471	1.2985
$\mu_{m,\ A}$ (h^{-1})	0.2719	0.0805	0.0929
K_{NH} (mg N L^{-1})	0.9329	1.0894	1.1189
Nitrite-oxidizing bacteria			
Y_{NOB} (mg O_2 mg N^{-1})	0.7791	0.5380	0.8197
$\mu_{m,\ NOB}$ (h^{-1})	0.1124	0.0936	0.5369
K_{NOB} (mg N L^{-1})	0.4364	0.8158	2.1670
Total bacteria			
k_d (d^{-1})	0.0304	0.0340	0.0362

This meant that they produced the highest amounts of heterotrophic bacteria per substrate oxidized. Plattes et al. (2007) obtained similar values of Y_H. On the other hand, the MBR and hybrid MBBR-MBR_b had the lowest values of the yield coefficient for nitrifying bacteria (Y_A) with values of 1.0389 mg O_2 mg N^{-1} and 1.2985 mg O_2 mg

N^{-1}, respectively, as shown in Table VII.4. Therefore, they required the lowest quantities of oxygen to oxidize the same amount of substrate. The values of Y_A reported by Seifi and Fazaelipoor (2012) were lower than those obtained in this study. Moreover, the hybrid MBBR-MBR_a showed a yield coefficient for NOB lower than the values of the other WWTPs (0.5380 mg O_2 mg N^{-1}).

Table VII.4 also shows the rest of the parameters which fit the Monod model for the heterotrophic, nitrifying and nitrite-oxidizing bacteria contained in each of the bioreactors. The hybrid MBBR-MBR_b showed the best performance from the point of view of the heterotrophic bacteria kinetics with values of $\mu_{m,\ H} = 0.0267\ h^{-1}$ and K_M = 8.8808 mg O_2 L^{-1}. Therefore, the heterotrophic bacteria in the hybrid MBBR-MBR_b required less time for organic matter oxidation under the operational conditions used in this research. Furthermore, this meant that the μ_m was achieved with less available substrate in the hybrid MBBR-MBR_b and less time was required to reach the steady state under the experimental conditions of this study. Additionally, these results support the highest COD removal efficiency of the hybrid MBBR-MBR_b (87.62±2.82%), as indicated in Table VII.2. Canziani et al. (2006) and Seifi and Fazaelipoor (2012) had similar values to those obtained in this research regarding $\mu_{m,\ H}$ and K_M, respectively.

According to the kinetic parameters for nitrifying bacteria, the MBR showed the best performance (Table VII.4). Nitrifying biomass from the MBR required less time for the oxidation of nitrogen contained in the influent under the operational conditions. The μ_m was achieved with less available substrate in the MBR and less time was required to reach the steady state under the experimental conditions of this study. The hybrid MBBR-MBR_b had better behavior than the hybrid MBBR-MBR_a when the substrate degradation rate (r_{su}) was evaluated, taking into account the kinetic parameters for nitrifying bacteria. These findings also support the highest TN removal efficiency in the hybrid MBBR-MBR_b (56.58±11.51%), as indicated in Table VII.2. Similar values of $\mu_{m,\ A}$ and K_{NH} were obtained by Seifi and Fazaelipoor (2012) and Henze et al. (1987), respectively.

From the point of view of NOB, the hybrid MBBR-MBR_b showed the best kinetic performance with values of $\mu_{m,\ NOB} = 0.5369\ h^{-1}$ and $K_{NOB} = 2.1670$ mg N L^{-1} (Pambrun et al., 2006). This supported the fact that the nitrate concentration in the effluent from the hybrid MBBR-MBR_b was higher than that from the MBR (Table VII.2).

Consequently, the MBR could have a better kinetic behavior regarding AOB because, as a whole, the kinetics of nitrifying bacteria was better, as previously mentioned, and the MBR had the highest nitrite concentration in its effluent (Table VII.2).

The values of the decay coefficient for total bacteria, k_d, are provided in Table VII.4. The results are similar for the three WWTPs, although the biomass contained in the bioreactor of the hybrid MBBR-MBR_b had the highest value, i.e. 3.62% of the total quantity of biomass was oxidized per day. The identical value of the SRT in the different WWTPs (11.7 days) was supported by the similarity of the values of k_d.

A common limitation of the activated sludge models (ASM) is the representation of nitrification dynamics as a single-step process (Iacopozzi et al., 2007). Kinetic modeling and microbiological study have enhanced the basic ASM3 model by introducing two-step nitrification. In this way, the characterization of the biological process and the control of the operational parameters of the WWTP will be improved. Therefore, operating costs could be optimized concerning the necessity of nitrification, using a suitable oxygen concentration.

4. Conclusions

The following conclusions were drawn:

1. All three systems showed similar performance in terms of pollutant removal although the hybrid MBBR-MBR systems (hybrid MBBR-MBR_a and hybrid MBBR-MBR_b) had the greatest nitrifying activities and potential capacities to remove TN because of the highest total concentration of nitrifying and denitrifying bacteria, respectively. Moreover, the hybrid MBBR-MBR_b showed the best performance from the point of view of the kinetics of heterotrophic and nitrite-oxidizing bacteria. It supported the efficiencies of COD and TN removals and the concentrations of nitrite and nitrate in the different effluents.

2. A common limitation of the activated sludge models (ASM) is the representation of nitrification dynamics as a single-step process. Kinetic modeling and microbiological study have enhanced the basic ASM3 model by introducing two-step nitrification. In this way, the characterization of the

biological process and the control of the operational parameters of the WWTP will be improved. Therefore, operating costs could be optimized concerning the necessity of nitrification, using a suitable oxygen concentration.

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VIII. CHAPTER 5

Kinetic study of the combined processes of a membrane bioreactor and a hybrid moving bed biofilm reactor-membrane bioreactor with advanced oxidation processes as a post-treatment stage for wastewater treatment (operational conditions of HRT=18 h and high biomass concentrations).

Abstract

Two membrane bioreactors (MBRs), MBR_a and MBR_b, with different mixed liquor suspended solids (MLSS) concentrations, and a hybrid moving bed biofilm reactor-membrane bioreactor (hybrid MBBR-MBR_b) which contained carriers only in the aerobic zone of the bioreactor were used in parallel with the same urban wastewater and compared. The bioreactors operated with a hydraulic retention time (HRT) of 18 h. Kinetic parameters for heterotrophic, autotrophic and nitrite-oxidizing bacteria were evaluated and related to organic matter and nitrogen removals. Three different advanced oxidation process (AOP) technologies, i.e. H_2O_2/UV, Fe^{2+}/H_2O_2/UV and TiO_2/H_2O_2/UV systems, at two H_2O_2 concentrations of 1 g L^{-1} and 2 g L^{-1}, were used to treat the effluents of each biological treatment in batch and were assessed regarding the kinetic performance. The hybrid MBBR-MBR_b had the best kinetic behavior from the point of view of heterotrophic and autotrophic biomass with a value of TN removal of 72.39±7.57%. The maximum rate of total organic carbon (TOC) degradation ($\eta_{max, TOC}$) was higher in the TiO_2/H_2O_2/UV system for a constant H_2O_2 concentration, and was independent of the effluent. The Fe^{2+}/H_2O_2/UV process was more suitable for the effluent from the hybrid MBBR-MBR_b since $\eta_{max, TOC}$ was higher at the two H_2O_2 concentrations used, i.e. 83.07% and 81.54% at 1 g L^{-1} and 2 g L^{-1}, respectively.

1. Introduction

Advanced technologies regarding wastewater treatment are necessary to preserve water quality and to satisfy the current discharge limits imposed on the effluents from municipal wastewater treatment plants (WWTPs) by the Water Framework Directive (Chave, 2001). Particularly, it is difficult to remove the most persistent pollutants, e.g., phenols, pesticides, solvents, etc., from wastewater. Currently used tertiary treatment systems include microfiltration, ultrafiltration, reverse osmosis, activated carbon adsorption and sand filters (Moreno et al., 2005), although none of these treatment methods is effective enough to produce water with acceptable levels of these organic compounds (Mantzavinos and Psillakis, 2004). Therefore, a further treatment stage is often necessary to attain this objective. This stage can entail the application of an advanced oxidation process (AOP), which is recommended when wastewater components have a high chemical stability and/or low biodegradability (Poyatos et al., 2010).

In this sense, a combination of a biological process and chemical oxidation method is usually required for an effective treatment (Wiszniowski et al., 2006; Renou et al., 2008) since biological systems are not adequate as the sole treatment of wastewater due to the fact that the persistent pollutants pass unaltered through the wastewater treatment plant (WWTP) (Badawy et al., 2009).

In this study, a hybrid technology between a moving bed biofilm reactor (MBBR) and a membrane bioreactor (MBR) called hybrid moving bed biofilm reactor-membrane bioreactor (hybrid MBBR-MBR) system, which combines suspended and attached biomass, was analyzed together with two membrane bioreactors (MBRs). The hybrid MBBR-MBR is based on the addition of carriers inside the bioreactor for biofilm growth (Ødegaard, 2006). These elements have a slightly lower density than water and they keep moving inside the reactor. This movement can be driven by aeration in an aerobic reactor or by a mechanical stirrer in an anaerobic or anoxic reactor. This process has been found to be a very simple and efficient technology in municipal wastewater treatment (Hem et al., 1994; Rusten et al., 1995).

The original wastewater contained a considerable amount of biodegradable compounds, so a pre-oxidation step would only cause unnecessary consumption of

chemicals. Thus, the biological treatment (removing biodegradable compounds) was followed by an AOP (oxidizing the organic compounds which are resistant to biological treatment) (Hörsch et al., 2003; Vidal et al., 2004), which was applied to the wastewater as a polishing step integrated with the biological process in order to increase the overall treatment efficiency (Balcioglu et al., 2003). Advanced oxidation processes (AOPs) are of particular interest and are widely recognized as being highly efficient for wastewater treatment of the most persistent pollutants (Comninellis et al., 2008; Klavarioti et al., 2009). These processes are based on the generation of the hydroxyl free radical ($HO^{\bullet}$) by the photolysis of H_2O_2 when ultraviolet (UV) radiation is applied (García-Montaño et al., 2006); the hydroxyl radical is very reactive, has a very high oxidation potential and is able to non-selectively oxidize almost all pollutant organic compounds, as stated in some key publications (Comninellis et al., 2008; Shannon et al., 2008). Therefore, a chemical wastewater treatment using AOPs can produce the complete mineralization of pollutants to CO_2, water, and inorganic compounds, or at least their transformation into more innocuous products (Poyatos et al., 2010):

$$\text{AOPs} \rightarrow {}^{\cdot}\text{OH} \xrightarrow{\text{pollutant}} CO_2 + H_2O + \text{inorganic ions}$$

Unfortunately, if applied as the only treatment, AOPs would render the treatment process economically expensive, as they usually imply a high demand of energy (radiation, ozone, etc.) and chemical reagents (catalysts and oxidizers) (Bauer and Fallmann, 1997; Muñoz et al., 2005). Thus, AOPs should be applied after the biological stage in order to make sure that the chemical oxidant is only used on recalcitrant compounds (Sarria et al., 2002).

Three different AOP technologies were evaluated and compared after the biological process in this research: an H_2O_2/UV system, a photo-Fenton (Fe^{2+}/H_2O_2/UV) process and a TiO_2/H_2O_2/UV system. The H_2O_2/UV system combines hydrogen peroxide and UV radiation and entails the formation of hydroxyl radicals generated by the photolysis of H_2O_2 and the corresponding propagation reactions. The photolysis of hydrogen peroxide occurs when UV radiation is applied and its rate is not dependent on the pH. An H_2O_2/UV system can totally mineralize any organic compound, reducing it to CO_2 and H_2O (Vogelpohl, 2007). The photo-Fenton process uses UV light for the reduction of Fe(III) oxalate back to Fe(II) oxalate, resulting in a

drastic reduction in sludge waste. The size of the reactor can be reduced because the velocity of the reaction is very high (Vogelpohl, 2007). However, it is necessary to exhaustively control the pH of the medium; the pH range should be between 2.6 and 3 for the best performance of the system. The $TiO_2/H_2O_2/UV$ system is based on heterogeneous photocatalysis where titanium dioxide is used as a catalyst and is combined with hydrogen peroxide and UV radiation. A larger number of oxidizing species can appear in this process. Data concerning chemical oxygen demand (COD) reduction indicate that this mineralization process is very effective with reduction levels higher than 90%. The fact that this process totally consumes the added peroxide and leads to a final non-toxic residue is an additional advantage of this process (García et al., 2007). However, there are limitations concerning energy transfer, and another problem is that photocatalysts are not readily available.

These systems have been shown to effectively degrade and remove specific pollutants, which otherwise would be extremely difficult to eliminate with conventional processes since many of these compounds are not biodegradable. For this reason, nowadays and in the future, they can be regarded as a technologically efficient tool for the treatment of water with persistent residues.

The aim of this research was to determine the kinetic parameters relating to the heterotrophic, autotrophic and nitrite-oxidizing bacteria in two MBR systems and a hybrid MBBR-MBR process and to relate them to the removal of organic matter and nitrogen, respectively, with a hydraulic retention time (HRT) of 18 h. Furthermore, the effluents of each biological system were subjected to three different AOP technologies at two different H_2O_2 concentrations to determine the kinetics of each process and to evaluate the effect of a biological process combined with an AOP technology as a post-treatment stage.

2. Materials and methods

2.1. Description of the wastewater treatment plants

Three pilot WWTPs were fed by a feeding peristaltic pump (323S, Watson-Marlow Pumps Group, USA) with municipal wastewater from a sewage storage tank. The WWTPs worked in parallel and real wastewater came from the outlet of the primary settler of a WWTP in Granada, Spain. The WWTPs consisted of two MBRs,

MBR_a and MBR_b (Figure VIII.1a and Figure VIII.1b, respectively), and a hybrid MBBR-MBR_b which combined an MBBR with an MBR and contained carriers only in the aerobic zone of the bioreactor (Figure VIII.1c). Three different AOP technologies, at two different H_2O_2 concentrations, treated the effluents of each biological treatment in batch. The reactor zones, the membrane tank, the effluent tank, some peristaltic pumps and the chemical oxidation reactor are shown in Figure VIII.1d.

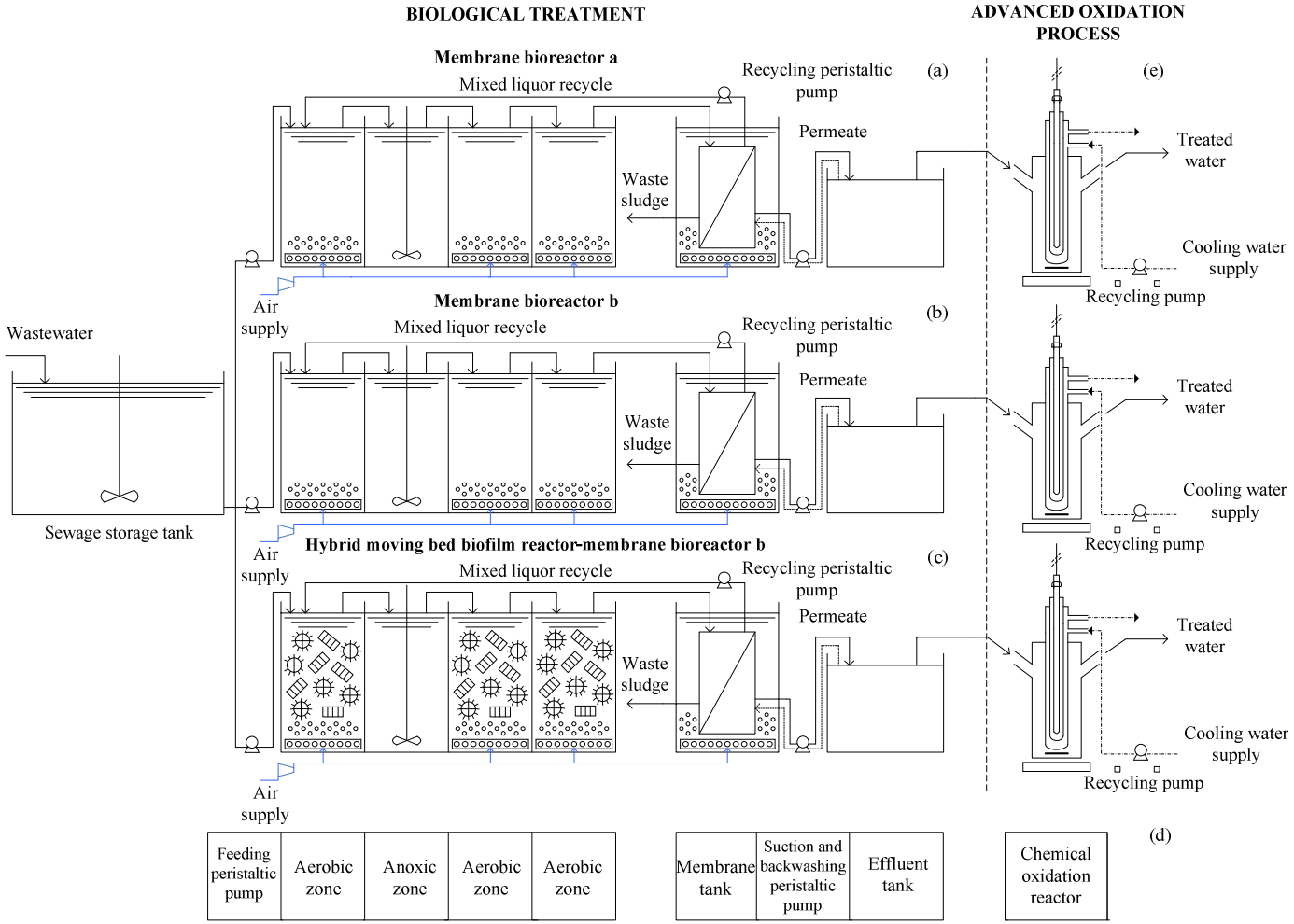

Figure VIII.1. Schematic diagram of the three urban WWTPs. (a) Membrane bioreactor a (MBR_a). (b) Membrane bioreactor b (MBR_b). (c) Hybrid moving bed biofilm reactor-membrane bioreactor containing carriers only in the aerobic zone of the bioreactor (Hybrid MBBR-MBR_b). (d) Nomenclature concerning the reactor zones, membrane tank, effluent tank, peristaltic pumps and chemical oxidation reactor. (e) Chemical oxidation reactor for the different AOP technologies.

The only differences between MBR_a and MBR_b were the concentration of the mixed liquor suspended solids (MLSS) and the sludge retention time (SRT) (Table VIII.1). The MBRs included a bioreactor divided into four zones, i.e. one anoxic zone and three aerobic ones (Figure VIII.1a and Figure VIII.1b). The dimensions of the bioreactor were 50 cm long, 12 cm wide and 60 cm high. The total volume was 36 L and the working volume was 24 L (Table VIII.1).

Table VIII.1. Operation conditions and stabilization concentrations of MLSS and attached BD of the biological reactors of the experimental plants. HRT (hydraulic retention time), SRT (sludge retention time), MLSS (mixed liquor suspended solids), BD (biofilm density).

Parameter	MBR_a		MBR_b		Hybrid MBBR-MBR_b	
	Aerobic zone	Anoxic zone	Aerobic zone	Anoxic zone	Aerobic zone	Anoxic zone
Working volume (L)	18	6	18	6	18	6
Filling ratio with carriers (%)	0	0	0	0	35	0
Flow rate (L h^{-1})	1.6		1.6		1.6	
HRT (h)	18		18		18	
SRT (day)	141.6		25.2		141.6	
Membrane flux (L m^{-2} h^{-1})	8		8		8	
MLSS (mg L^{-1})	6,405.56±365.36		2,739.68±211.75		4,369.84±232.79	
MLVSS (mg L^{-1})	5,326.87±303.84		2,121.49±163.97		3,526.81±187.88	
BD (mg L^{-1})	-		-		2,008.93±171.15	
VBD (mg L^{-1})	-		-		1,693.69±144.30	

Urban wastewater was pumped into the first aerobic chamber of the bioreactor from the sewage storage tank. It went through the anoxic zone and then it reached the second and third aerobic compartments through a communicating vessel system. The anoxic zone was in the second compartment to avoid recycling from the membrane tank, which contained a higher dissolved oxygen concentration to prevent membrane fouling; this could change the anoxic conditions. Therefore, the anoxic zone was set between the first and the third aerobic chambers with dissolved oxygen concentrations which could be adjusted to values that were not too high. The recirculation rate was two times the influent flow rate for the MBR_a and hybrid MBBR-MBR_b, and it was three times and a half the influent rate for the MBR_b.

Subsequently, the outlet of the bioreactor was led into a membrane tank which was designed to be an external submerged unit. It was cylindrical, had a diameter of 10 cm and was 65 cm high. The total volume of this tank was 6.7 L, whereas the working volume was 4.32 L. The membrane module consisted of a vertically oriented submerged module of hollow-fiber ultrafiltration membranes (Micronet Porous Fiber, SL, Spain) with a total membrane area of 0.20 m^2. The suction process was carried out from the outside to the inner side. The hollow fibers were made of polyvinylidene fluoride, with an inner braid-reinforcement made of polyester with a pore size of 0.04 μm. An air compressor (ACO-500, Hailea, China) supplied aeration, which was applied to the base of the module by a coarse bubble disk diffuser (CAP 3, ECOTEC, SA, Spain). The air flow rate had a value of 100 L h^{-1} and the air was supplied at a constant pressure and temperature of 0.5 bar and 20ºC, respectively. The permeate was extracted through the membrane using a suction-backwashing peristaltic pump (323U, Watson-Marlow Pumps Group, USA) to collect it into the permeate tank. The cyclic mode of operation consisting of production and backwashing periods of 9 min and 1 min, respectively, and the transmembrane pressures (TMP) varied between 0.1 and 0.5 bar. A fraction of the permeate was led into the chemical oxidation reactor to evaluate the effectiveness of each AOP technology in a batch process.

A specific volume of the retentate was removed from the membrane tank as waste sludge. Recycling was carried out from the membrane tank to pump out the aerobic mixed liquor into the first aerobic chamber through a recycling peristaltic pump (323S, Watson-Marlow Pumps Group, USA); then, the anoxic chamber received the mixed liquor. This allowed for maintaining the working MLSS concentration inside the bioreactor and facilitated nitrogen removal.

The hybrid MBBR-MBR_b combined an MBBR with an MBR (Figure VIII.1c). The dimensions and operation of the biological reactor and the membrane tank were identical to those described for the MBR (Table VIII.1). Biomass grew as suspended and attached biomass in the hybrid MBBR-MBR_b. Attached biomass grew on carriers which moved freely in the mixed liquor of the bioreactor by aeration in the aerobic zone and by a mechanical stirrer in the anoxic one. The carrier used was called K1 and was developed and supplied by AnoxKaldnes AS (Norway). This carrier has been widely studied in similar experiments (Leiknes and Ødegaard, 2007; Di Trapani et al., 2008).

The K1 media filling-fraction and the working reactor volumes are shown in Table VIII.1. Recycling was carried out from the membrane tank to the anoxic chamber to maintain the working MLSS concentration inside the bioreactor and to allow for nitrogen removal.

All anoxic zones had variable speed stirrers (Multi Mixer MM-1000, Biosan Laboratories, Inc., USA) which kept the biofilm media moving in the hybrid MBBR-MBR_b. The sewage storage tank also had a variable speed propeller (identical to the previous ones) to homogenize the municipal wastewater. The normal propeller speed was 320 rpm. Aerobic zones were equipped with a fine bubble disk diffuser (AFD 270, ECOTEC, SA, Spain) at the bottom of the bioreactor. An air compressor (ACO-500, Hailea, China) supplied an air flow rate of 30 L h^{-1} (at a constant pressure and temperature of 0.5 bar and 20ºC) to the aerobic zone of the bioreactors; it was measured and regulated by a rotameter (2100 Model, Tecfluid, SA, Spain). Both the stirrer in the anoxic zone and the diffuser in the aerobic one had the function of homogenizing the mixed liquor and keeping the carriers moving inside the reactor in the hybrid MBBR-MBR_b.

2.2. Advanced oxidation processes

Three different AOP technologies were evaluated after each of the biological treatments (MBR_a, MBR_b and hybrid MBBR-MBR_b). An H_2O_2/UV system, a photo-Fenton (Fe^{2+}/H_2O_2/UV) process and a TiO_2/H_2O_2/UV system treated the effluent from the different biological treatments at pH 3 and at two H_2O_2 concentrations, 1 and 2 g L^{-1}, according to Schrank et al. (2007), to study the behavior of the different AOP technologies. The concentration of Fe^{2+} ($FeSO_4 \cdot 7H_2O$) was 40 mg L^{-1} and the concentration of TiO_2 was 200 mg L^{-1} (Poyatos et al., 2010). The AOP was carried out in a batch chemical oxidation reactor (laboratory-scale UV-Consulting Peschl® photoreactor) with a volume of 800 mL (Figure VIII.1e). This reactor consisted of a cylindrical quartz glass with a 150-W medium-pressure mercury lamp enclosed in a quartz glass. The temperature was controlled with a cooling tube to remove the heat produced from the lamp maintaining it at a constant temperature of 25.0±0.5°C. The photoreactor was covered with an opaque material to avoid interference from other external radiation and was placed on a magnetic stirrer in order to maintain sample homogeneity (López-López et al., 2013).

2.3. Experimental procedure and analytical determinations

Samples were collected every day from the influent, the three effluents and the anoxic and aerobic zones of the bioreactors and the membrane tanks. The operation conditions of the biological treatment of the three pilot WWTPs are shown in Table VIII.1.

The chemical oxidation reactor was filled with the effluent of each biological treatment and the different H_2O_2 concentrations were added to the effluent when the temperature was constant at 25.0±0.5°C after the light from the lamp was turned on. During the degradation, no additional H_2O_2 was added. The effluent was maintained in constant agitation by a magnetic stirrer in order to have greater contact surface with the UV light. Samples were taken every 15 min through a tap and the experiments lasted 2 h (Bali et al., 2004; Schrank et al., 2007). The pH was adjusted to 3 for the different experiments using sulfuric acid (10%) and sodium hydroxide (1M) as required in the chemical oxidation reactor of the AOP.

Physical and chemical determinations were carried out in relation to the pH, conductivity, temperature, dissolved oxygen, chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD_5), total organic carbon (TOC), total suspended solids (TSS), volatile suspended solids (VSS), total phosphorus (TP), total nitrogen (TN) and the concentrations of ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) according to section **Materials and Methods**.

The kinetic parameters for heterotrophic, autotrophic and nitrite-oxidizing bacteria were evaluated (**Materials and Methods**). The kinetic model of pseudofirst order of the organic removal was used to adjust the kinetics of the different AOP technologies used according to Calero et al. (2011). The rate of degradation of the pseudofirst-order model, η (%), was calculated for every AOP technology, as shown in Eq. (1):

$$\frac{d\eta}{dt} = k \cdot \eta \rightarrow \eta = \eta_{max} \cdot (1 - e^{-k_1 \cdot t}) \qquad (1)$$

where k_1 is the rate constant of first order (min^{-1}) and η_{max} is the maximum rate of degradation of the pseudofirst-order model (%). This model was chosen as the

correlation coefficient between the empirical and theoretical data was the highest, indicated in a previous study carried out by López-López et al. (2013).

The evaluation of statistically significant differences between the results concerning COD, BOD_5, TOC, TSS, TN, TP and concentrations of NH_4^+, NO_2^- and NO_3^- was carried out according to section **Materials and Methods.**

3. Results and discussion

3.1. Evolution of the suspended and attached biomass

Figure VIII.2a, Figure VIII.2b and Figure VIII.2c show the increase in the MLSS concentration and the attached biofilm density (BD) for the experimental plants until the day 45, when the start-up phase ended. Subsequently, the steady state started as the working concentrations of MLSS and BD corresponding to the steady state were achieved; this phase had a duration of 69 days. The values of the concentration of MLSS and attached BD for the WWTPs in the steady state are shown in Table VIII.1.

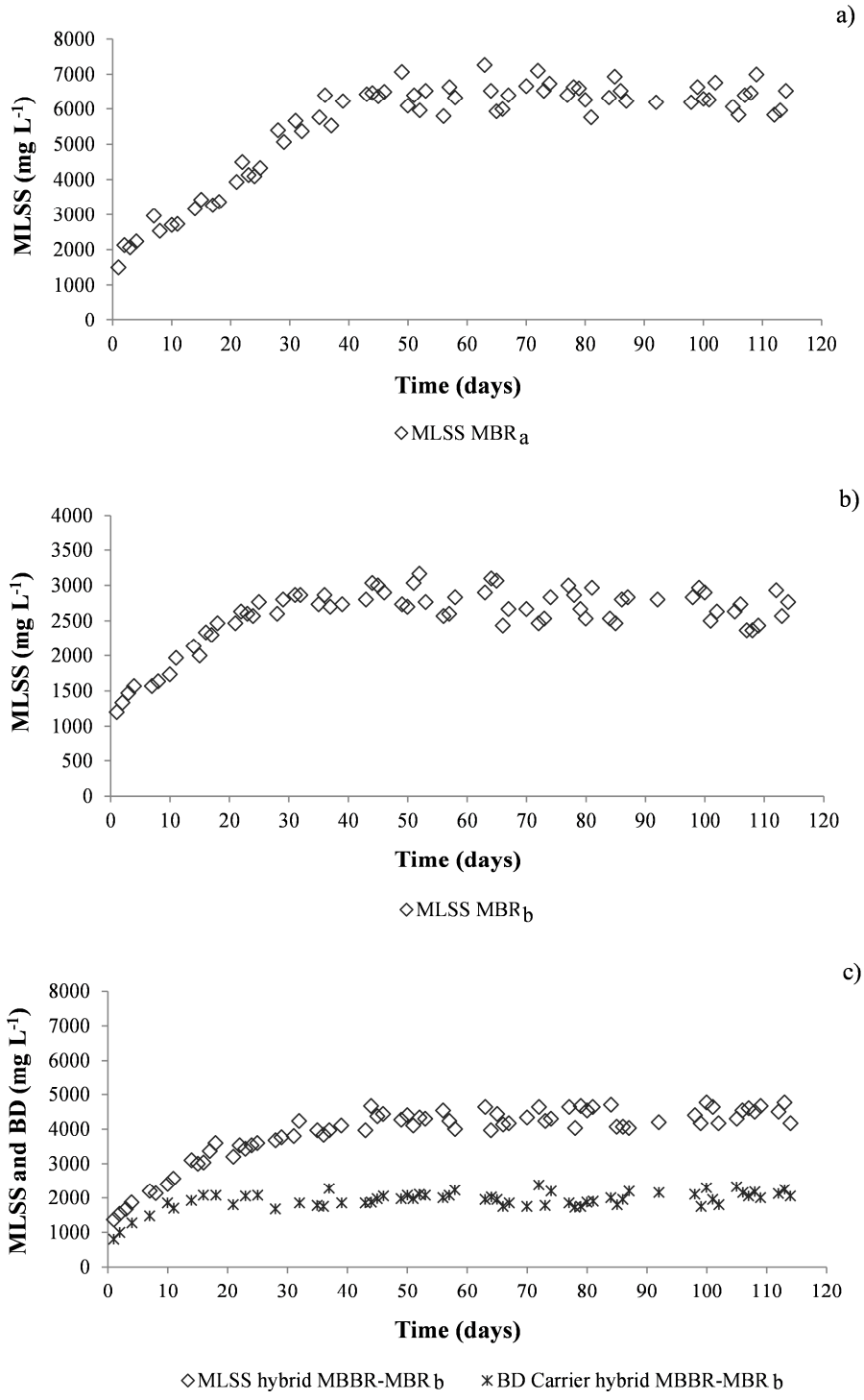

Figure VIII.2. Evolution of the mixed liquor suspended solids (MLSS) and attached biofilm density (BD). (a) MLSS of the MBR_a. (b) MLSS of the MBR_b. (c) MLSS and attached BD of the hybrid MBBR-MBR_b.

Mixed liquor volatile suspended solids (MLVSS) and volatile biofilm density (VBD) were used for the estimation of kinetic parameters. The MBR_a and the hybrid MBBR-MBR_b worked at similar biomass concentrations with the only difference being that the hybrid MBBR-MBR_b contained both suspended and attached biomass. The

biomass concentration in MBR_b was established at a lower value than in MBR_a to assess the operational differences. The concentration of MLSS in the MBR_a (6,405.56±365.36 mg L^{-1}) was higher than that in MBR_b (2,739.68±211.75 mg L^{-1}). Merayo et al. (2013) worked with similar concentrations of MLSS in MBR systems to those used in this research. Leyva-Díaz et al. (2014) also used a similar MLSS concentration than that in MBR_b. The concentration of MLSS in the hybrid MBBR-MBR_b, 4,369.84±232.79 mg L^{-1}, was lower than that in MBR_a, although this difference was compensated for by the attached BD on the carriers contained in the hybrid MBBR-MBR_b with a value of 2,008.93±171.15 mg L^{-1}. These values of the concentration of MLSS and BD were similar to those employed by Yang et al. (2009).

3.2. Physical and chemical parameters

Table VIII.2 shows the average values of pH, conductivity, temperature and dissolved oxygen concentration of the influent, effluents and mixed liquors of each bioreactor. The pH values in the biological reactors and the effluents were slightly acidic due to the nitrification process (Canziani et al., 2006). The temperature was 20.8±2.5ºC in the three WWTPs. Wang et al. (2006) recommend a concentration of dissolved oxygen over 2.0±0.1 mg O_2 L^{-1} to obtain an efficient removal of COD and an effective nitrification process, as occurred in the aerobic zone of the different bioreactors.

Table VIII.2. Average values of pH, conductivity, temperature and dissolved oxygen of the influent, effluents and mixed liquors of the biological reactors of the experimental plants.

Parameter	Sampling zone									
	Influent	MBR_a			MBR_b			Hybrid MBBR-MBR_b		
		Effluent	Anoxic zone	Aerobic zone	Effluent	Anoxic zone	Aerobic zone	Effluent	Anoxic zone	Aerobic zone
pH	8.11±0.10	6.91±0.96	6.63±0.71	6.49±0.65	6.69±0.87	6.81±0.53	6.33±0.58	6.14±0.91	6.01±0.82	5.74±0.79
Conductivity (µS cm^{-1})	997±238	769±199	1,045±89	1,039±87	778±184	1,059±86	1,053±84	817±204	1,093±88	1,094±85
Temperature (ºC)	20.8±2.5	20.8±2.5	20.8±2.5	20.8±2.5	20.8±2.5	20.8±2.5	20.8±2.5	20.8±2.5	20.8±2.5	20.8±2.5
Dissolved oxygen (mg O_2 L^{-1})	-	-	0.2±0.1	2.3±1.1	-	0.3±0.2	2.4±1.3	-	0.2±0.1	3.2±1.1

3.3. Organic matter and nutrient removal

The organic matter removal was very similar in the studied WWTPs, as can be observed in Table VIII.3 through the parameters COD, BOD_5 and TOC and the removal percentages of them during the steady state. The differences between the three WWTPs were not statistically significant regarding the removal percentages of COD, BOD_5 and TOC with an HRT of 18 h as the p-values obtained from the post-hoc procedure, Tukey's HSD, were higher than α=0.05. Similar percentages of COD removal, higher than 85%, were obtained by Jonoud et al. (2003) with an HRT of 20 h.

Table VIII.3. Average values of COD, BOD_5, TOC, TSS, TP, TN, NH_4^+, NO_2^- and NO_3^- of the influent and effluents of the experimental plants and removal percentages of COD, BOD_5, TOC, TSS, TP and TN during the steady state. COD (chemical oxygen demand), BOD_5 (five-day biochemical oxygen demand), TOC (total organic carbon), TSS (total suspended solids), TP (total phosphorus), TN (total nitrogen), NH_4^+ (concentration of ammonium), NO_2^- (concentration of nitrite), NO_3^- (concentration of nitrate).

Parameter	Sampling zone				Removal percentage	Wastewater treatment plant		
	Influent	Effluent MBR_a	Effluent MBR_b	Effluent Hybrid MBBR-MBR_b		MBR_a	MBR_b	Hybrid MBBR-MBR_b
COD (mg O_2 L^{-1})	256.54±67.56	29.55±9.56	28.91±9.56	30.84±8.49	**COD (%)**	88.48±4.51	88.73±4.28	87.98±4.04
BOD_5 (mg O_2 L^{-1})	126.80±34.61	4.35±2.90	4.25±1.88	3.94±2.16	**BOD_5 (%)**	96.57±3.01	96.65±2.22	96.89±2.47
TOC (mg C L^{-1})	98.62±29.91	15.33±1.35	15.04±1.50	14.44±1.51	**TOC (%)**	84.46±4.05	84.75±3.77	85.36±3.63
TSS (mg L^{-1})	111.79±32.59	5.40±3.52	6.80±3.52	7.79±4.43	**TSS (%)**	95.17±3.64	93.92±4.10	93.03±4.65
TP (mg P L^{-1})	10.05±1.58	5.84±2.01	5.61±1.40	5.50±1.21	**TP (%)**	41.88±16.27	44.13±13.74	45.30±7.85
TN (mg N L^{-1})	69.77±16.59	20.02±7.97	21.80±5.15	19.26±7.48	**TN (%)**	71.31±4.75	68.76±5.49	72.39±7.57
NH_4^+ (mg NH_4^+ L^{-1})	80.15±25.29	ND	ND	ND				
NO_2^- (mg NO_2^- L^{-1})	14.28±0.39	3.69±2.48	14.24±6.05	19.98±8.85				
NO_3^- (mg NO_3^- L^{-1})	13.64±6.89	83.69±32.32	77.35±21.26	58.37±17.45				

ND: Not Detected

The MBR_a, MBR_b and hybrid MBBR-MBR_b had TSS values for the effluents of 5.22±3.52 mg L^{-1}, 6.22±3.52 mg L^{-1} and 7.41±4.43 mg L^{-1}. There were no statistically significant differences between them as the three WWTPs contained a module including hollow-fiber ultrafiltration membranes in the MBR.

The concentrations of TN and TP in the influent and the effluents and the reduction percentages of TN and TP in the three WWTPs are indicated in Table VIII.3. The differences were not statistically significant regarding the removal percentages of TN and TP between the WWTPs with an HRT of 18 h as the p-values obtained were higher than α=0.05. In spite of this, the hybrid MBBR-MBR_b showed better performance than the other experimental plants regarding TN removal, with a value of 72.39±7.57%, as can be observed in Table VIII.3. Percentages of TN higher than 50%, and similar to those obtained in this study, were also obtained by Jonoud et al. (2003) with an HRT of 20 h. MBR_b had the lowest removal percentage of TN as the biomass concentration was lower than those in the MBR_a and hybrid MBBR-MBR_b (Table VIII.1). Thus, the hybrid MBBR-MBR_b is suitable to remove TN with an anoxic zone without carriers, which provides better contact between nitrate and the microorganisms (Larrea et al., 2007).

Dong et al. (2011) also carried out research into these systems with an HRT of 18 h using a ceramic biocarrier. They obtained COD removal efficiencies lower than those achieved in this study. However, the TN removal performance was better than those obtained in this research.

The removal percentages of TP were low in the WWTPs as there was not a strict anaerobic zone to initialize the process of biological phosphorus removal (Kermani et al., 2009). However, the creation of small anaerobic zones in the anoxic compartments of each bioreactor as well as the physical process of ultrafiltration made phosphorus removal possible.

3.4. Biological kinetic modeling of MBR_a, MBR_b and hybrid MBBR-MBR_b

3.4.1. Kinetic parameters for heterotrophic and autotrophic biomass of the biological treatment

The bioreactors in MBR_b and hybrid MBBR-MBR_b had the highest values of the yield coefficient for heterotrophic biomass (Y_H), i.e. 0.5889 mg VSS mg COD^{-1} and 0.5853 mg VSS mg COD^{-1}, respectively, as shown in Table VIII.4. These values were similar to those obtained by Plattes et al. (2007). Furthermore, these WWTPs had the highest values of the yield coefficient for autotrophic biomass (Y_A) with values of 1.7329 mg O_2 mg N^{-1} and 2.5385 mg O_2 mg N^{-1}, respectively (Table VIII.4). These values were slightly higher than those obtained by Seifi and Fazaelipoor (2012). Therefore, these experimental plants produced the highest amounts of heterotrophic bacteria per substrate oxidized and they required the highest quantities of oxygen to oxidize the same amount of substrate.

Table VIII.4. Kinetic parameters for the characterization of heterotrophic and autotrophic biomass. Y_H (yield coefficient for heterotrophic bacteria), $\mu_{m,\ H}$ (maximum specific growth rate for heterotrophic bacteria), K_M (half-saturation coefficient for organic matter), Y_A (yield coefficient for autotrophic bacteria), $\mu_{m,\ A}$ (maximum specific growth rate for autotrophic bacteria), K_{NH} (half-saturation coefficient for ammonia-nitrogen), Y_{NOB} (yield coefficient for nitrite-oxidizing bacteria), $\mu_{m,\ NOB}$ (maximum specific growth rate for nitrite-oxidizing bacteria), K_{NOB} (half-saturation coefficient for nitrite-nitrogen), k_d (decay coefficient for total bacteria).

Parameter	Sampling zone		
	MBR_a	MBR_b	Hybrid $MBBR\text{-}MBR_b$
Heterotrophic bacteria			
Y_H (mg VSS mg COD^{-1})	0.5338	0.5889	0.5853
$\mu_{m,\ H}$ (h^{-1})	0.0074	0.0380	0.0472
K_M (mg O_2 L^{-1})	6.2459	8.9815	9.0025
Autotrophic bacteria			
Y_A (mg O_2 mg N^{-1})	1.3567	1.7329	2.5385
$\mu_{m,\ A}$ (h^{-1})	0.0279	0.1213	0.0376
K_{NH} (mg N L^{-1})	0.6920	2.7288	0.8122
Nitrite-oxidizing bacteria			
Y_{NOB} (mg O_2 mg N^{-1})	0.5421	0.3659	0.5029
$\mu_{m,\ NOB}$ (h^{-1})	0.0610	0.0890	0.1911
K_{NOB} (mg N L^{-1})	0.6216	0.5267	1.7476
Total bacteria			
k_d (d^{-1})	0.0235	0.0282	0.0232

Table VIII.4 also shows the rest of the parameters which fit the Monod model for the heterotrophic, autotrophic and nitrite-oxidizing bacteria from the bioreactors. Similar values regarding the maximum specific growth rate for heterotrophic biomass ($\mu_{m,\ H}$) and the half-saturation coefficient for organic matter (K_M) were obtained by Canziani et al. (2006) and Seifi and Fazaelipoor (2012), respectively. Moreover, Plattes et al. (2007) and Ferrai et al. (2010) obtained similar values of the maximum specific growth rate for autotrophic biomass ($\mu_{m,\ A}$) and the half-saturation coefficient for ammonia-nitrogen (K_{NH}), respectively. The hybrid $MBBR\text{-}MBR_b$ showed the best kinetic behavior from the point of view of the heterotrophic and autotrophic biomass kinetics when r_{su} was evaluated depending on the kinetic parameters, biomass concentration and substrate concentration (Figure VIII.3a and Figure VIII.3b). The r_{su} was clearly higher for the heterotrophic biomass and slightly higher for the autotrophic biomass in the hybrid $MBBR\text{-}MBR_b$ under the operational conditions used in this study.

Therefore, the heterotrophic and autotrophic bacteria from the hybrid MBBR-MBR_b required less time for substrate oxidation, the μ_m was achieved with less available substrate and less time was required to reach the steady state. These results supported the highest TN removal performance of the hybrid MBBR-MBR_b (72.39±7.57%), as indicated in Table VIII.3. The best kinetic performance of the hybrid MBBR-MBR_b regarding heterotrophic biomass was not reflected in the COD removal efficiencies (Table VIII.3) as the HRT had a high value of 18 h.

Nevertheless, the MBR_a had the best kinetic performance regarding the nitrite-oxidizing bacteria (NOB) kinetics with values of Y_{NOB} = 0.5421 mg O_2 mg N^{-1}, $\mu_{m,\ NOB}$ = 0.0610 h^{-1} and K_{NOB} = 0.6216 mg N L^{-1} (Henze et al., 2000; Iacopozzi et al., 2007), as shown in Figure VIII.3c. This supported the fact that the nitrate concentration in the effluent from the MBR_a was higher than that from the hybrid MBBR-MBR_b with a value of 83.69±32.32 mg NO_3^- L^{-1} (Table VIII.3). Therefore, the hybrid MBBR-MBR_b could have a better kinetic behavior regarding the ammonium-oxidizing bacteria (AOB) since, as a whole, the kinetics of autotrophic bacteria was better, as previously indicated, and the hybrid MBBR-MBR_b had the highest nitrite concentration in its effluent with a value of 19.98±8.85 mg NO_2^- L^{-1} (Table VIII.3). There were statistically significant differences regarding nitrite and nitrate formations between the MBR_a and hybrid MBBR-MBR_b with an HRT of 18 h as the p-values obtained were less than α=0.05, p-value $_{\text{MBRa-Hybrid MBBR-MBRb}}$ (NO_2^-) = 0.00833 and p-value $_{\text{MBRa-Hybrid MBBR-MBRb}}$ (NO_3^-) = 0.03148. Leyva-Díaz et al. (2015) obtained similar conclusions in a study carried out with similar configurations of WWTPs under an HRT of 9.5 h, although the hybrid MBBR-MBR_b showed the best kinetic performance regarding the NOB and the MBR had the best kinetic behavior in relation to the autotrophic biomass.

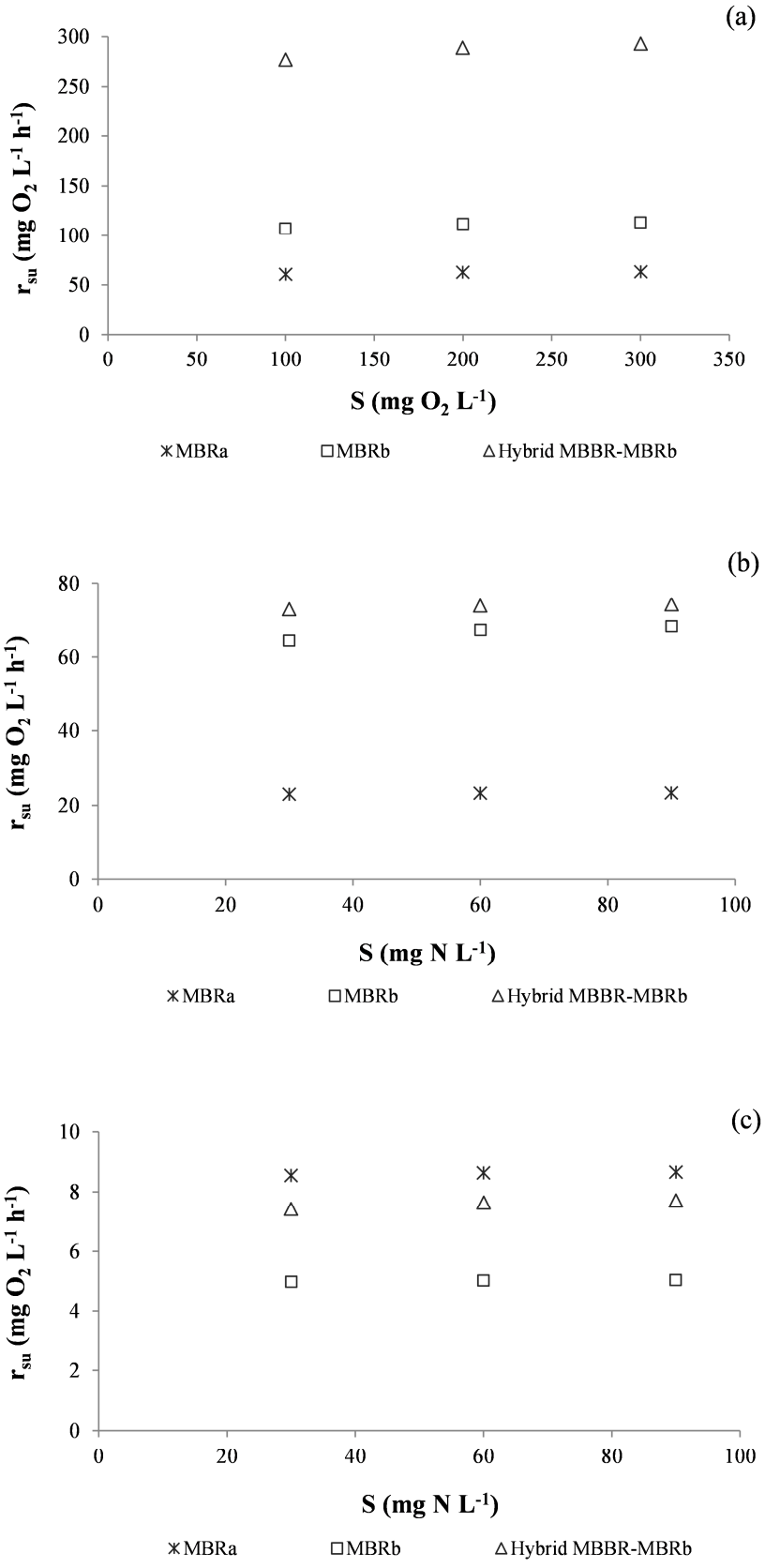

Figure VIII.3. Substrate degradation rate (r_{su}) obtained in the biological kinetic study depending on the substrate concentration for the different bioreactors from the WWTPs. (a) Heterotrophic bacteria. (b) Autotrophic bacteria. (c) Nitrite-oxidizing bacteria.

The values of k_d are also indicated in Table VIII.4. The decay coefficient for the biomass contained in the MBR_b was the highest, i.e. 2.82% of the total quantity of biomass was oxidized per day. The SRT in MBR_b was the lowest with a value of 25.2 days as the flow rate of waste sludge had to be higher than those corresponding to MBR_a and hybrid MBBR-MBR_b in order to maintain a MLSS concentration of 2,739.68±211.75 mg L^{-1} (Table VIII.1). Therefore, the biomass decay rate will be higher because the organic loading rate was identical in the three WWTPs, but the MLSS concentration was lower in MBR_b. The values of k_d concerning MBR_a and the hybrid MBBR-MBR_b were very similar as the SRT was identical and the biomass concentrations were almost the same (Table VIII.1).

3.4.2. Chemical kinetic modeling of AOP technologies as a post-treatment in the MBR_a, MBR_b and hybrid MBBR-MBR_b

Figure VIII.4 shows the evolution of the rate of TOC removal of the pseudofirst-order model (η_{TOC}) at two different H_2O_2 concentrations, 1 g L^{-1} and 2 g L^{-1}, for the different AOP technologies.

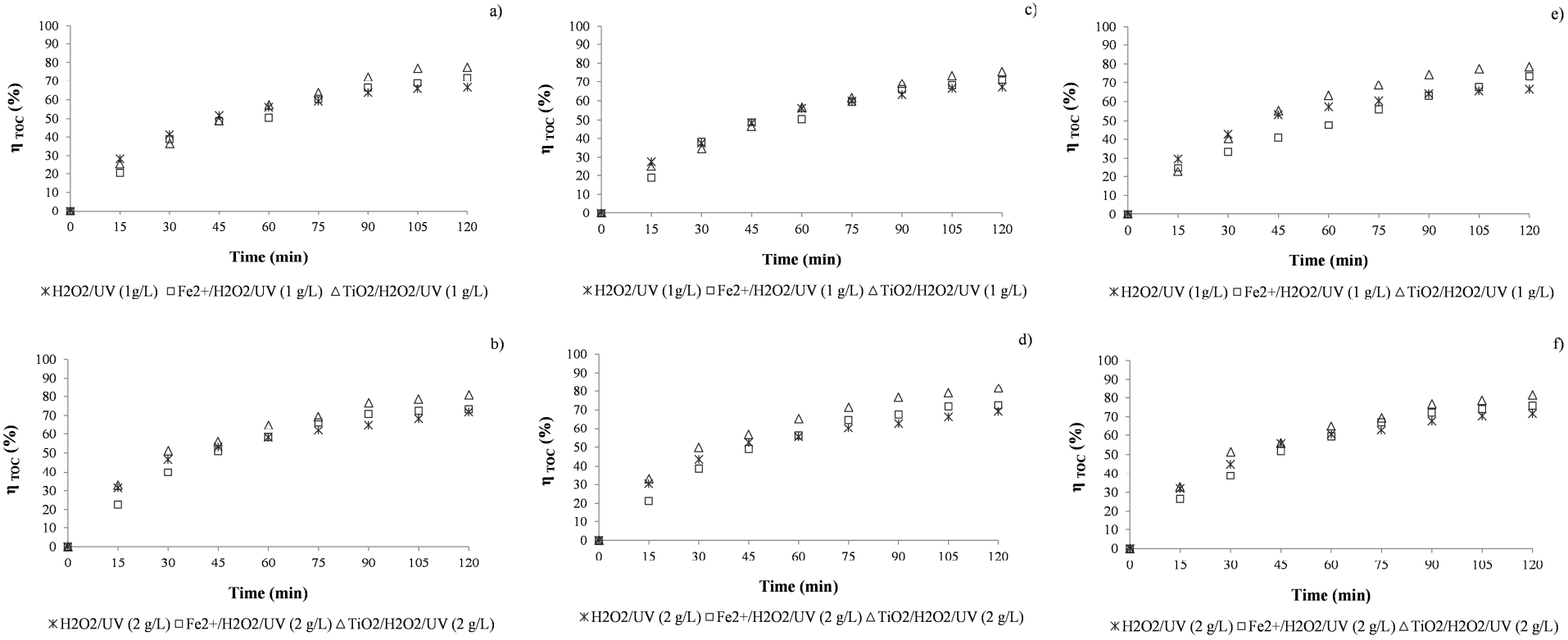

Figure VIII.4. Rate of TOC removal of the pseudofirst-order model (η_{TOC}) of the different AOP technologies. (a) Effluent from MBR_a for an H_2O_2 concentration of 1 g L^{-1}. (b) Effluent from MBR_a for an H_2O_2 concentration of 2 g L^{-1}. (c) Effluent from MBR_b for an H_2O_2 concentration of 1 g L^{-1}. (d) Effluent from MBR_b for an H_2O_2 concentration of 2 g L^{-1}. (e) Effluent from the hybrid MBBR-MBR_b for an H_2O_2 concentration of 1 g L^{-1}. (f) Effluent from the hybrid MBBR-MBR_b for an H_2O_2 concentration of 2 g L^{-1}.

The corresponding values of the kinetic parameters of this model are shown in Table VIII.5.

Table VIII.5. Kinetic parameters of the pseudofirst-order model for the determination of the effectiveness of the different AOP technologies used.

Advanced oxidation process	H_2O_2 concentration (g L^{-1})	Kinetic parameters					
		Effluent MBR_a		Effluent MBR_b		Effluent Hybrid MBBR-MBR_b	
		$\mu_{máx, TOC}$ (%)	$k_{1, TOC}$ (min^{-1})	$\mu_{máx, TOC}$ (%)	$k_{1, TOC}$ (min^{-1})	$\mu_{máx, TOC}$ (%)	$k_{1, TOC}$ (min^{-1})
H_2O_2/UV	1	66.41	0.03	69.04	0.03	66.87	0.04
	2	67.00	0.03	69.02	0.03	70.98	0.03
Fe^{2+}/H_2O_2/UV	1	77.11	0.02	76.78	0.02	83.07	0.02
	2	79.64	0.02	79.13	0.02	81.54	0.02
TiO_2/H_2O_2/UV	1	87.43	0.02	84.88	0.02	85.70	0.02
	2	80.90	0.03	82.29	0.03	81.19	0.03

The values of the rate constant for TOC degradation, $k_{1, TOC}$, were almost independent of the AOP technology used and the effluent considered. The maximum rate of TOC degradation, $\eta_{max, TOC}$, was higher in the TiO_2/H_2O_2/UV system for a constant H_2O_2 concentration, and was independent of the effluent (Figure VIII.4); it occurred since this AOP technology totally consumed the added H_2O_2 and the mineralization process was more effective than in the H_2O_2/UV and Fe^{2+}/H_2O_2/UV systems (García et al., 2007). The $\eta_{max, TOC}$ was higher for the effluents from the MBR_b and hybrid MBBR-MBR_b at H_2O_2 concentrations of 1 g L^{-1} and 2 g L^{-1} in the H_2O_2/UV system. The photolysis rate increased in this AOP technology under higher values of conductivity (Glaze et al., 1987) and were higher for the effluents from the MBR_b and hybrid MBBR-MBR_b, i.e. 778±184 μS cm^{-1} and 817±204 μS cm^{-1} (Table VIII.2), respectively. The Fe^{2+}/H_2O_2/UV process was more suitable for the effluent from the hybrid MBBR-MBR_b since $\eta_{max, TOC}$ was higher at the two H_2O_2 concentrations used, i.e. 83.07% and 81.54% at 1 g L^{-1} and 2 g L^{-1} of H_2O_2, respectively. It was caused by the lowest value of BOD_5 for the effluent from the hybrid MBBR-MBR_b, i.e. 3.40±2.16 mg O_2 L^{-1} (Table VIII.3), so the concentration of biodegradable organic compounds was lower than in the effluents from MBR_a and MBR_b and the consumption of chemicals was more effective for oxidizing the organic compounds which were resistant to biological treatment. On the other hand, the TiO_2/H_2O_2/UV system did not improve the

TOC removal when the H_2O_2 concentration increased in any WWTP, so this process must only be used at an H_2O_2 concentration of 1 g L^{-1}. Higher H_2O_2 doses led to an enhancement in the proportion of organic matter (intermediates) susceptible to biodegradation (Poyatos et al., 2010) and resulted in the unnecessary consumption of chemical reagents for oxidizing it, with a loss in the effectiveness of the treatment for the most persistent pollutants.

4. Conclusions

The following conclusions were drawn:

1. The hybrid MBBR-MBR_b showed the best kinetic performance from the point of view of heterotrophic and autotrophic biomass. It supported the efficiency of TN removal with a value of 72.39±7.57% for the hybrid MBBR-MBR_b, but the organic matter removal was very similar in the three WWTPs as the HRT was 18 h. The MBR_a had the best behavior regarding the kinetics of nitrite-oxidizing bacteria, which supported the concentrations of nitrite and nitrate in the different effluents.

2. The $\eta_{max,\ TOC}$ was higher in the $TiO_2/H_2O_2/UV$ system for a constant H_2O_2 concentration, and was independent of the effluent as the H_2O_2 was totally consumed and the mineralization process was more effective than in the H_2O_2/UV and $Fe^{2+}/H_2O_2/UV$ systems. Furthermore, the $TiO_2/H_2O_2/UV$ process did not improve the TOC removal when the H_2O_2 concentration increased in any WWTP. The $Fe^{2+}/H_2O_2/UV$ system was more suitable for the effluent from the hybrid MBBR-MBR_b with values of $\eta_{max,\ TOC}$ of 83.07% and 81.54% at H_2O_2 concentrations of 1 g L^{-1} and 2 g L^{-1}, respectively, as the effluent from hybrid MBBR-MBR_b had the lowest value of BOD_5 and the consumption of chemical reagents was more effective for oxidizing the most persistent pollutants.

3. Among the different alternatives studied, the combined process of hybrid MBBR-MBR_b with $TiO_2/H_2O_2/UV$ as a post-treatment stage showed the best performance from the point of view of the biological and chemical kinetics.

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IX. CHAPTER 6

Study of kinetic modeling, nitrifying and denitrifying microbial populations, and organic matter and nitrogen removal in a pure MBBR-MBR system for wastewater treatment (operational conditions of 9.5 h and 6 h of HRT and low biomass concentrations).

Abstract

The moving bed biofilm reactor-membrane bioreactor (MBBR-MBR) is a novel solution to conventional processes. In this study, two membrane bioreactors (MBR_a and MBR_b), a hybrid MBBR-MBR_b and a pure MBBR-MBR were compared. The hybrid MBBR-MBR_b contained suspended and attached biomass, while the pure MBBR-MBR mainly had attached biomass. The reactors operated with two hydraulic retention times (HRTs) of 9.5 h and 6 h. The kinetic parameters for heterotrophic and autotrophic biomasses, mainly nitrite-oxidizing bacteria (NOB), were evaluated and related to organic matter and nitrogen removals. The analysis of the bacterial community structure of the AOB, NOB and denitrifying bacteria (DeNB) from the pure MBBR-MBR operating under an HRT of 9.5 h was carried out by means of pyrosequencing to detect and quantify the contribution of the nitrifying bacteria in the total bacterial community. The pure MBBR-MBR had the highest efficiency of total nitrogen (TN) removal with a value of 71.91±16.04% under a hydraulic retention time (HRT) of 9.5 h and 63.21±11.01% under an HRT of 6 h. The hybrid MBBR-MBR_b showed the highest chemical oxygen demand (COD) removal efficiencies under the two working HRTs, with values of 87.39±6.01% for 9.5 h and 84.10±2.25% for 6 h. The kinetic study supported the efficiencies of COD and TN removals as the hybrid MBBR-MBR_b and pure MBBR-MBR showed the best performances from the point of view of the kinetics of the heterotrophic and autotrophic biomass, respectively. The presence of the attached biomass improved the organic matter and nitrogen removals in a hybrid MBBR-MBR_b and pure MBBR-MBR, respectively.

1. Introduction

Industrial development, increase in urbanization and changes in farming practices have caused a huge rise in the consumption of water resources and a deterioration of their quality. In an effort to control stricter effluent limits or upgrade existing overloaded activated sludge plants, advanced technologies for wastewater treatment have been proposed (Wang et al., 2006).

The MBR is of primary interest in the field of wastewater treatment (Visvanathan et al., 2000). The conventional MBR uses suspended biomass to degrade wastewater constituents and membrane filtration to separate biomass, typically through microfiltration or ultrafiltration (Zhou and Smith, 2002). Several advantages are attributed to MBR treatment such as a smaller footprint, a higher effluent quality, a good disinfection capability and the capacity for higher volumetric loading rates (Le-Clech et al., 2006). However, maintaining membrane permeability and preventing fouling are major problems in operation (Judd, 2006). Membrane fouling leads to a decline in the permeate flux, an increase in the trans-membrane pressure and a reduction in the performance of the treatment process (Hasan et al., 2012).

Moving bed biofilm reactor (MBBR) represents a different spectrum in advanced wastewater treatment. MBBR is operated similarly to the activated sludge process with the addition of freely moving carrier media (Ødegaard, 2006). Carrier geometry, to promote attached biomass growth, has included smooth cylinders, cylinders with internal crosses and external fins (Ødegaard, 2006), rectangles, cubes and spheres (Valdivia et al., 2007). Additionally, several materials have been used for biomass support including porous ceramic, reticulated foam, polyvinyl alcohol, polyurethane, plastic foam and high-density polyethylene (Ødegaard, 2006). A filling fraction below 70% for cylindrical plastic carriers is recommended by Ødegaard (2006); Di Trapani et al. (2008) and Mannina and Viviani (2009) analyzed the performance of nutrient removal of MBBR at 33% and 66% and noticed little variation in terms of the removal of wastewater constituents.

Some of the most important advantages of the MBBR process compared with the conventional activated sludge process include better oxygen transfer, a shorter hydraulic retention time (HRT), higher organic loading rates, a higher nitrification rate and a

larger surface area for mass transfer (Chan et al., 2009). According to Ivanovic and Leiknes (2008), MBBR can process high organic loading rates at relatively short hydraulic retention times (HRTs), in the range of 4 h, while producing consistently high quality effluent with respect to the five-day biochemical oxygen demand (BOD_5), total nitrogen (TN) and total suspended solids (TSS). However, the settleability of biosolids is the largest challenge in MBBR design as the production of filamentous bacteria and poorly settling biomass often hinder solid separation in a secondary clarifier (Ødegaard, 2000).

Three main phases are included in the operation of MBBR: the discrete solid phase of inert carriers with immobilized microbial cells, the discrete air bubble phase and the continuous aqueous phase (Chan et al., 2009). As reported for other microbial processes, cell immobilization has various advantages (Petruccioli et al., 1994; Juárez-Jiménez et al., 2012), among them seamlessly integrated simultaneous nitrification and denitrification (Yang et al., 2009). This is based on the fact that there are dissolved oxygen concentration gradients within the microbiological flocs as a result of diffusion limitations from the aqueous phase into the immobilized biofilm. The aerobic liquid provides an oxidizing environment where soluble BOD_5 is removed and ammonia is nitrified (Posmanik et al., 2014). Nitrite and nitrate produced during nitrification diffuses to the inner parts of the biofilm where there is an anoxic micro-zone. This micro-zone harbors heterotrophic denitrifiers that produce nitrogen gas in the traditional manner (Yang et al., 2009; Ji et al., 2013). In this way, the elimination of nitrogen from wastewater is driven by ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) under aerobic conditions, and denitrifying bacteria (DeNB) under anoxic conditions (Leyva-Díaz et al., 2015). Therefore, the identification of AOB, NOB and DeNB is a necessary method for the evaluation of the nitrification-denitrification process in wastewater treatment systems. Next-generation sequencing techniques are a widely used molecular biology tool for the research of microbial community assemblages in natural and engineered environments (González-Martínez et al., 2014; Wei et al., 2014). In this study, the analysis of AOB, NOB and DeNB has been developed by means of pyrosequencing and a Bray-Curtis cluster analysis. The identification and quantification of the microbial population present in the pure MBBR-MBR system was carried out according to the research which was developed by Leyva-Díaz et al. (2015) for the MBR and hybrid MBBR-MBR systems.

As a result of the disadvantages of the MBR and MBBR systems, an alternative configuration called “moving bed biofilm reactor-membrane bioreactor” (MBBR-MBR) was developed by Leiknes and Ødegaard (2007). This system aims to partially mitigate the fouling concerns in relation to MBR systems and the settleability issues regarding MBBR ones. It becomes economically attractive when compact technology is required to accommodate space constraints or stringent effluent quality requirements are mandatory (Yang et al., 2006; Reboleiro-Rivas et al., 2013).

There are two ways of working in an MBBR-MBR system. In a pure MBBR-MBR process, the biofilm grows attached to small carrier elements suspended in constant motion throughout the entire volume of the reactor; there is no recycling from the MBR to the MBBR and the MLSS concentration in the bioreactor is similar to the influent concentration (Falletti et al., 2009). A hybrid MBBR-MBR process combines suspended and attached biomass as there is recycling from the MBR to the MBBR (De la Torre et al., 2013; Mannina and Viviani, 2009).

Kinetic modeling allows for describing and verifying the biological processes that occur in wastewater treatment. Furthermore, it is a very useful tool for predicting the behavior of the biological processes, applicable to their design, evaluation and control. There are some uncertainties regarding the kinetic performance of MBBR-MBR systems as it has been less studied than in other systems. The coexistence of two kinds of biomass, suspended and attached, could lead to a modification of the kinetic parameters of the system, compared to processes involving pure suspended or attached biomass (Di Trapani et al., 2010). Several studies have been carried out to improve the knowledge of the modeling of these systems in the last few years (Mannina et al., 2011; Leyva-Díaz et al., 2015).

The aim of this research was to determine the kinetic parameters relating to the heterotrophic, autotrophic and nitrite-oxidizing bacteria in two MBR systems, a hybrid MBBR-MBR and a pure MBBR-MBR and to relate them to the removal of organic matter and nitrogen. Furthermore, populations of nitrifying bacteria were identified and quantified for the pure MBBR-MBR system to support the previous results.

2. Materials and methods

2.1. Description of the experimental pilot plants

Three pilot wastewater treatment plants (WWTPs), working in parallel, were fed by a feeding peristaltic pump (323S, Watson-Marlow Pumps Group, USA) with urban wastewater from a sewage storage tank. Real wastewater came from the outlet of the primary settler of a wastewater treatment plant (WWTP) located in Granada (Spain). The WWTPs consisted of a membrane bioreactor (MBR) (Figure IX.1a), a hybrid MBBR-MBR_b (Figure IX.1b) and a pure MBBR-MBR (Figure IX.1c). Figure IX.1d shows the reactor zones, the membrane tank, the effluent tank and some peristaltic pumps.

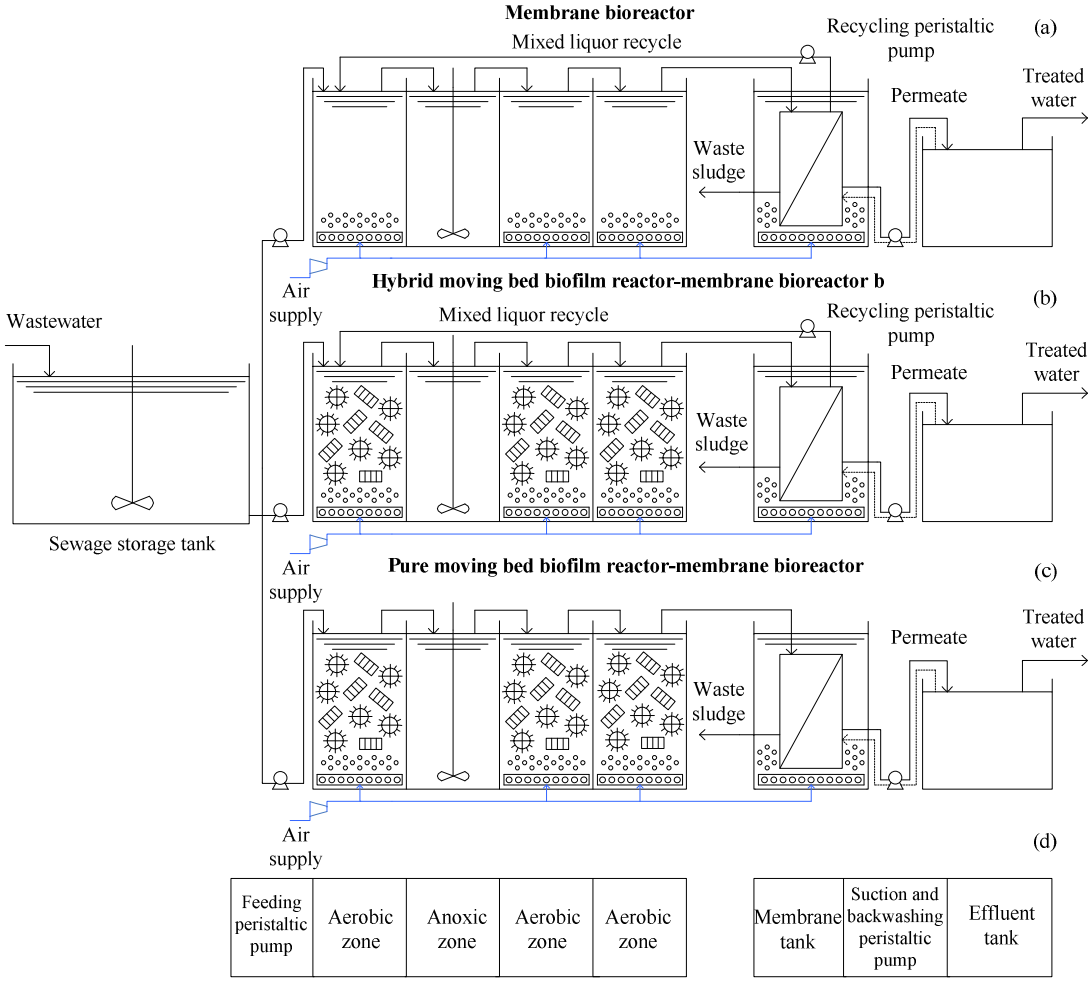

Figure IX.1. Diagram of the wastewater treatment plants used in the study. (a) Membrane bioreactor (MBR_a and MBR_b). (b) Hybrid moving bed biofilm reactor-membrane bioreactor (Hybrid MBBR-MBR_b). (c) Pure moving bed biofilm reactor-membrane bioreactor (Pure MBBR-MBR). (d) Nomenclature concerning the reactor zones, membrane tank, effluent tank and some peristaltic pumps.

These WWTPs operated under two different HRTs, 9.5 h and 6 h, and the MBR worked at two different biomass concentrations. The MBR that worked at a lower biomass concentration (2,820.59±243.87 mg L^{-1} and 2,777.78±282.27 mg L^{-1} for the HRTs of 9.5 h and 6 h, respectively) was named MBR_a and the MBR that operated at a higher biomass concentration (6,656.67±445.02 mg L^{-1} and 6,566.67±255.73 mg L^{-1} for the HRTs of 9.5 h and 6 h, respectively, as shown in Table IX.1) was called MBR_b.

The MBR, hybrid MBBR-MBR_b and pure MBBR-MBR all included a biological reactor divided into four zones: one anoxic zone and three aerobic ones. The dimensions of the bioreactor were 50 cm long, 12 cm wide and 60 cm high and the total volume was 36 L. The working volume was 24 L because the reactor had a security percentage with a value of 33% in relation to the total volume. The volume of the anoxic zone was 6 L. Table IX.1 shows the operating conditions of the WWTPs.

Table IX.1. Operation conditions and stabilization concentrations of MLSS, MLVSS, attached BD and VBD of the experimental plants. HRT (hydraulic retention time), SRT (sludge retention time), MLSS (mixed liquor suspended solids), MLVSS (mixed liquor volatile suspended solids), BD (biofilm density), VBD (volatile biofilm density).

Parameter	MBR_a		MBR_b		Hybrid $MBBR\text{-}MBR_b$		Pure MBBR-MBR	
	Aerobic zone	Anoxic zone	Aerobic zone	Anoxic zone	Aerobic zone	Anoxic zone	Aerobic zone	Anoxic zone
Volume (L)	18	6	18	6	18	6	18	6
Filling ratio with carriers (%)	0	0	0	0	35	0	35	0
HRT=9.5 h								
Flow rate ($L\ h^{-1}$)	3.00		3.00		3.00		3.00	
Membrane flux ($L\ m^{-2}\ h^{-1}$)	15		15		15		15	
SRT (day)	7.2		119.5		7.2		6	
MLSS ($mg\ L^{-1}$)	2,820.59±243.87		6,656.67±445.02		2,041.90±258.37		208.00±61.30	
MLVSS ($mg\ L^{-1}$)	2,328.98±201.37		5,714.84±382.06		1,629.58±206.20		153.62±45.27	
BD ($mg\ L^{-1}$)	-		-		997.73±124.62		1,920.45±127.16	
VBD ($mg\ L^{-1}$)	-		-		868.84±108.52		1,615.25±106.95	
HRT=6 h								
Flow rate ($L\ h^{-1}$)	4.70		4.70		4.70		4.70	
Membrane flux ($L\ m^{-2}\ h^{-1}$)	23.5		23.5		23.5		23.5	
SRT (day)	5.7		102		5.4		4.5	
MLSS ($mg\ L^{-1}$)	2,777.78±282.27		6,566.67±255.73		2,243.75±216.95		258.75±79.99	
MLVSS ($mg\ L^{-1}$)	2,229.59±226.56		5,677.18±221.09		1,893.67±183.10		196.01±60.60	
BD ($mg\ L^{-1}$)	-		-		748.53±111.97		2,070.00±202.97	
VBD ($mg\ L^{-1}$)	-		-		667.41±99.84		1,814.51±177.92	

Municipal wastewater, which came from the sewage storage tank, was pumped into the first aerobic chamber of the bioreactor. Then, it went through the anoxic compartment and subsequently reached the second and third aerobic chambers through a communicating vessel system. Aerobic zones were equipped with a fine bubble disk diffuser (AFD 270, ECOTEC, SA, Spain) at the bottom of the reactor. Air to the aerobic zone was supplied by an air compressor (ACO-500, Hailea, China). The airflow to the reactor was measured by a rotameter (2100 Model, Tecfluid, SA, Spain) and regulated by a manual valve. The air flow rate in each of the biological reactors had a value of 30 $L\ h^{-1}$ and the air was supplied at a constant pressure and temperature of 0.5 bar and

20ºC. The anoxic zone was in the second compartment in order to avoid the possibility that recycling from the membrane tank, which contained a higher dissolved oxygen concentration to prevent membrane fouling, could change the anoxic conditions. Therefore, the anoxic zone was set between the first and the third aerobic zones with dissolved oxygen concentrations that could be adjusted to values that were not too high.

The outlet of the bioreactor was led into a membrane tank that was designed as an external submerged unit. It was cylindrical, had a diameter of 10 cm and was 65 cm high. The total volume of this tank was 6.7 L, whereas the working volume was 4.32 L. The membrane module consisted of a vertically oriented submerged module of hollow-fiber ultrafiltration membranes (Micronet Porous Fiber, SL, Spain). The membrane was flowed from the outside to the inner side through a sucking process. The total membrane area was 0.20 m^2. The hollow fibers were made of polyvinylidene fluoride and had an inside braid-reinforcement made of polyester. The fibers had an outer diameter of 2.45 mm, an inner diameter of 1.10 mm and a pore size of 0.04 μm. Aeration, with an air flow rate of 100 L h^{-1}, was applied to the base of the module by a coarse bubble disk diffuser (CAP 3, ECOTEC, SA, Spain) and was supplied and regulated in a similar way to the bioreactor. The membranes were continuously aerated with a tangential air current to prevent any organic or inorganic solids from settling on their surface. The permeate was extracted through the membrane using a suction-backwashing peristaltic pump (323U, Watson-Marlow Pumps Group, USA) to collect it in the permeate tank. The cyclic mode of operation consisted of production and backwashing periods of 9 min and 1 min, respectively. The transmembrane pressures (TMP) varied between 0.1 and 0.5 bar. The operating parameters such as permeate flow, permeation and backwashing times could be adjusted by a control panel.

A small volume of the retentate was removed from the membrane tank as excess sludge. A recycling was carried out from the membrane tank to pump out the aerobic mixed liquor into the anoxic chamber through a recycling peristaltic pump (323S, Watson-Marlow Pumps Group, USA) in the MBR systems and hybrid MBBR-MBR_b. The anoxic chamber received the recycling flow from the membrane tank after passing through the first aerobic chamber. This allowed the working mixed liquor suspended solids (MLSS) concentration to be maintained inside the bioreactor and nitrogen removal to be achieved. The recycling rate was three times and a half the influent flow

rate for the MBR_a and hybrid MBBR-MBR_b, and it was two times the influent flow rate for the MBR_b.

The hybrid MBBR-MBR_b combined an MBBR with an MBR. Biomass grew as suspended flocs and as a biofilm in the hybrid MBBR-MBR_b. Biofilm grew on carriers that moved freely in the water volume due to aeration in the aerobic zone and a mechanical stirrer in the anoxic one. This kind of carrier is called K1 and was developed and supplied by AnoxKaldnes AS (Norway). The K1 carrier has been widely studied in similar experiments (Leiknes and Ødegaard, 2007; Di Trapani et al., 2008). The K1 medium filling-fraction (percentage of the reactor volume occupied by carriers in an empty tank) had a value of 35% in the aerobic zone, whereas the anoxic zone had no carriers. A biomass recycling was carried out from the membrane tank to the anoxic chamber to obtain the working MLSS concentration inside the bioreactor and the nitrogen removal.

The pure MBBR-MBR also combined an MBBR with an MBR. It had the same characteristics as the hybrid MBBR-MBR_b. The difference between the pure MBBR-MBR and hybrid MBBR-MBR_b was the fact that the biomass growth was mainly developed on carriers in the first system as there was no biomass recycling from the membrane tank to the MBBR and the MLSS concentration in the bioreactor was similar to that in the influent.

Sampling ports were provided in each bioreactor for sample collection. All anoxic zones had variable speed stirrers (Multi Mixer MM-1000, Biosan Laboratories, Inc., USA), which kept the biofilm medium moving in the anoxic zone. The sewage storage tank also had a variable speed propeller to homogenize urban wastewater; this stirrer was identical to the previous ones. The normal propeller speed was 320 rpm in both the anoxic zone and in the feeding tank. Both the stirrer in the anoxic zone and the diffuser in the aerobic one had the function of keeping the carriers moving inside the reactor and homogenizing the mixed liquor.

2.2. Experimental procedure and analytical determinations

Samples were collected every 24 h from the influent, the effluents and the anoxic and aerobic zones of the bioreactors and the membrane tank. Biomass samples were

collected from the biofilm developed on the carriers and the mixed liquor belonging to the pure MBBR-MBR under an HRT of 9.5 h for the pyrosequencing process.

Firstly, the MBR_a, hybrid MBBR-MBR_b and pure MBBR-MBR operated under an HRT of 9.5 h. Then, the MBR_b operated at a higher biomass concentration under an HRT of 9.5 h. Afterwards, the MBR_a, hybrid MBBR-MBR_b and pure MBBR-MBR worked under an HRT of 6 h. Then, the MBR_b operated at a higher biomass concentration under an HRT of 6 h. A level indicator connected to the feeding pump controlled the influent in each bioreactor to ensure that the level in the system was correct and that the membranes were covered by the mixed liquor.

Physical and chemical determinations were carried out concerning the pH, conductivity, temperature, dissolved oxygen, chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), volatile suspended solids (VSS), total phosphorus (TP), total nitrogen (TN) and the concentrations of ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) according to section **Materials and Methods**.

The microbial communities of the pure MBBR-MBR under an HRT of 9.5 h were analyzed by 454 pyrosequencing methods in order to detect and quantify the contribution of nitrifying bacteria (AOB and NOB) and denitrifying bacteria in the total bacterial community. Furthermore, the kinetic parameters for heterotrophic, autotrophic and nitrite-oxidizing bacteria were evaluated (**Materials and Methods**).

The evaluation of statistically significant differences between the results concerning COD, BOD_5, TSS, TN, TP and concentrations of NH_4^+, NO_2^- and NO_3^- was carried out according to section **Materials and Methods.** Moreover, a Bray-Curtis similarity analysis for the OTUs identified as AOB, NOB and DeNB was performed in the pure MBBR-MBR under an HRT of 9.5 h (**Materials and Methods**).

3. Results and discussion

3.1. Biomass formation and physical and chemical parameters

The values of the concentration of MLSS, mixed liquor volatile suspended solids (MLVSS), attached biofilm density (BD) and attached volatile biofilm density (VBD) from the MBR_a, MBR_b, hybrid MBBR-MBR_b and pure MBBR-MBR under the HRTs

of 9.5 h and 6 h are shown in Table IX.1. MLVSS and VBD were used for the estimation of the kinetic parameters.

Table IX.2. Average values of pH, conductivity, temperature and dissolved oxygen of the influent, effluents and mixed liquors of the biological reactors of the experimental plants under the working HRTs of 9.5 h and 6 h**.** HRTs (hydraulic retention times).

Parameter	Sampling zone												
	Influent	MBR_a			MBR_b			Hybrid MBBR-MBR_b			Pure MBBR-MBR		
		Effluent	Anoxic zone	Aerobic zone	Effluent	Anoxic zone	Aerobic zone	Effluent	Anoxic zone	Aerobic zone	Effluent	Anoxic zone	Aerobic zone
HRT=9.5 h													
pH	8.17±0.23 8.21±0.15(1)	8.09±0.54	7.03±0.35	6.87±0.33	7.18±0.47	7.46±0.56	7.06±0.54	7.71±0.68	6.69±0.35	6.59±0.35	8.12±0.81	7.25±0.28	7.23±0.32
Conductivity ($\mu S\ cm^{-1}$)	1,171±330 1,340±128(1)	914±268	849±308	828±306	1,114±107	1,075±117	1,074±107	871±251	788±284	758±276	1,008±331	984±360	953±375
Temperature (ºC)	14.7±1.1 14.4±1.3(1)	14.7±1.1	14.6±1.2	14.6±1.2	14.7±1.3	14.6±1.2	14.7±1.1	14.7±1.1	14.7±1.1	14.7±1.1	14.7±1.1	14.7±1.1	14.7±1.1
Dissolved oxygen ($mg\ O_2\ L^{-1}$)	-	-	0.3±0.2	2.8±1.1	-	0.3±0.1	2.0±1.0	-	0.3±0.1	3.4±1.1	-	0.4±0.2	3.6±1.0
HRT=6 h													
pH	8.16±0.15 8.01±0.10(2)	7.10±0.54	7.39±0.52	7.05±0.59	7.63±0.83	7.91±0.61	7.19±0.72	6.71±0.35	7.17±0.57	6.59±0.73	7.49±0.50	7.80±0.06	7.67±0.08
Conductivity ($\mu S\ cm^{-1}$)	1,313±103 1,206±102(2)	1,025±79	1,085±83	1,051±84	996±107	963±97	970±107	1,031±64	1,053±53	1,033±59	1,271±133	1,376±142	1,342±134
Temperature (ºC)	20.2±1.3 20.4±1.3(2)	20.7±0.8	20.7±0.8	20.7±0.8	20.7±1.3	20.7±1.2	20.7±1.3	20.7±1.0	20.7±0.9	20.7±0.9	20.7±1.3	20.7±1.2	20.7±1.3
Dissolved oxygen ($mg\ O_2\ L^{-1}$)	-	-	0.3±0.2	2.0±1.1	-	0.2±0.1	1.8±1.1	-	0.2±0.1	1.8±0.8	-	0.3±0.1	2.1±0.5

(1) Average values of pH, conductivity and temperature for the influent of the MBR_b with an HRT of 9.5 h.
(2) Average values of pH, conductivity and temperature for the influent of the MBR_b with an HRT of 6 h.

Sriwiriyarat and Randall (2005) carried out their study with similar values of MLSS and BD. The concentration of MLSS in the MBR_a was slightly higher than that in the hybrid MBBR-MBR_b with the two operational HRTs. This difference was compensated by the attached biofilm on the carriers contained in the hybrid MBBR-MBR_b. The pure MBBR-MBR had mainly attached BD and the MLSS concentration in the bioreactor was similar to that of the influent. This attached BD was similar to those reported by Martín-Pascual et al. (2012).

The average values of conductivity, pH, temperature and dissolved oxygen concentration of the influent, effluents and mixed liquors of each bioreactor of the pilot plants of municipal wastewater treatment are shown in Table IX.2. The values of conductivity of the effluent and mixed liquor of the pure MBBR-MBR were similar to that of the influent under the two working HRTs as there was no recycling from the membrane tank to the MBBR in the pure MBBR-MBR. They were slightly higher than those of the MBR_a and hybrid MBBR-MBR_b. The temperature was 14.7±1.1ºC under an HRT of 9.5 h and 20.7±1.1ºC under an HRT of 6 h in the WWTPs. Rutt et al. (2006) developed other studies of MBBR in winter with similar temperature values to those obtained in this study for an HRT of 9.5 h. The dissolved oxygen concentration in the aerobic zone of the bioreactors was usually over 2.0 mg O_2 L^{-1}, which is recommended to achieve the efficient removal of COD and an effective nitrification process, according to Wang et al. (2006).

3.2. Organic matter and nutrient removal

The values of COD and BOD_5 obtained from the influent and effluents relating to the MBR_a, MBR_b, hybrid MBBR-MBR_b and pure MBBR-MBR under the two working HRTs for the steady state are shown in Table IX.3. These values were similar to those reported by Ahl et al. (2006).

Table IX.3. Average values of COD, BOD_5, TSS, TP, TN, NH_4^+, NO_2^- and NO_3^- of the influent and effluents of the experimental plants and removal percentages of COD, BOD_5, TSS, TP and TN during the steady state under the working HRTs of 9.5 h and 6 h. COD (chemical oxygen demand), BOD_5 (five-day biochemical oxygen demand), TSS (total suspended solids), TP (total phosphorus), TN (total nitrogen), NH_4^+ (concentration of ammonium), NO_2^- (concentration of nitrite), NO_3^- (concentration of nitrate), HRTs (hydraulic retention times).

Parameter	Sampling zone					Removal percentage	Wastewater treatment plant			
	Influent	Effluent MBR_a	Effluent MBR_b	Effluent Hybrid MBBR-MBR_b	Effluent Pure MBBR-MBR		MBR_a	MBR_b	Hybrid MBBR-MBR_b	Pure MBBR-MBR
HRT=9.5 h										
COD (mg O_2 L^{-1})	224.08±102.30 234.00±46.10(1)	34.62±15.12	29.87±9.17	28.26±10.67	42.66±18.74	**COD (%)**	84.55±5.77	87.23±4.62	87.39±6.01	80.96±7.67
BOD_5 (mg O_2 L^{-1})	111.00±52.10 116.67±43.20(1)	5.93±4.49	2.31±1.61	2.82±2.63	7.91±4.02	**BOD_5 (%)**	94.66±3.11	98.02±1.44	97.46±1.52	92.87±4.63
TSS (mg L^{-1})	86.16±35.58 90.00±17.86(1)	2.38±2.59	4.40±2.96	3.53±2.61	7.26±2.53	**TSS (%)**	97.24±2.52	95.11±3.33	95.90±3.71	91.57±6.95
TP (mg TP L^{-1})	8.38±0.94 10.07±1.80(1)	4.02±0.88	7.98±1.31	4.56±0.85	4.19±1.10	**TP (%)**	52.00±11.63	20.75±2.07	45.61±12.27	50.03±12.82
TN (mg TN L^{-1})	65.47±32.18 91.54±8.81(1)	19.29±7.63	45.72±8.15	25.23±9.62	18.39±9.60	**TN (%)**	70.54±14.57	50.05±7.50	61.46±11.87	71.91±16.04
NH_4^+ (mg NH_4^+ L^{-1})	81.29±25.65 115.86±36.56(1)	ND	ND	ND	ND					
NO_2^- (mg NO_2^- L^{-1})	3.71±0.09 3.03±0.08(1)	0.27±0.18	0.95±0.64	40.88±18.10	45.11±19.16					
NO_3^- (mg NO_3^- L^{-1})	4.93±2.49 2.22±1.12(1)	85.05±32.84	201.21±77.69	56.64±16.93	20.64±5.67					
HRT=6 h										
COD (mg O_2 L^{-1})	207.61±38.79 226.36±51.20(2)	33.20±3.76	29.64±7.19	33.01±6.20	41.99±7.12	**COD (%)**	84.01±2.15	86.90±4.28	84.10±2.25	79.78±4.60
BOD_5 (mg O_2 L^{-1})	104.29±15.01 113.33±13.66(2)	4.79±0.67	2.36±0.82	4.60±1.23	4.40±0.98	**BOD_5 (%)**	95.41±0.96	97.92±0.73	95.58±0.87	95.78±0.82
TSS (mg L^{-1})	83.07±20.26 75.09±18.27(2)	4.58±2.38	2.80±2.04	5.41±2.46	3.14±2.62	**TSS (%)**	94.49±3.65	96.27±3.78	93.49±3.66	96.21±2.71
TP (mg TP L^{-1})	9.15±1.27 9.13±1.70(2)	4.98±0.70	6.83±1.44	5.24±0.72	5.31±1.48	**TP (%)**	45.55±11.67	25.22±10.20	42.71±6.91	41.98±9.95
TN (mg TN L^{-1})	80.21±8.50 84.70±4.74(2)	43.43±8.71	44.50±4.42	41.28±13.44	29.51±3.93	**TN (%)**	45.86±10.69	47.46±6.76	48.53±16.71	63.21±11.01
NH_4^+ (mg NH_4^+ L^{-1})	100.45±31.70 106.45±33.59(2)	ND	ND	ND	ND					
NO_2^- (mg NO_2^- L^{-1})	0.81±0.02 3.20±0.08(2)	8.42±5.67	2.03±1.37	18.16±8.04	28.87±12.26					
NO_3^- (mg NO_3^- L^{-1})	8.14±4.11 4.12±2.08(2)	180.97±69.88	194.34±75.04	158.36±47.34	91.77±25.22					

ND: Not Detected
(1) Average values of COD, BOD_5, TSS, TP, TN, NH_4^+, NO_2^- and NO_3^- of the influent of the MBR_b with an HRT of 9.5 h.
(2) Average values of COD, BOD_5, TSS, TP, TN, NH_4^+, NO_2^- and NO_3^- of the influent of the MBR_b with an HRT of 6 h.

The hybrid MBBR-MBR_b had a higher performance than the MBR_a and pure MBBR-MBR regarding COD and BOD_5 removal under an HRT of 9.5 h. The removal percentages of COD and BOD_5 were 87.39±6.01% and 97.46±1.52%, respectively, in the hybrid MBBR-MBR_b under an HRT of 9.5 h, as indicated in Table IX.3. Di Trapani et al. (2010) obtained BOD_5 removal efficiencies similar to those shown in this study with similar HRT and SRT in a hybrid MBBR. The differences between the hybrid MBBR-MBR_b and the MBR_a and pure MBBR-MBR systems regarding the removal percentages of COD and BOD_5 were statistically significant with an HRT of 9.5 h, since the p-values obtained from the post hoc procedure, Tukey´s HSD, were lower than α=0.05 under an HRT of 9.5 h, as shown in Table IX.4. The hybrid MBBR-MBR_b also showed a better behavior than the MBR_a and pure MBBR-MBR regarding COD removal under an HRT of 6 h with a value of 84.10±2.25%. The differences between the hybrid MBBR-MBR_b and MBR_a regarding the COD removal were not statistically significant under an HRT of 6 h, although the kinetic performance for heterotrophic biomass was much better for the hybrid MBBR-MBR_b compared to the MBR_a. However, these differences were statistically significant between the hybrid MBBR-MBR_b and pure MBBR-MBR with a p-value of 0.00030 (Table IX.4). The improvement regarding the removal of organic matter in the hybrid MBBR-MBR_b was probably due to the presence of suspended and attached biomass, as MBR_a only contained suspended biomass, while the pure MBBR-MBR mainly had attached biomass.

Furthermore, the MBR_b performed better than the MBR_a regarding the COD and BOD_5 removal under the operational HRTs of 9.5 h and 6 h, as shown in Table IX.3. The differences between the two systems were statistically significant (Table IX.4), except for the COD removal under an HRT of 6 h, as the MBR_b had a higher biomass concentration in the bioreactor under the two working HRTs (Table IX.1).

The removal percentages of COD were slightly lower for the WWTPs under an HRT of 6 h as the organic loading rate was higher.

Table IX.4. P-values of sequential comparison (ANOVA analysis) of removal percentages of COD, BOD_5, TSS, TN and TP between the different experimental plants. COD (chemical oxygen demand), BOD_5 (five-day biochemical oxygen demand), TSS (total suspended solids), TN (total nitrogen). TP (total phosphorus).

Wastewater treatment plants		Parameter				
		COD	BOD_5	TSS	TN	TP
HRT=9.5 h						
MBR_a	**Hybrid MBBR-MBR_b**	**0.04647**	**0.02697**	0.49540	0.08853	0.74847
MBR_a	**Pure MBBR-MBR**	0.06573	0.21543	0.12308	0.79388	0.97190
MBR_a	**MBR_b**	**0.04804**	**0.00525**	0.48026	**0.01225**	0.05120
Hybrid MBBR-MBR_b	**Pure MBBR-MBR**	**0.00029**	**0.00016**	0.25946	**0.03126**	0.86867
HRT=6 h						
MBR_a	**Hybrid MBBR-MBR_b**	0.06519	0.79999	0.99110	0.89674	0.99995
MBR_a	**Pure MBBR-MBR**	**0.00450**	0.22043	0.78543	**0.04478**	0.99956
MBR_a	**MBR_b**	0.06309	**0.00520**	0.93273	0.89994	0.21317
Hybrid MBBR-MBR_b	**Pure MBBR-MBR**	**0.00030**	0.89992	0.23252	**0.03480**	0.99999

The physical process of ultrafiltration must naturally have a minimum flow of suspended solids through the membrane, as can be seen in the values of TSS for the effluents of the MBR_a, MBR_b, hybrid MBBR-MBR_b and pure MBBR-MBR under the two working HRTs from Table IX.3. The differences between the pilot plants regarding the removal percentage of TSS were not statistically significant with the working HRTs of 9.5 h and 6 h, as the p-values obtained from the post hoc procedure, Tukey's HSD, were higher than α=0.05, as indicated in Table IX.4. This is logical as all the pilot plants contained a module of hollow-fiber ultrafiltration membranes in the membrane tank.

The concentrations of TN and TP in the influent and the effluents of the municipal WWTPs and the removal percentages of these nutrients under the two working HRTs are indicated in Table IX.3. The pure MBBR-MBR had the best performance in relation to TN removal if it was compared with the performance of the MBR_a and hybrid MBBR-MBR_b under the two working HRTs. The removal percentage of TN had a value of 71.91±16.04% under an HRT of 9.5 h and 63.21±11.01% under an HRT of 6 h in the pure MBBR-MBR, as shown in Table IX.3. The difference between the pure MBBR-MBR and hybrid MBBR-MBR_b regarding the removal percentage of TN was statistically significant with an HRT of 9.5 h, and the differences between the pure MBBR-MBR and the MBR_a and hybrid MBBR-MBR_b systems were also statistically significant under an HRT of 6 h as the p-values obtained were lower than α=0.05 (Table

IX.4). These results indicate that the nitrification and denitrification processes were more effective in the pure MBBR-MBR. The MLSS concentrations in the pure MBBR-MBR were very low under the two working HRTs (208.00±61.30 mg L^{-1} and 258.75±79.99 mg L^{-1} for an HRT of 9.5 h and 6 h, respectively) in relation to those of the MBR_a and hybrid MBBR-MBR_b. The biomass growth was mainly developed on carriers as attached biomass, with values of BD of 1,920.45±127.16 mg L^{-1} and 2,070.00±202.97 mg L^{-1} for an HRT of 9.5 h and 6 h, respectively, and involved a better contact between nitrate and the microorganisms (Rusten et al., 1995). The nitrification took place at the carrier interface, which was an aerobic layer, and denitrification occurred in the deeper layer of the biofilm, where anoxic conditions were present. As a result, the removal efficiency of TN was the highest in the pure MBBR-MBR under the two working HRTs (Yang et al., 2009). Di Trapani et al. (2010) generally obtained similar performances in a hybrid MBBR with respect to TN removal, with similar values of HRT and SRT.

Furthermore, the MBR_b showed a slightly higher performance than the MBR_a regarding the TN removal under an HRT of 6 h, as shown in Table IX.3, without statistically significant differences. However, the MBR_b had a lower performance than the MBR_a concerning the TN removal with an HRT of 9.5 h (Table IX.3) and the differences between the two systems were statistically significant, as seen in Table IX.4, despite the fact that the MBR_b had a higher biomass concentration in the bioreactor (Table IX.1). This was probably due to the presence of a higher concentration of TN in the influent of the MBR_b with a value of 91.54±8.81 for an HRT of 9.5 h (Table IX.3).

The removal percentages of TN were lower for the WWTPs under an HRT of 6 h as the ammonium loading rate was higher.

The experimental plants also removed TP as shown in Table IX.3. The creation of small anaerobic zones in the anoxic compartments of each bioreactor as well as the physical process of ultrafiltration made the TP removal possible. However, these systems did not have a strict anaerobic zone to initialize the process of biological phosphorus removal (Kermani et al., 2009). Therefore, the differences between the pilot plants, regarding the removal percentage of TP, were not statistically significant with the two working HRTs, as the p-values obtained from the post hoc procedure, Tukey´s HSD, were higher than α=0.05, as indicated in Table IX.4.

3.3. Study of the nitrifying and denitrifying microbial populations in the pure MBBR-MBR system: Importance of AOB, NOB and DeNB

In this study, the identification and quantification of the nitrifying and denitrifying microbial populations present in the pure MBBR-MBR system under an HRT of 9.5 h was carried out to complement the research that was developed by Leyva-Díaz et al. (2015) for an MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b (**Chapter 4**). In this way, all the possible configurations regarding MBR and MBBR-MBR have been analyzed from the point of view of the nitrifying and denitrifying microbial populations.

The relative abundances of AOB, NOB, DeNB and other bacterial species growing in the mixed liquor and on the carriers of the pure MBBR-MBR system are shown in Figure IX.2.

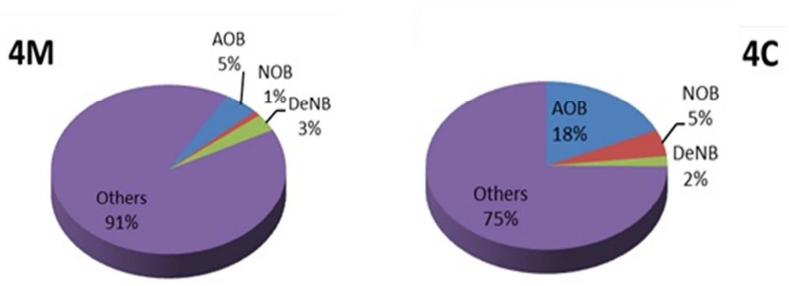

Figure IX.2. Percentage of AOB, NOB, DeNB and other bacteria in relation to the total bacteria in MLSS (M) and BD attached to carriers (C) in the pure MBBR-MBR. AOB (ammonium-oxidizing bacteria), NOB (nitrite-oxidizing bacteria), DeNB (denitrifying bacteria).

The AOB was more represented in the fixed biofilm with respect to the mixed liquor. The NOB showed a similar pattern to that of the AOB. On the other hand, the DeNB showed a higher relative abundance in the mixed liquor than on the carrier. The differences regarding the relative abundances of these four phylotype groups between the different systems studied by Leyva-Díaz et al. (2015) and the pure MBBR-MBR under an HRT of 9.5 h were smaller for the AOB and NOB. The relative abundance of the DeNB on the carriers showed that the hybrid MBBR-MBR_a, which contained carriers in the anoxic and aerobic zones of the bioreactor (Leyva-Díaz et al., 2015), accounted for the highest DeNB representation and the pure MBBR-MBR system accounted for the lowest DeNB representation. Nevertheless, the potential of the DeNB was higher in the hybrid MBBR-MBR_b containing carriers only in the aerobic zone of the bioreactor than in the other two systems of the study carried out by Leyva-Díaz et al.

(2015) and the pure MBBR-MBR system under an HRT of 9.5 h within the mixed liquor of the bioreactors of the different WWTPs. The results showed that the DeNB community structure was much more volatile than those corresponding to the AOB and NOB. In light of this, the stability of the AOB and NOB bacterial community structure with respect to the DeNB at the different operational conditions was suggested.

The differences in the bacterial community structure between the MBR and the two hybrid MBBR-MBR systems studied by Leyva-Díaz et al. (2015) were driven by the different operational conditions. In these cases, the AOB communities increased substantially with the addition of carriers, while the NOB communities did not experience any change. On the other hand, the DeNB were favored by the addition of carriers in the anoxic and aerobic zones of the bioreactor, while the addition of carriers only in the aerobic zone did not change their relative abundance substantially. In the case of the pure MBBR-MBR system under an HRT of 9.5 h, the working temperature changed (14.7±1.1ºC) and the decrease in the temperature (the three systems studied by Leyva-Díaz et al. (2015) worked at 17.2±1.9ºC) affected the AOB, NOB and DeNB. The AOB communities decreased in the suspended biomass but those developed on the carriers increased. For the NOB communities, no significant changes were found, but the DeNB experienced a strong decrease in the relative abundance in the suspended and attached biomass.

The TN removal turned out to be significantly higher in the pure MBBR-MBR than that obtained in the MBR_a and hybrid MBBR-MBR_b. This pattern could be explained by the higher AOB and NOB relative abundance on the carriers with respect to the other configurations studied by Leyva-Díaz et al. (2015).

3.4. Diversity and relative abundance of AOB, NOB and DeNB in the pure MBBR-MBR system

OTUs identified through pyrosequencing were related to important ecological roles in the nitrogen cycle inside the different WWTPs. Among them, ecological roles of ammonium oxidation, nitrite oxidation and nitrate/nitrite/nitrous oxide reduction were carried out by the AOB, NOB and DeNB, respectively. The diversity and relative abundance of these bacteria in the suspended biomass and fixed biofilm for the MBR and hybrid MBBR-MBR systems can be found in Leyva-Díaz et al. (2015) and for the

pure MBBR-MBR in Figure IX.2. It can be seen that the AOB accounted for higher relative abundance values than NOB or DeNB. Moreover, a Bray-Curtis similarity analysis comparing the AOB, NOB and DeNB community structure of all the WWTPs can be seen in Figure IX.3.

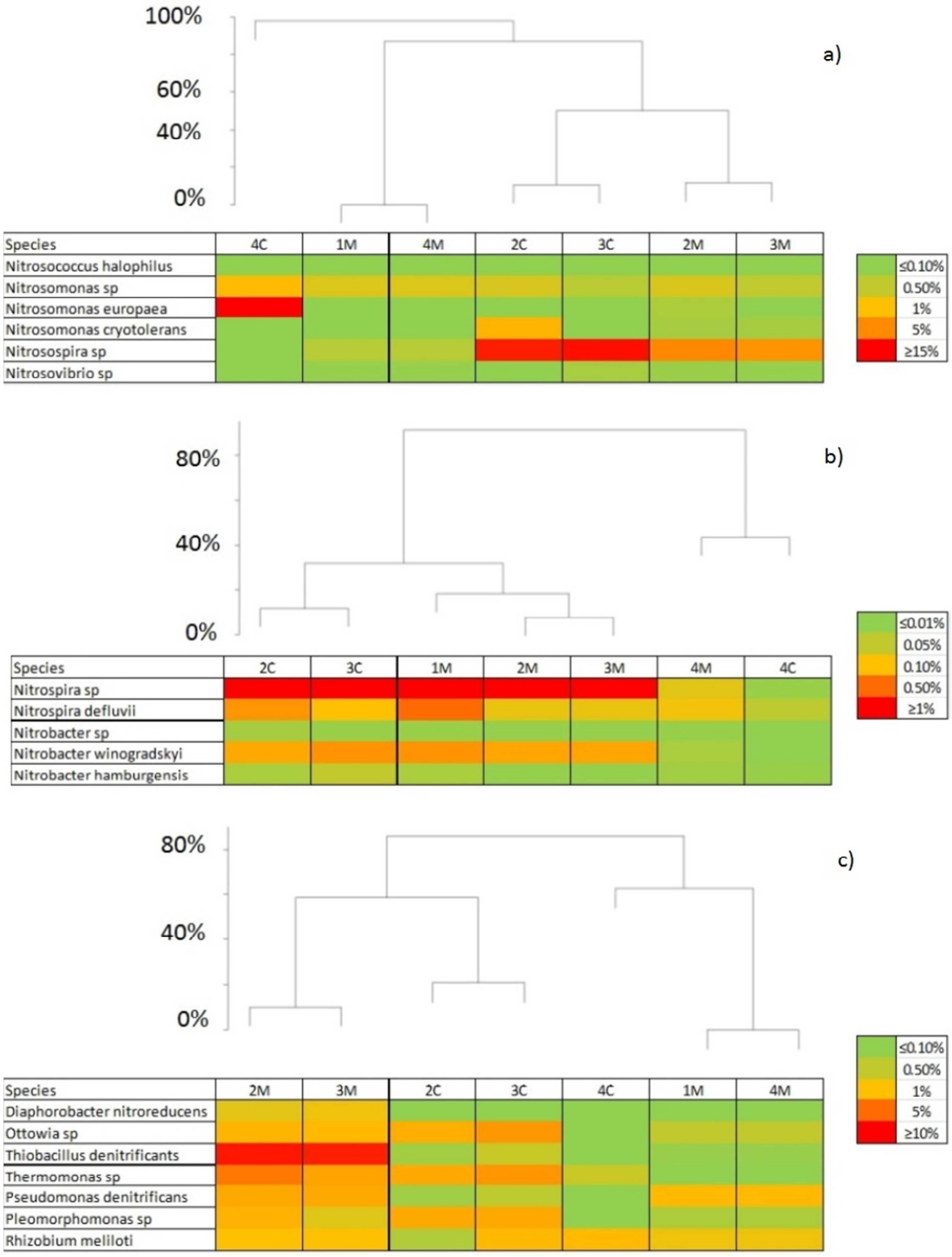

Figure IX.3. Bacterial community structure of AOB (a), NOB (b) and DeNB (c) in MLSS (M) and BD attached to carriers (C) in the MBR (1), hybrid MBBR-MBR_a (2) and hybrid MBBR-MBR_b (3) studied by Leyva-Díaz et al. (2015) and the pure MBBR-MBR (4) under an HRT of 9.5 h.

Samples from the same WWTP, i.e. planktonic biomass and fixed biofilm samples, had a remarkable similarity, with the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b being a consistent cluster, and the MBR and pure MBBR-MBR becoming

another. In general, the community structures of the AOB, NOB and DeNB showed clear similarities for the same growth conditions (planktonic growth and fixed biofilm growth). In this regard, the development of the AOB, NOB and DeNB was driven by the growth conditions of the biomass inside the WWTPs in a more important fashion than by the environmental conditions that characterized the different WWTPs.

This study showed that nitrifying populations were heterogeneous in the pure MBBR-MBR system with a large number of different species (Figure IX.4).

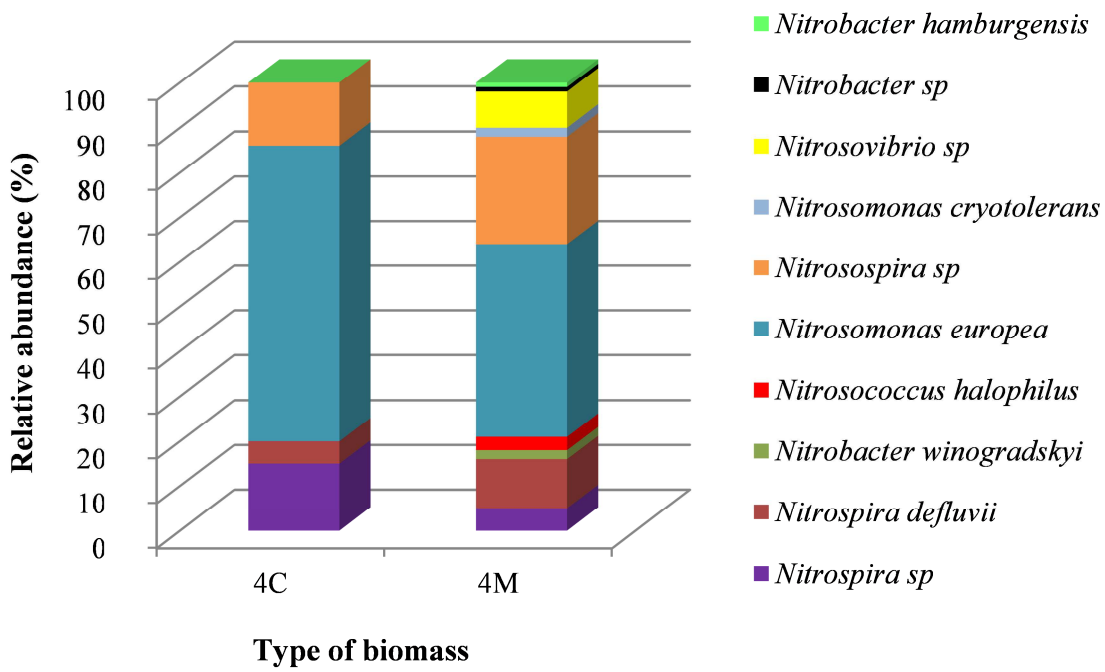

Figure IX.4. Relative abundance of the total nitrifying bacteria in MLSS (M) and BD attached to carriers (C) in the pure MBBR-MBR (4).

Among the AOB, species from the genera *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira* and *Nitrosovibrio* could be found in the pure MBBR-MBR system. All these phylotypes have been reported as AOB (González-Martínez et al., 2011). Nevertheless, the genera *Nitrosococcus* and *Nitrosovibrio* accounted for a low relative abundance. Therefore, *Nitrosomonas*- and *Nitrosospira*-related species were the most important bacteria driving the ammonium oxidation in the pure MBBR-MBR system. *Nitrosospira* sp. was the dominant AOB in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, while *Nitrosomonas europaea* and *Nitrosomonas* sp. were dominant in the MBR and pure MBBR-MBR (Leyva-Díaz et al., 2015). It has been reported that *Nitrosomonas* species are r-strategists, while *Nitrosospira* species are k-strategists

(Terada et al., 2013). As a result, *Nitrosomonas* will be favored by high ammonium concentrations in the environment.

The diversity of NOB in the pure MBBR-MBR showed species belonging to the genera *Nitrospira* or *Nitrobacter*, which have been reported as NOB (González-Martínez et al., 2011). The *Nitrospira* genus dominated over *Nitrobacter* in the bioreactors of all the WWTPs. *Nitrospira* sp. largely represented the most important NOB for the WWTPs. It has been found that high nitrite concentrations promote the growth of *Nitrobacter* species over *Nitrospira* species (Ter Haseborg et al., 2010). This was in accordance with the low nitrite concentrations shown in Table IX.3 of this study and those obtained by Leyva-Díaz et al. (2015), which could be the reason why *Nitrospira* sp. was the most important NOB in the WWTPs.

The DeNB were more diverse than the AOB and NOB in the pure MBBR-MBR system, with species from seven genera thriving within the bioreactor, i.e. *Diaphorobacter*, *Ottowia*, *Thiobacillus*, *Thermomonas*, *Pseudomonas*, *Pleomorphomonas* and *Rhizobium*. In the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, *Ottowia* sp., *Thermomonas* sp., *Pseudomonas denitrificans*, *Rhizobium melitoti* and *Pleomorphomonas* sp. were the dominant DeNB. The species of *Ottowia* has been reported for nitrate and nitrite reduction (Spring et al., 2004; Geng et al., 2014). FISH analysis of nitrifying communities suggests that the *Thermomonas* species thrives on the metabolites produced by AOB and NOB (Dolinšek et al., 2013) and has been reported for nitrite and nitrate reduction (Mergaert et al., 2003). The reduction of nitrate by *Pseudomonas denitrificans* has been proved (Parvanova-Mancheva and Beschkov, 2009). The *Pleomorphomonas* genus strain type *Pleomorphomonas oryzae* has shown nitrate reduction ability (Xie and Yokota, 2005). In the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, there were differences between the planktonic biomass and fixed biofilm, with a much higher relative abundance of *Thiobacillus denitrificans* in the mixed liquor than on the carriers. *Thiobacillus denitrificans* has been identified as sulfur-oxidizing denitrifying bacteria (Sahinkaya et al., 2013; Kim et al., 2014). In this regard, a high *Thiobacillus denitrificans* relative abundance in the mixed liquor could be related to its higher sulfur concentration with respect to the fixed biofilm. The dominant DeNB in the MBR and pure MBBR-MBR were *Rhizobium melitoti* and *Pseudomonas denitrificans*.

The higher TN removal in the pure MBBR-MBR in comparison to the other WWTPs might also reside in the different bacterial assemblages in the fixed biofilm on the carriers. The Bray-Curtis similarity analysis showed the bacterial community structure of the fixed biofilm of the pure MBBR-MBR to be different for AOB, NOB and DeNB with respect to the other WWTPs.

3.5. Kinetic modeling of MBR_a, MBR_b, hybrid MBBR-MBR_b and pure MBBR-MBR

The concentrations of nitrifying bacteria and heterotrophic bacteria were necessary for the assessment of the different kinetic parameters. Table IX.5 is based on the MLVSS and the VBD (Table IX.1) as well as the percentages of nitrifying bacteria (AOB and NOB), denitrifying bacteria and heterotrophic bacteria in the MLSS and BD attached to the carriers for the MBR and hybrid MBBR-MBR systems (Leyva-Díaz et al., 2015) and the pure MBBR-MBR system under an HRT of 9.5 h (Figure IX.2).

Table IX.5. Total concentration of nitrifying bacteria (AOB and NOB), denitrifying bacteria (DeNB) and heterotrophic bacteria as MLVSS concentration and attached VBD in the experimental plants. AOB (ammonium-oxidizing bacteria), NOB (nitrite-oxidizing bacteria), MLVSS (mixed liquor volatile suspended solids), VBD (volatile biofilm density).

Microbial population	Total biomass concentration					
	MBR_a	MBR_b	Hybrid $MBBR-MBR_b$		Pure MBBR-MBR	
	MLVSS (mg L^{-1})	MLVSS (mg L^{-1})	MLVSS (mg L^{-1})	VBD (mg L^{-1})	MLVSS (mg L^{-1})	VBD (mg L^{-1})
HRT=9.5 h						
Nitrifying bacteria	69.87±8.52	171.45±14.04	130.37±16.49	156.40±19.54	9.22±2.71	371.51±24.60
Ammonium-oxidizing bacteria (AOB)	23.29±2.84	57.15±4.68	114.07±14.43	130.33±16.28	7.68±2.26	290.75±19.25
Nitrite-oxidizing bacteria (NOB)	46.58±5.68	114.30±9.36	16.30±2.06	26.07±3.26	1.54±0.45	80.76±5.35
Denitrifying bacteria (DeNB)	93.16±11.36	228.59±18.72	325.92±41.24	69.51±8.68	4.61±1.36	32.31±2.14
Heterotrophic bacteria	1,816.60±221.47 (78%)	4,457.58±364.97 (78%)	1,254.78±158.77 (77%)	642.94±80.30 (74%)	112.14±33.05 (73%)	1,098.37±72.73 (68%)
HRT=6 h						
Nitrifying bacteria	66.89±9.21	170.31±6.63	151.50±14.65	120.13±17.98	11.76±3.64	417.34±40.93
Ammonium-oxidizing bacteria (AOB)	22.30±3.07	56.77±2.21	132.56±12.82	100.11±14.98	9.80±3.03	326.61±32.03
Nitrite-oxidizing bacteria (NOB)	44.59±6.14	113.54±4.42	18.94±1.83	20.02±3.00	1.96±0.61	90.73±8.90
Denitrifying bacteria (DeNB)	89.18±12.27	227.09±8.84	378.73±36.62	53.39±7.99	5.88±1.82	36.29±3.56
Heterotrophic bacteria	1,739.08±239.33 (78%)	4,428.20±172.45 (78%)	1,458.13±140.99 (77%)	493.88±73.88 (74%)	143.09±44.23 (73%)	1,233.86±120.98 (68%)

3.5.1. Kinetic parameters for heterotrophic biomass

Table IX.6 shows the parameters that fit the Monod model for the heterotrophic biomass contained in each of the bioreactors under the operational HRTs of 9.5 h and 6 h, the yield coefficient (Y_H), the maximum specific growth rate ($\mu_{m,\ H}$) and the half-saturation coefficient for organic matter (K_M).

Table IX.6. Kinetic parameters for the characterization of heterotrophic and autotrophic biomass. Y_H (yield coefficient for heterotrophic biomass), $\mu_{m,\ H}$ (maximum specific growth rate for heterotrophic biomass), K_M (half-saturation coefficient for organic matter), Y_A (yield coefficient for autotrophic biomass), $\mu_{m,\ A}$ (maximum specific growth rate for autotrophic biomass), K_{NH} (half-saturation coefficient for ammonia nitrogen), Y_{NOB} (yield coefficient for nitrite-oxidizing bacteria), $\mu_{m,\ NOB}$ (maximum specific growth rate for nitrite-oxidizing bacteria), K_{NOB} (half-saturation coefficient for nitrite-nitrogen), k_d (decay coefficient for autotrophic and heterotrophic biomass).

Parameter	Sampling zone			
	MBR_a	MBR_b	Hybrid MBBR-MBR_b	Pure MBBR-MBR
HRT=9.5 h				
Heterotrophic bacteria				
Y_H (mg VSS mg COD^{-1})	0.4994	0.6016	0.5519	0.5093
$\mu_{m,\ H}$ (h^{-1})	0.0135	0.0173	0.0155	0.0181
K_M (mg O_2 L^{-1})	6.7662	9.1926	4.2454	2.6791
Autotrophic bacteria				
Y_A (mg O_2 mg N^{-1})	1.5568	2.9304	1.5891	2.3465
$\mu_{m,\ A}$ (h^{-1})	0.1328	0.2169	0.1434	0.7169
K_{NH} (mg N L^{-1})	0.8913	0.8622	1.5984	2.0748
Nitrite-oxidizing bacteria				
Y_{NOB} (mg O_2 mg N^{-1})	0.6044	0.8484	0.4918	0.5897
$\mu_{m,\ NOB}$ (h^{-1})	0.0992	0.0967	0.0912	0.0336
K_{NOB} (mg N L^{-1})	1.2118	0.3989	0.3370	0.1404
Total bacteria				
k_d (d^{-1})	0.0440	0.0309	0.0750	0.1150
HRT=6 h				
Heterotrophic bacteria				
Y_H (mg VSS mg COD^{-1})	0.5632	0.5809	0.5756	0.5941
$\mu_{m,\ H}$ (h^{-1})	0.0255	0.0114	0.0658	0.0292
K_M (mg O_2 L^{-1})	7.0629	5.5141	18.9121	2.9681
Autotrophic bacteria				
Y_A (mg O_2 mg N^{-1})	1.9591	2.5174	2.2366	2.3657
$\mu_{m,\ A}$ (h^{-1})	0.1607	0.4669	0.2250	0.3591
K_{NH} (mg N L^{-1})	0.3887	2.8433	2.4179	3.1582
Nitrite-oxidizing bacteria				
Y_{NOB} (mg O_2 mg N^{-1})	0.5746	0.8943	0.4918	0.4989
$\mu_{m,\ NOB}$ (h^{-1})	0.1482	0.1774	0.2478	0.1828
K_{NOB} (mg N L^{-1})	0.5809	1.1449	1.0025	1.2601
Total bacteria				
k_d (d^{-1})	0.0366	0.0337	0.0390	0.0982

The biomass of the hybrid MBBR-MBR_b showed a performance that was better than those corresponding to the MBR_a and pure MBBR-MBR under the two working HRTs according to the evaluation of the substrate degradation rate (r_{su}), depending on the kinetic parameters and the substrate and biomass concentrations, shown in Figure IX.5a and Figure IX.5d. Thus, the heterotrophic biomass of the hybrid MBBR-MBR_b required less time for organic matter oxidation under the operational conditions of this research. Moreover, this meant that the detection of the μ_m could be carried out with less available substrate in the hybrid MBBR-MBR_b and less time would be required to accomplish a steady state under the experimental conditions of this study. This was in accordance with the highest COD removal efficiencies of the hybrid MBBR-MBR_b under the two working HRTs, with values of 87.39±6.01% for 9.5 h and 84.10±2.25% for 6 h, as indicated in Table IX.3. Figure IX.5a and Figure IX.5d also show that the MBR_b had a better kinetic behavior than the MBR_a under the two operational HRTs as the MLSS concentration was higher in the MBR_b, which supported the removal percentages of COD of the MBR_a and MBR_b.

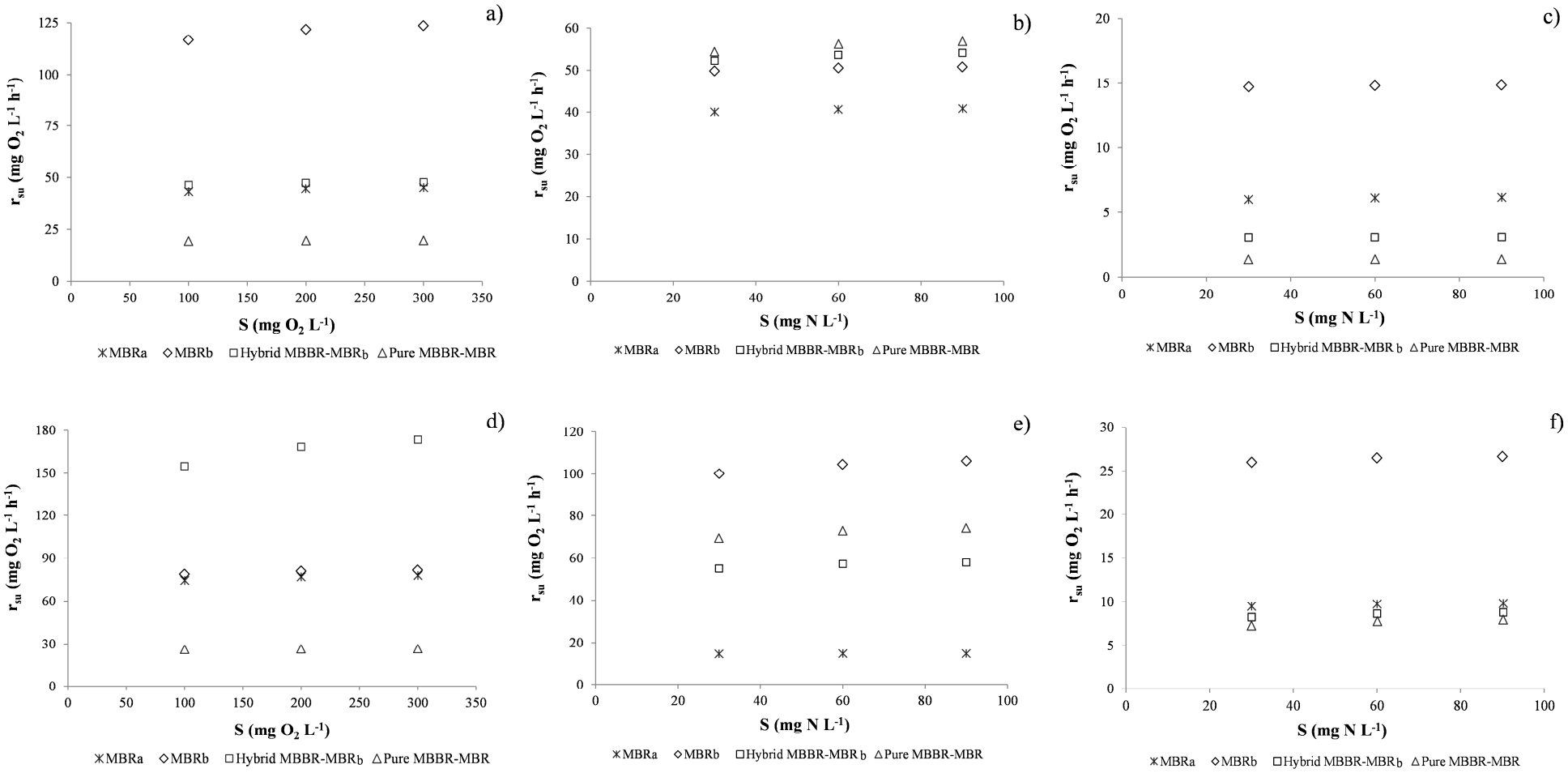

Figure IX.5. Substrate degradation rate (r_{su}) obtained in the biological kinetic study depending on the substrate concentration for the different bioreactors from the WWTPs. (a) Heterotrophic bacteria under an HRT of 9.5 h. (b) Autotrophic bacteria under an HRT of 9.5 h. (c) Nitrite-oxidizing bacteria under an HRT of 9.5 h. (d) Heterotrophic bacteria under an HRT of 6 h. (e) Autotrophic bacteria under an HRT of 6 h. (f) Nitrite-oxidizing bacteria under an HRT of 6 h.

Henze el al. (1987) and Plattes et al. (2007) obtained similar values of Y_H in an MBBR. Gujer et al. (1999) proposed 2 d^{-1} at 20ºC as the default $\mu_{m,\ H}$ value in ASM3. This value was higher than those obtained in this study. However, Canziani et al. (2006) and Seifi and Fazaelipoor (2012) reported similar values to those obtained in this research regarding $\mu_{m,\ H}$ and K_M, respectively, in an MBR and an MBBR.

3.5.2. Kinetic parameters for autotrophic biomass

Table IX.6 also shows the parameters that fit the Monod model for the autotrophic biomass contained in each of the bioreactors, the yield coefficient (Y_A), the maximum specific growth rate ($\mu_{m,\ A}$) and the half-saturation coefficient for ammonia nitrogen (K_{NH}). According to these kinetic parameters and the assessment of the r_{su}, the biomass of the pure MBBR-MBR showed a performance that was better than those corresponding to the MBR_a and hybrid MBBR-MBR_b under the two working HRTs (Figure IX.5b and Figure IX.5e). Therefore, the autotrophic biomass of the pure MBBR-MBR required less time for the oxidation of nitrogen contained in the influent under the operational conditions. The detection of the μ_m was carried out with less available substrate in the pure MBBR-MBR and less time would be required to accomplish a steady state under the experimental conditions of this study. This was supported by the fact that there was no competition between attached and suspended biomass as the MLSS concentration was very low in the pure MBBR-MBR. Therefore, the attached biomass had an enormous quantity of available substrate and there was a better accessibility to it by this kind of biomass in the pure MBBR-MBR. In general, these results were in accordance with the performances of TN removal of the WWTPs. The pure MBBR-MBR was the pilot plant with the highest percentage of TN removal (71.91±16.04% under an HRT of 9.5 h and 63.21±11.01% under an HRT of 6 h), as shown in Table IX.3, as it had the best kinetic behavior when the r_{su} was evaluated taking into account the kinetic parameters for autotrophic biomass. Figure IX.5b and Figure IX.5e also show that the MBR_b had a better kinetic performance than the MBR_a under the two operational HRTs as occurred in the heterotrophic kinetics.

The values of Y_A reported by Seifi and Fazaelipoor (2012) and Di Trapani et al. (2008) were slightly lower than those obtained in this study. Seifi and Fazaelipoor (2012) and Henze el al. (1987) had similar values to those obtained in this research regarding $\mu_{m,\ A}$ and K_{NH}, respectively, in an MBBR.

3.5.3. Kinetic parameters for nitrite-oxidizing bacteria

From the point of view of the NOB, the MBR_a showed the best kinetic performance under the two operational HRTs, with values of Y_{NOB} = 0.6044 mg O_2 mg N^{-1}, $\mu_{m,\ NOB}$ = 0.0992 h^{-1} and K_{NOB} = 1.2118 mg N L^{-1} for an HRT of 9.5 h and Y_{NOB} = 0.5746 mg O_2 mg N^{-1}, $\mu_{m,\ NOB}$ = 0.1482 h^{-1} and K_{NOB} = 0.5809 mg N L^{-1} for an HRT of 6 h (Henze et al., 2000; Pambrun et al., 2006; Iacopozzi et al., 2007), as shown in Figure IX.5c and Figure IX.5f. This supported the fact that the nitrate concentration in the effluent from the MBR_a was higher than those from the hybrid MBBR-MBR_b and pure MBBR-MBR under the two working HRTs (Table IX.3). Consequently, the pure MBBR-MBR could have a better kinetic behavior regarding the AOB because, on the whole, the kinetics of autotrophic bacteria was better in this system, as previously mentioned, and the pure MBBR-MBR had the highest nitrite concentration in its effluent (Table IX.3). Furthermore, Figure IX.5c and Figure IX.5f show that the MBR_b had a higher r_{su} than the MBR_a due to its higher MLSS concentration. There were statistically significant differences regarding nitrite and nitrate formations between the MBR_a and pure MBBR-MBR as the p-values obtained were less than α=0.05, p-value $_{\text{MBRa-Pure MBBR-MBR}}$ (NO_2^-) = 0.00027 and p-value $_{\text{MBRa-Pure MBBR-MBR}}$ (NO_3^-) = 0.01095 for an HRT of 9.5 h and p-value $_{\text{MBRa-Pure MBBR-MBR}}$ (NO_2^-) = 0.01315 and p-value $_{\text{MBRa-Pure MBBR-MBR}}$ (NO_3^-) = 0.02289 for an HRT of 6 h. Similar conclusions were drawn in **Chapter 3** and **Chapter 5** with similar configurations of WWTPs under an HRT of 18 h.

3.5.4. Decay coefficient for autotrophic and heterotrophic biomass

The values of k_d under the working HRTs of 9.5 h and 6 h are also indicated in Table IX.6. The k_d for the biomass contained in the bioreactor of the pure MBBR-MBR was the highest under the two working HRTs. This meant that 11.50% (for an HRT of 9.5 h) and 9.82% (for an HRT of 6 h) of the total quantity of biomass represented the quantity of biomass oxidized per day. The values of SRT in the pure MBBR-MBR were the lowest compared to the MBR_a and hybrid MBBR-MBR_b, with a value of 6 days under an HRT of 9.5 h and 4.5 days under an HRT of 6 h, as the flow rate of waste sludge had to be higher than those corresponding to the MBR_a and hybrid MBBR-MBR_b in order to maintain a very low MLSS concentration inside the bioreactor of the pure MBBR-MBR. Therefore, the biomass decay rate will be higher because the organic

loading rate was identical in the other WWTPs, but the MLSS concentration was lower in the pure MBBR-MBR. The same reason explained the higher values of k_d for the MBR_a (low MLSS concentration) in relation to the MBR_b (high MLSS concentration). The values of k_d obtained in this study were in the range reported in the literature (Metcalf, 2003).

4. Conclusions

The following conclusions were drawn:

1. This study demonstrated that the pure MBBR-MBR had the highest potential to remove TN from the municipal wastewater, with efficiencies of TN removal of 71.91±16.04% and 63.21±11.01% for an HRT of 9.5 h and 6 h, respectively, as the attached biomass had an enormous quantity of available substrate and there was a better accessibility to it by this kind of biomass. The hybrid MBBR-MBR_b showed the best performance of COD removal as this system had a better heterotrophic kinetic performance for the two working HRTs. The effect of the attached biomass enhanced the organic matter and total nitrogen removal, but a pure MBBR-MBR without biomass recycling was necessary to obtain the highest efficiency of total nitrogen removal.

2. The microbial ecology analysis showed that the AOB and NOB populations were more stable in terms of community structure than the DeNB in the MBR, hybrid MBBR-MBR_b and pure MBBR-MBR. The growth state of the biomass influenced the AOB, NOB and DeNB community structure as the fixed biofilm from the pure MBBR-MBR had a higher relative abundance of AOB and NOB, which supported the best kinetic performance for the autotrophic biomass in the pure MBBR-MBR, while DeNB throve better in planktonic biomass. The differences between the AOB, NOB and DeNB community structure were more related to the growth state of the biomass than to the operational conditions in the different plants. The Bray-Curtis similarity analysis showed the bacterial community structure of the fixed biofilm of the pure MBBR-MBR to be different for AOB, NOB and DeNB with respect to the other WWTPs, which could explain the higher total nitrogen removal.

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X. CHAPTER 7

Biological phosphorus removal from municipal wastewater in hybrid moving bed biofilm reactor-membrane bioreactor systems (operational conditions of HRT=18 h and high biomass concentrations).

Abstract

A membrane bioreactor (MBR_p), a hybrid moving bed biofilm reactor-membrane bioreactor containing carriers in the anaerobic, anoxic and aerobic zones (hybrid MBBR-MBR_{ap}) and a hybrid moving bed biofilm reactor-membrane bioreactor which contained carriers only in the anaerobic and anoxic compartments (hybrid MBBR-MBR_{bp}) were used in parallel and compared regarding the nutrient and organic matter removal from municipal wastewater. The hydraulic retention time (HRT) was 18 h. A kinetic study for the heterotrophic and autotrophic bacteria, mainly nitrite-oxidizing bacteria (NOB), was carried out and related to the nutrient and organic matter removal. The hybrid MBBR-MBR_{ap} performed best regarding chemical oxygen demand (COD) and total phosphorus (TP) removals, with values of 85.82±2.12% and 81.42±3.85%, respectively. This system had a higher phosphorus release under anaerobic conditions and a higher phosphorus uptake under aerobic conditions. The highest TN removal efficiency was obtained for the hybrid MBBR-MBR_{bp}, with a value of 61.39±10.71%. Moreover, the effluent from the MBR_p contained the highest concentration of nitrate, with a value of 153.45±59.25 mg NO_3^- L^{-1}. These results were supported by the kinetic study for the heterotrophic, autotrophic and nitrite-oxidizing bacteria.

1. Introduction

Wastewater with high levels of phosphorus and nitrogen can be the main reason for several problems when released into the environment, such as oxygen consumption, eutrophication and toxicity (Luostarinen et al., 2006). In this way, biological nutrient removal (BNR) processes have been developed to remove these nutrients from wastewater, which is crucial to the environmental protection (Mulkerrins et al., 2004; Yang et al., 2010). Phosphorus removal techniques can be divided into three main categories: physical, chemical and biological. Physical methods have proved to be either too expensive, as in the cases of electrodialysis and reverse osmosis, or inefficient, removing only 10% of the total phosphorus (TP) (Yeoman et al., 1988). Chemical removal techniques have the problem of the chemical costs and the high sludge production (Helness and Ødegaard, 1999). Biological methods can remove up to 98% of the TP. These processes, with their economic advantages over physical and chemical treatment methods, have been widely used in existing wastewater treatment plants (WWTPs) to overcome the eutrophication problem in receiving waters.

The BNR process could include an enhanced biological phosphorus removal (EBPR) process with the application of an anaerobic-aerobic sequence enabling the growth of polyphosphate accumulative organisms (PAOs), which store large amounts of phosphorus as polyphosphates. In light of this, some improved wastewater treatment processes based on the anaerobic/anoxic/oxic (AAO) system have been developed to remove phosphorus and nitrogen from wastewater (Esakki Raj et al., 2013; Uan et al., 2013). Additionally, nitrogen removal can be achieved in an AAO system containing an anoxic-aerobic sequence which allows for removing the nitrogen as a final product of nitrogen gas by the combination of nitrification by autotrophs under aerobic conditions and denitrification by heterotrophs under anoxic conditions (Wang et al., 2006). According to current knowledge of biological phosphorus removal, under anaerobic conditions PAOs use the energy released from the hydrolysis of intracellular polyphosphate to transport volatile fatty acids (VFAs) across their cell membranes and, hence, produce polyhydroxybutyrate (PHB). The phosphate is released in connection with the storage of organic matter under anaerobic conditions. Under aerobic or anoxic conditions, PHB serves as an energy source for cell growth and storage of excess polyphosphate. The accumulation of phosphate beyond that needed for normal cell

growth is commonly known as EBPR since it results in a net uptake of phosphorus over the cycle (Yang et al., 2010).

As a consequence, advanced technologies for wastewater treatment have been developed to control stricter effluent limits regarding phosphorus and nitrogen as well as organic matter or upgrade existing overloaded activated sludge plants (Wang et al., 2006). The membrane bioreactor (MBR) is an efficient technology for treating domestic wastewater to a very high quality. This system combines a biological process and a membrane filtration process, the latter using either microfiltration or ultrafiltration (Kim et al., 2011). The MBR enjoys many advantages over the conventional activated sludge process for wastewater treatment, in terms of both treatment efficiency and process control (Miura et al., 2007). Despite all the advantages of the MBR, it has the problem of the membrane fouling which increases the operational costs (high energy and chemical costs) and shortens the life of the membrane (Rahimi et al., 2011a; Huyskens et al., 2012). An alternative to the MBR is the use of the moving bed biofilm reactor-membrane bioreactor (MBBR-MBR), which has not the problems of the activated sludge processes and may reduce the problems of the MBR regarding the effect of membrane fouling by high biomass concentrations (Leiknes and Ødegaard, 2007). This technology has emerged as a promising process for the enhancement of nitrification, denitrification and phosphorus removal, particularly when there are space limitations or necessary modifications that will require large moneraty expenses (Hooshyari et al., 2009). Several studies have evaluated the performance and affecting factors of coupling membrane filtration with a moving bed biofilm reactor (MBBR) (Melin et al., 2005; Lee et al., 2006). The MBBR-MBR has many advantages over the MBR such as less sludge production rate due to high sludge retention time (SRT), higher organic loading rates, less suspended solids concentration that results in less membrane fouling, better oxygen transfer, higher biological reaction rates through the accumulation of high concentrations of active biomass, simultaneous nitrification-denitrification and phosphorus removal due to oxygen gradient in biomass layer attached to the carrier, a larger surface area for mass transfer and high resistance of attaches biomass to overloading and toxic compounds (Hasar, 2009; Rahimi et al., 2011b). In this study, two hybrid MBBR-MBR systems were used with different distribution of carriers in the different zones of each bioreactor; they combined suspended and attached biomass

inside the bioreactor since there was recycling between the membrane tank and the MBBR (Mannina and Viviani, 2009).

In this context, biofilms, which grow on the carriers of the MBBR-MBR systems, bear great potential for the simultaneous and efficient removal of organic carbon and nutrients like phosphorus and nitrogen in wastewater treatment (Watanabe et al., 1995; Pastorelli et al., 1999). They are spatially heterogeneous, providing space for anaerobic, anoxic and aerobic processes; they are well suited for nitrification since attached growth of the slow-growing nitrifying bacteria protects them from washout and the combination of aerobic and anoxic conditions facilitates the denitrification process; and they can be exposed to alternating anaerobic and aerobic conditions as necessary for EBPR (Gieseke et al., 2002). First examples of multiple nutrient removal in biofilm systems have already been demonstrated (Pastorelli et al., 1999; Helness, 2007). As nitrification and phosphorus removal both consume oxygen, organisms in such a system are potentially subjected to competition for oxygen. Nitrifying bacteria in pure culture are known to have a lower affinity for oxygen compared to heterotrophic bacteria as, e.g., the PAOs, which may result in problems when integrating nitrifying activity (Prosser, 1989). However, recent studies suggest the affinities of certain nitrifying bacteria for oxygen to be relatively high (Schramm et al., 2000). Furthermore, the phosphorus and nitrogen removal require chemical oxygen demand (COD), which is often the limiting substrate in the incoming wastewater, in BNR systems. Thus, the denitrification could complicate the EBPR since the denitrifying bacteria (DeNB) consume a portion of the substrate before the substrate can be utilized by the PAOs, in other words, the transfer of nitrate into the anaerobic phase could inhibit the phosphate release (Kuba et al., 1994; Akin and Ugurlu, 2004). The use of carriers or aerobic granular sludge instead of activated sludge can resolve this conflict (Wang et al., 2009). Attached-growth biofilm can form aerobic, anoxic and anaerobic zones along the direction of the mass transfer, providing favorable environment for the simultaneous nitrification and denitrification (Puznana et al., 2000; Yang et al., 2009). It could be assumed that the biofilm can improve the nitrogen removal in the aerobic phase, and inhibit the transfer of nitrate into the anaerobic phase. As a result, the conflict on simultaneous nitrogen and phosphorus removal could be resolved. Therefore, understanding the underlying mechanisms of coexistence and competition of the organisms involved is essential to reliably set up nitrification and denitrification in the EBPR system (Gieseke et al., 2002).

Additionally, there are still some uncertainties regarding the kinetic behavior of the MBBR-MBR, particularly the kinetic behavior of nitrite-oxidizing bacteria (NOB) which are involved in the nitrification process (Rongsayamanont et al., 2010), due to the coexistence of suspended and attached biomass which could modify the kinetics of both biomasses, compared with processes involving pure suspended or attached biomass (Di Trapani et al., 2010). In this regard, it should be noted the importance of the kinetic modeling for the design, evaluation, control and prediction of the behavior of the biological processes which take part in the wastewater treatment (Hvala et al., 2002). Thus, a kinetic study was carried out for the heterotrophic, autotrophic and nitrite-oxidizing bacteria.

The aim of this study was to evaluate the phosphorus and nitrogen removal by applying three lab-scale WWTPs based on the AAO system. The WWTPs consisted of an MBR and two hybrid MBBR-MBR processes which were operated for 133 days in parallel under the same operational and environmental conditions. The performances regarding the phosphorus, nitrogen and organic matter removal were investigated, evaluated and supported by a kinetic study for heterotrophic, autotrophic and nitrite-oxidizing bacteria.

2. Materials and methods

2.1. Description of the wastewater treatment plants

The experiments were conducted by using three lab-scale WWTPs, working in parallel, which were fed with municipal wastewater from an influent tank by a feeding peristaltic pump (323S, Watson-Marlow Pumps Group, USA). Real wastewater came from the outlet of the primary settler of a wastewater treatment plant (WWTP) in Granada (Spain). The WWTPs consisted of an MBR_p (Figure X.1a), a hybrid MBBR-MBR system containing carriers in the anaerobic, anoxic and aerobic zones of the bioreactor (hybrid MBBR-MBR_{ap}) (Figure X.1b) and a hybrid MBBR-MBR system which contained carriers only in the anaerobic and anoxic zones of the bioreactor (hybrid MBBR-MBR_{bp}) (Figure X.1c). The carrier used in the hybrid MBBR-MBR systems was called K1 and was previously studied in similar experiments (Melin et al., 2005; Di Trapani et al., 2008).

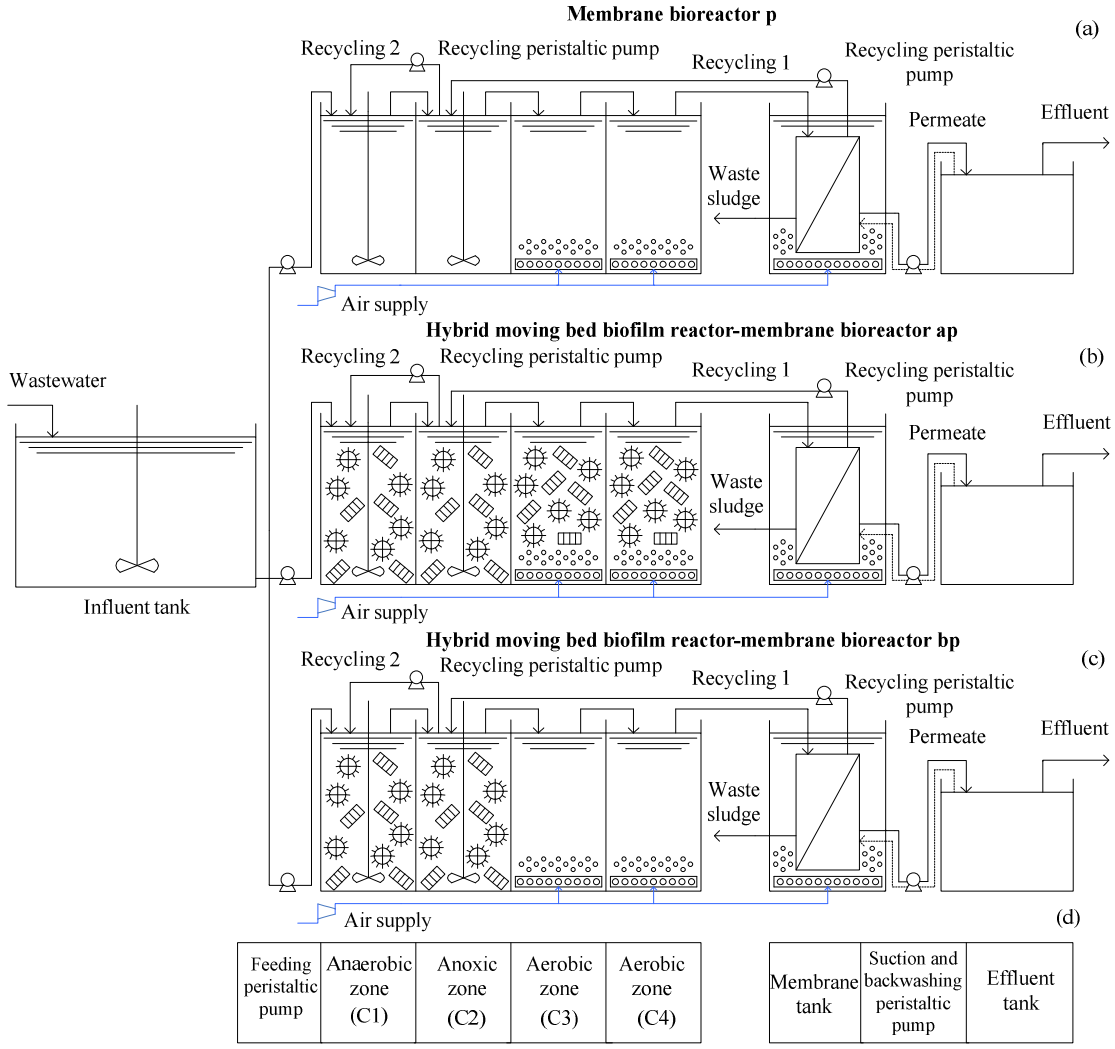

Figure X.1. Diagram of the experimental pilot plants. (a) Membrane bioreactor (MBR_p). (b) Hybrid moving bed biofilm reactor-membrane bioreactor containing carriers in the anaerobic, anoxic and aerobic zones of the bioreactor (hybrid MBBR-MBR_{ap}) (c) Hybrid moving bed biofilm reactor-membrane bioreactor containing carriers in the anaerobic and anoxic zones of the bioreactor (hybrid MBBR-MBR_{bp}).

The reactor zones, the membrane tank, the effluent tank and some peristaltic pumps are shown in Figure X.1d. The bioreactors of the WWTPs were divided into four zones (C1, C2, C3 and C4), i.e. one anaerobic zone (C1), one anoxic zone (C2) and two aerobic zones (C3 and C4). The dimensions of the bioreactor were 50 cm long, 12 cm wide and 60 cm high and the working volume was 24 L. The membrane tank was cylindrical, had a diameter of 10 cm, a height of 65 cm and a working volume of 4.32 L.

Municipal wastewater was pumped into the first anaerobic chamber (C1) of the bioreactor from the influent tank. Phosphate was released and COD was partially consumed under anaerobic conditions. Then, it went through the anoxic zone (C2) and

the rest of the aerobic compartments by a communicating vessel system. The aerobic zones (C3 and C4) had the purpose of the organic matter oxidation, nitrification and phosphate accumulation. Recycling 1 consisted of a nitrate recirculation from the membrane tank to the anoxic chamber of the bioreactor which allowed the nitrogen removal and minimized the effect of nitrate in wastewater entering the anaerobic zone. Recycling 2 based on an anoxic recirculation which increased the organic matter utilization and provided the optimal conditions for fermentation uptake in the anaerobic compartment. The recirculation rates of Recycling 1 and Recycling 2 were two times the influent flow rate (Kermani et al., 2009), which had a value of 1.6 L h^{-1} (Table X.1). Furthermore, Recycling 1 and Recycling 2 were necessary for maintaining the working mixed liquor suspended solids (MLSS) concentration inside each bioreactor.

The outlet of the bioreactor was led into the membrane tank and the permeate was extracted through the membrane by a suction-backwashing peristaltic pump (323U, Watson-Marlow Pumps Group, USA) to collect it into the effluent tank. A cyclic mode of operation was carried out by production and backwashing periods of 9 min and 1 min, respectively. The operational conditions of the WWTPs are shown in Table X.1.

Table X.1. Operational conditions and working concentrations of MLSS and attached BD in the steady state of the experimental plants. MLSS (mixed liquor suspended solids), BD (biofilm density).

Parameter	MBR_p			Hybrid $MBBR\text{-}MBR_{ap}$			Hybrid $MBBR\text{-}MBR_{bp}$		
	Anaerobic zone	Anoxic zone	Aerobic zone	Anaerobic zone	Anoxic zone	Aerobic zone	Anaerobic zone	Anoxic zone	Aerobic zone
Volume (L)	6	6	12	6	6	12	6	6	12
Filling ratio with carriers (%)	0	0	0	35	35	35	35	35	0
Flow rate (L h^{-1})	1.6			1.6			1.6		
HRT (h)	18			18			18		
SRT (day)	120			120			120		
MLSS (mg L^{-1})	6,431.67±256.94			4,419.30±254.42			4,485.00±336.39		
MLVSS (mg L^{-1})	5,472.23±218.62			3,775.16±217.34			3,756.86±281.77		
BD (mg L^{-1})	-			2,028.95±149.13			1,991.25±154.17		
VBD (mg L^{-1})	-			1,826.94±134.28			1,639.40±126.93		

Both the stirrers (Multi Mixer MM-1000, Biosan Laboratories, Inc., USA) in the anaerobic and anoxic zones and the fine bubble disk diffusers (AFD 270, ECOTEC, SA, Spain) in the aerobic zones had the objectives of homogenizing the mixed liquor and keeping the carriers moving in the hybrid MBBR-MBR systems. An air flow rate of 30 L h^{-1} was supplied to the aerobic zones of the bioreactors by an air compressor (ACO-500, Hailea, China). Normal propeller speed was 320 rpm in the anaerobic and anoxic zones and in the influent tank.

The membrane module consisted of a vertically oriented submerged module of hollow-fiber ultrafiltration membranes (Micronet Porous Fiber, SL, Spain) with a filtration area of 0.20 m^2 and a pore size of 0.04 μm. Another air compressor (ACO-500, Hailea, China) supplied aeration, which was applied to the base of the module by a coarse bubble disk diffuser (CAP 3, ECOTEC, SA, Spain) with an air flow rate of 100 L h^{-1}.

2.2. Experimental procedure and analytical determinations

Samples were collected from the influent, the three effluents, the anaerobic, anoxic and aerobic zones of the bioreactors, the effluent of the anaerobic zone and the membrane tanks every day. Physical and chemical determinations were carried out in relation to the pH, conductivity, temperature, dissolved oxygen, chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), volatile suspended solids (VSS), total phosphorus (TP), total nitrogen (TN) and the concentrations of ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) according to section **Materials and Methods**.

The biomass concentrations concerning the heterotrophic, autotrophic and nitrite-oxidizing bacteria were estimated by considering the values of mixed liquor volatile suspended solids (MLVSS) and volatile biofilm density (VBD) from Table X.1 and supposing the percentages of heterotrophic, autotrophic and nitrite-oxidizing bacteria in the MLSS and BD attached to the carriers which were determined by Leyva-Díaz et al. (2015) for an MBR and two hybrid MBBR-MBR systems and a hydraulic retention time (HRT) of 9.5 h (Table X.2). Table X.2 also shows the concentration of DeNB.

Table X.2. Total concentration of nitrifying bacteria (AOB and NOB), denitrifying bacteria (DeNB) and heterotrophic bacteria as MLVSS concentration and attached VBD in the experimental plants. AOB (ammonium-oxidizing bacteria), NOB (nitrite-oxidizing bacteria), MLVSS (mixed liquor volatile suspended solids), VBD (volatile biofilm density).

Microbial population	Total biomass concentration				
	MBR_p	Hybrid MBBR-MBR_{ap}		Hybrid MBBR-MBR_{bp}	
	MLVSS (mg L^{-1})	MLVSS (mg L^{-1})	VBD (mg L^{-1})	MLVSS (mg L^{-1})	VBD (mg L^{-1})
Nitrifying bacteria	164.16±6.56	339.76±19.56	328.85±24.17	300.55±22.54	295.09±22.85
Ammonium-oxidizing bacteria (AOB)	54.72±2.19	264.26±15.21	274.04±20.14	262.98±19.72	245.91±19.04
Nitrite-oxidizing bacteria (NOB)	109.44±4.37	75.50±4.35	54.81±4.03	37.57±2.82	49.18±3.81
Denitrifying bacteria (DeNB)	218.89±8.74	226.51±13.04	200.96±14.77	751.37±56.35	131.15±10.15
Heterotrophic bacteria	4,268.34±170.52 (78%)	2,982.37±171.70 (79%)	1,406.74±103.40 (77%)	2,892.78±216.97 (77%)	1,213.16±93.93 (74%)

Furthermore, the kinetic parameters for heterotrophic, autotrophic and nitrite-oxidizing bacteria were evaluated (**Materials and Methods**).

The evaluation of statistically significant differences between the results concerning COD, BOD_5, TSS, TN, TP and concentrations of NH_4^+, NO_2^- and NO_3^- was carried out according to section **Materials and Methods.**

3. Results and discussion

3.1. Evolution of the suspended and attached biomass

Figure X.2a, Figure X.2b and Figure X.2c show the evolution of the MLSS concentration and the attached biofilm density (BD) for the MBR_p, hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp}. The steady state started the day 50 when the working concentrations of MLSS and BD were achieved; this phase had a duration of 83 days. The values of the concentration of MLSS and attached BD for the WWTPs in the steady state are shown in Table X.1.

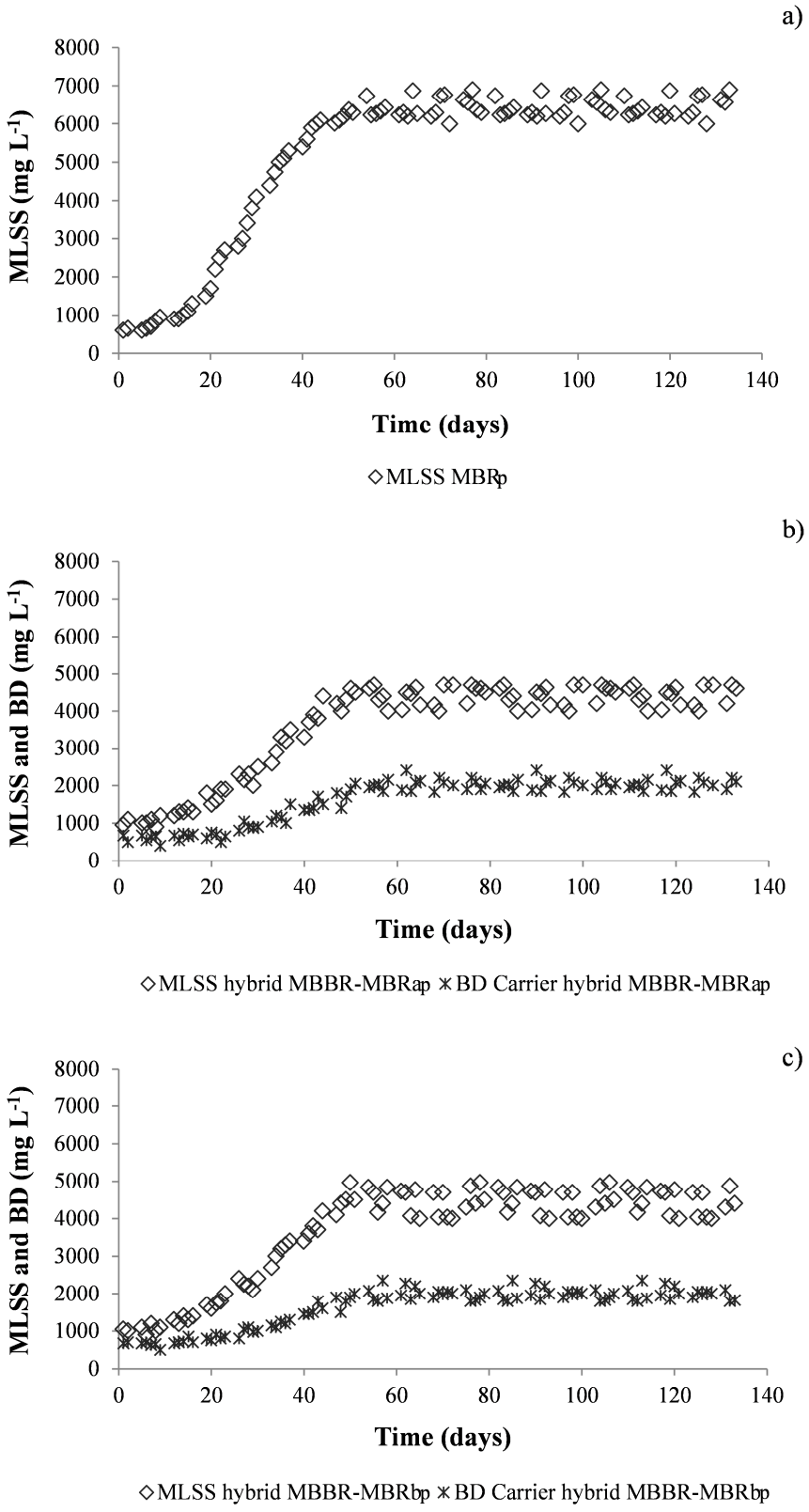

Figure X.2. Evolution of the suspended and attached biomass as mixed liquor suspended solids (MLSS) and biofilm density (BD), respectively, in the bioreactors of the WWTPs. (a) MLSS of the MBR_p. (b) MLSS and BD of the hybrid MBBR-MBR_{ap}. (c) MLSS and BD of the hybrid MBBR-MBR_{bp}.

The MBR_p, hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp} worked at similar biomass concentrations with the only difference being that the hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp} contained both suspended and attached biomass. The biomass concentration in the MBR_p was established at 6,431.67±256.94 mg L^{-1}. Merayo et al. (2013) worked with similar concentrations of MLSS in MBR systems to those used in this research. The concentration of MLSS in the hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp}, 4,419.30±254.42 mg L^{-1} and 4,485.00±336.39 mg L^{-1}, respectively, were lower than that in the MBR_p, although this difference was compensated for by the attached BD on the carriers contained in the hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp}, with values of 2,028.95±149.13 mg L^{-1} and 1,991.25±154.17 mg L^{-1}, respectively (Table X.1). It allowed for studying the differences regarding the microbial kinetics and the organic matter and nutrient removal between the three WWTPs. These values of the concentration of MLSS and BD were similar to those employed by Yang et al. (2009). Furthermore, MBR and hybrid MBBR-MBR systems with similar biomass concentrations were studied in **Chapter 5** and **Chapter 6**.

3.2. Physical and chemical parameters

Table X.3 shows the average values of pH, conductivity, temperature and dissolved oxygen concentration of the influent, effluents and mixed liquors of each bioreactor. The nitrification process caused a slight drop in the pH values in the mixed liquors of the bioreactors and the effluents according to Canziani et al. (2006), as shown in Table X.3.

Table X.3. Average values of pH, conductivity, temperature and dissolved oxygen of the influent, effluents and mixed liquors of the biological reactors of the experimental plants.

Parameter	Sampling zone											
	Influent	MBR_p				Hybrid MBBR-MBR_{ap}				Hybrid MBBR-MBR_{bp}			
		Effluent	Anaerobic zone	Anoxic zone	Aerobic zone	Effluent	Anaerobic zone	Anoxic zone	Aerobic zone	Effluent	Anaerobic zone	Anoxic zone	Aerobic zone
pH	8.00±0.06	6.80±0.77	7.14±0.61	6.99±0.81	6.80±0.82	6.25±0.45	6.65±0.42	6.46±0.62	6.02±0.46	6.32±1.08	6.86±0.52	6.64±0.67	6.41±0.80
Conductivity ($\mu S\ cm^{-1}$)	1,323±110	1,079±78	1,077±90	1,074±87	1,080±92	1,094±103	1,052±40	1,050±41	1,054±39	1,098±143	1,100±152	1,103±147	1,096±157
Temperature (ºC)	14.7±0.7	15.3±0.5	14.8±0.5	14.8±0.5	14.8±0.5	15.3±0.3	14.9±0.5	14.8±0.7	14.8±0.7	15.2±0.6	14.9±0.5	14.9±0.5	14.9±0.5
Dissolved oxygen ($mg\ O_2\ L^{-1}$)	-	-	ND	0.3±0.1	3.3±1.3	-	ND	0.4±0.1	2.5±1.0	-	ND	0.3±0.1	2.2±1.6

ND: Not Detected

The temperature was 14.9±0.5ºC in the three WWTPs as the study was carried out between the months of December and March. The temperature was softened due to the fact that the WWTPs were not out in the open, but they were inside a laboratory. This implied that the sludge was partially digested and converted into carbon dioxide. This could be the reason why the SRT had values of 120 days for the WWTPs. The anaerobic compartments of the different bioreactors did not contain dissolved oxygen to initialize the process of biological phosphorus removal (Kermani et al., 2009). The concentrations of dissolved oxygen in the anoxic zone of the bioreactors were 0.3±0.1 mg O_2 L^{-1}, 0.4±0.1 mg O_2 L^{-1} and 0.3±0.1 mg O_2 L^{-1} for the MBR_p, hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp}, respectively. The aerobic chambers of the different bioreactors contained concentrations of dissolved oxygen which were higher than 2.0±0.1 mg O_2 L^{-1}, according to the suggestion of Wang et al. (2006) to obtain efficient processes of organic matter oxidation and nitrification (Table X.3).

3.3. Organic matter and nitrogen removal

The removal percentages of organic matter and nitrogen in the steady state were very similar in the WWTPs studied, as can be observed in Table X.4 through the parameters COD, BOD_5 and TN. The differences between the three WWTPs were not statistically significant regarding the removal percentages of COD, BOD_5 and TN with an HRT of 18 h as the p-values obtained from the post-hoc procedure, Tukey's HSD, were higher than α=0.05. In spite of this, the hybrid MBBR-MBR_{ap} showed a slightly higher performance than the other experimental plants regarding the COD and BOD_5 removal, with values of 85.82±2.12% and 97.74±0.84%, respectively (Table X.4). The hybrid MBBR-MBR_{bp} had the best efficiency regarding the TN removal, with a value of 61.39±10.71% (Table X.4). It is probably due to the higher concentration of DeNB in the MLSS of the hybrid MBBR-MBR_{bp} (Table X.2). Similar percentages of COD and TN removal, higher than 85% and higher than 50%, respectively, were obtained by Jonoud et al. (2003) with an HRT of 20 h.

Table X.4. Average values of COD, BOD_5, TSS, TN, NH_4^+, NO_2^- and NO_3^- of the influent and effluents of the experimental plants and removal percentages of COD, BOD_5, TSS and TN during the steady state under an HRT of 18 h. COD (chemical oxygen demand), BOD_5 (five-day biochemical oxygen demand), TSS (total suspended solids), TN (total nitrogen), NH_4^+ (concentration of ammonium), NO_2^- (concentration of nitrite), NO_3^- (concentration of nitrate), HRT (hydraulic retention time).

Parameter	Sampling zone				Removal percentage	Wastewater treatment plant		
	Influent	Effluent MBR_p	Effluent Hybrid $MBBR\text{-}MBR_{ap}$	Effluent Hybrid $MBBR\text{-}MBR_{bp}$		MBR_p	Hybrid $MBBR\text{-}MBR_{ap}$	Hybrid $MBBR\text{-}MBR_{bp}$
COD (mg O_2 L^{-1})	185.80±45.81	32.14±3.13	26.34±3.94	31.26±5.42	**COD (%)**	82.70±2.47	85.82±2.12	83.18±2.11
BOD_5 (mg O_2 L^{-1})	73.50±22.14	1.91±0.48	1.66±0.71	1.72±0.58	**BOD_5 (%)**	97.41±0.96	97.74±0.84	97.67±0.29
TSS (mg L^{-1})	65.47±19.33	3.39±2.20	2.76±2.34	3.49±1.82	**TSS (%)**	94.82±2.84	95.78±2.88	94.66±2.37
TN (mg N L^{-1})	85.76±10.54	35.19±6.09	35.91±6.16	33.11±5.91	**TN (%)**	58.96±8.38	58.13±9.01	61.39±10.71
NH_4^+ (mg NH_4^+ L^{-1})	107.36±33.88	ND	ND	ND				
NO_2^- (mg NO_2^- L^{-1})	3.09±0.08	1.78±1.20	17.86±7.62	35.54±15.73				
NO_3^- (mg NO_3^- L^{-1})	5.85±2.96	153.45±59.25	134.95±37.08	98.74±29.52				

ND: Not Detected

Rahimi et al. (2011b) also obtained similar percentages of COD and TN removal in a study about the simultaneous nitrification-denitrification and phosphorus removal in a fixed bed sequencing batch reactor. Yang et al. (2010) obtained slightly higher performances regarding the organic matter and nitrogen removal in a study about the simultaneous nitrogen and phosphorus removal in a moving bed membrane bioreactor. That study showed that the major part of COD was consumed during the anaerobic phase and the removal efficiency was 84.0%. It was also similar to other nitrogen and phosphorus removal researches (Cassidy and Beliab, 2005). According to Yang et al. (2010), COD was mainly removed during the anaerobic phase and microorganisms stored COD as PHB under anaerobic conditions for using it later during the aerobic phase. At the end of the aerobic phase, the average COD removal efficiency was 93.5% and the TN removal efficiency averaged at 86.6%.

Furthermore, the removal percentages of COD and TN were lower than those obtained for an MBR and a hybrid MBBR-MBR_b under an HRT of 18 h and similar biomass concentrations, as shown in Table VIII.3 (**Chapter 5**). It is probably due to the fact that the aerobic zone could not be long enough for the heterotrophic and nitrifying bacteria to become established entirely (Gieseke et al., 2002) as the volume of the aerobic zone is 12 L (Table X.1) in the WWTPs of this study, while the volume of the aerobic zone was 18 L in the previous research as observed in Table VIII.1 (**Chapter 5**).

The reduction percentages of TSS are also indicated in Table X.4. There were no statistically significant differences regarding this parameter between the three systems studied as they all contained a module including hollow-fiber ultrafiltration membranes in the membrane tank.

3.4. Phosphorus removal

The concentrations of TP in the influent, effluents of the anaerobic zone and effluents of the experimental plants as well as the removal percentages of TP in the steady state are indicated in Table X.5.

Table X.5. Average values of TP of the influent, effluents of the anaerobic zone and effluents of the experimental plants and removal percentages of TP of the three systems during the steady state under an HRT of 18 h. TP (total phosphorus), HRT (hydraulic retention time).

Parameter	Sampling zone						
	Influent	MBR_p		Hybrid MBBR-MBR_{ap}		Hybrid MBBR-MBR_{bp}	
		Effluent anaerobic zone	Effluent experimental plant	Effluent anaerobic zone	Effluent experimental plant	Effluent anaerobic zone	Effluent experimental plant
TP (mg P L^{-1})	5.98±0.80	6.86±0.79	1.53±0.38	7.51±0.73	1.11±0.24	7.05±0.96	1.41±0.31
TP removal (%)	-	74.38±3.90		81.42±3.85		76.44±3.04	

These performances of TP removal were quite higher than those obtained in MBR and hybrid MBBR-MBR systems working with similar biomass concentrations under an HRT of 18 h, which ranged from 41.88±16.27% to 45.30±7.85% of TP removal, as shown in Table VIII.3 (**Chapter 5**). It is due to the existence of an anaerobic zone which enables the phosphorus removal in this study.

The hybrid MBBR-MBR systems had the highest efficiencies of TP removal. In light of this, the hybrid MBBR-MBR_{ap} showed the highest concentration of TP in the effluent of the anaerobic zone and the lowest concentration of TP in the effluent of the WWTP with values of 7.51±0.73 mg P L^{-1} and 1.11±0.24 mg P L^{-1}, respectively. The performance regarding the TP removal was the highest in the hybrid MBBR-MBR_{ap}, with a value of 81.42±3.85%. There were statistically significant differences regarding the removal percentages of TP between the three WWTPs with an HRT of 18 h as the p-values obtained from the post-hoc procedure, Tukey's HSD, were less than α=0.05, p-value $_{\text{Hybrid MBBR-MBRap-MBRp}}$ (TP removal) = 0.03341, p-value $_{\text{Hybrid MBBR-MBRap-Hybrid MBBR-MBRbp}}$ (TP removal) = 0.03734 and p-value $_{\text{Hybrid MBBR-MBRbp-MBRp}}$ (TP removal) = 0.04465.

Since production of PHB necessary for phosphate uptake is linked to phosphate release, a high phosphate release is an advantage with respect to achieving a high net phosphate removal (Helness and Ødegaard, 1999; Kermani et al., 2009). It explains the highest TP removal efficiency for the hybrid MBBR-MBR_{ap}. This system showed the highest phosphorus release under anaerobic conditions and the highest phosphorus uptake under aerobic conditions, as shown in Figure X.3.

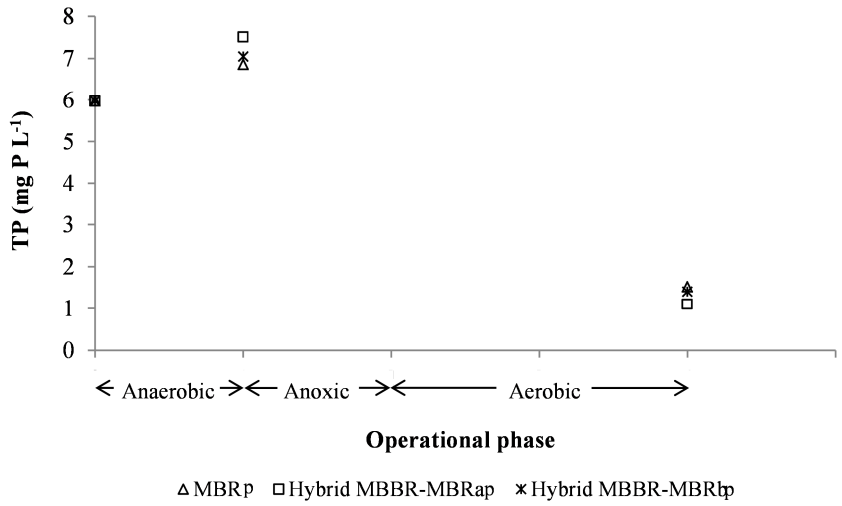

Figure X.3. Evolution of total phosphorus (TP) with the phosphorus release and phosphorus uptake during the anaerobic and aerobic stages, respectively, in each bioreactor of the MBR_p, hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp}.

It should be noted that the COD is the primary source of VFAs for the PAOs. The conversion of COD to VFAs occurs quickly through fermentation in the anaerobic chamber. Thus, the more organic matter removal, the more cell growth, and, thus, a higher phosphorus removal will take place (Tchobanoglous et al., 2003; Kermani et al., 2009). It supports the highest performance regarding the COD removal for the hybrid MBBR-MBR_{ap}, with a value of 85.82±2.12% (Table X.4) and the resulting highest phosphorus removal for this system.

The highest phosphorus removal for the hybrid MBBR-MBR_{ap} could explain the fact that this system had a performance regarding the TN removal slightly lower than the other two systems, with a value of 58.13±9.01% (Table X.4). Nitrifying bacteria and PAOs are potentially subjected to competition for oxygen, although nitrifying bacteria are known to have a lower affinity for oxygen compared to heterotrophic bacteria such as, e.g., the PAOs, which may result in problems concerning the nitrifying activity (Prosser, 1989; Gieseke et al., 2002).

The MBR_p showed the lowest value of TP removal. This is possibly due to the higher concentration of nitrate in this system, 153.45±59.25 mg NO_3^- L^{-1} (Table X.4), as the phosphorus and nitrogen removal requires a consumption of COD. In this way, the transfer of a higher concentration of nitrate into the anaerobic zone could consume a portion of the substrate as COD before the substrate is utilized by the PAOs, which might partially inhibit the phosphate release in the MBR_p (Kuba et al., 1994; Akin and

Ugurlu, 2004). The use of carriers as well as the lower concentration of nitrate in the hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp} (Table X.4) could solve the conflict on simultaneous nitrogen and phosphorus removal. Attached biomass can form aerobic, anoxic and anaerobic zones which provide favorable conditions for the simultaneous nitrification and denitrification (Puznana et al., 2000; Yang et al., 2009). Thus, the biofilm can improve the nitrogen removal in the aerobic zone and avoid the transfer of nitrate into the anaerobic chamber.

The results obtained regarding the TP removal in this study were in the range reported by the literature. Yang et al. (2014) obtained a performance regarding the TP removal of 85.05±8.02% in a membrane-coupled MBBR with similar concentrations of attached biomass, which is slightly higher than the performance obtained in this research. Additionally, the TP removal efficiency averaged at 84.1% in a sequencing batch moving bed membrane bioreactor which was analyzed by Yang et al. (2010). Rahimi et al. (2011b) carried out their research in a fixed bed sequencing batch reactor and the TP removal rates were 77-90%. Moreover, Kermani et al. (2008) and Kermani et al. (2009) evaluated the TP removal efficiency in a lab-scale MBBR system, which showed performances of 87.92% and 89.73% on average, respectively.

3.5. Kinetic modeling of MBR_p, hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp}

Table X.6 shows the kinetic parameters which fit the Monod model for the heterotrophic, autotrophic and nitrite-oxidizing bacteria from the different systems.

Table X.6. Kinetic parameters for the characterization of heterotrophic and autotrophic biomass. Y_H (yield coefficient for heterotrophic biomass), $\mu_{m,\ H}$ (maximum specific growth rate for heterotrophic biomass), K_M (half-saturation coefficient for organic matter), Y_A (yield coefficient for autotrophic biomass), $\mu_{m,\ A}$ (maximum specific growth rate for autotrophic biomass), K_{NH} (half-saturation coefficient for ammonia nitrogen), Y_{NOB} (yield coefficient for nitrite-oxidizing bacteria), $\mu_{m,\ NOB}$ (maximum specific growth rate for nitrite-oxidizing bacteria), K_{NOB} (half-saturation coefficient for nitrite-nitrogen), k_d (decay coefficient for autotrophic and heterotrophic biomass).

Parameter	Sampling zone		
	MBR_p	**Hybrid MBBR-MBR_{ap}**	**Hybrid MBBR-MBR_{bp}**
		Heterotrophic bacteria	
Y_H (mg VSS mg COD^{-1})	0.7100	0.5665	0.5441
$\mu_{m,\ H}$ (h^{-1})	0.0070	0.0073	0.0072
K_M (mg O_2 L^{-1})	3.4397	2.0673	3.0155
		Autotrophic bacteria	
Y_A (mg O_2 mg N^{-1})	2.5337	3.3034	2.1976
$\mu_{m,\ A}$ (h^{-1})	0.1466	0.0421	0.0937
K_{NH} (mg N L^{-1})	0.6438	0.6693	1.0514
		Nitrite-oxidizing bacteria	
Y_{NOB} (mg O_2 mg N^{-1})	0.5538	0.9875	0.6387
$\mu_{m,\ NOB}$ (h^{-1})	0.1008	0.0968	0.2177
K_{NOB} (mg N L^{-1})	1.0553	0.6757	1.2574
		Total bacteria	
k_d (d^{-1})	0.0257	0.0174	0.0242

The hybrid MBBR-MBR_{ap} showed the best kinetic behavior regarding the heterotrophic biomass when the r_{su} was assessed depending on the kinetic parameters and substrate and biomass concentrations (Figure X.4a). It supported the highest removal percentages of COD and BOD_5 for this system, as can be observed in Table X.4. The values of the yield coefficient for heterotrophic biomass (Y_H) were similar to those obtained by Plattes et al. (2007). Moreover, similar values concerning the maximum specific growth rate for heterotrophic biomass ($\mu_{m,\ H}$) and the half-saturation coefficient for organic matter (K_M) were obtained by Canziani et al. (2006) and Seifi and Fazaelipoor (2012), respectively.

In relation to the autotrophic biomass, the hybrid MBBR-MBR_{bp} had the highest values of r_{su} (Figure X.4b) which is the reason why this system showed the best efficiency of TN removal (Table X.4) under the operational conditions used in this

study. The values of the yield coefficient for autotrophic biomass (Y_A) were slightly higher than those obtained by Seifi and Fazaelipoor (2012). Similar values concerning the maximum specific growth rate for autotrophic biomass ($\mu_{m,\ A}$) and the half-saturation coefficient for ammonia-nitrogen (K_{NH}) were obtained by Plattes et al. (2007) and Ferrai et al. (2010), respectively.

Therefore, the required time for the substrate oxidation was lower in the heterotrophic biomass from the hybrid MBBR-MBR_{ap} and the autotrophic biomass from the hybrid MBBR-MBR_{bp}; the μ_m was obtained with less available substrate and the steady state was reached in less time for the heterotrophic and autotrophic biomasses from the hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp}, respectively.

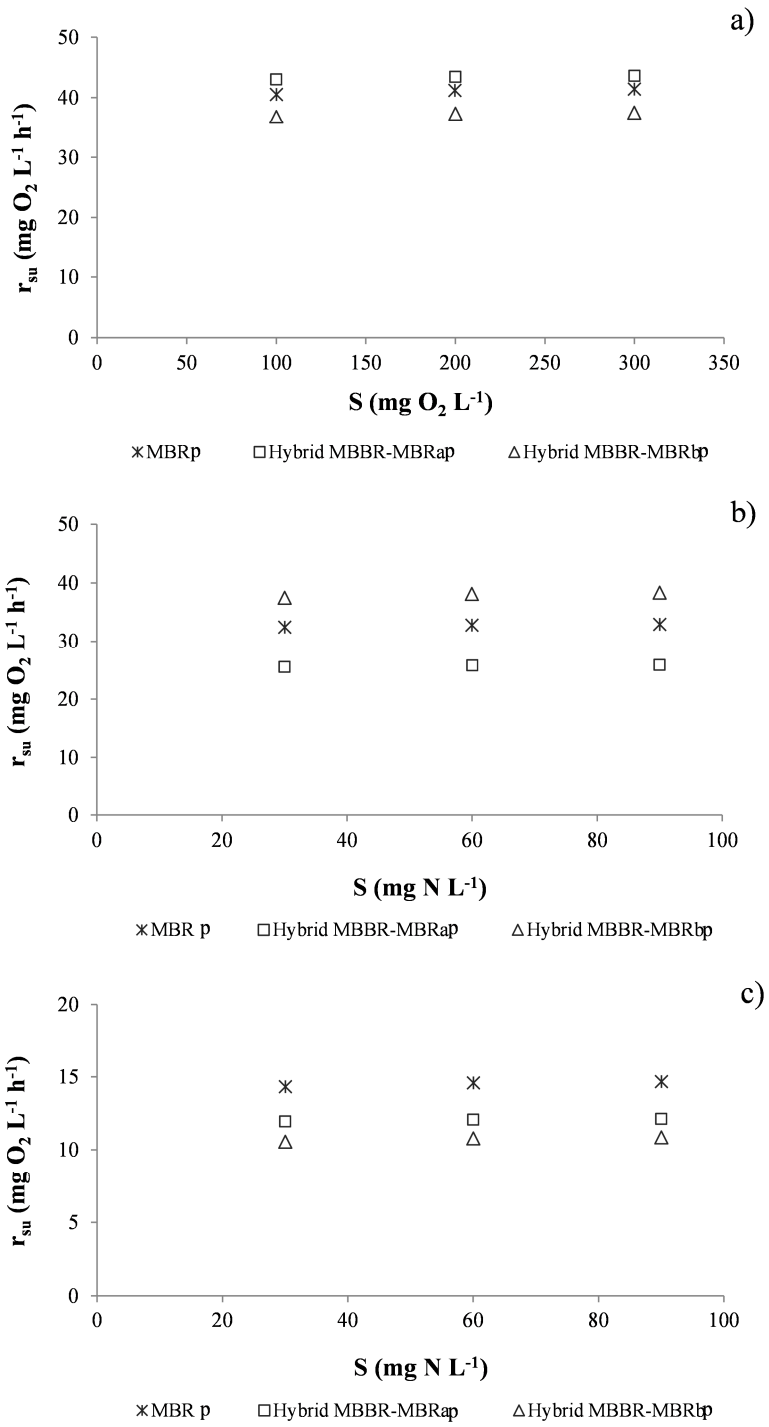

Figure X.4. Substrate degradation rate (r_{su}) obtained in the biological kinetic study depending on the substrate concentration for the different bioreactors from the WWTPs. (a) Heterotrophic bacteria. (b) Autotrophic bacteria. (c) Nitrite-oxidizing bacteria.

However, the MBR_p had the best kinetic performance concerning the NOB kinetics with values of Y_{NOB} = 0.5538 mg O_2 mg N^{-1}, $\mu_{m,\ NOB}$ = 0.1008 h^{-1} and K_{NOB} = 1.0553 mg N L^{-1} (Pambrun et al., 2006; Iacopozzi et al., 2007), as shown in Figure X.4c. This supported the fact that the nitrate concentration in the effluent from the MBR_p was

higher than those from the hybrid MBBR-MBR systems, with a value of 153.45±59.25 mg NO_3^- L^{-1} (Table X.4). Therefore, the hybrid MBBR-MBR_{bp} could have a better kinetic behavior regarding the ammonium-oxidizing bacteria (AOB) because, overall, the kinetics of autotrophic bacteria was better, as previously mentioned, and the hybrid MBBR-MBR_{bp} had the highest nitrite concentration in its effluent, with a value of 35.54±15.73 mg NO_2^- L^{-1}, as indicated in Table X.4. There were statistically significant differences regarding the nitrite and nitrate formations between the MBR_p and hybrid MBBR-MBR_{bp} with an HRT of 18 h as the p-values obtained were less than α=0.05, p-value $_{MBRp\text{-}Hybrid\ MBBR\text{-}MBRbp}$ (NO_2^-) = 0.00226 and p-value $_{MBRp\text{-}Hybrid\ MBBR\text{-}MBRbp}$ (NO_3^-) = 0.02904. Similar conclusions were obtained with similar configurations of WWTPs and values of MLSS and BD under an HRT of 18 h, as shown in Figure VIII.3c (**Chapter 5**), since the MBR system showed the best kinetic behavior regarding the NOB.

The values of k_d are also indicated in Table X.6. These values of k_d concerning the MBR_p, hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp} were very similar as the SRT was identical and the biomass concentrations were almost the same in the three systems (Table X.1).

4. Conclusions

The following conclusions can be drawn from this study through a comparison of the nutrient and organic matter removal in an MBR_p and two hybrid MBBR-MBR systems (hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp}) working in parallel:

1. The hybrid MBBR-MBR_{ap}, which contained carriers in the anaerobic, anoxic and aerobic zones of the bioreactor, showed an improvement trend regarding the performance of TP removal compared to the other WWTPs, with a value of 81.42±3.85%. It involved a higher phosphorus release under anaerobic conditions and a higher phosphorus uptake under aerobic conditions.

2. The hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp} showed higher TP removal efficiencies than the MBR_p as attached biomass can form aerobic, anoxic and anaerobic zones, so the biofilm can enhance the TN removal in the aerobic zone and avoid the transfer of nitrate into the anaerobic compartment, which could consume COD and inhibit the TP removal.

3. The hybrid MBBR-MBR_{ap} had the highest performance concerning the COD removal, with a value of 85.82±2.12%, which was supported by its better kinetic behavior for the heterotrophic biomass. It explained the best phosphorus removal for this system as a higher organic matter removal implied an increase in the cell growth as the COD is the primary source of VFAs for the PAOs.

4. The hybrid MBBR-MBR_{bp} showed the best efficiency in relation to the TN removal, with a value of 61.39±10.71%, which could possibly be due to the higher concentration of denitrifying bacteria in the mixed liquor as well as the better kinetic performance for the autotrophic biomass. Nevertheless, the hybrid MBBR-MBR_{ap} had a TN removal efficiency lower than the other two systems, probably due to the competition for the oxygen between nitrifying bacteria and PAOs since nitrifying bacteria have a lower affinity for oxygen compared to the PAOs, which resulted in a higher phosphorus removal and some problems concerning the nitrifying activity.

5. The MBR_p showed the best performance in relation to the kinetics of NOB, which supported the concentrations of nitrate and nitrite in the different effluents, with values of concentration of nitrate and nitrite of 153.45±59.25 mg NO_3^- L^{-1} and 35.54±15.73 mg NO_2^- L^{-1} for the MBR_p and hybrid MBBR-MBR_{bp}, respectively.

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XI. OVERALL DISCUSSION

1. Organic matter removal

The type of wastewater treatment plant (WWTP), the hydraulic retention time (HRT) and the biomass concentration, as mixed liquor suspended solids (MLSS) or biofilm density (BD) attached to carriers, were the variables studied in this research, as shown in Table XI.1. Furthermore, the temperature, chemical oxygen demand (COD) and total nitrogen (TN) in the influent are also presented in Table XI.1. Thus, Table XI.1 complement Table III.2.

Table XI.1. Operational conditions of the experimental plants regarding HRT, concentrations of MLSS and BD of the bioreactors, temperature, COD of the influent and TN of the influent. HRT (hydraulic retention time), MLSS (mixed liquor suspended solids), BD (biofilm density), COD (chemical oxygen demand), TN (total nitrogen).

Wastewater treatment plant	Operational condition	Parameter						
		HRT (h)	MLSS (mg L^{-1})	BD (mg L^{-1})	Total biomass (mg L^{-1})	Temperature (ºC)	COD influent (mg O_2 L^{-1})	TN influent (mg N L^{-1})
MBR	1	30.4	2,691.30±114.99	-	2,691.30±114.99	21.1±4.1	336.08±104.48	99.17±36.50
	2	26.5	4,383.86±316.01	-	4,383.86±316.01	14.9±1.3	437.73±112.90	147.76±68.43
	3	18.0	3,574.34±175.26	-	3,574.34±175.26	23.3±1.5	320.16±63.63	95.48±46.01
	4	18.0	6,405.56±365.36	-	6,405.56±365.36	20.8±2.5	256.54±67.56	69.77±16.59
	5	18.0	2,739.68±211.75	-	2,739.68±211.75	20.8±2.5	256.54±67.56	69.77±16.59
	6	9.5	3,326.83±233.95	-	3,326.83±233.95	17.2±1.9	257.47±73.57	100.37±27.60
	7	9.5	2,820.59±243.87	-	2,820.59±243.87	14.7±1.1	224.08±102.30	65.47±32.18
	8	9.5	6,656.67±445.02	-	6,656.67±445.02	14.7±1.1	234.00±46.10	91.54±8.81
	9	6.0	2,777.78±282.27	-	2,777.78±282.27	20.7±1.1	207.61±38.79	80.21±8.50
	10	6.0	6,566.67±255.73	-	6,566.67±255.73	20.7±1.1	226.36±51.20	84.70±4.74
Hybrid MBBR-MBR_a	11	30.4	1,569.87±82.01	1,228.18±75.89	2,798.05±157.90	21.1±4.1	336.08±104.48	99.17±36.50
	12	26.5	2,553.75±293.42	1,000.35±345.26	3,554.10±238.68	14.9±1.3	437.73±112.90	147.76±68.43
	13	18.0	2,028.93±155.52	1,610.83±73.60	3,639.76±229.12	23.3±1.5	320.16±63.63	95.48±46.01
	14	9.5	2,498.25±138.40	1,270.19±81.55	3,768.44±219.95	17.2±1.9	257.47±73.57	100.37±27.60
Hybrid MBBR-MBR_b	15	30.4	1,823.99±51.11	880.00±43.01	2,703.99±94.12	21.1±4.1	336.08±104.48	99.17±36.50
	16	26.5	2,999.14±400.18	675.00±175.39	3,674.14±275.57	14.9±1.3	437.73±112.90	147.76±68.43
	17	18.0	2,306.66±112.93	1,207.50±76.61	3,514.16±189.54	23.3±1.5	320.16±63.63	95.48±46.01
	18	18.0	4,369.84±232.79	2,008.93±171.15	6,378.77±403.94	20.8±2.5	256.54±67.56	69.77±16.59
	19	9.5	2,457.58±156.90	1,250.00±66.51	3,707.58±223.41	17.2±1.9	257.47±73.57	100.37±27.60
	20	9.5	2,041.90±258.37	997.73±124.62	3,039.63±282.99	14.7±1.1	224.08±102.30	65.47±32.18
	21	6.0	2,243.75±216.95	748.53±111.97	2,992.28±228.92	20.7±1.1	207.61±38.79	80.21±8.50
Pure MBBR-MBR	22	9.5	208.00±61.30	1,920.45±127.16	2,128.45±188.46	14.7±1.1	224.08±102.30	65.47±32.18
	23	6.0	258.75±79.99	2,070.00±202.97	2,328.75±182.96	20.7±1.1	207.61±38.79	80.21±8.50

The COD and TN removal and the kinetic parameters for heterotrophic and autotrophic biomass under the different operational conditions are indicated in Table XI.2.

Table XI.2. COD and TN removals and kinetic parameters for heterotrophic and autotrophic biomass, Y_H, $\mu_{m,\,H}$, K_M, Y_A, $\mu_{m,\,A}$, K_{NH}, k_d, under the operational conditions shown in Table XI.1. COD (chemical oxygen demand), TN (total nitrogen), Y_H (yield coefficient for heterotrophic biomass), $\mu_{m,\,H}$ (maximum specific growth rate for heterotrophic biomass), K_M (half-saturation coefficient for organic matter), Y_A (yield coefficient for autotrophic biomass), $\mu_{m,\,A}$ (maximum specific growth rate for autotrophic biomass), K_{NH} (half-saturation coefficient for ammonia nitrogen). k_d (decay coefficient for autotrophic and heterotrophic biomass).

Wastewater treatment plant	Operational condition	COD removal (%)	Kinetic parameters for heterotrophic biomass							
			$\mu_{m,\,H}$ (h^{-1})	K_M (mg O_2 L^{-1})	Y_H (mg VSS mg COD^{-1})	TN removal (%)	$\mu_{m,\,A}$ (h^{-1})	K_{NH} (mg N L^{-1})	Y_A (mg O_2 mg N^{-1})	k_d (d^{-1})
MBR	1	90.75±3.30	0.0028	4.7464	0.2798	63.06±8.42	-	-	-	0.0333
	2	91.97±2.96	0.0086	2.3659	0.5040	65.17±7.41	0.0765	1.3070	0.9714	0.0484
	3	90.29±2.05	0.0068	8.6103	0.4235	63.73±8.05	0.0530	15.2472	0.9852	0.0165
	4	88.48±4.51	0.0074	6.2459	0.5338	71.31±4.75	0.0279	0.6920	1.3567	0.0235
	5	88.73±4.28	0.0380	8.9815	0.5889	68.76±5.49	0.1213	2.7288	1.7329	0.0282
	6	86.77±3.24	0.0192	16.4736	0.4609	58.03±16.87	0.2719	0.9329	1.0389	0.0304
	7	84.55±5.77	0.0135	6.7662	0.4994	70.54±14.57	0.1328	0.8913	1.5568	0.0440
	8	87.23±4.62	0.0173	9.1926	0.6016	50.05±7.50	0.2169	0.8622	2.9304	0.0309
	9	84.01±2.15	0.0255	7.0629	0.5632	45.86±10.69	0.1607	0.3887	1.9591	0.0366
	10	86.90±4.28	0.0114	5.5141	0.5809	47.46±6.76	0.4669	2.8433	2.5174	0.0337
Hybrid MBBR-MBR_a	11	90.83±3.53	0.0044	10.8310	0.3453	61.80±11.95	-	-	-	0.0230
	12	90.97±2.55	0.0048	0.9597	0.5041	63.84±15.81	0.0263	0.7617	0.7772	0.0314
	13	89.80±2.25	0.0065	6.6382	0.3960	62.72±8.22	0.0840	5.7525	1.8887	0.0311
	14	87.05±4.60	0.0214	9.8251	0.5331	54.84±11.61	0.0805	1.0894	1.5471	0.0340
Hybrid MBBR-MBR_b	15	91.71±2.59	0.0031	3.5491	0.3025	64.07±8.69	-	-	-	0.0207
	16	90.74±3.69	0.0012	1.2417	0.3967	67.34±11.22	0.0331	0.5327	0.6595	0.0326
	17	90.24±2.87	0.0110	9.0178	0.4338	63.96±7.00	0.0861	3.1287	2.1970	0.0307
	18	87.98±4.04	0.0472	9.0025	0.5853	72.39±7.57	0.0376	0.8122	2.5385	0.0232
	19	87.62±2.82	0.0267	8.8808	0.5498	56.58±11.51	0.0929	1.1189	1.2985	0.0362
	20	87.39±6.01	0.0155	4.2454	0.5519	61.46±11.87	0.1434	1.5984	1.5891	0.0750
	21	84.10±2.25	0.0658	18.9121	0.5756	48.53±16.71	0.2250	2.4179	2.2366	0.0390
Pure MBBR-MBR	22	80.96±7.67	0.0181	2.6791	0.5093	71.91±16.04	0.7169	2.0748	2.3465	0.1150
	23	79.78±4.60	0.0292	2.9681	0.5941	63.21±11.01	0.3591	3.1582	2.3657	0.0982

The differences between the membrane bioreactor (MBR), the hybrid moving bed biofilm reactor-membrane bioreactor containing carriers in the aerobic and anoxic zones of the bioreactor (hybrid MBBR-MBR_a) and the hybrid moving bed biofilm reactor-membrane bioreactor which contained carriers only in the aerobic zone of the bioreactor (hybrid MBBR-MBR_b) were not statistically significant regarding the COD removal with the hydraulic retention times (HRTs) of 30.4 h (**Chapter 1**), 26.5 h (**Chapter 2**), 18 h (**Chapter 3** and **Chapter 5**) and 9.5 h (**Chapter 4**) as the p-values obtained from the post hoc procedure, Tukey´s HSD, were higher than α=0.05. It is supported by Figure XI.1, which shows similar values of COD removal (%) for these wastewater treatment plants (WWTPs) under the HRT considered. It occurred with the highest HRTs as the systems were in the asymptotic zone of organic matter removal due to the fact that the organic loading rate was lower. In general, the hybrid MBBR-MBR_b had COD removal efficiencies slightly higher than the hybrid MBBR-MBR_a.

However, the hybrid MBBR-MBR_b was the best system concerning the COD removal under the HRTs of 9.5 h and 6 h (**Chapter 6)** and similar biomass concentrations, with efficiencies slightly higher than the MBR systems. The pure MBBR-MBR showed the lowest performance in relation to the COD removal (Figure XI.1); the differences were statistically significant between the hybrid MBBR-MBR_b and pure MBBR-MBR under both HRTs, as shown in Table IX.3 (**Chapter 6**). The improvement regarding the organic matter removal in the hybrid MBBR-MBR_b under the two lowest HRTs, which implied higher organic loading rates, was probably due to the presence of suspended and attached biomass, as the MBR only contained suspended biomass, while the pure MBBR-MBR mainly had attached biomass.

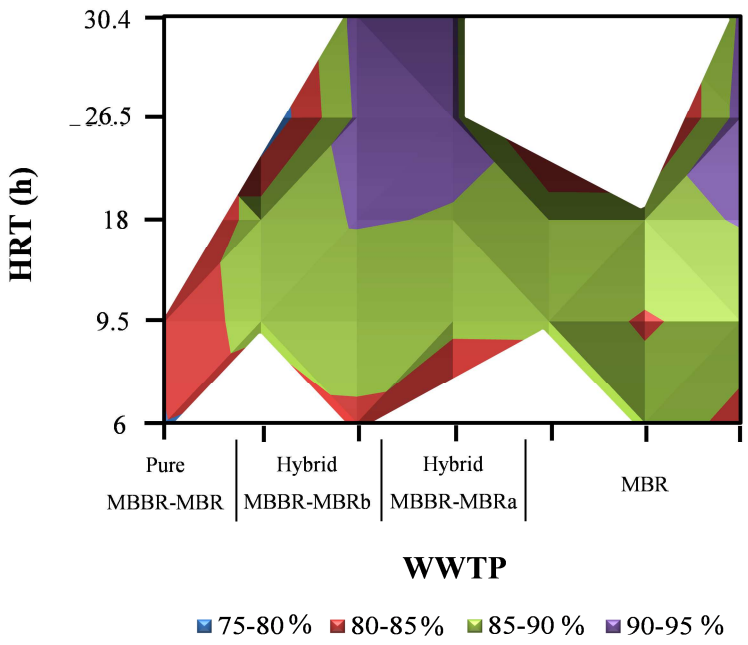

Figure XI.1. COD removal (%) depending on the WWTP and the HRT. COD (chemical oxygen demand), WWTP (wastewater treatment plant), HRT (hydraulic retention time).

It should be noted that the efficiency of organic matter removal usually decreased when the HRT was lower for each WWTP as the organic loading rate was higher.

Figure XI.2 shows the COD removal (%) for the different WWTPs with three total biomass concentrations. The lower biomass concentrations around average values of 2,700 mg L^{-1} and 3,700 mg L^{-1} had the highest efficiencies of COD removal, as can be observed in the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b (the pure MBBR-MBR only worked with the lowest biomass concentrations). The high biomass concentrations around an average value of 6,500 mg L^{-1} did not usually improve the COD removal. It could be due to a higher localized competition between the suspended and attached biomass, which could cause an inhibitory effect on the COD removal (Okabe et al., 1996).

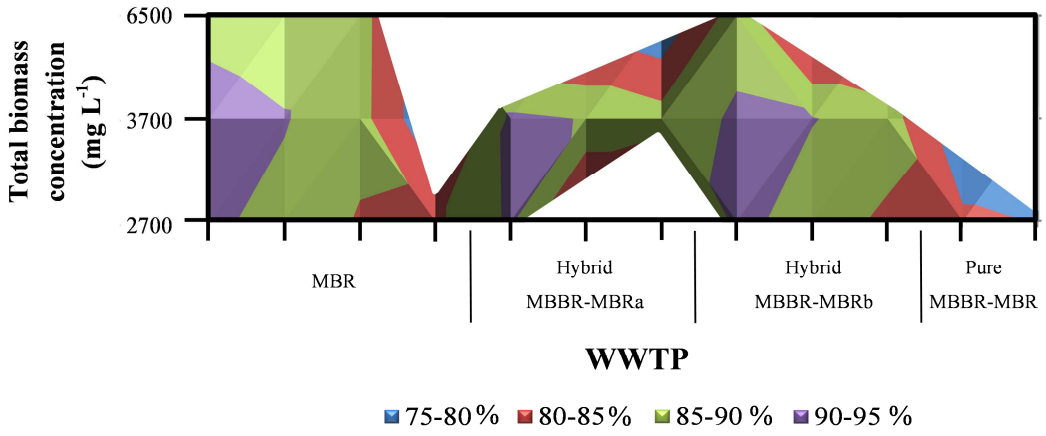

Figure XI.2. COD removal (%) depending on the WWTP and the total biomass concentration. COD (chemical oxygen demand), WWTP (wastewater treatment plant).

The results of the multivariable statistical analysis for organic matter removal are shown in Figure XI.3. This analysis shows four triplot diagrams for the MBR (Figure XI.3a), hybrid MBBR-MBR_a (Figure XI.3b), hybrid MBBR-MBR_b (Figure XI.3c) and pure MBBR-MBR (Figure XI.3d).

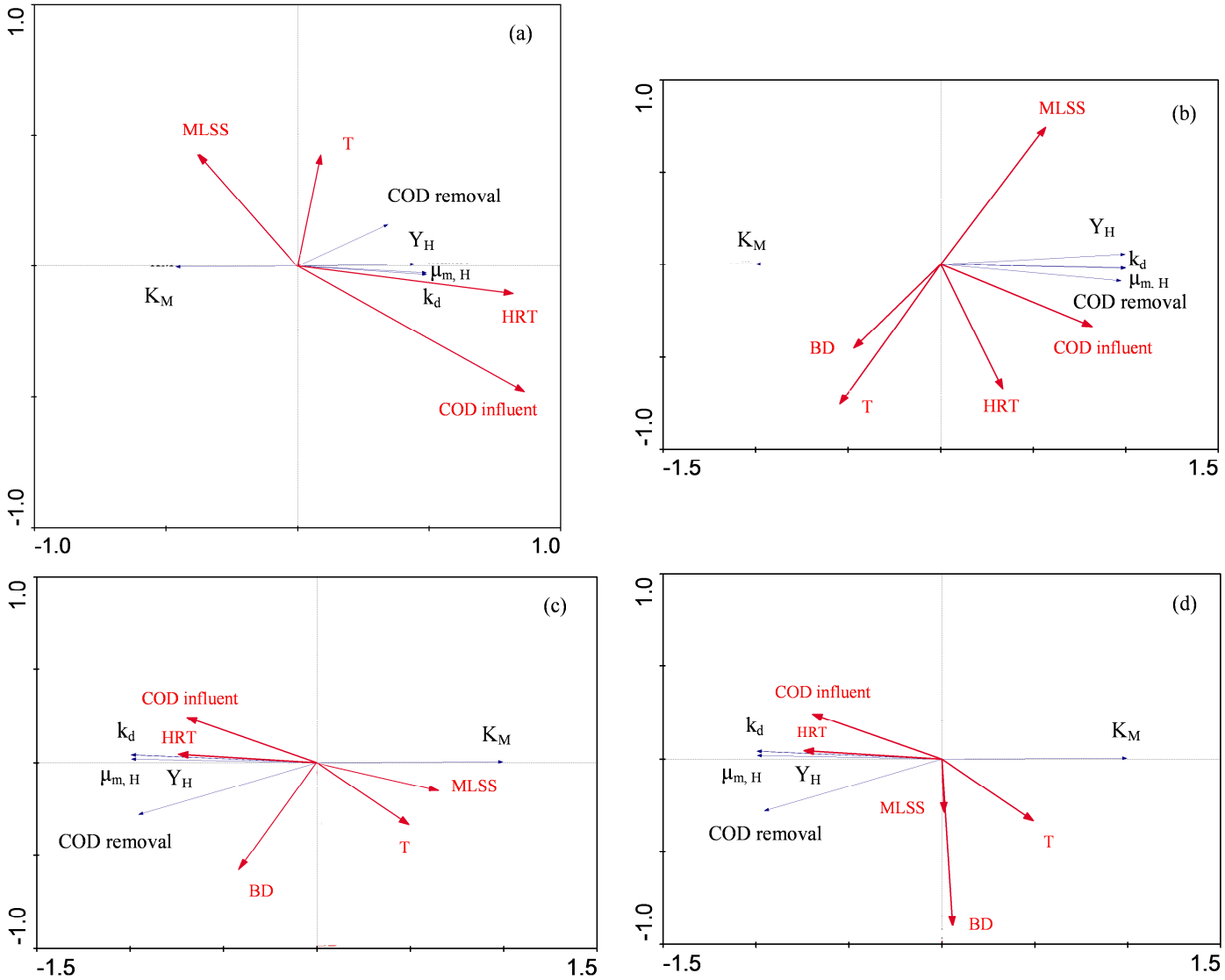

Figure XI.3. Triplot diagram of the Redundancy Analysis (RDA) of the kinetic parameters for heterotrophic biomass (Table XI.2) and chemical oxygen demand (COD) removal in relation to the variables COD of the influent, temperature (T), hydraulic retention time (HRT), mixed liquor suspended solids (MLSS) and biofilm density (BD) in the MBR (a), hybrid MBBR-MBR_a (b), hybrid MBBR-MBR_b (c) and pure MBBR-MBR (d).

Bearing in mind the length and the angles between the different vectors represented in Figure XI.3, the influence of each variable, COD of the influent, temperature, HRT, MLSS and BD (Table XI.1), can be independently analyzed on the different species, COD removal and kinetic parameters for heterotrophic biomass (Table XI.2).

In this sense, it should be noted that the MLSS and BD do not almost have influence on the COD removal and the kinetic parameters for heterotrophic biomass as the angles between these vectors are approximately 90º. This explained that the high biomass concentrations around an average value of 6,500 mg L^{-1} did not usually improve the COD removal (Figure XI.2). However, the BD showed a positive correlation with the COD removal and heterotrophic kinetic parameters in the hybrid MBBR-MBR_b. Therefore, the attached biomass had a higher influence on the COD removal and kinetic parameters for heterotrophic biomass in the hybrid MBBR-MBR_b; it supported the highest performance of COD removal under the HRTs of 9.5 h and 6 h in this system, as previously indicated.

The HRT presented a positive correlation with the COD removal and the kinetic parameters for heterotrophic biomass. It was in accordance with Figure XI.1 as the efficiency of COD removal increased when the HRT was higher.

The temperature did not affect the different systems studied regarding the organic matter removal (the angles were approximately 90º) as its effect was decreased due to the fact that the WWTPs were not out in the open, but they were inside a laboratory. On the other hand, Figure XI.3d shows a higher length for the vector of BD in relation to the vector of MLSS in the pure MBBR-MBR as this system mainly contained attached biomass.

Moreover, the COD removal and the kinetic parameters for heterotrophic biomass had a positive correlation in the four systems, so the kinetic study for heterotrophic biomass supported the results of organic matter removal.

2. Nitrogen removal

The differences between the MBR, the hybrid MBBR-MBR_a and the hybrid MBBR-MBR_b were not statistically significant regarding the TN removal with the HRTs of 30.4 h (**Chapter 1**), 26.5 h (**Chapter 2**), 18 h (**Chapter 3** and **Chapter 5**) and

9.5 h (**Chapter 4**) as the p-values obtained from the post hoc procedure, Tukey´s HSD, were higher than α=0.05. It is supported by Figure XI.4, which shows similar values of TN removal (%) for these WWTPs under a specific HRT.

Nevertheless, the hybrid MBBR-MBR_b usually showed a slightly higher efficiency concerning TN removal under the working HRTs. These results indicated that the nitrification and denitrification processes in the hybrid MBBR-MBR systems were more effective than in the MBR, but an anoxic zone without carriers was necessary to provide better contact between nitrate and the microorganisms (Rusten et al., 1995; Rusten et al., 2000; Larrea et al., 2007).

The pure MBBR-MBR had the highest percentages of TN removal, under the HRTs of 9.5 h and 6 h, if it was compared with the performance of the MBR_a and hybrid MBBR-MBR_b (**Chapter 6**), as observed in Figure XI.4. The difference between the pure MBBR-MBR and hybrid MBBR-MBR_b regarding the removal percentage of TN was statistically significant with an HRT of 9.5 h, p-value $_{\text{Pure MBBR-MBR-Hybrid MBBR-MBRb}}$ (TN removal) = 0.03126, and the differences between the pure MBBR-MBR and the systems MBR_a and hybrid MBBR-MBR_b were also statistically significant under an HRT of 6 h, p-value $_{\text{Pure MBBR-MBR-MBRa}}$ (TN removal) = 0.04478 and p-value $_{\text{Pure MBBR-MBR-Hybrid MBBR-MBRb}}$ (TN removal) = 0.03480, as the p-values obtained were lower than α=0.05, as observed in Table IX.3 (**Chapter 6**).

The efficiency of TN removal generally decreased when the HRT was lower for each WWTP as the ammonium loading rate was higher.

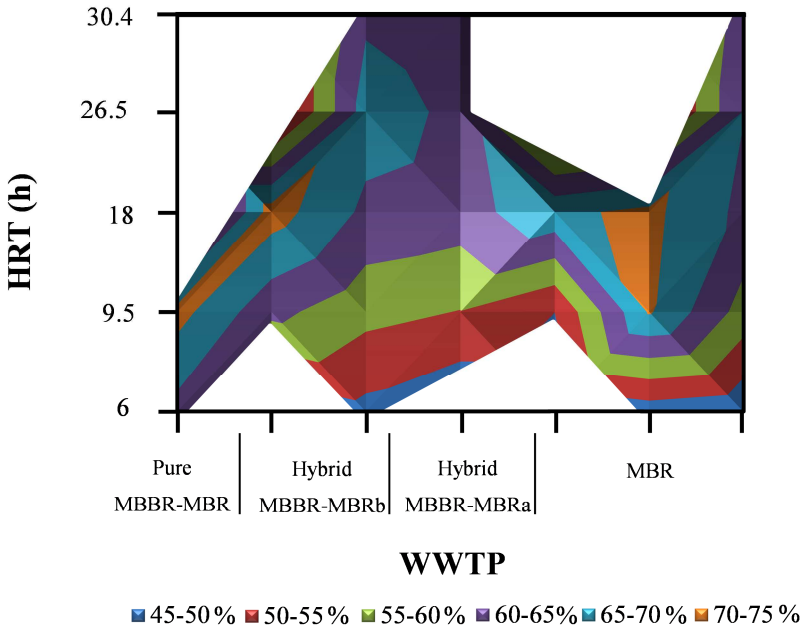

Figure XI.4. TN removal (%) depending on the WWTP and the HRT. TN (total nitrogen), WWTP (wastewater treatment plant), HRT (hydraulic retention time).

Figure XI.5 shows the TN removal (%) for the different WWTPs with three total biomass concentrations. The high biomass concentrations around an average value of 6,500 mg L^{-1} had usually the highest performances of TN removal, as can be observed in the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b (the pure MBBR-MBR only worked with low biomass concentrations around an average value of 2,700 mg L^{-1}).

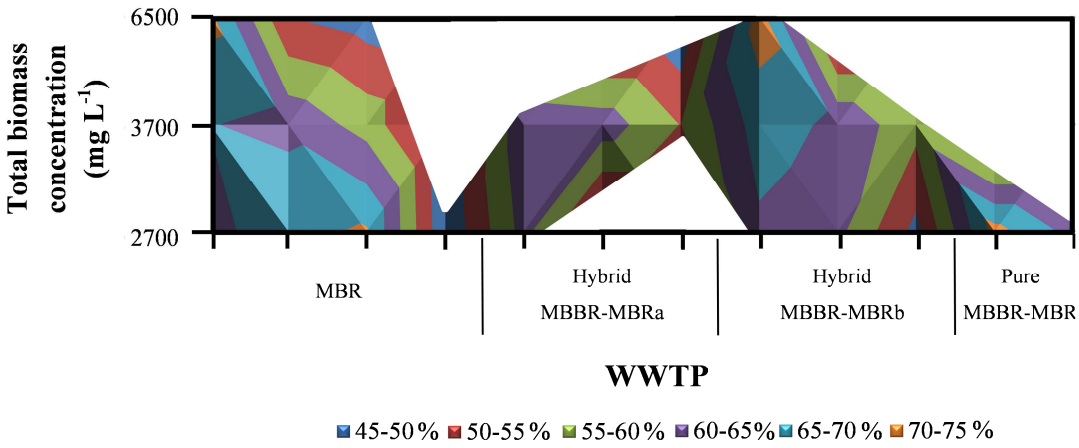

Figure XI.5. TN removal (%) depending on the WWTP and the total biomass concentration. TN (total nitrogen), WWTP (wastewater treatment plant).

It should be noted that there are four points in Figure XI.4 and Figure XI.5 with TN removal ranging from 70% to 75%. It supports the best efficiency regarding the TN removal for the pure MBBR-MBR. This system showed percentages of TN removal which ranged from 70% to 75% under an HRT of 9.5 h and a low total biomass concentration, 208.00±61.30 mg L^{-1} for MLSS and 1,920.45±127.16 mg L^{-1} for BD (Table XI.1). Nevertheless, the MBR had similar TN removal performances under an HRT of 18 h and a high total biomass concentration of 6,405.56 mg L^{-1} for MLSS, and with an HRT of 9.5 h and a biomass concentration of 2,820.59 mg L^{-1} for MLSS. In the first case, the MBR worked with a lower ammonium loading rate and a higher biomass concentration than the pure MBBR-MBR, and in the second case, the MBR worked with a higher biomass concentration than the pure MBBR-MBR (Table XI.1). In this sense, the hybrid MBBR-MBR_b had also the same TN removal efficiency under an HRT of 18 h and a biomass concentration of 4,369.84 mg L^{-1} for MLSS and 2,008.93 mg L^{-1} for BD (Table XI.1). However, the pure MBBR-MBR got the same performance regarding TN removal with a higher ammonium loading rate and a lower biomass concentration (Table XI.1). It also occurred with TN removal ranging from 65% to 70%.

These results indicated that the nitrification and denitrification processes were more effective in the pure MBBR-MBR. The MLSS concentrations in the pure MBBR-MBR were very low under the two working HRTs (208.00±61.30 mg L^{-1} and 258.75±79.99 mg L^{-1} for an HRT of 9.5 h and 6 h, respectively) in relation to those of the MBR_a and hybrid MBBR-MBR_b. The biomass was mainly developed on carriers as attached biomass, with values of biofilm density (BD) of 1,920.45±127.16 mg L^{-1} and 2,070.00±202.97 mg L^{-1} for an HRT of 9.5 h and 6 h, respectively, and involved a better contact between nitrate and the microorganisms (Rusten et al., 1995). The nitrification took place at the carrier interface, which was an aerobic layer, and denitrification occurred in the deeper layer of the biofilm, where anoxic conditions were present. As a result, the removal efficiency of TN was the highest in the pure MBBR-MBR under the two working HRTs (Yang et al., 2009).

The results of the multivariable statistical analysis for nitrogen removal are shown in Figure XI.6. This analysis shows four triplot diagrams for the MBR (Figure XI.6a), hybrid MBBR-MBR_a (Figure XI.6b), hybrid MBBR-MBR_b (Figure XI.6c) and pure MBBR-MBR (Figure XI.6d).

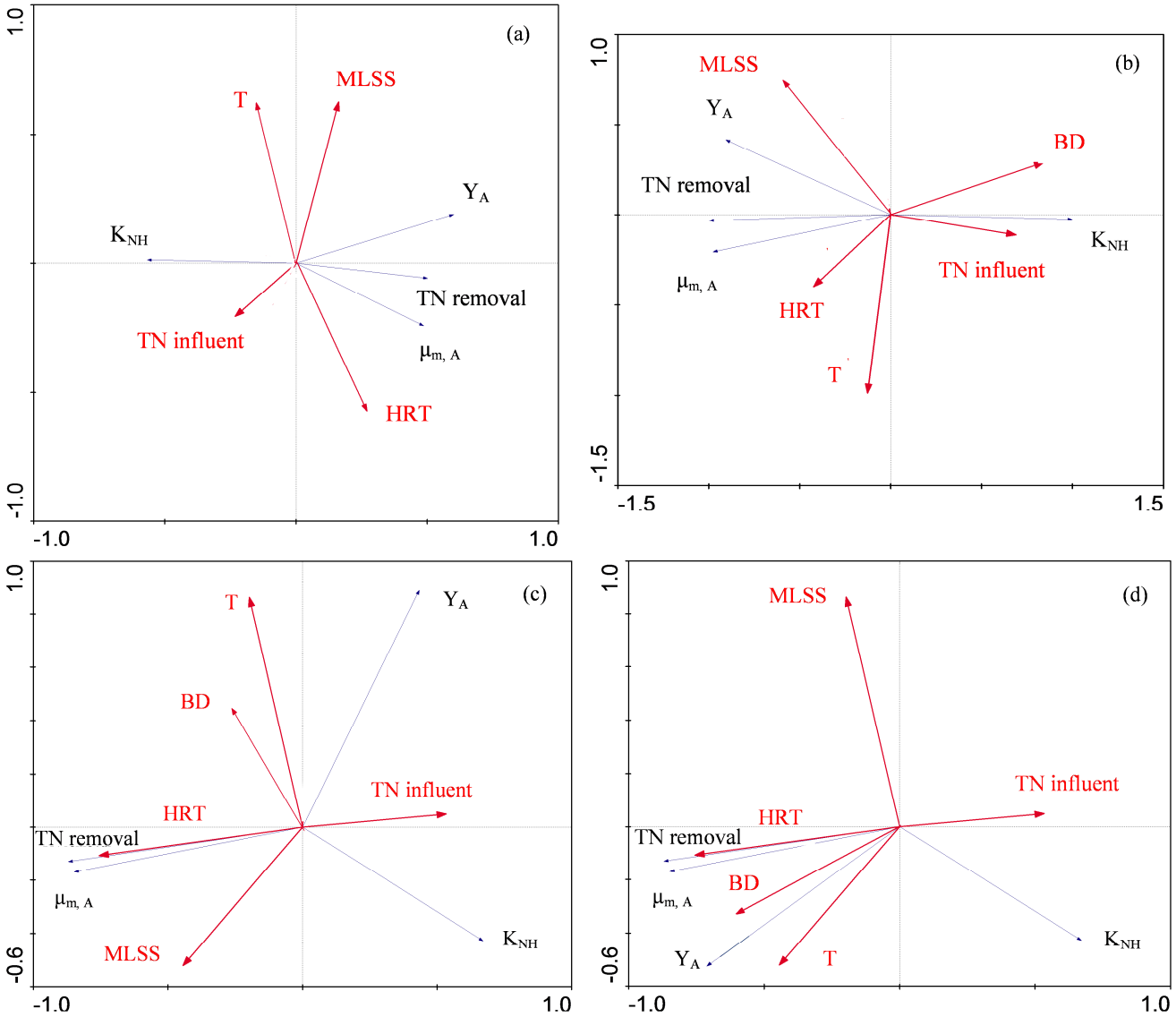

Figure XI.6. Triplot diagram of the Redundancy Analysis (RDA) of the kinetic parameters for autotrophic biomass (Table XI.2) and total nitrogen (TN) removal in relation to the variables TN of the influent, temperature (T), hydraulic retention time (HRT), mixed liquor suspended solids (MLSS) and biofilm density (BD) in the MBR (a), hybrid MBBR-MBR_a (b), hybrid MBBR-MBR_b (c) and pure MBBR-MBR (d).

Bearing in mind the length and the angles between the different vectors represented in Figure XI.6, the influence of each variable, TN of the influent, temperature, HRT, MLSS and BD (Table XI.1), can be independently analyzed on the different species, TN removal and kinetic parameters for autotrophic biomass (Table XI.2).

The HRT and MLSS concentration presented a positive correlation with the TN removal and the kinetic parameters for autotrophic biomass. It was in accordance with Figure XI.4 and Figure XI.5 as the efficiency of TN removal increased when the HRT and biomass concentration were higher. In this sense, the BD showed a strongly positive correlation with the TN removal and the autotrophic kinetic parameters for the pure MBBR-MBR (Figure XI.6d), which implies that the attached biomass had a higher influence on the nitrogen removal and the autotrophic kinetics than those obtained for the hybrid MBBR-MBR_b and hybrid MBBR-MBR_a as these systems presented a slightly positive correlation (Figure XI.6c) and a strongly negative correlation (Figure XI.6b) with the TN removal and the kinetic parameters for autotrophic biomass, respectively. It supported the higher TN removal in the pure MBBR-MBR under the HRTs lower than 9.5 h. Moreover, the effect of the attached biomass was higher in the hybrid MBBR-MBR_b in relation to the hybrid MBBR-MBR_a, which confirmed its higher TN removal under the HRTs higher than 9.5 h.

The temperature did not affect the different systems studied regarding the nitrogen removal as its effect was decreased due to the fact that the WWTPs were not out in the open, but they were inside a laboratory.

In general, the TN removal and the kinetic parameters for autotrophic biomass had a positive correlation in the four systems, so the kinetic study for autotrophic biomass supported the results of nitrogen removal.

As a conclusion, Figure XI.7 shows that the type of system, HRT and BD are the main variables which have influence on the performance of the different biological processes as they had a positive correlation with the organic matter and nitrogen removal and the heterotrophic and autotrophic kinetics of the different bioreactors.

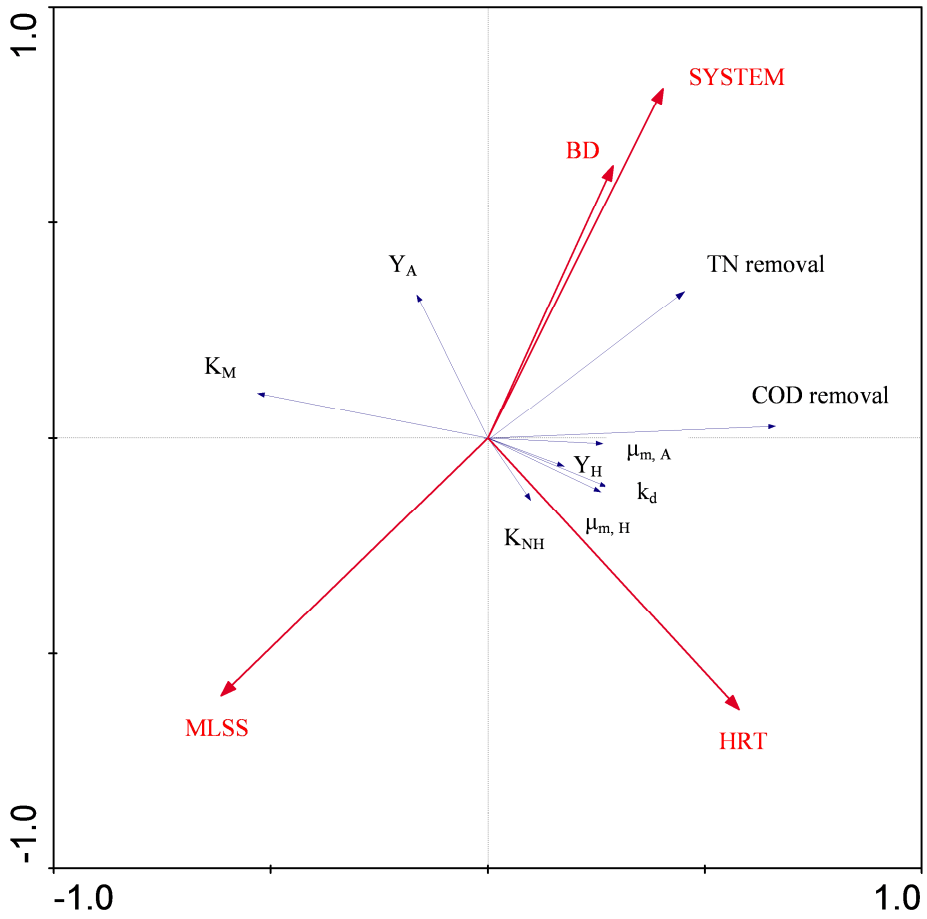

Figure XI.7. Triplot diagram of the Redundancy Analysis (RDA) of the kinetic parameters for autotrophic and heterotrophic biomass and chemical oxygen demand (COD) and total nitrogen (TN) removal in relation to the variables wastewater treatment technology (system), hydraulic retention time (HRT), mixed liquor suspended solids (MLSS) and biofilm density (BD).

Bearing in mind the two-step nitrification process, the nitrite-oxidizing bacteria (NOB) kinetics was studied under the HRTs of 18 h (**Chapter 3** and **Chapter 5**), 9.5 h (**Chapter 4** and **Chapter 6**) and 6 h (**Chapter 6**) for the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b. Furthermore, the kinetic behavior for NOB was analyzed under an HRT of 18 h for the MBR_p, hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp} (**Chapter 7**).

The MBR had usually the best kinetic behavior regarding the NOB kinetics under the different HRTs, e.g., with values of Y_{NOB} = 0.5682 mg O_2 mg N^{-1}, $\mu_{m, NOB}$ = 0.2032 h^{-1} and K_{NOB} = 0.9370 mg N L^{-1} under an HRT of 18 h, as shown in Figure VI.3c (**Chapter 3**). The MBR_p also showed the best kinetic performance concerning the NOB kinetics with values of Y_{NOB} = 0.5538 mg O_2 mg N^{-1}, $\mu_{m, NOB}$ = 0.1008 h^{-1} and K_{NOB} = 1.0553 mg N L^{-1}, as shown in Figure X.4c (**Chapter 7**). This supported the fact that the

nitrate concentration in the effluent from the MBR systems was higher than in the other processes, as shown in Table VI.3 (**Chapter 3**), Table VIII.3 (**Chapter 5**), Table IX.2 (**Chapter 6**) and Table X.4 (**Chapter 7**). Pambrun et al. (2006) and Iacopozzi et al. (2007) obtained similar values for the NOB kinetics.

As a consequence of this, the hybrid MBBR-MBR_b and hybrid MBBR-MBR_{bp} under an HRT of 18 h (**Chapter 3**, **Chapter 5** and **Chapter 7**), and the pure MBBR-MBR with the HRTs of 9.5 h and 6 h (**Chapter 6**) could have a better kinetic behavior regarding the ammonium-oxidizing bacteria (AOB) because, as a whole, the kinetics of autotrophic bacteria was better in these systems and the nitrite concentrations in their effluents were higher than those obtained in the MBR system, as indicated in Table VI.3 and Table VIII.3 for the hybrid MBBR-MBR_b (**Chapter 3 and Chapter 5**), Table X.4 for the hybrid MBBR-MBR_{bp} (**Chapter 7**) and Table IX.2 for the pure MBBR-MBR (**Chapter 6**).

There were statistically significant differences regarding nitrite and nitrate formations between the different experimental plants mentioned previously since the p-values obtained were lower than α=0.05, as shown in Table XI.3.

Table XI.3. P-values of sequential comparison (ANOVA analysis) of concentrations of nitrite (NO_2^-) and nitrate (NO_3^-) in the effluents between the different experimental plants. NO_2^- (concentration of nitrite), NO_3^- (concentration of nitrate).

Wastewater treatment plant		Parameter	
		NO_2^-	NO_3^-
HRT=18 h			
MBR	**Hybrid MBBR-MBR_b**	0.01959	0.03435
MBR_a	**Hybrid MBBR-MBR_b**	0.00833	0.03148
MBR_p	**Hybrid MBBR-MBR_{bp}**	0.00226	0.02904
HRT=9.5 h			
MBR_a	**Pure MBBR-MBR**	0.00027	0.01095
HRT=6 h			
MBR_a	**Pure MBBR-MBR**	0.01315	0.02289

3. Phosphorus removal

The differences between the MBR_p, the hybrid MBBR-MBR_{ap} and the hybrid MBBR-MBR_{bp} were not statistically significant regarding the organic matter and nitrogen removal under an HRT of 18 h (**Chapter 7**) as the p-values obtained from the post hoc procedure, Tukey´s HSD, were higher than α=0.05. In spite of this, the hybrid MBBR-MBR_{ap} showed a slightly higher performance regarding the COD removal, with a value of 85.82±2.12%, and the hybrid MBBR-MBR_{bp} had the best efficiency regarding the TN removal, with a value of 61.39±10.71% (Table X.4; **Chapter 7**). Additionally, the removal percentages of COD and TN were lower than those obtained for the MBR_a and hybrid MBBR-MBR_b under an HRT of 18 h and similar biomass concentrations, as shown in Table VIII.3 (**Chapter 5**). It is probably due to the fact that the aerobic zone could not be long enough for the heterotrophic and nitrifying bacteria to establish entirely (Gieseke et al., 2002) as the volume of the aerobic zone is 12 L in the MBR_p, hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp} (Table X.1; **Chapter 7**), while the volume of the aerobic zone was 18 L in the MBR_a and hybrid MBBR-MBR_b (Table VIII.1; **Chapter 5**).

The performances of total phosphorus (TP) removal for the MBR_p, hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp} (Chapter 7) were quite higher than those obtained in MBR and hybrid MBBR-MBR systems working with similar biomass concentrations under an HRT of 18 h, which ranged from 41% to 46% of TP removal, as shown in Table VI.3 (**Chapter 3**) and Table VIII.3 (**Chapter 5**). It is due to the existence of an anaerobic zone which enables the phosphorus removal.

The hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp} had the highest TP removal efficiencies. In this sense, the hybrid MBBR-MBR_{ap} showed the highest performance regarding the TP removal, with a value of 81.42±3.85% (Table X.5; **Chapter 7**). This system had the highest phosphorus release under anaerobic conditions according to Table X.5 (**Chapter 7**), and a high phosphate release is an advantage with respect to achieving a high net phosphate removal as production of polyhydroxybutyrate (PHB) necessary for phosphate uptake is linked to phosphate release (Helness and Ødegaard, 1999; Kermani et al., 2009). Furthermore, the highest performance regarding the COD removal for the hybrid MBBR-MBR_{ap}, with a value of 85.82±2.12% (Table X.4; **Chapter 7**), also supported its best behavior concerning the TP removal. The more

organic matter removal, the more cell growth for the polyphosphate accumulative organisms (PAOs), and a higher phosphorus removal will take place (Tchobanoglous et al., 2003; Kermani et al., 2009), since the COD is the primary source of volatile fatty acids (VFAs) for the PAOs.

There were statistically significant differences regarding the removal percentages of TP between the three WWTPs with an HRT of 18 h as the p-values obtained from the post-hoc procedure, Tukey's HSD, were less than α=0.05, p-value $_{\text{Hybrid MBBR-MBRap-MBRp}}$ (TP removal) = 0.03341, p-value $_{\text{Hybrid MBBR-MBRap-Hybrid MBBR-MBRbp}}$ (TP removal) = 0.03734 and p-value $_{\text{Hybrid MBBR-MBRbp-MBRp}}$ (TP removal) = 0.04465.

In this sense, the highest phosphorus removal for the hybrid MBBR-MBR_{ap} could explain that this system had a performance regarding the TN removal slightly lower than the MBR_p and hybrid MBBR-MBR_{bp} (Table X.4; **Chapter 7**). Nitrifying bacteria and PAOs compete for oxygen, and nitrifying bacteria have a lower affinity for oxygen, which may result in problems regarding the nitrifying activity (Prosser, 1989; Gieseke et al., 2002).

The MBR_p showed the lowest value of TP removal. It is possibly due to the higher concentration of nitrate in this system, 153.45±59.25 mg NO_3^- L^{-1} (Table X.4; Chapter 7), which could consume a portion of COD before the it is utilized by the PAOs. It could partially inhibit the phosphate release in the MBR_p (Kuba et al., 1994; Akin and Ugurlu, 2004).

The use of carriers, as well as the lower concentration of nitrate in the hybrid MBBR-MBR_{ap} and hybrid MBBR-MBR_{bp} (Table X.4; **Chapter 7**), could solve the conflict on simultaneous nitrogen and phosphorus removal. Attached biomass can form aerobic, anoxic and anaerobic zones which provide favorable conditions for the simultaneous nitrification and denitrification (Puznana et al., 2000; Yang et al., 2009). Thus, the biofilm can improve the nitrogen removal in the aerobic zone and avoid the transfer of nitrate into the anaerobic chamber.

4. Microbiological studies

4.1. Enzymatic activities of α-glucosidase, acid phosphatase and alkaline phosphatase

The values of α-glucosidase, acid phosphatase and alkaline phosphatase enzymatic activities of suspended and attached biomass of the microbial communities in the four chambers (C1, C2, C3 and C4) of the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b (Figure III.1a, Figure III.1b and Figure III.1c, respectively, of the section **Materials and Methods**) were studied under 30.4 h, 26.5 h and 18 h of HRT and similar biomass concentrations for each one of the HRTs (Table III.2 of the section **Materials and Methods**).

Table XI.4 shows the values of the α-glucosidase, acid and alkaline phosphatase enzymatic activities in the suspended and attached biomass of the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b under the HRTs of 30.4 h, 26.5 h and 18 h.

Table XI.4. Average values of the enzymatic activities of α-glucosidase, acid phosphatase and alkaline phosphatase in the suspended and attached biomass of the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b under the HRTs of 30.4 h, 26.5 h and 18 h. HRTs (hydraulic retention times).

Enzymatic activity	Sampling zone						
	MBR	Hybrid MBBR-MBR_a			Hybrid MBBR-MBR_b		
	Suspended biomass	Suspended biomass	Attached biomass	Total biomass	Suspended biomass	Attached biomass	Total biomass
HRT=30.4 h							
α-glucosidase (mM g VSS^{-1} min^{-1})	**0.1481±0.0068**	0.1412±0.0162	0.1585±0.0129	**0.2997±0.0291**	0.2197±0.0253	0.5150±0.0381	**0.7347±0.0634**
acid phosphatase (mM g VSS^{-1} min^{-1})	**2.6692±0.3991**	2.6429±0.3950	1.3069±0.1393	**3.9498±0.5343**	2.7650±0.4054	4.4769±0.3373	**7.2419±0.7427**
alkaline phosphatase (mM g VSS^{-1} min^{-1})	**10.0904±0.7878**	7.9465±0.6918	2.9025±0.2911	**10.8490±0.9829**	9.3361±0.1704	16.0685±0.1474	**25.4046±0.3178**
HRT=26.5 h							
α-glucosidase (mM g VSS^{-1} min^{-1})	**0.2064±0.0210**	0.2400±0.0322	0.0304±0.0049	**0.2704±0.0371**	0.2954±0.0266	0.2132±0.0212	**0.5086±0.0478**
acid phosphatase (mM g VSS^{-1} min^{-1})	**4.2315±0.2828**	5.5489±0.3398	0.7431±0.0654	**6.2920±0.4052**	5.0840±0.3504	2.6041±0.3006	**7.6881±0.6510**
alkaline phosphatase (mM g VSS^{-1} min^{-1})	**5.0313±0.3468**	2.9807±0.3271	0.3620±0.0554	**3.3427±0.3825**	3.1925±0.2288	2.2401±0.2066	**5.4326±0.4354**
HRT=18 h							
α-glucosidase (mM g VSS^{-1} min^{-1})	**0.4650±0.0430**	0.2791±0.0307	0.0142±0.0009	**0.2933±0.0316**	0.2128±0.0256	0.1982±0.0158	**0.4110±0.0414**
acid phosphatase (mM g VSS^{-1} min^{-1})	**8.6876±0.6129**	5.8527±0.7331	0.2946±0.0311	**6.1473±0.7642**	6.4976±0.6558	2.0179±0.1644	**8.5155±0.8202**
alkaline phosphatase (mM g VSS^{-1} min^{-1})	**4.3872±0.2845**	1.7947±0.1674	0.1677±0.0203	**1.9624±0.1877**	1.6962±0.1016	0.5091±0.0644	**2.2053±0.1660**

The differences were statistically significant regarding the three enzymatic activities between the hybrid MBBR-MBR_b and the other experimental plants studied under the HRT of 30.4 h as the p-values obtained from the post hoc procedure, Tukey´s HSD, were lower than α=0.05, but there were not statistically significant differences concerning the enzymatic activities between the experimental plants under the HRTs of 26.5 h and 18 h as the p-values obtained from the post hoc procedure were higher than α=0.05.

In spite of the fact that there were not statistically significant differences regarding the COD and TN removals with the HRTs of 30.4 h (**Chapter 1**), 26.5 h (**Chapter 2**), 18 h (**Chapter 3**), the hybrid MBBR-MBR_b usually showed a slightly higher efficiency concerning the TN removal under the working HRTs. These results were supported by the highest values of α-glucosidase, acid phosphatase and alkaline phosphatase in the hybrid MBBR-MBR_b (Table XI.4) as the bacterial activity is closely related to the enzymatic activity within an ecosystem (Nybroe et al., 1992). The hybrid MBBR-MBR_b did not show the best values of enzymatic activities under an HRT of 18 h although these values were similar to those obtained in the MBR, without statistically significant differences between both systems.

The enzymatic activities of α-glucosidase, acid and alkaline phosphatase showed different values in relation to the biomass configuration in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, with higher values for suspended biomass than for attached biomass, except for the attached biomass from the hybrid MBBR-MBR_b which had higher values of the enzymatic activities under an HRT of 30.4 h (Table XI.4). This might be caused by the low diffusivity that extracellular polymeric substances (EPS) layers provide to the attached biomass, which impedes the release of extracellular microbial enzymes so the enzymatic activity is reduced (Reboleiro-Rivas et al., 2013).

In this sense, it should be noted that the suspended biomass had similar values regarding the enzymatic activities for the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, while the attached biomass from the hybrid MBBR-MBR_b showed higher enzymatic activities of α-glucosidase, acid and alkaline phosphatase than the attached biomass from the hybrid MBBR-MBR_a. This improvement in the enzymatic activities of the attached biomass from the hybrid MBBR-MBR_b could have been caused by differences in the structure of the biofilm which was developed on the carriers, or

differences in the microbial community as a consequence of the existence of an anoxic zone without carriers. It was in accordance with the best performance of this system regarding the TN removal, explained previously.

4.2. TGGE fingerprint analysis

TGGE fingerprint band analysis regarding suspended and attached biomass communities for chambers C1, C2, C3 and C4 of the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b (Figure III.1a, Figure III.1b and Figure III.1c, respectively, of the section **Materials and Methods**) was carried out under 30.4 h, 26.5 h and 18 h of HRT and similar biomass concentrations for each one of the HRTs (Tabla III.2 of the section **Materials and Methods**).

Fingerprints belonging to the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b were clearly differentiated. Differences in the configuration of the bioreactors were shown in the TGGE fingerprints of their bacterial communities. However, differences in the disposition of bacterial communities seemed to not be remarkable in the different chambers of the three bioreactors, as indicated in the study of the enzymatic activities. In this sense, the TGGE fingerprints suggest similar diversity regarding the bacterial communities of the suspended and attached biomasses.

Therefore, the different configurations of the bioreactors led to differences in the bacterial communities developed in each of the WWTPs.

4.3. Analysis of biofilm communities by SEM

The SEM analysis was carried out to determine the morphology of the biofilm developed on the carriers of the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b (Figure III.1b and Figure III.1c, respectively, of the section **Materials and Methods**) under two HRTs of 26.5 h and 18 h. The SEM results demonstrated the presence of microorganisms with different structures such as bacilli, cocci, filamentous bacteria and also the appearance of extracellular polymeric substances (EPS) layers, which are the basis for biofilm development (Calderon et al., 2011; Calderon et al., 2012), as shown in Figure V.9 and Figure V.10 (**Chapter 2**) for an HRT of 26.5 h, and Figure VI.8 (**Chapter 3**) for an HRT of 18 h.

4.4. Study of the nitrifying and denitrifying microbial populations

The diversity and relative abundance of the nitrifying and denitrifying microbial populations in the suspended biomass and fixed biofilm for the MBR, hybrid MBBR-MBR_a, hybrid MBBR-MBR_b and pure MBBR-MBR were carried out under an HRT of 9.5 h (**Chapter 4 and Chapter 6**). The relative abundances of AOB, NOB, denitrifying bacteria (DeNB) and other bacterial species growing in the mixed liquor and on the carriers of these systems are shown in Figure XI.8.

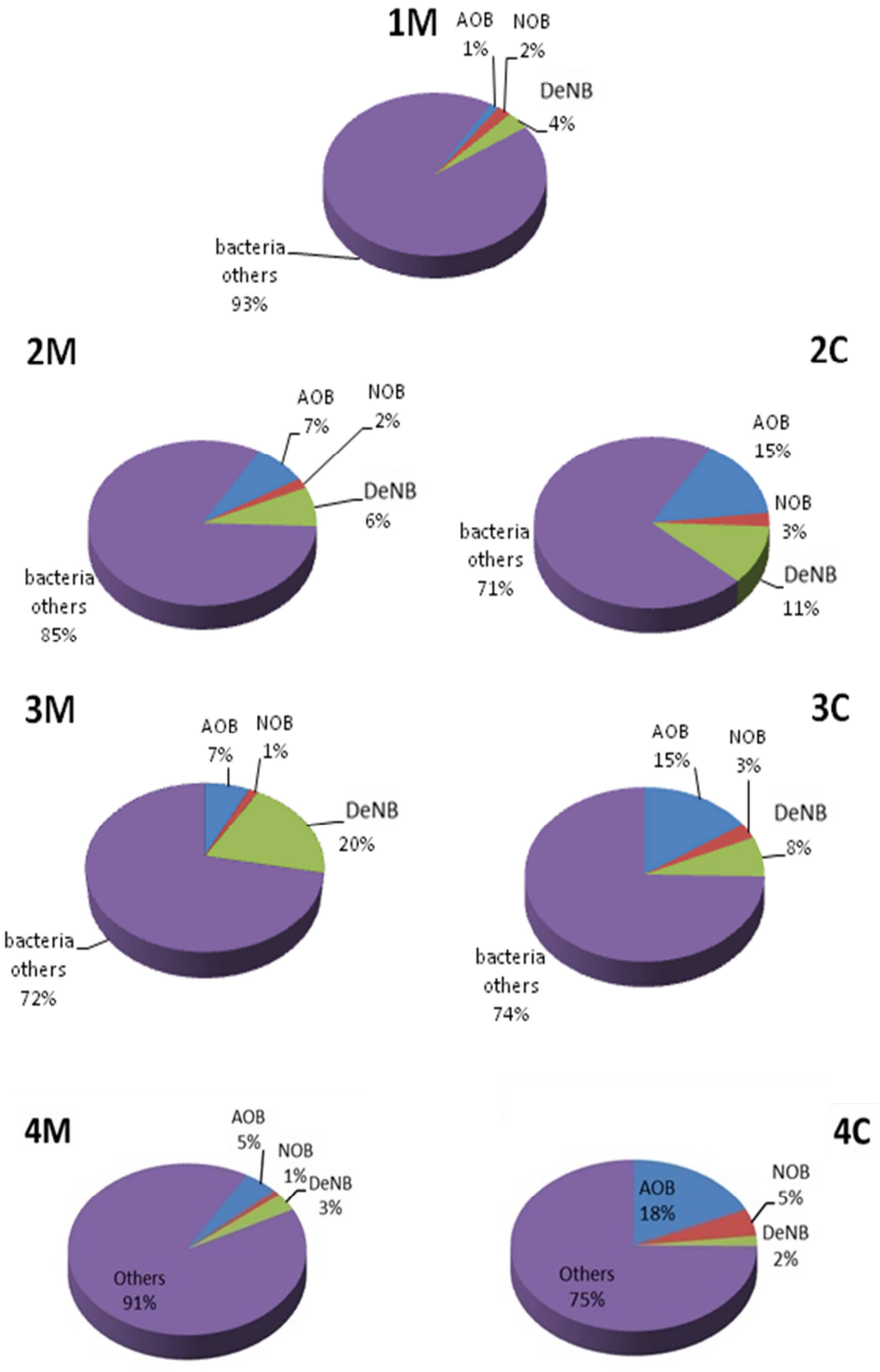

Figure XI.8. Percentage of AOB, NOB, DeNB and other bacteria in relation to the total bacteria in MLSS (M) and BD attached to carriers (C) in the MBR (1), hybrid MBBR-MBR$_a$ (2), hybrid MBBR-MBR$_b$ (3) and pure MBBR-MBR (4). AOB (ammonium-oxidizing bacteria), NOB (nitrite-oxidizing bacteria), DeNB (denitrifying bacteria).

The AOB was more represented in the fixed biofilm with respect to the mixed liquor. The NOB showed a similar pattern than that obtained for the AOB. On the other hand, the DeNB showed a higher relative abundance in the mixed liquor than on the carrier. The relative abundance of the DeNB on the carriers showed that the hybrid MBBR-MBR_a, which contained carriers in the anoxic and aerobic zones of the bioreactor, accounted for the highest DeNB representation and the pure MBBR-MBR system accounted for the lowest DeNB representation. Nevertheless, the potential of the DeNB was higher in the hybrid MBBR-MBR_b, containing carriers only in the aerobic zone of the bioreactor, compared to the other systems within the mixed liquor of the bioreactors of the different WWTPs. The high concentrations of DeNB detected in the hybrid MBBR-MBR_b could be explained by the absence of carriers in the anoxic zone. Attached growth on the surfaces of supporting materials has certain advantages, such as the protection of microorganisms in a hostile environment (Simões et al., 2010), e.g. in the presence of antimicrobial agents, ultraviolet light, and oxygen (Lyon, 2008). Consequently, the absence of attached biomass can reduce the growth of some aerobic microorganisms in the anoxic compartment of the bioreactor, resulting in the enrichment of denitrifying bacteria under these environmental conditions. The results showed that the DeNB community structure was much more volatile than those corresponding to the AOB and NOB. In light of this, the stability of the AOB and NOB bacterial community structure with respect to the DeNB at the different operational conditions was suggested.

The differences in the bacterial community structure between the MBR and the two hybrid MBBR-MBR systems were driven by the different operational conditions. In these cases, the AOB communities increased substantially with the addition of carriers, while the NOB communities did not experience any change. On the other hand, the DeNB were favored by the addition of carriers in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b. In the case of the pure MBBR-MBR, the working temperature changed (14.7±1.1ºC) and the decrease in the temperature (the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b worked at 17.2±1.9ºC) affected the AOB, NOB and DeNB. The AOB communities decreased in the suspended biomass but those developed on the carriers increased in relation to the hybrid MBBR-MBR systems. For the NOB communities, no significant changes were found, but the DeNB experienced a strong decrease in the relative abundance in the suspended and attached biomass.

The TN removal turned out to be significantly higher in the pure MBBR-MBR than that obtained in the MBR_a, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b under an HRT of 9.5 h (Table XI.2). This pattern could be explained by the higher AOB and NOB relative abundance on the carriers of the pure MBBR-MBR with respect to the other configurations.

Operational taxonomic units (OTUs) identified through pyrosequencing were related to important ecological roles in the nitrogen cycle inside the different WWTPs. Among them, ecological roles of ammonium oxidation, nitrite oxidation and nitrate/nitrite/nitrous oxide reduction were carried out by the AOB, NOB and DeNB, respectively. A Bray-Curtis similarity analysis comparing the AOB, NOB and DeNB community structure of all the WWTPs can be seen in Figure IX.3 (**Chapter 6**). Samples from the same WWTP, i.e. planktonic biomass and fixed biofilm samples, had a remarkable similarity, with the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b being a consistent cluster, and the MBR and pure MBBR-MBR becoming another. In general, the community structures of the AOB, NOB and DeNB showed clear similarities for the same growth conditions (planktonic growth and fixed biofilm growth). In this regard, the development of the AOB, NOB and DeNB was driven by the growth conditions of the biomass inside the WWTPs in a more important manner than by the environmental conditions which characterized the different WWTPs.

Additionally, this study showed that nitrifying populations were heterogeneous in the different systems with a large number of different species (Figure XI.9).

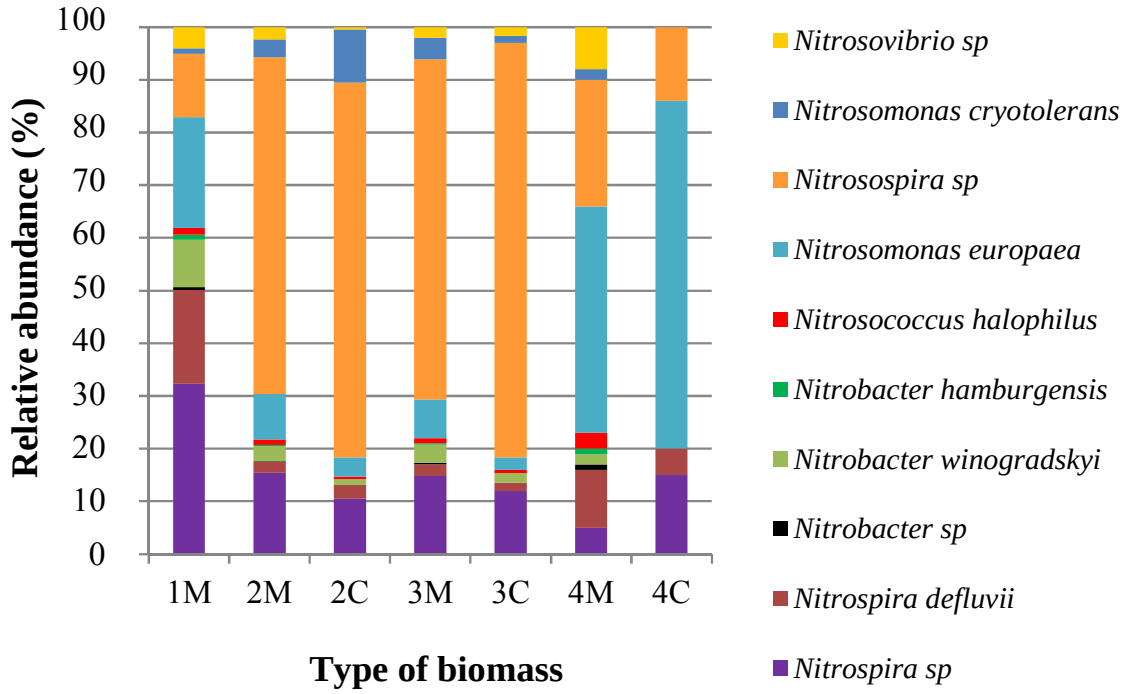

Figure XI.9. Relative abundance of the total nitrifying bacteria in MLSS (M) and BD attached to carriers (C) in the MBR (1), hybrid MBBR-MBR$_a$ (2), hybrid MBBR-MBR$_b$ (3) and pure MBBR-MBR (4).

Among the AOB, species from the genera *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira* and *Nitrosovibrio* could be found in the pure MBBR-MBR system. All these phylotypes have been reported as AOB (González-Martínez et al., 2011). Nevertheless, the genera *Nitrosococcus* and *Nitrosovibrio* accounted for a low relative abundance. In this way, *Nitrosomonas*- and *Nitrosospira*-related species were the most important bacteria driving the ammonium oxidation in the pure MBBR-MBR system. *Nitrosospira* sp. was the dominant AOB in the hybrid MBBR-MBR$_a$ and hybrid MBBR-MBR$_b$, while *Nitrosomonas europaea* was the dominant in the MBR and pure MBBR-MBR (Leyva-Díaz et al., 2015). It has been reported that *Nitrosomonas* species are r-strategists, while *Nitrosospira* species are k-strategists (Terada et al., 2013). As a result, *Nitrosomonas* will be favored by high ammonium concentrations in the environment.

The diversity of NOB in the pure MBBR-MBR showed species belonging to the genera *Nitrospira* or *Nitrobacter*, which have been reported as NOB (González-Martínez et al., 2011). *Nitrospira* genus dominated over *Nitrobacter* in the bioreactors of all the WWTPs. It is well-known that species of these genera are the key NOB in WWTPs (Schramm et al., 1998; Kim and Kim, 2006; Vanparys et al., 2007). *Nitrospira*

sp. largely represented the most important NOB for the WWTPs. It has been found that high nitrite concentrations promote the growth of *Nitrobacter* species over *Nitrospira* species (Ter Haseborg et al., 2010). It was in accordance with the low nitrite concentrations showed in Table IX.2 (**Chapter 6**) for the pure MBBR-MBR and those obtained in Table VII.2 (**Chapter 4**) for the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, which could be the reason why *Nitrospira* sp. was the most important NOB in the WWTPs.

The nitrifying population was very similar in the suspended and attached biomass in the hybrid MBBR-MBR_a, hybrid MBBR-MBR_b and pure MBBR-MBR (Figure XI.9).

The DeNB were more diverse than the AOB and NOB in the pure MBBR-MBR system, with species from seven genera thriving within the bioreactor, i.e. *Diaphorobacter*, *Ottowia*, *Thiobacillus*, *Thermomonas*, *Pseudomonas*, *Pleomorphomonas* and *Rhizobium*. In the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, *Ottowia* sp., *Thermomonas* sp., *Pseudomonas denitrificans*, *Rhizobium melitoti* and *Pleomorphomonas* sp. were the dominant DeNB. The species of *Ottowia* has been reported for nitrate and nitrite reduction (Spring et al., 2004; Geng et al., 2014). FISH analysis of nitrifying communities suggests that *Thermomonas* species thrives on the metabolites produced by AOB and NOB (Dolinšek et al., 2013) and has been reported for nitrite and nitrate reduction (Mergaert et al., 2003). The reduction of nitrate by *Pseudomonas denitrificans* has been proved (Parvanova-Mancheva and Beschkov, 2009). The *Pleomorphomonas* genus strain type *Pleomorphomonas oryzae* has shown nitrate reduction ability (Xie and Yokota, 2005). In the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b, there were differences between the planktonic biomass and fixed biofilm, with a much higher relative abundance of *Thiobacillus denitrificans* in the mixed liquor than on the carriers. *Thiobacillus denitrificans* has been identified as sulfur-oxidizing denitrifying bacteria (Sahinkaya et al., 2013; Kim et al., 2014). The dominant DeNB in the MBR and pure MBBR-MBR were *Rhizobium melitoti* and *Pseudomonas denitrificans*.

The higher TN removal in the pure MBBR-MBR compared with the other WWTPs might also reside in the different bacterial assemblages in the fixed biofilm on the carriers. The Bray-Curtis similarity analysis showed the bacterial community

structure of the fixed biofilm of the pure MBBR-MBR to be different for AOB, NOB and DeNB with respect to the other WWTPs.

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XII. CONCLUSIONES/CONCLUSIONS

CONCLUSIONES

Se estudiaron diferentes plantas de tratamiento de aguas residuales (WWTPs) en base a la eliminación de materia orgánica, nitrógeno y fósforo bajo diferentes tiempos de retención hidraúlico (TRHs) y concentraciones de biomasa, que iban de 6 h hasta 30.4 h y de 2,128 mg L^{-1} a 6,656 mg L^{-1}, respectivamente. Las WWTPs para la eliminación de materia orgánica y nitrógeno consistían en un bioreactor de membrana (MBR), un biorreactor de membrana con lecho móvil (MBBR-MBR) híbrido que presentaba relleno en las zonas anóxica y aeróbica del bioreactor (MBBR-MBR $híbrido_a$), un MBBR-MBR híbrido que contenía material soporte solamente en la zona aeróbica del bioreactor (MBBR-MBR $híbrido_b$) y un MBBR-MBR puro que también tenía relleno sólo en la zona aeróbica del reactor biológico y el crecimiento de la biomasa se desarrollaba principalmente sobre el material soporte. Las WWTPs para la eliminación de materia orgánica, nitrógeno y fósforo consistían en un biorreactor de membrana (MBR_p), un MBBR-MBR híbrido que contenía relleno en las zonas anaeróbica, anóxica y aeróbica del biorreactor (MBBR-MBR $híbrido_{ap}$) y un MBBR-MBR híbrido que presentaba material soporte solamente en las zonas anaeróbica y anóxica del biorreactor (MBBR-MBR $híbrido_{bp}$).

De acuerdo con los resultados obtenidos, así como con la revisión bibliográfica llevada a cabo, se presentan las siguientes conclusiones respecto a los biorreactores de membrana con y sin lecho móvil analizados en este estudio:

1. El sistema MBBR-MBR $híbrido_b$ era el mejor respecto a la eliminación de demanda química de oxígeno (DQO) para TRHs por debajo de 9.5 h (87.39±6.01% y 84.10±2.25% para 9.5 h y 6 h, respectivamente) debido a la presencia de biomasa suspendida y adherida, con diferencias estadísticamente significativas en relación al resto de sistemas, mientras que el sistema MBBR-MBR puro tenía el rendimiento más bajo respecto a la eliminación de DQO con valores de 80.96±7.67% y 79.78±4.60% a 9.5 h y 6 h, respectivamente. No había diferencias estadísticamente significativas respecto a la eliminación de DQO entre las diferentes configuraciones para TRHs mayores de 18 h. La eficacia de eliminación de DQO normalmente disminuía cuando la concentración de biomasa mostraba los valores más altos alrededor de un valor medio de 6,500 mg L^{-1} y cuando el tiempo de retención hidráulico

(TRH) era más bajo. El estudio cinético para la biomasa heterótrofa estaba en consonancia con estos resultados.

2. El rendimiento en eliminación de nitrógeno total (NT) era ligeramente mayor en el sistema MBBR-MBR híbrido$_b$ para TRHs mayores de 9.5 h. El sistema MBBR-MBR puro tenía los rendimientos más elevados en cuanto a eliminación de NT para TRHs inferiores a 9.5 h, con diferencias estadísticamente significativas, ya que la biomasa se desarrollaba principalmente sobre el material soporte como biomasa adherida. En consecuencia, una zona anóxica sin relleno facilitaba la interacción entre el nitrato y los microorganismos. En general, la eficacia de eliminación de NT aumentaba cuando la concentración de biomasa mostraba los valores más elevados en torno a un valor medio de 6,500 mg L^{-1} y cuando el TRH era más alto. Estos resultados estaban en consonancia con el estudio cinético llevado a cabo para la biomasa autótrofa.

3. La introducción de una zona anaeróbica en el biorreactor mejoraba la eliminación de fósforo total (PT), que pasaba de valores en el rango 41.88±16.27% - 45.30±7.85% para sistemas MBR y MBBR-MBR híbridos sin una cámara anaeróbica hasta valores en el rango 74.38±3.90% - 81.42±3.85% para sistemas MBR y MBBR-MBR híbridos con una zona anaeróbica, bajo un TRH de 18 h y concentraciones de biomasa similares. En este sentido, el sistema MBBR-MBR híbrido$_{ap}$ mostraba una tendencia de mejora en cuanto al rendimiento de eliminación de PT, con diferencias estadísticamente significativas en relación al resto de sistemas, debido a su mayor liberación de fósforo bajo condiciones anaeróbicas, lo cual constituye una ventaja respecto a la consecución de una mayor eliminación neta de fosfato.

4. Los sistemas MBR y MBR$_p$ presentaban, en general, el mejor comportamiento cinético respecto a la cinética de bacterias oxidadoras de nitrito (NOB) bajo las condiciones operacionales de este estudio, lo cual implicaba que la concentración de nitrato en el efluente de los sistemas MBR fuera mayor que en los demás procesos. Los sistemas MBBR-MBR híbrido$_b$ y MBBR-MBR híbrido$_{bp}$ con un TRH de 18 h, y el sistema MBBR-MBR puro

con TRHs de 9.5 h y 6 h podrían tener un mejor comportamiento cinético en relación a las bacterias oxidadoras de amonio (AOB) ya que la cinética global de bacterias autótrofas era mejor en estos sistemas y las concentraciones de nitrito en sus efluentes eran mayores que aquellas obtenidas en los sistemas MBR.

5. Una limitación común de los modelos de fangos activos (ASM) es la representación del proceso de nitrificación en una única etapa. El modelado cinético y el estudio microbiológico han mejorado el modelo básico ASM3 considerando el proceso de nitrificación en dos etapas. De esta forma, la caracterización del proceso biológico y el control de los parámetros operacionales de las WWTPs serán mejorados. Por lo tanto, los costes operacionales podrían ser optimizados respecto a las necesidades de nitrificación del sistema, usando la concentración adecuada de oxígeno.

6. La eliminación de material soporte de la zona anóxica del biorreactor (configuración MBBR-MBR híbrido$_b$) determinó un incremento de las actividades enzimáticas estudiadas (α-glucosidasa, fosfatasa ácida y fosfatasa alcalina), así como de la capacidad de eliminación de NT, en relación a las otras configuraciones ensayadas (MBR y MBBR-MBR híbrido$_a$) con TRHs de 18 h, 26.5 h y 30.4 h. Estos resultados fueron especialmente significativos al establecerse condiciones operacionales con altos TRHs (30.4 h).

7. La diversidad de la comunidad bacteriana presente en los biorreactores de los sistemas MBR, MBBR-MBR híbrido$_a$ y MBBR-MBR híbrido$_b$ se veía afectada por la configuración de los mismos, con independencia del TRH ensayado. No obstante, en todos los casos se detectaron comunidades complejas tanto en la biomasa suspendida como adherida al soporte (biofilm). En cuanto a la diversidad bacteriana analizada mediante técnicas moleculares como la electroforesis en gel con gradiente de temperatura (TGGE), se puede establecer que la misma era análoga en la biomasa suspendida y adherida en cada configuración, por lo que se puede afirmar que es la configuración del sistema la que influye de una forma directa sobre la diversidad microbiana, más que el hecho de que la biomasa estuviera adherida o no a un soporte.

8. La ausencia de recirculación de licor mezcla desde el tanque de membranas al reactor biológico, que caracteriza al sistema MBBR-MBR puro, originó un aumento de la población microbiana de *Nitrosomonas europaea* como género de AOB más representativo en relación con *Nitrosospira* sp., que constituía la población de AOB principal en los sistemas MBBR-MBR híbrido$_a$ y MBBR-MBR híbrido$_b$. En todos los sistemas estudiados, *Nitrospira* era el género de NOB dominante, cuyo crecimiento era favorecido por las bajas concentraciones de nitrito observadas en las diferentes configuraciones analizadas a un TRH de 9.5 h.

Conclusiones de aplicación

1. Entre los diferentes procesos estudiados, el sistema MBBR-MBR híbrido$_b$ es el proceso que presenta un mejor rendimiento de eliminación de materia orgánica para TRHs inferiores a 9.5 h, con lo que serviría para la rehabilitación de plantas de fangos activos y biorreactores de membrana (MBRs) que por algún motivo no cumplieran con la Directiva 91/271/CEE.

2. El sistema MBBR-MBR híbrido$_b$, con TRHs superiores a 9.5 h, serviría para adaptar plantas de tratamiento de aguas residuales cuyo vertido fuera a zonas sensibles donde está limitada la concentración de NT. Esta adaptación respecto al rendimiento de eliminación de NT se llevaría a cabo de la siguiente manera:

   a. A mayor concentración total de biomasa, el rendimiento aumenta.
   b. A mayor TRH, el rendimiento aumenta.

3. Los diferentes sistemas estudiados, bajo las condiciones de concentración de biomasa total y TRH analizadas, cumplen con la Directiva 91/271/CEE en cuanto a eliminación de materia orgánica. Esto puede ser debido a la existencia de un proceso físico de separación con membranas de ultrafiltración que mejora sustancialmente los rendimientos.

4. Se ha comprobado que en un sistema MBBR-MBR puro, sin recirculación, con el 35% de relleno en la zona aeróbica, que supone el 75% del reactor, y sin relleno en la zona anóxica, prácticamente toda la biomasa está adherida al

relleno, comparándose su funcionamiento con un lecho inundado parcialmente. Cabe destacar que los tiempos de retención celulares (TRCs) de 6 días y 4.5 días para TRHs de 9.5 h y 6 h, respectivamente, no permiten el uso de este sistema para la eliminación de materia orgánica, en cambio sí que se mostró muy eficaz para la eliminación de NT a TRHs inferiores a 9.5 h. En consecuencia, el sistema MBBR-MBR puro se podría usar para adaptar plantas de tratamiento de aguas residuales cuyo influente contenga una baja concentración de materia orgánica y también cuando su efluente no cumpla con la Directiva 91/271/CEE en relación a las concentraciones de nitrógeno y fósforo.

5. El uso de material soporte en las zonas anaeróbica, anóxica y aeróbica del biorreactor (configuración MBBR-MBR híbrido$_{ap}$) mejoraba el rendimiento en eliminación de PT, a un TRH de 18 h, en relación al resto de sistemas estudiados, por lo que podría utilizarse para adaptar plantas de tratamiento de aguas residuales cuyo vertido fuera a zonas sensibles donde está limitada la concentración de PT.

6. El modelado cinético y el estudio microbiológico han mejorado el modelo básico ASM3 considerando el proceso de nitrificación en dos etapas. De esta forma, la caracterización del proceso biológico y el control de los parámetros operacionales de las WWTPs serán mejorados. Por lo tanto, los costes operacionales podrían ser optimizados respecto a las necesidades de nitrificación del sistema, usando la concentración adecuada de oxígeno.

CONCLUSIONS

Different wastewater treatment plants (WWTPs) were studied in relation to the organic matter, nitrogen and phosphorus removal under different hydraulic retention times (HRTs) and biomass concentrations, which ranged from 6 h to 30.4 h and from 2,128 mg L^{-1} to 6,656 mg L^{-1}, respectively. The WWTPs for organic matter and nitrogen removal consisted of a membrane bioreactor (MBR), a hybrid moving bed biofilm reactor-membrane bioreactor (MBBR-MBR) containing carriers both in the anoxic and aerobic zones of the bioreactor (hybrid MBBR-MBR_a), a hybrid MBBR-MBR which contained carriers only in the aerobic zone of the bioreactor (hybrid MBBR-MBR_b) and a pure MBBR-MBR which also contained carriers only in the aerobic zone of the biological reactor and the biomass growth was mainly developed on the carriers. The WWTPs for organic matter, nitrogen and phosphorus removal consisted of an MBR_p, a hybrid MBBR-MBR containing carriers in the anaerobic, anoxic and aerobic zones of the bioreactor (hybrid MBBR-MBR_{ap}) and a hybrid MBBR-MBR which contained carriers only in the anaerobic and anoxic zones of the bioreactor (hybrid MBBR-MBR_{bp}).

Based on the results obtained, as well as the literature review carried out, the following conclusions are presented regarding the MBBR-MBR systems and membrane bioreactors (MBRs) analyzed throughout the study:

1. The hybrid MBBR-MBR_b was the best system concerning the chemical oxygen demand (COD) removal for HRTs lower than 9.5 h (87.39±6.01% and 84.10±2.25% for 9.5 h and 6 h, respectively) due to the presence of suspended and attached biomass, with statistically significant differences regarding the other systems, while the pure MBBR-MBR showed the lowest performance in relation to the COD removal with values of 80.96±7.67% and 79.78±4.60% for 9.5 h and 6 h, respectively. There were not statistically significant differences regarding the COD removal between the different configurations for HRTs higher than 18 h. The efficiency of COD removal usually decreased when the biomass concentration showed the highest values around an average value of 6,500 mg L^{-1} and when the hydraulic retention time (HRT) decreased. The kinetic study for heterotrophic biomass supported these data.

2. The efficiency concerning the total nitrogen (TN) removal was slightly higher in the hybrid MBBR-MBR_b for HRTs higher than 9.5 h. The pure MBBR-MBR had the highest percentages of TN removal for HRTs lower than 9.5 h, with statistically significant differences, as the biomass was mainly developed on carriers as attached biomass. Consequently, an anoxic zone without carriers provided better contact between nitrate and the microorganisms. The efficiency of TN removal generally increased when the biomass concentration showed the highest values around an average value of 6,500 mg L^{-1} and when the HRT increased. It was in accordance with the kinetic study for autotrophic biomass.

3. The introduction of an anaerobic zone in the bioreactor improved the total phosphorus (TP) removal, which ranged from 41.88±16.27% - 45.30±7.85% for MBR and hybrid MBBR-MBR systems without an anaerobic compartment to 74.38±3.90 - 81.42±3.85% for MBR and hybrid MBBR-MBR systems with an anaerobic zone, under an HRT of 18 h and similar biomass concentrations. In light of this, the hybrid MBBR-MBR_{ap} showed an improvement trend regarding the performance of TP removal, with statistically significant differences in relation to the other systems, due to its higher phosphorus release under anaerobic conditions, which is an advantage with respect to achieving a high net phosphate removal.

4. The MBR and MBR_p had usually the best kinetic behavior regarding the nitrite-oxidizing bacteria (NOB) kinetics under the operational conditions of this study, which implied that the nitrate concentration in the effluent from the MBR systems was higher than in the other processes. The hybrid MBBR-MBR_b and hybrid MBBR-MBR_{bp} under an HRT of 18 h, and the pure MBBR-MBR with the HRTs of 9.5 h and 6 h could have a better kinetic behavior regarding the ammonium-oxidizing bacteria (AOB) because, as a whole, the kinetics of autotrophic bacteria was better in these systems and the nitrite concentrations in their effluents were higher than those obtained in the MBR system.

5. A common limitation of the activated sludge models (ASM) is the representation of nitrification dynamics as a single-step process. The kinetic

modeling and microbiological study have enhanced the basic ASM3 model considering the two-step nitrification. In this way, the characterization of the biological process and the control of the operational parameters of the WWTPs will be improved. Therefore, operating costs could be optimized concerning the necessity of nitrification, using a suitable oxygen concentration.

6. The removal of carrier from the anoxic zone of the bioreactor (hybrid MBBR-MBR_b) involved an increase of the enzymatic activities studied (α-glucosidase, acid phosphatase and alkaline phosphatase), as well as the capacity to remove TN in relation to the other configurations tested (MBR and hybrid MBBR-MBR_a) under HRTs of 18 h, 26.5 h and 30.4 h. These results were statistically significant under high HRTs (30.4 h).

7. The bacterial community diversity from the MBR, hybrid MBBR-MBR_a and hybrid MBBR-MBR_b was influenced by the different configurations, independently of the HRT assayed. Nevertheless, complex communities were detected in the suspended and attached biomass (biofilm) in all the systems. Regarding the bacterial diversity analyzed by molecular methods such as temperature gradient gel electrophoresis (TGGE), it should be noted that it was similar in the suspended and attached biomass of each configuration, thus, it can be concluded that the system configuration directly affected the microbial diversity, more than the fact that the biomass was attached or not to carriers.

8. The lack of mixed liquor recycling from the membrane tank to the biological reactor, which characterizes the pure MBBR-MBR, caused an increase in the microbial population of *Nitrosomonas europaea* as the most representative AOB compared with *Nitrosospira* sp., which was the main AOB population in the hybrid MBBR-MBR_a and hybrid MBBR-MBR_b. In all the systems studied, *Nitrospira* was the dominant NOB genus as the low nitrite concentrations favored its growth in the different configurations analyzed under an HRT of 9.5 h.

Implementation conclusions

1. Among the different processes studied, the hybrid MBBR-MBR_b shows the best efficiency of organic matter removal for HRTs lower than 9.5 h, so this system would enable the rehabilitation of activated sludge plants and membrane bioreactors (MBRs) which, for any reason, did not comply with the Directive 91/271/EEC.

2. The hybrid MBBR-MBR_b, under HRTs higher than 9.5 h, could be used to adapt WWTPs whose effluent was flowed into sensitive zones where the TN concentration is restricted. This adaptation concerning the performance of TN removal could be carried out in the following way:

   a. The higher the total biomass concentration is, the higher the performance is.
   b. The higher the HRT is, the higher the performance is.

3. The different systems studied, under the conditions of total biomass concentration and HRT assayed, obey the Directive 91/271/EEC regarding organic matter removal. This could be caused by the existence of a physical separation process with ultrafiltration membranes, which substantially improve the performance of organic matter removal.

4. It has been proved that in a pure MBBR-MBR, without recycling, with 35% of carrier in the aerobic zone, which represents 75% of the bioreactor, and without carrier in the anoxic zone, the biomass was mainly developed on carriers as attached biomass, comparing its performance to a partially submerged filter. It should be noted that the sludge retention times (SRTs) of 6 days and 4.5 days for HRTs of 9.5 h and 6 h, respectively, do not allow the use of this system for organic matter removal, while the pure MBBR-MBR was very efficient for TN removal under HRTs lower than 9.5 h. Consequently, the pure MBBR-MBR could be used to adapt WWTPs whose influent contains a low organic matter concentration and also when its effluent does not comply with the Directive 91/271/EEC regarding the nitrogen and phosphorus concentrations.

5. The use of carrier in the anaerobic, anoxic and aerobic zones of the bioreactor (hybrid MBBR-MBR_{ap} configuration) improved the performance regarding TP removal, under an HRT of 18 h, compared to the other systems studied, so this system could be used to adapt WWTPs whose effluent was flowed into sensitive zones where the TP concentration is restricted.

6. The kinetic modeling and microbiological study have enhanced the basic ASM3 model considering the two-step nitrification. In this way, the characterization of the biological process and the control of the operational parameters of the WWTPs will be improved. Therefore, operating costs could be optimized concerning the necessity of nitrification, using a suitable oxygen concentration.

XIII. LÍNEAS FUTURAS DE INVESTIGACIÓN/FUTURE RESEARCH LINES

LÍNEAS FUTURAS DE INVESTIGACIÓN

Una vez estudiado el comportamiento de los biorreactores de membrana con y sin lecho móvil, se pueden destacar las siguientes líneas de investigación futuras:

- Análisis complementario de la eliminación biológica de fósforo bajo diferentes condiciones operacionales en biorreactores de membrana con lecho móvil (sistemas MBBR-MBR).

- Evaluación del ensuciamiento de la membrana bajo diferentes concentraciones de biomasa suspendida y adherida en sistemas MBBR-MBR.

- Estudio de la influencia de la salinidad del agua residual en el funcionamiento de los sistemas MBBR-MBR, analizando el efecto sobre el ensuciamiento de la membrana y la biología del sistema.

- Estudio del escalado de los sistemas MBBR-MBR para tratar aguas residuales urbanas.

- Análisis de costes de implantación y explotación de reactores de biopelícula de lecho móvil (MBBRs) con y sin tecnología de membranas, y comparativa de costes a escala real entre MBBRs y biorreactores de membrana (MBRs).

FUTURE RESEARCH LINES

Having studied the performance of moving bed biofilm reactor-membrane bioreactor (MBBR-MBR) systems and membrane bioreactors (MBRs), the following future research lines should be highlighted:

- Supplementary analysis of the biological phosphorus removal under different operational conditions in MBBR-MBR systems.
- Evaluation of the membrane fouling under different concentrations of suspended and attached biomass in MBBR-MBR systems.
- Study of the influence of wastewater salinity on the performance of the MBBR-MBR systems, by assessing its effect on membrane fouling and system biology.
- Study of scale-up of the MBBR-MBR systems for municipal wastewater treatment.
- Analysis of investment and operating costs of moving bed biofilm reactors (MBBRs) with and without membrane technology, and comparative study of costs at full scale between MBBRs and MBRs.

XIV. SCIENTIFIC CONTRIBUTIONS

The results presented in the Doctoral Thesis have been partially published both in international scientific journals and international and national congresses. Moreover, the PhD student has taken part in different research articles and congresses in the field of wastewater treatment.

1. Research articles in international scientific journals

1.1. Research articles of the PhD student

- **Leyva-Díaz, J.C.**, González-Martínez, A., González-López, J., Muñío, M.M., Poyatos, J.M., 2015. Kinetic modeling and microbiological study of two-step nitrification in a membrane bioreactor and hybrid moving bed biofilm reactor-membrane bioreactor for wastewater treatment. Chemical Engineering Journal 259, 692-702.

- **Leyva-Díaz, J.C.**, Martín-Pascual, J., Muñío, M.M., González-López, J., Hontoria, E., Poyatos, J.M., 2014. Comparative kinetics of hybrid and pure moving bed reactor-membrane bioreactors. Ecological Engineering 70, 227-234.

- **Leyva-Díaz, J.C.**, Martín-Pascual, J., González-López, J., Hontoria, E., Poyatos, J.M., 2013. Effects of scale-up on a hybrid moving bed biofilm reactor-membrane bioreactor for treating urban wastewater. Chemical Engineering Science 104, 808-816.

- **Leyva-Díaz, J.C.**, Calderón, K., Rodríguez, F.A., González-López, J., Hontoria, E., Poyatos, J.M., 2013. Comparative kinetic study between moving bed biofilm reactor-membrane bioreactor and membrane bioreactor systems and their influence on organic matter and nutrients removal. Biochemical Engineering Journal 77, 28-40.

- Start-up of membrane bioreactor and hybrid moving bed biofilm reactor-membrane bioreactor: Kinetic study **(Sent)**.

- Microbial kinetics and enzymatic activities in hybrid moving bed biofilm reactor-membrane bioreactor systems **(Sent)**.

- Kinetic study of the combined processes of a membrane bioreactor and a hybrid moving bed biofilm reactor-membrane bioreactor with advanced oxidation processes as a post-treatment stage for wastewater treatment **(Sent)**.

1.2. Collaborations of the PhD student

- Rodríguez, F.A., **Leyva-Díaz, J.C.**, Reboleiro-Rivas, P., González-López, J., Hontoria, E., Poyatos, J.M., 2014. Influence of sludge retention time and temperature on the sludge removal in a submerged membrane bioreactor: Comparative study between pure oxygen and air to supply aerobic conditions. Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering 49(2), 243-251.

- Martín-Pascual, J., Reboleiro-Rivas, P., López-López, C., **Leyva-Díaz, J.C.**, Jóver, M., Muñío, M.M., González-López, J., Poyatos, J.M., 2014. Effect of the filling ratio, MLSS, hydraulic retention time, and temperature on the behavior of the hybrid biomass in a hybrid moving bed membrane bioreactor plant to treat urban wastewater. Journal of Environmental Engineering. DOI: 10.1061/(ASCE)EE.1943-7870.0000939.

- Martín-Pascual, J., **Leyva-Díaz, J.C.**, López-López, C., Muñío, M.M., Hontoria, E., Poyatos, J.M., 2013. Effects of temperature on the permeability and critical flux of the membrane in a moving bed membrane bioreactor. Desalination and Water Treatment. DOI: 10.1080/19443994.2013.873879.

- Bermúdez de Castro, F.H., Blázquez, G., Calero de Hoces, M., Martín-Lara, M.A., **Leyva-Díaz, J.C.**, 2009. Biosorción de plomo con hueso de aceituna en columna de lecho fijo. Afinidad 66, 365-371.

- Biofouling associated to calcite and struvite biominerals precipitation in a pure moving bed biofilm reactor-membrane bioreactor: Isolation and metagenomic characterization of involved bacteria **(Sent)**.

- Combined treatment of textile wastewater by coagulation-flocculation and advanced oxidation processes **(Sent)**.

2. Contributions to international and national congresses

2.1. Contributions of the PhD student

- **Leyva-Díaz, J.C.**, Muñío, M.M., Poyatos, J.M. Study of the kinetic modeling in hybrid and pure moving bed biofilm reactor-membrane bioreactor systems for wastewater treatment. International Congress on Project Management and Engineering, Granada (Spain), July 15-17, 2015.

- **Leyva-Díaz, J.C.**, Poyatos, J.M. Kinetic study of membrane bioreactor and hybrid moving bed biofilm reactor-membrane bioreactor. IWA World Water Congress & Exhibition, Lisbon (Portugal), September 21-26, 2014.

- **Leyva-Díaz, J.C.**, Muñío, M.M., González-López, J., Hontoria, E., Poyatos, J.M. Comparison between a membrane bioreactor, a hybrid moving bed biofilm reactor-membrane bioreactor and a pure moving bed biofilm reactor-membrane bioreactor in wastewater treatment. Interdisciplinary Water Congress, Vigo (Spain), June 2-6, 2014.

2.2. Collaborations of the PhD student

- García-Mesa, J.J., Rodríguez, F.A., **Leyva-Díaz, J.C.**, Delgado-Ramos, F., Hontoria, E., Poyatos, J.M. Quality characterization in real wastewater treatment systems by particle size distribution. 12th Mediterranean Congress of Chemical Engineering, Barcelona (Spain), November 15-18, 2011.

- Calero, M., Hernáinz, F., Blázquez, G., Martín-Lara, M.A., **Leyva-Díaz, J.C.** Uso de hueso de aceituna para la biosorción de plomo mediante columna de relleno. XIV Simposium Científico-Técnico Expoliva, Jaén (España), Mayo 13-15, 2009.

RELACIÓN DE TABLAS Y FIGURAS/TABLES AND FIGURES

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Y_{NOB} (yield coefficient for nitrite-oxidizing bacteria), $\mu_{m,\ NOB}$ (maximum specific growth rate for nitrite-oxidizing bacteria), K_{NOB} (half-saturation coefficient for nitrite-nitrogen), k_d (decay coefficient for total bacteria).

Figures

Figures

taxonomic affiliation at the species level. Samples were clustered by similarities in dominant nitrifying OTUs distribution. The scale at the bottom represents the contribution of a particular OTU and is expressed as a percentage of the total. The closest bacterial relative is shown on the left side of the map.

VIII. CHAPTER 5

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IX. CHAPTER 6

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