Title

Starch-soiled stainless steel cleaning using surfactants and α-amylase

Authors

E. Jurado-Alameda, O. Herrera-Márquez, Juan F. Martínez-Gallegos, José M. Vicaria*

Address

Chemical Engineering Department, Faculty of Sciences, University of Granada, Avda.Fuentenueva, s/n, 18071 Granada, Spain

Corresponding author

*José M. Vicaria, Chemical Engineering Department, Faculty of Sciences, University of Granada, Avda.Fuentenueva, s/n, 18071 Granada, Spain. Phone: +34 958241389. Fax number: +34 958 24 89 92. email: vicaria@ugr.es

Abstract

The cleaning of dry starch adhered to stainless steel has been studied in a device which simulates a CIP system. The influence of an α-amylase, two polyoxyethylene lauryl ether carboxylic acids, a linear alkyl benzene sulfonate, a fatty ethoxylated alcohol, an alkylpolyglycoside, and two polyoxyethylene mono- and diglycerides has been analysed. The variables analysed were temperature, enzyme concentration, and different surfactants. The enzyme allowed for milder washing conditions improving starch removal. Surfactants, including the anionic ones, did not meaningfully alter the enzyme activity. Furthermore, they did not significantly modify the detergency in the presence or absence of enzyme, except for ethoxylated alcohol and polyoxyethylene(3) lauryl ether carboxylic acid solutions which decreased the detergency of the enzyme solutions. Temperature increase improved detergency either in the presence or absence of enzyme or surfactants. The experimental results advised interactions between those surfactants, the enzyme and the substrate, which could affect washing performance, basically at high washing times.

Keywords

Starch, amylase, detergency, enzymatic activity, anionic surfactant, non-ionic surfactant

1. Introduction

Starch is a widespread feedstock for industrial processes, especially in food manufacturing and processing, where it performs multiple functions such as water retention, bulking and gelling agent, thickener, and colloidal stabiliser (Singh et al., 2007). In industrial processes involving starches or their derivatives, these products often adhere to the surfaces inside pipes and accessories and are difficult to eliminate, since starch residues show strong soil-substrate bonds to hard surfaces (Din and Bird, 1996).

The cleaning process in the food industry is considered a critical operation. Food establishments have to market high-quality products that are pathogen and toxin free, and thus cleaning and disinfecting need to be repeated regularly at short time intervals (Wildbrett, 1990). Generally, these procedures are standardised and are usually similar without taking into account the type of specific soiling agent to eliminate. However, quite often it becomes necessary to develop specific formulations that optimise the cleaning and reduce the total cost of the process.

The addition of enzymes to the detergent formulations brings multiple advantages: lower washing temperatures, energy savings, reduction or replacement of chemicals harmful to the environment (Bravo Rodríguez et al., 2006a), increased soil removal, improved surfactant action, better washing performance (Galante and Formantici, 2003; Hmidet et al., 2009; Roy and Mukherjee, 2013), and milder washing conditions compared to enzyme-free detergents (Gupta et al., 2003). Amylases are the second most frequently used enzymes in detergency (Mitidieri et al., 2006). They hydrolyse starch, producing lower-molecular-weight dextrins, oligosaccharides, and sugars, which are more soluble than the original starch, thus making it easier to remove starchy deposits (Olsen and Falholt, 1998; Pongsawasdi and Murakami, 2010) and avoiding their redeposition (Hmidet et al., 2009). The α-amylase from *Bacillus licheniformis* is the one most widely used in detergents due to its thermostability (Bravo Rodriguez et al., 2006b).

The performance of α-amylases in detergents is affected by their compositions (Hmidet et al., 2009; Roy and Mukherjee, 2013). Among other components, surfactants usually alter the catalytic activities and storage stability of enzymes. Frequently enzymes, such as α-amylases, are unstable in solutions of anionic surfactants, including linear alkyl benzene sulfonates (LAS), and lose enzymatic activity (Tanaka and Hoshino, 1999, 2002; Bravo Rodriguez et al., 2006b; Hmidet et al., 2009; Shafiei et al., 2011; Roy and Mukherjee, 2013). On the contrary, non-ionic surfactants rarely diminish their enzymatic activity and usually do not modify or even increase it, as has been found for alkylpolyglycosides, fatty alcohol ethoxylates, and other ethoxylated surfactants (Hoshino and Tanaka, 2003; Mitidieri et al., 2006; Bravo Rodriguez et al., 2006b; Hmidet et al., 2009; Shafiei et al., 2011). It has also been verified that fatty alcohol ethoxylates

stabilise proteases in the presence of LAS (Russell and Britton, 2002), and alkylpolyglycosides are capable of increasing enzyme stability in liquid-detergent formulations (Von Rybinski, 1998). In addition, the formation of micelles can also modify the surfactant effect on the enzyme kinetics (Hoshino and Tanaka, 2003; Tanaka and Hoshino, 2002). Therefore alkylpolyglycosides, fatty alcohol ethoxylates, and other non-ionic ethoxylated surfactants may improve the α-amylase performance in detergents compared to anionic surfactants such as LAS.

Formation of surfactant-starch complexes can also affect the efficiency of the washing process. Both amylose and amylopectin, the constituents of starch, have given inclusion complexes with ionic and non-ionic surfactants (Bravo Rodríguez et al., 2008; Gudmundsson, 1990, 1992; Hoshino and Tanaka, 2003; Hui et al., 1983; Kim and Robinson, 1979; Lundqvist et al., 2002a, 2002b, 2002c; Martínez-Gallegos et al., 2011; Svensson et al, 1996; Tanaka and Hoshino, 2002; Wangsakan et al., 2004; Yamamoto et al., 1983). These complexes may affect the enzymatic hydrolysis of starch by amylases, either hindering (Kim and Robinson, 1979) or favouring it (Hoshino and Tanaka, 2003). Furthermore, surfactant-polymer complexes may increase polymer solubility, i.e. starch solubility, but also raise surface tension below the critical micelle concentration (CMC) (Goddard, 1986), thereby modifying the detergency of the washing liquor.

As can be seen, the efficacy of the cleaning process depends on numerous factors such as the properties and concentration of the soiling agent, the properties of the substrate, the characteristics of the washing device, temperature, detergent formulation, hydrodynamic forces and the duration of the process (Von Rybinski, 2007). Therefore, experimental work is indispensable to assess the performance of surfactants and enzymes on starch soil removal. To simulate and evaluate the washing process on hard surfaces the Bath-Substrate-Flow laboratory device (BSF) can be used (Jurado et al., 2003).

So far, most studies on starch soil removal with surfactants and amylases concern laundry detergents for textile cleaning (Hmidet et al., 2009; Hoshino and Tanaka, 2003; Roy and Mukherjee, 2013; St. Laurent et al., 2007; Tanaka and Hoshino, 1999). However, little work has been done involving hard surfaces (Jurado Alameda et al., 2011) and none on stainless steel, a predominant material for pipes and processing equipment in the food industry. In addition, virtually all these studies have been performed with wet starch, but not with dry starch, this being one of the most common forms in which starch can be found when such equipment becomes soiled.

Therefore, the aim of the present work is to analyse the washing process of dry starch adhered to stainless steel, using detergent formulations based on α-amylase and different anionic

and nonionic surfactants. The effect of temperature, enzyme concentration and surfactant concentration on detergency is also analysed.

2. Materials and Methods

2.1 Materials

Commercial cornstarch called Maizena® was used as the soiling agent. Soluble potato starch was supplied by Panreac. **Table 1** summarizes the characteristics of the surfactants assayed and their abbreviated names. LAS was supplied by Petresa (Cádiz, Spain), APG by Henkel KgaA, (Düsseldorf, Germany) and the remaining tested surfactants by Kao Corporation S.A. (Barcelona, Spain). The concentrations of the aqueous solutions of surfactants are expressed as dry weight. The surfactants studied were selected primarily on the basis of environmental criteria. All the surfactants selected are readily biodegradable under aerobic conditions (**Table 1**).

A commercial preparation of thermostable exo-amylase 4-α-D-glucanglucanohydrolase, EC 3.2.1.1 from *B. licheniformis* was obtained from Sigma (A3403-500KU), with an optimal pH range of 7-9. All washing assays with α-amylase were performed in 0.1 M phosphate buffer solution, pH=7. Enzymatic activity was measured regularly to assess the α-amylase stability during the testing period.

2.2 Soiling agent and substrate

The solid substrate was a set of spherical wads of stainless steel fibres (**Figure 1**). The wads measured roughly 2 cm in diameter and weighed between 0.80-0.85 g (fibers diameter was 0.51 mm; free volume fraction of wads was 82% and 93% with and without starch soiling, respectively). The soiling agent was an aqueous solution of gelatinized cornstarch (8% w/w) produced by heating the solution at 70ºC for an hour with constant stirring (Souza and Andrade, 2002). The gel thus prepared was allowed to cool at room temperature and left to stand for at least 12 h before being used. The spherical stainless steel wads were soiled with starch gel in the following way: 1) the surface of the wads was uniformly impregnated with the soil by submersion in the starch gel; 2) the soiled wads were placed on a grate and dried for 12h in an oven at 60ºC; 3) the dried wads were removed and weighed. The quantity of starch retained was determined by the weight difference between unsoiled and soiled wads. This quantity should be as constant as possible. Eight wads, each containing 2.0 ± 0.2 g of dry starch, were used in every washing test. **Table 2** summarizes the composition of the dry starch. Moisture was determined

by drying at 110 ºC on an infrared balance (model AD-4714A from AND) to a constant weight. Protein was determined by the Kjeldahl method using a conversion factor of 6.25. Fat was determined by the Soxhlet method after acid hydrolysis. The carbohydrate content was determined by arithmetic difference from the rest of the components. Salts were determined by ICP-OES from the ashes. For the analysis of Ca, Mg, K, and Na, the samples (15 g of soil), placed in ceramic crucibles, were calcined in a furnace at 550ºC for 1 h. The ashes were weighed (0.1 g), placed in a solution of 6 mL HNO_3/HF (1/1) and heated in an oven at 160ºC to dryness. Then 4 mL of HNO_3 were added, kept 1 h at 80ºC, and (after cooling) diluted to 100 mL with distilled water. Then the minerals were analysed using a Perkin Elmer Optima 8300 ICP-OES Spectrometer.

2.3 Detergency evaluation

The cleaning assays were made in a Bath-Substrate-Flow system (BSF) proposed by Jurado et al.(2007) that simulates a CIP system (**Figure 2**).

Operating conditions were as follows: pH 7 (0.1 M phosphate buffer) or 13 (4.1 g/L KCl, 5.8 g/L NaOH), volume of wash-bath solution (500 mL), stirring speed (60 rpm), flow rate (30 L/h upward), testing time (45 min), temperature (40-60ºC), and enzyme concentration in the washing solution (0.00-1.00 g/L); experiments were performed with 1.0 g/L of surfactant or in its absence.

The washing procedure was as follows: 1) the prepared washing solution (pH, type of surfactant, surfactant concentration, enzyme concentration) was added to the tank and experimental temperature was set with the thermostatic bath; 2) the steel-fibre wads, already soiled and dried, were placed in the column; 3) the pump was turned on to start the washing process; 4) washing samples were withdrawn periodically for 45 min; 5) the starch concentration in the samples was analysed. Experiments were repeated at least 3 times.

The effectiveness of the washing or detergency (De, %), was calculated according to Eq.(1):

$$De = \frac{m_{washing}}{m_{initial}} 100 \qquad (1)$$

Where $m_{washing}$ is the starch mass present in the washing solution, and $m_{initial}$ is the total quantity of starch adhered to the steel wads at the beginning of the process. For the application of this equation, the composition of the washing solution in the BSF was considered constant throughout the system. The volume of the samples removed was also considered negligible with respect to the total washing volume.

2.4 Enzymatic activity in the presence of surfactants

The α-amylase activity was determined by measuring the formation of reducing sugars released during starch hydrolysis in the presence of different anionic and non-ionic surfactants. The substrate used was soluble potato starch; a stock solution of 6.00 g/L in pH 7 0.1 M phosphate buffer was prepared by boiling it for 15 min and afterwards cooling to room temperature. Stock solutions with a concentration of 3.00 g/L were prepared with each of the surfactants studied, as well as a solution of α-amylase of 0.18 g/L, all of them in pH 7 0.1 M phosphate buffer. The activity in this α-amylase stock solution was 2480 units/L, where one unit will liberate 1.0 mg of maltose from starch in 3 minutes at pH 6.9 at 20 ºC (as defined by Sigma).

Samples containing 1 mL of the stock solution of potato starch and 1 mL of surfactant solution were placed in a thermostatically controlled bath at 60ºC. When the temperature was stable 1 mL of the stock solution of enzyme was added, beginning the activity assay, which lasted 5 min. The final concentration of the samples (2.00 g/L potato starch, 1.00 g/L surfactant, 0.06 g/L enzyme) is representative of the washing formulations used in this work. Thus, enzymatic activity in these solutions can be related to the detergency found in the BSF device, as similar formulations are used in both tests. Five replicas were made in each experiment.

The amount of reducing sugar released was determined by the dinitrosalicylic (DNS) acid method (Bernfeld, 1955), following the protocol for enzymatic assay of α-amylase proposed by Sigma Aldrich (1997). Before dilution and measurement of absorbance, the samples were centrifuged for 10 min, 9000 g (Universal 320R, Hettich). In order to calculate the residual enzyme activities, the activity of the crude enzyme incubated under similar conditions without any surfactant in solution was taken as 100%.

2.5 Analysis of the starch in the washing solution

The total soluble carbohydrates in the washing solution were analysed by the phenol-sulphur colorimetric method (DuBois et al., 1956). The washing samples taken at different times were added to test tubes containing 1 mL of 2 N sulphuric acid and then placed in a digester (Spectroquant TR320, Merck) at 100°C for 30 min to hydrolyse the starch in solution. Subsequently, the samples were cooled in an ice bath. After applying the necessary dilution, the phenol-sulphur determination was performed by adding 0.5 mL of the sample to 0.5 ml of a phenol solution at 5% (w/v) and 2.5 ml of concentrated sulphuric acid (96%). The absorbance of samples was measured 15 min later at 490 nm, using a spectrophotometer Cary 100 Bio UV-Visible (Varian). The concentration of the starch in the solution was determined from a

calibration curve with glucose, multiplying by a correction factor of 0.9, which considered the stoichiometric relation between starch and glucose in the acid hydrolysis of the starch (Lampitt et al., 1947).

3. Results and Discussion

3.1 Activity of α-amylase with surfactants

As described in section 1, surfactants are able to alter the α-amylase activity due to both enzyme-surfactant and/or surfactant-starch interactions, therefore affecting the removal of starchy soil in food-industry equipment. Thus, it is important to ascertain the effect of surfactants on the enzyme activity in order to understand what happens in more complex processes such as cleaning. To this end, experiments of enzyme activity were performed in the presence of the surfactants used in the detergency tests (**Table 1)**. The experimental conditions assayed considered ranges of enzyme concentration and surfactant concentration similar to those found in real industrial cleaning processes. **Figure 3** shows the results found. Enzymatic activity results were assessed by analysis of variance (ANOVA) with a p-value ≤ 0.05. Regarding the anionic surfactant LAS, the activity of the α-amylase seemed to decrease in its presence (**Figure 3**). However, the ANOVA showed no significant difference between the aqueous solutions of α-amylase without surfactant (100% activity) and the residual activities of the enzyme in the presence of LAS. Therefore, the enzyme can be considered stable in the presence of LAS under the conditions tested. Many enzymes, including α-amylases, are unstable and lose biocatalytic activity in solutions of anionic surfactants, e.g. sodium lauryl sulphate (SDS), caused by the electrostatic and hydrophobic interactions brought about between the surfactant monomers or their micelles and the proteins (Hagihara et al., 2002; Montserret et al., 2000; Tanaka and Hoshino, 2002), and so the secondary and tertiary structures of the enzymes can be altered (Bravo Rodriguez et al., 2006b). LAS has also been found to destabilise proteases (Russell and Britton, 2002) and to significantly reduce the activity of the α-amylase studied (Bravo Rodriguez et al., 2006b). The stability towards LAS found in the present work is important because stable enzymes in the presence of anionic surfactants have rarely been observed. Examples of this unusual behaviour were reported by Jaiswal and Prakash (2013) and Tanaka and Hoshino (2002). These latter authors found greater enzymatic activity for SDS concentrations below its critical micelle concentration, being due to the preferential formation of the enzyme-substrate complex. In agreement with this statement, the stability of the α-amylase within the solutions containing

LAS could depend on their concentration with respect to the critical micelle concentration of LAS at the assayed temperature.

Enzymes are usually more stable in aqueous solutions of non-ionic surfactants than in anionic surfactants ones, as it has been pointed out in section 1. Concerning the alkylpolyglycoside assayed, the statistical analysis of the experimental results indicated that the α-amylase activity in APG aqueous solutions was similar to that without any surfactant (**Figure 3**). Bravo Rodriguez et al.(2006b) have also observed a slight increase in the α-amylase activity in aqueous solutions of APGs, these results agreeing with those found for other non-ionic surfactants such as fatty alcohol ethoxylates (Hoshino and Tanaka, 2003). Moreover, von Rybinski and Hill (1998) indicated that APGs were capable of increasing enzyme stability in liquid-detergent formulations. Furthermore, Bravo Rodríguez et al. (2008) reported that APGs formed complexes with the starch since their early addition, which may also alter the enzymatic activity as commented before, and would justify the slight increase in the enzymatic activity observed under their experimental conditions (Bravo Rodriguez et al., 2006b). Under our conditions these complexes seemed not to alter the α-amylase activity.

For the fatty ethoxylated alcohol assayed, the ANOVA test showed that the activity of the α-amylase was similar with or without AE (**Figure 3)**. Bravo Rodríguez et al. (2006b) showed that the AE assayed reduced the α-amylase activity only very slightly, either above or below its CMC, following the well-known ability of AEs to stabilize proteases even in the presence of other anionic surfactants (Russell and Britton, 2002). Like APGs, AEs have also been found to form–complexes with starch, their amount being proportional to the total added surfactant, although AEs showed a weaker tendency to form complexes with starch compared with APGs and other non-ionic surfactants (Martínez-Gallegos et al., 2011). Thus, under the experimental conditions of the present work, either no complexes between AE and starch were formed, or if formed they did not appear to meaningfully modify the enzymatic activity as also supposed with APG.

From the statistical analysis of the experimental results of the other surfactants assayed, polyoxyethylene lauryl ether carboxylic acids (LEC-OE3 and LEC-OE10) and polyoxyethylene mono- and diglycerides (PGE–OE2 and PGE–OE17), it could be inferred that the α-amylase activity was unaffected by any of them (**Figure 3**). Although no information has been found in the literature on the activity that α-amylases show in their aqueous solutions, it might have been expected for the LEC surfactants to have induced a reduction in the enzyme activity due to their anionic nature, but they did not, being a remarkable fact as also noticed with LAS; meanwhile

PGE surfactants, being non-ionic, should not affect or in any case increase the biocatalytic activity as it was observed.

3.2 Detergent formulations with α-amylase for the cleaning of dry starch

The detergency of dry starch adhered to stainless steel was analysed as a function of time. As an example, **Figure 4** shows, for washing times of 45 min and different temperatures, the detergency achieved in the BSF with solutions of pH=13 in the absence of enzyme, in cases of absence of surfactants, with AE solutions of 1.00 g/L, and with APG solutions of 1.00 g/L. In the best of cases, which took place at high temperature, the detergency reached was 47%. Surfactants only significantly increased detergency at the lowest temperature assayed, 30ºC, but no effect was detected at the highest temperature, 60ºC, and even they somewhat reduced detergency at the intermediate temperature, 40ºC. It was deduced that, in the absence of enzyme, the cleaning of dry starch adhered to stainless steel was difficult, requiring a high pH, a long time period, and a high temperature.

Experiments using exclusively enzymatic solutions in the absence of surfactants were also performed. As an example, **Figure 5** shows the detergency reached, at pH=7 and 40 ºC and 60ºC, as a function of time with solutions containing different enzyme concentrations. In general, it was observed that higher detergency resulted when higher concentrations of enzymes were used, notably increasing the detergency with washing time and temperature. According to this result, the optimal concentration of α-amylase in a commercial detergent should be determined by economic criteria that balance the efficiency of washing with the cost of the enzyme used as a feedstock.

The experimental detergency results found for the different enzyme concentrations were satisfactorily fitted with time to linear equations, except for 1.00 g/L enzyme concentration, where data only fitted a straight line within 0 to 20 min (**Figure 5)**. It can be assumed that the final detergency will be the sum of the hydrolysis caused by the enzyme, the drag caused by the flow, which is higher as the enzymatic hydrolysis progresses, and at longer times, the feasible negative effect of starch redeposition. At high enzyme concentrations and high detergency values, the effects of drag and redeposition would be more pronounced, together with decreasing enzymatic reaction rate with time due to fast substrate depletion, and therefore the time course of the detergency may not always follow the same trend. At 60 ºC, the slopes of the straight line fittings showed a linear dependence with respect to enzyme concentration, and therefore the detergency under these conditions could be evaluated from the equation:

$$De = (0.56 + 3.08\,Ce)t \qquad (2)$$

where Ce is the enzyme concentration. The model adequately reproduces the experimental results found under the tested conditions.

Comparing these enzymatic washing experiments (**Figure 5**) with the previous ones done in the absence of enzyme, with or without surfactants, and under more drastic washing conditions, i.e. pH=13, (**Figure 4**), it was found that the same or greater detergency would be achieved with an intermediate enzyme concentration at pH=7, thus improving the washing process allowing for milder conditions.

Regarding temperature effect, an intermediate enzyme concentration, 0.06 g/L, was assayed at 40 ºC and 60 ºC (**Figure 5**). It was observed that the temperature increased at least 2-fold the detergency at any time considered. In agreement with these results, a highly positive effect of temperature on detergency has also been described for washing wet starch soiling when α-amylase was used (Jurado Alameda et al., 2011). It is well known that temperature has an important influence on soil removal, promoting dragging and improving starch dissolution. Higher temperature breaks the intermolecular hydrogen bonds and allows water penetration, diminishing viscosity and augmenting detergency (Bertuzzi et al., 2007). Furthermore high temperature may induce dry starch reswelling which facilitates both the hydrolysis action of the α-amylase (Olsen and Falholt, 1998) and the starch dissolution thus raising detergency. Finally, it should also be taken in account that enzymatic activity rises with increasing temperature when enzyme denaturation is not significant.

3.3 Influence of surfactants on the enzymatic formulations

With the aim of increasing detergency of the enzyme solutions, the influence of the addition of different surfactants was studied. Washing experiments were performed assaying the surfactants given in **Table 1**, with concentrations of 0.06 g/L and 1.00 g/L for the α-amylase and the surfactants, respectively, at 40-60ºC and pH=7. As an example, **Figure 6** shows the detergency dependence with time for the non-ionic surfactants tested. In addition, **Figure 7** summarises the detergency values obtained for all surfactants at 60 ºC after 45 min; these 45 min data were assessed by analysis of variance (ANOVA) and by Fisher's Least Significant Difference test as a multiple comparison procedure with a p-value ≤ 0.05.

No significant differences were detected using surfactants at 40ºC at any time, with respect to enzyme solutions assays in their absence, the final detergency being roughly 12% (**Figure 6**). However, once again a significant increase of detergency was found with temperature, as previously commented in washing experiments only with enzyme (**Figure 5**), and as observed for the same temperatures at pH=13 using AE and APG in the absence of

enzyme (**Figure 4**). The reasons for that increase could be those ones already pointed out in section 3.2.: temperature promotes starch dragging, reswelling and dissolution, diminishes viscosity, and raises enzymatic activity when thermal deactivation is negligible. Jurado et al. (2011) studied the cleaning of wet starch soiling retained on glass spheres and expanded polyurethane discs between 30 and 60ºC, using α-amylase, LAS, and APG solutions. They observed that, in the absence of enzyme, temperature had no a significant effect on detergency when surfactant solutions and glass spheres were used, but higher temperatures produced higher detergency because the discs had porous surfaces and the viscosity of the starch film was decisive. Thus, in the present work, the effect of temperature in the viscosity of the washing liquor inside de porous stainless steel wads could be an important factor with respect to inner cleaning action as mechanical shear would be limited by the wad structure. In addition, Jurado et al. (2011) found that detergency also increased with temperature when α-amylase was used. Notwithstanding, in our work, the detergency experiments were made with dry starch. The structure of dry starch differs from that of wet starch and is more difficult to remove from hard surfaces. Furthermore, the soiled surface (stainless steel) is very different from that used by Jurado et al. (2011), and more appropriate to simulate the interaction starch-substrate found in food industry, since the strength of the adhesion depends on surface characteristics (Liu et al., 2006). Thus, it seems that the detergency process between both studies could greatly differ too.

Regarding the surfactants effects on the enzyme solutions detergency at 60 ºC, the ANOVA shows $p=0.0008$ (<0.05) after 45min washing and thus a significant difference was found at that time (**Figure 7**), as oppositely described for 40 ºC. The results of the multiple-range test indicated that only the presence of AE or LEC-OE3 in the α-amylase solution produced a statistically significant, although limited, decrease in detergency. Apparently, the best detergency results were obtained with LEC-OE10, PGE-OE2 and LAS, but they did not significantly differ from those found only with enzyme. Jurado et al. (2011) has also found that both anionic and non-ionic surfactants had the same ability to clean wet starch soiled surfaces; furthermore, their washing performance increased with both surfactants only when glass spheres were used as a substrate, i.e. non-porous material, but the detergency values did not differ from those found without any surfactant when polyurethane discs were used, i.e. porous substrate; these results seem to support our findings with the porous stainless steel wads, while being studies with different cleaning conditions as commented before.

Analysing the AE effect on detergency described, although no statistically significant variation in enzyme activity was found for AE, a small decrease was observed in its mean value (**Figure 3**); moreover, Bravo Rodríguez et al. (2006b) have also found a slight decrease in the α-

amylase activity with this AE, while under different experimental conditions. Therefore this small enzyme activity decrease could justify the little detergency reduction observed with AE at 45min washing. This decline in AE washing effectiveness appears to indicate that there might have been some removal of the enzyme from the medium by the surfactant or that there might have been some competition between the surfactant and enzyme for the substrate, such as complexation. In addition, this effect seemed to be time-dependent as it was only noticed for washing times higher than 30 min (**Figure 6**).

Since at 60ºC the detergency of the solutions with α-amylase and AE proved lower than those attained with solutions containing only enzyme (without surfactants) (**Figure 6)**, an assay with 0.06 g/L of enzyme and progressive addition of AE was performed: in the first 20 min of the test, the washing solution contained only enzyme; at 20 min, AE was added up to 1.00 g/L in the washing solution; at 40 min, AE was added up to 2.00 g/L. **Figure 8** shows the results of this test together with the results of the washing tests carried out at the same enzyme concentration without surfactant and with a constant concentration of AE of 1 g/L. It appeared that the detergency diminished with the progressive addition of AE. Bravo Rodríguez et al. (2006b) have also found a slight raising reduction in the α-amylase activity with increasing concentration of this AE. Therefore, once again, this may suggest an interference between the surfactant and the enzyme that affects starch removal, supporting the results already commented for the AE.

Regarding LEC-OE3 and its reduced detergency, no previous studies have been found related to enzymatic activity or starch complexation, and no statistically significant effects over the α-amylase activity were observed under the experimental conditions tested (**Figure 3**). However its homologous surfactant LEC-OE10 did not modify the detergency compared with enzyme solutions without surfactants (**Figure 7**). The LEC-OE3 lower detergency values could be related to its lower number of oxyethylene groups compared with LEC-OE10 (**Table 1**), signifying lower hydrophibicity which could alter the washing properties.

With respect to APG, although its detergency was not different from that of the enzyme solution at any time (**Figure 6**), it was statistically significantly lower at 45min compared to LEC-OE10, PGE-OE2, PGE-OE17 and LAS (**Figure 7**). Since no differences in enzymatic activity was found with APG nor with any of the surfactants assayed with respect to enzyme in the absence of surfactants (**Figure 3**), thus, only the high tendency of APG to form complexes with starch compared to other ethoxylated surfactants (Martínez-Gallegos et al., 2011) could likely justify this behaviour, although the complexation capacity of LEC-OE10, PGE-OE2, PGE-OE17 and LAS is unknown.

For the solutions of polyoxyethylene mono- and diglycerides, the detergency found seemed to be slightly greater with PGE-OE2 solution than with PGE-OE17 or lacking surfactants at washing times higher than 20 min (**Figure 6**), although this difference was no statistically significant at 45 min (**Figure 7**). This effect could be related to the wettability of the surfactants, as aqueous solutions of PGE–OE2 have showed higher wettability than PGE–OE17 (Jurado et al., 2011b).

4. Conclusions

The enzymatic activity and detergency performance of an α-amylase in the presence or absence of several non-ionic and anionic surfactants was tested and compared.

Surfactants effects on enzymatic activity were considered practically negligible under the assayed conditions. This is an outstanding finding for the anionic surfactants tested, a linear alkyl benzene sulfonate and two polyoxyethylene alkyl ether carboxylic acids, since usually anionic surfactants diminish enzymes activities, and so, experimental conditions for stable enzymes in their presence have barely been reported. Therefore, all the assayed surfactants could be included in enzyme-based detergent formulations without a significant loss in the enzyme efficiency when used at the conditions tested.

The washing experiments in the absence of enzyme showed that cleaning of dry starch adhered to stainless steel demanded high pH and temperature, and a long time period. Under these hard conditions surfactants did not improve detergency. Using α-amylase solutions at relatively low concentrations allowed for equal of better starch removal under milder operation conditions thus improving the washing process. A detergency mathematical model was proposed which properly fitted the detergency data versus enzyme concentration and washing time. When surfactants were added to the enzyme solutions, the detergency levels reached did not significantly differ from those found with solutions that contained only α-amylase, but for AE or LEC-OE3 which registered lower detergency values; LEC-OE10, PGE-OE2 and LAS showed the best surfactants results. Increasing temperature noticeably ameliorate washing performance of enzyme solutions with or without surfactants.

All these results suggest that, together with temperature, the interactions between surfactants, enzyme and substrate could affect the washing performance, i.e., the dry starch removal from stainless steel food process equipment, most especially at elevated washing times.

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References

Bertuzzi, M. A., Armada, M., Gottifredi, J.C., 2007. Physicochemical characterization of starch based films. J. Food Eng. 82, 17–25. doi:10.1016/j.jfoodeng.2006.12.016

Bernfeld, P., 1955. Amylases, α and β. Methods Enzymol. 1, 149–158. doi:10.1016/0076-6879(55)01021-5

Bravo Rodríguez, V., Jurado Alameda, E., Martínez Gallegos, J.F., Reyes Requena, A., García López, A I., 2008. Formation of complexes between alkyl polyglycosides and potato starch. Colloids Surf. B. Biointerfaces 65, 92–97. doi:10.1016/j.colsurfb.2008.03.001

Bravo Rodríguez, V., Jurado Alameda, E., Martínez Gallegos, J.F., Reyes Requena, A., García López, A.I., 2006a. Thermal deactivation of a commercial α-amylase from Bacillus licheniformis used in detergents. Biochem. Eng. J. 27, 299–304. doi:10.1016/j.bej.2005.08.018

Bravo Rodriguez, V., Jurado Alameda, E., Martinez Gallegos, J.F., Reyes Requena, A., Garcia Lopez, A.I., Cabral, J.M.S., Fernandes, P., Fonseca, L.J.P.D., 2006b. Modification of the activity of an α-amylase from Bacillus licheniformis by several surfactants. Electron. J. Biotechnol. 9, 566-571. doi:10.2225/vol9-issue5-fulltext-16

Bravo Rodriguez, V., Jurado Alameda, E., Reyes Requena, A., García López, A. I., Bailón-Moreno, R., Cuevas Aranda, M., 2005. Determination of average molecular weight of commercial surfactants: Alkylpolyglucosides and fatty alcohol ethoxylates. J. Surfactants Deterg. 8, 341–346. doi:10.1007/s11743-005-0366-y

Din, R.A., Bird, M.R., 1996. In Proceedings of the Second European Conference for Young Researchers in Chemical Enginering Vol. 1 (pp. 187-189). Leeds, UK.

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric Method for Determination of Sugars and Related Substances. Anal. Chem. 28, 350–356. doi:10.1021/ac60111a017

Galante, Y., Formantici, C., 2003. Enzyme Applications in Detergency and in Manufacturing Industries. Curr. Org. Chem. 7, 1399–1422. doi:10.2174/1385272033486468

Goddard, E.D., 1986. Polymer—surfactant interaction Part I. uncharged water-soluble polymers and charged surfactants. Colloids Surf. 19, 255–300. doi:10.1016/0166-6622(86)80340-7

Gudmundsson, M., 1992. Effects of an added inclusion-amylose complex on the retrogradation of some starches and amylopectin. Carbohydr. Polym. 17, 299–304. doi:10.1016/0144-8617(92)90173-N

Gudmundsson, M., Eliasson, A.-C., 1990. Retrogradation of amylopectin and the effects of amylose and added surfactants/emulsifiers. Carbohydr. Polym. 13, 295–315. doi:10.1016/0144-8617(90)90061-V

Gupta, R., Gigras, P., Mohapatra, H., Goswami, V.K., Chauhan, B., 2003. Microbial α-amylases: a biotechnological perspective. Process Biochem. 38, 1599–1616. doi:10.1016/S0032-9592(03)00053-0

Hagihara, Y., Hong, D.-P., Hoshino, M., Enjyoji, K., Kato, H., Goto, Y., 2002. Aggregation of β 2 -Glycoprotein I Induced by Sodium Lauryl Sulfate and Lysophospholipids. Biochemistry 41, 1020–1026. doi:10.1021/bi015693q

Hmidet, N., El-Hadj Ali, N., Haddar, A., Kanoun, S., Alya, S.-K., Nasri, M., 2009. Alkaline proteases and thermostable α-amylase co-produced by Bacillus licheniformis NH1: Characterization and potential application as detergent additive. Biochem. Eng. J. 47, 71–79. doi:10.1016/j.bej.2009.07.005

Hoshino, E., Tanaka, A., 2003. Enhancement of Enzymatic Catalysis of Bacillus amyloliquefaciens α -Amylase by Nonionic Surfactant Micelles. J. Surfactants Deterg. 6, 299–303. doi: 10.1007/s11743-003-0273-2

Hui, Y., Russell, J.C., Whitten, D.G., 1983. Photochemical reactivity in organized assemblies. 33. Host-guest interactions in amylose inclusion complexes: photochemistry of surfactant stilbenes in helical cavities of amylose. J. Am. Chem. Soc. 105, 1374–1376. doi:10.1021/ja00343a053

Jaiswal, N., Prakash, O., 2013. Immobilization of Soybean α-amylase on Gelatin and its Application as a Detergent Additive. Acta Biochim. Pol. 60, 337-346.

Jurado Alameda, E., Vicaria, J.M., Altmajer Vaz, D., Luzón, G., Jiménez Pérez, J.L., Moya-Ramírez, I., 2012. Ozone degradation of alkylbenzene sulfonate in aqueous solutions using a stirred tank reactor with recirculation. J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng. 47, 2205–12. doi:10.1080/10934529.2012.707537

Jurado Alameda, E., Bravo Rodríguez, V., Altmajer Vaz, D., de Cassia Siqueira Curto Valle, R., 2011. Effectiveness of starch removal in a Bath-Substrate-Flow (BSF) device using surfactants and α-amylase. Food Hydrocoll. 25, 647–653. doi:10.1016/j.foodhyd.2010.07.031

Jurado Alameda, E., Bravo Rodríguez, V., Bailón Moreno, R., Núñez Olea, J., Altmajer Vaz, D., 2007. Patent ES 2 251 269. Método BSF (Baño-Sustrato-Flujo) y dispositivo para la evaluación de la eficacia detersiva y dispersante de tensioactivos, de coadyuvantes de la detergencia y de composiciones detergentes de superficies duras.

Jurado, E., Fernández-Serrano, M., Ríos, F., Lechuga, M. (2013), in: Chamy, R.; Rosenkranz, F. (Eds.), Biodegradation - Life of Science (pp. 63-81). InTech, Rijeka, Croatia.

Jurado, E., Fernández-Serrano, M., Lechuga, M., Ríos, F., 2012. Environmental Impact of Ether Carboxylic Derivative Surfactants. J. Surfactants Deterg. 15, 1–7. doi:10.1007/s11743-011-1278-z

Jurado, E., Fernández-Serrano, M., Núñez-Olea, J., Lechuga, M., Jiménez, J.., Ríos, F., 2011a. Effect of Concentration on the Primary and Ultimate Biodegradation of Alkylpolyglucosides in Aerobic Biodegradation Tests. Water Environ. Res. 83, 154–161. doi:10.2175/106143010X12780288628336

Jurado, E., Vicaria, J.M., García-Martín, J.F., García-Román, M., 2011b. Wettability of Aqueous Solutions of Eco-Friendly Surfactants (Ethoxylated Alcohols and Polyoxyethylene Glycerin Esters). J. Surfactants Deterg. 15, 251–258. doi:10.1007/s11743-011-1312-1

Jurado, E., Bravo, V., Bailón, R., Núñez, J., Altmajer, D., 2003. Bath - Substrate - Flow Method for Evaluating the Detersive and Dispersant Performance of Hard-Surface Detergents. Ind. Eng. Chem. Res. 42, 4303–4310.

Kim, Y.J., Robinson, R.J., 1979. Effect of Surfactants on Starch in a Model System. Starch - Stärke 31, 293–300. doi:10.1002/star.19790310904

Lampitt, L.H., Fuller, C.H.F., Goldenberg, N., 1947. Starches and starch fractions. Part II. The determination of starch by hydrolysis with hydrochloric acid. J. Soc. Chem. Ind. 66, 117–121. doi:10.1002/jctb.5000660403

Lechuga, M., Fernández-Arteaga, A., Fernández-Serrano, M., Jurado, E., Burgos, A., Ríos, F., 2014. Combined Use of Ozonation and Biodegradation of Anionic and Non-ionic Surfactants. J. Surfactants Deterg. 17, 363–370. doi:10.1007/s11743-013-1480-2

Liu, W., Fryer, P.J., Zhang, Z., Zhao, Q., Liu, Y., 2006. Identification of cohesive and adhesive effects in the cleaning of food fouling deposits. Innov. Food Sci. Emerg. Technol. 7, 263–269. doi:10.1016/j.ifset.2006.02.006

Lundqvist, H., Eliasson, A.-C., Olofsson, G. 2002a. Binding of hexadecyltrimethylammonium bromide to starch polysaccharides. Part I. Surface tension measurements. Carbohydr. Polym. 49, 43–55. doi:10.1016/S0144-8617(01)00299-5

Lundqvist, H., Eliasson, A.-C., Olofsson, G., 2002b. Binding of hexadecyltrimethylammonium bromide to starch polysaccharides. Part II. Calorimetric study. Carbohydr. Polym. 49, 109–120. doi:10.1016/S0144-8617(01)00326-5

Lundqvist, H., Nilsson, G.S., Eliasson, A.-C., Gorton, L., 2002c. Changing the Amylopectin-Sodium Dodecyl Sulphate Interaction by Modifying the Exterior Chain Length. Starch -

Stärke 54, 100–107. doi:10.1002/1521-379X(200204)54:3/4<100::AID-STAR100>3.0.CO;2-Y

Martinez Gallegos, J.F., 2005. PhD Thesis. Utilización de α-amilasas en la formulacion de detergentes comerciales.Universidad de Granada, Spain, 2005.

Martínez-Gallegos, J.F., Bravo-Rodríguez, V., Jurado-Alameda, E., García-López, A.I., 2011. Polyoxyethylene alkyl and nonyl phenol ethers complexation with potato starch. Food Hydrocoll. 25, 1563–1571. doi:10.1016/j.foodhyd.2011.01.010

Mitidieri, S., Souza Martinelli, A.H., Schrank, A., Vainstein, M.H., 2006. Enzymatic detergent formulation containing amylase from Aspergillus niger: a comparative study with commercial detergent formulations. Bioresour. Technol. 97, 1217–24. doi:10.1016/j.biortech.2005.05.022

Montserret, R., McLeish, M.J., Böckmann, A., Geourjon, C., Penin, F., 2000. Involvement of Electrostatic Interactions in the Mechanism of Peptide Folding Induced by Sodium Dodecyl Sulfate Binding. Biochemistry 39, 8362–8373. doi:10.1021/bi000208x

Olsen, H.S., Falholt, P., 1998. The role of enzymes in modern detergency. J. Surfactants Deterg. 1, 555–567. doi:10.1007/s11743-998-0058-7

Pongsawasdi, P., Murakami, S., 2010. In: Hagen, E.T. (Ed.) Detergents: Types, Components and Uses (pp. 71-95). Nova Science Publishers, New York.

Roy, J.K., Mukherjee, A.K., 2013. Applications of a high maltose forming, thermo-stable α-amylase from an extremely alkalophilic Bacillus licheniformis strain AS08E in food and laundry detergent industries. Biochem. Eng. J. 77, 220–230. doi:10.1016/j.bej.2013.06.012

Russell, G.L., Britton, L.N., 2002. Use of certain alcohol ethoxylates to maintain protease stability in the presence of anionic surfactants. J. Surfactants Deterg. 5, 5–10. doi:10.1007/s11743-002-0198-9

Shafiei, M., Ziaee, A.-A., Amoozegar, M.A., 2011. Purification and characterization of an organic-solvent-tolerant halophilic α-amylase from the moderately halophilic Nesterenkonia sp. strain F. J. Ind. Microbiol. Biotechnol. 38, 275–81. doi:10.1007/s10295-010-0770-1

Sigma-Aldrich, 1997. Enzymatic Assay of α-amylase (EC 3.2.1.1).

Singh, J., Kaur, L., McCarthy, O.J., 2007. Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications—A review. Food Hydrocoll. 21, 1–22. doi:10.1016/j.foodhyd.2006.02.006

Souza, R.C.R., Andrade, C.T., 2002. Investigation of the gelatinization and extrusion processes of corn starch. Adv. Polym. Technol. 21, 17–24. doi:10.1002/adv.10007

St. Laurent, J.B., de Buzzaccarini, F., De Clerck, K., Demeyere, H., Labeque, R., Lodewick, R., van Langenhove, L., 2007. In Johansson, I., Somasundaran, P. (Eds.), Handbook for Cleaning/Decontamination of Surfaces, Elsevier. doi:10.1016/B978-044451664-0/50003-6

Svensson, E., Gudmundsson, M., Eliasson, A.-C., 1996. Binding of sodium dodecylsulphate to starch polysaccharides quantified by surface tension measurements. Colloids Surf., B. 6, 227–233. doi:10.1016/0927-7765(95)01260-5

Tanaka, A., Hoshino, E., 1999. Study on the substrate specificity of α-amylases that contribute to soil removal in detergents. J. Surfactants Deterg. 2, 193–199. doi:10.1007/s11743-999-0073-8

Tanaka, A., Hoshino, E., 2002. Thermodynamic and activation parameters for the hydrolysis of amylose with Bacillus α-amylases in a diluted anionic surfactant solution. J. Biosci. Bioeng. 93, 485–490. doi:10.1016/S1389-1723(02)80096-2

Von Rybinski, W., 2007. In: Johansson, I., Somasundaran, P. (Eds.), Handbook for Cleaning/Decontamination of Surfaces. Elsevier. doi:10.1016/B978-044451664-0/50002-4

Von Rybinski, W., Hill, K., 1998. Alkyl Polyglycosides—Properties and Applications of a new Class of Surfactants. Angew. Chemie Int. Ed. 37, 1328–1345. doi:10.1002/(SICI)1521-3773(19980605)37:10<1328::AID-ANIE1328>3.0.CO;2-9

Wangsakan, A., Chinachoti, P., McClements, D.J., 2004. Effect of Surfactant Type on Surfactant−Maltodextrin Interactions: Isothermal Titration Calorimetry, Surface Tensiometry, and Ultrasonic Velocimetry Study. Langmuir 20, 3913–3919. doi:10.1021/la0361619

Wildbrett, G., 1990. Reinigung und Desinfektion lebensmittelberuehrender Oberflaechen - Erfordernisse und Risiken. Bayer. Landwirtsch. Jahrbuch, Sonderh.

Yamamoto, M., Sano, T., Harada, S., Yasunaga, T., 1983. Cooperativity in the binding of sodium dodecyl sulfate to amylose. Bull. Chem. Soc. Jpn. 56, 2643–2646. doi:10.1246/bcsj.56.2643

Table captions

Table 1. Properties of the commercial surfactants assayed.

Table 2. Composition of dry starch.

Figure captions

Figure 1. Spherical wads of stainless-steel fibre with dry starch adhered.

Figure 2. Scheme of the BSF device: (1) stirred tank (volume 400 mL) for the washing solution, (2) packed column (volume 50 mL, diameter 2.5 cm, height 8.5 cm) with soiled substrate, (3) thermostatically controlled bath, (4) peristaltic pump, and (5) paddle stirrer.

Figure 3. α-amylase (*B. licheniformis*) activity with several surfactants (pH=7, 60 ºC). Different letters denote statistical difference between the experimental conditions using the Fisher's Least Significant Difference test with a 95.0% confidence level.

Figure 4. Detergency in BSF at different temperatures after 45 min with pH=13 solutions, pH=13 solutions with AE 1.00 g/L, and pH=13 solutions with APG 1.00 g/L (flow rate 30 L/h; the error bars represent ±SD of at least 3 replicates).

Figure 5. Detergency in BSF with α-amylase. Influence of temperature (40 ºC closed circles, 60 ºC open symbols) and enzyme concentration (0.03–1.00 g/L at 60 ºC) as a function of time (pH=7, flow rate 30 L/h, the error bars represent ±SD of at least 3 replicates)

Figure 6. Detergency in BSF with α-amylase. Influence of surfactants and temperature vs. time. pH=7, flow rate 30 L/h, 0.06 g/L α-amylase concentration and 1 g/L surfactant concentration. The error bars represent ±SD of at least 3 replicates.

Figure 7. Detergency assays in BSF. Influence of the surfactant at 45 min of the cleaning process at pH=7, flow rate 30 L/h, 0.06 g/L enzyme, 60ºC, and 1.00 g/L of surfactant concentration (the error bars represent ±SD of at least 3 replicates; different letters denote statistical difference between the experimental conditions using the Fisher's Least Significant Difference test with a 95.0% confidence level).

Figure 8. Detergency of 0.06 g/L α-amylase solutions vs. time. Comparison without surfactant, with AE 1 g/L and with gradual addition of AE from 0 to 2 g/L (0 g/L at 0 min; 1 g/L at 20 min; 2 g/L at 50 min). pH=7, flow rate 30 L/h, average values of at least 3 replicates.

Figure1

Figure2

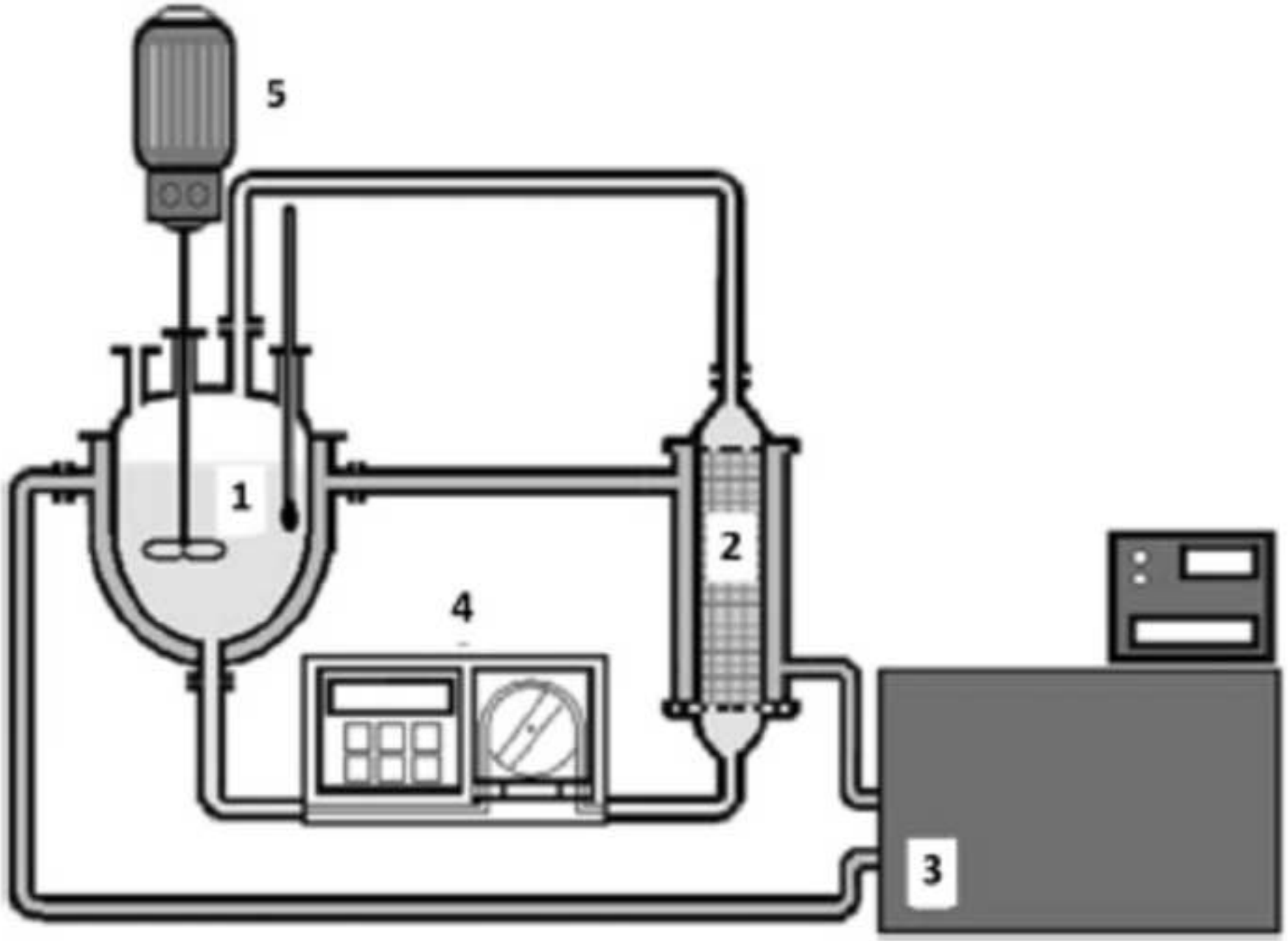


Figure3

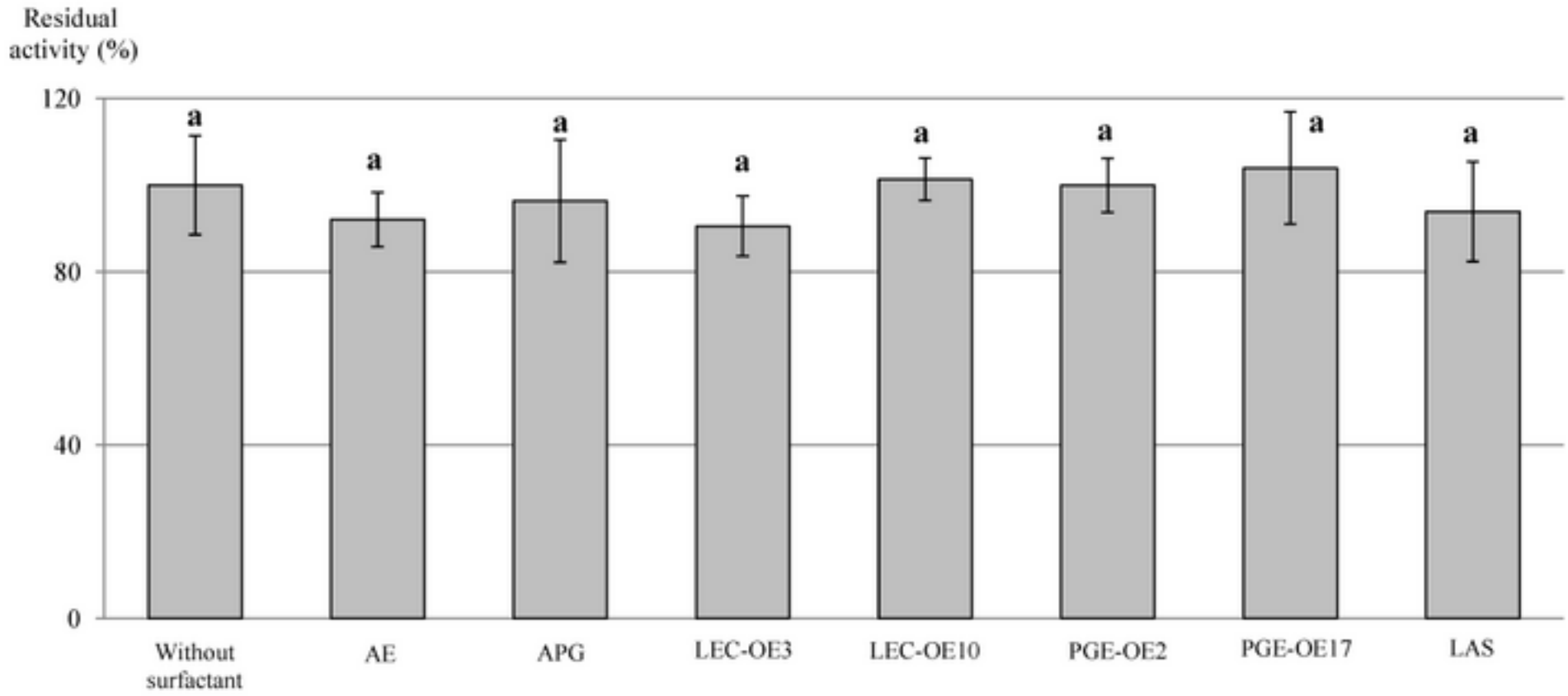

Residual activity (%)
120
80
40
0
a
a
a
a
a
a
a
a
Without surfactant
AE
APG
LEC-OE3
LEC-OE10
PGE-OE2
PGE-OE17
LAS

Figure4

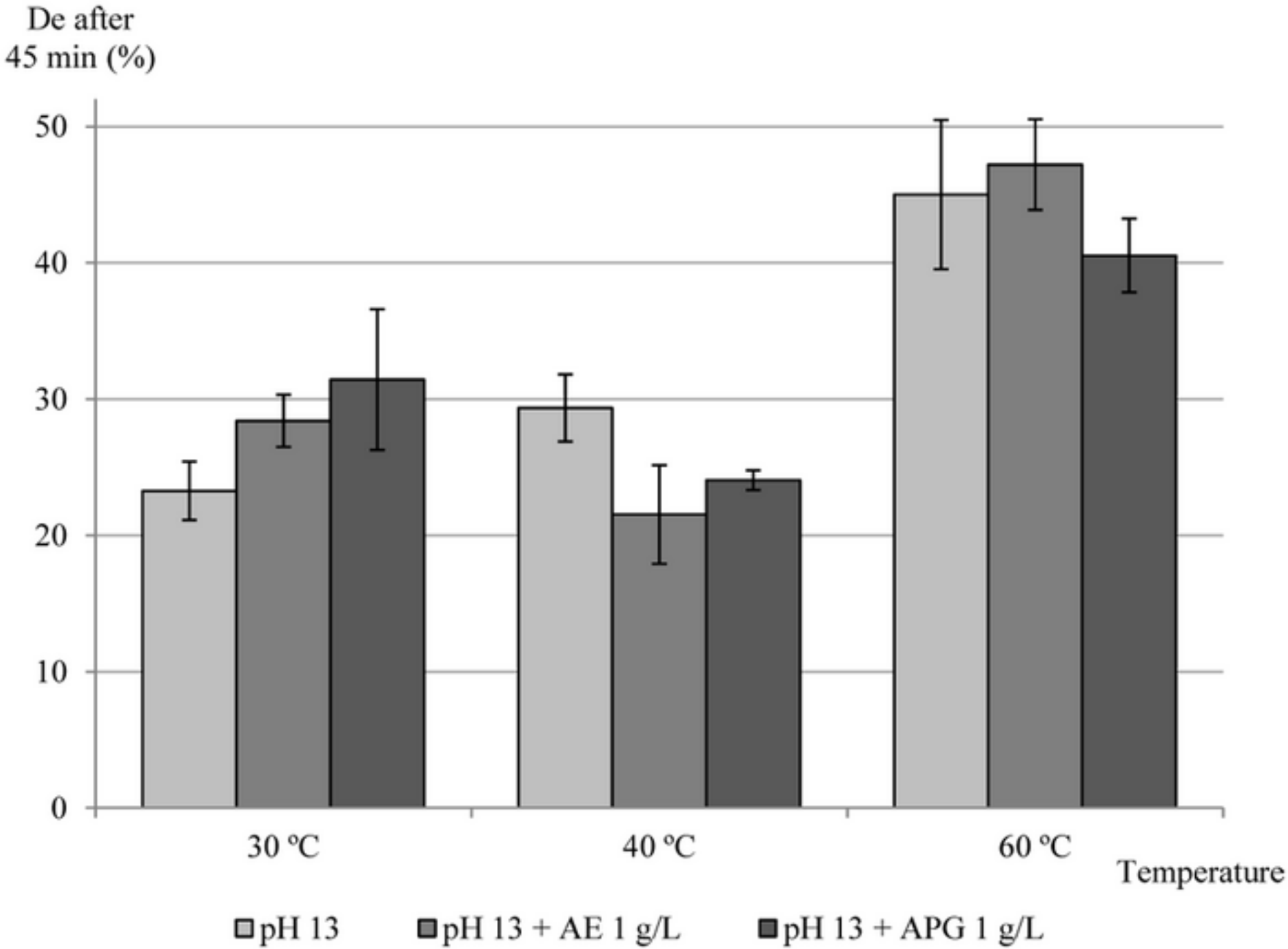

De after
45 min (%)
50
40
30
20
10
0
30 °C
40 °C
60 °C
Temperature
pH 13
pH 13 + AE 1 g/L
pH 13 + APG 1 g/L

Figure5

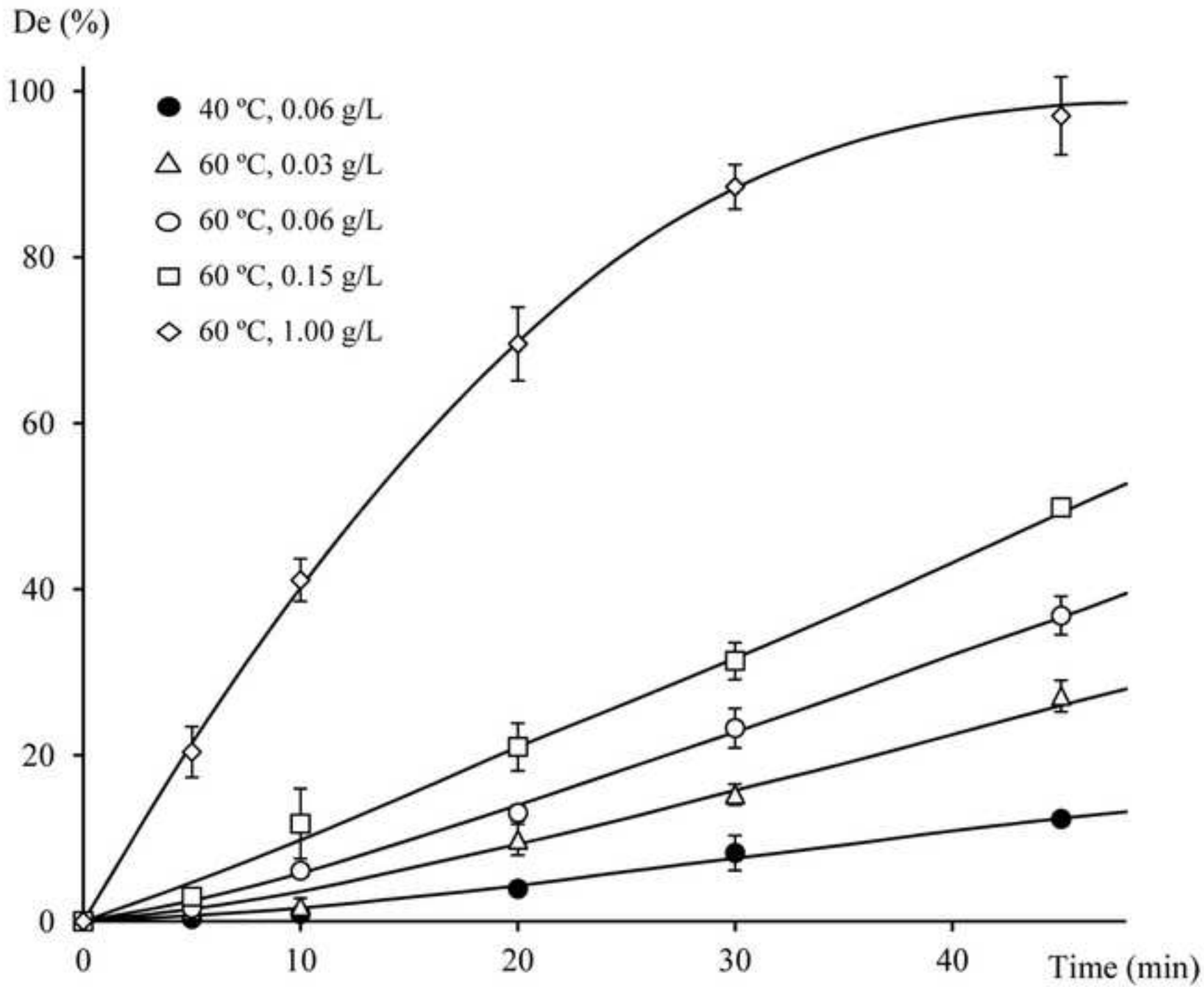

De (%)
100
80
60
40
20
0
40 °C, 0.06 g/L
60 °C, 0.03 g/L
60 °C, 0.06 g/L
60 °C, 0.15 g/L
60 °C, 1.00 g/L
0
10
20
30
40
Time (min)

Figure6

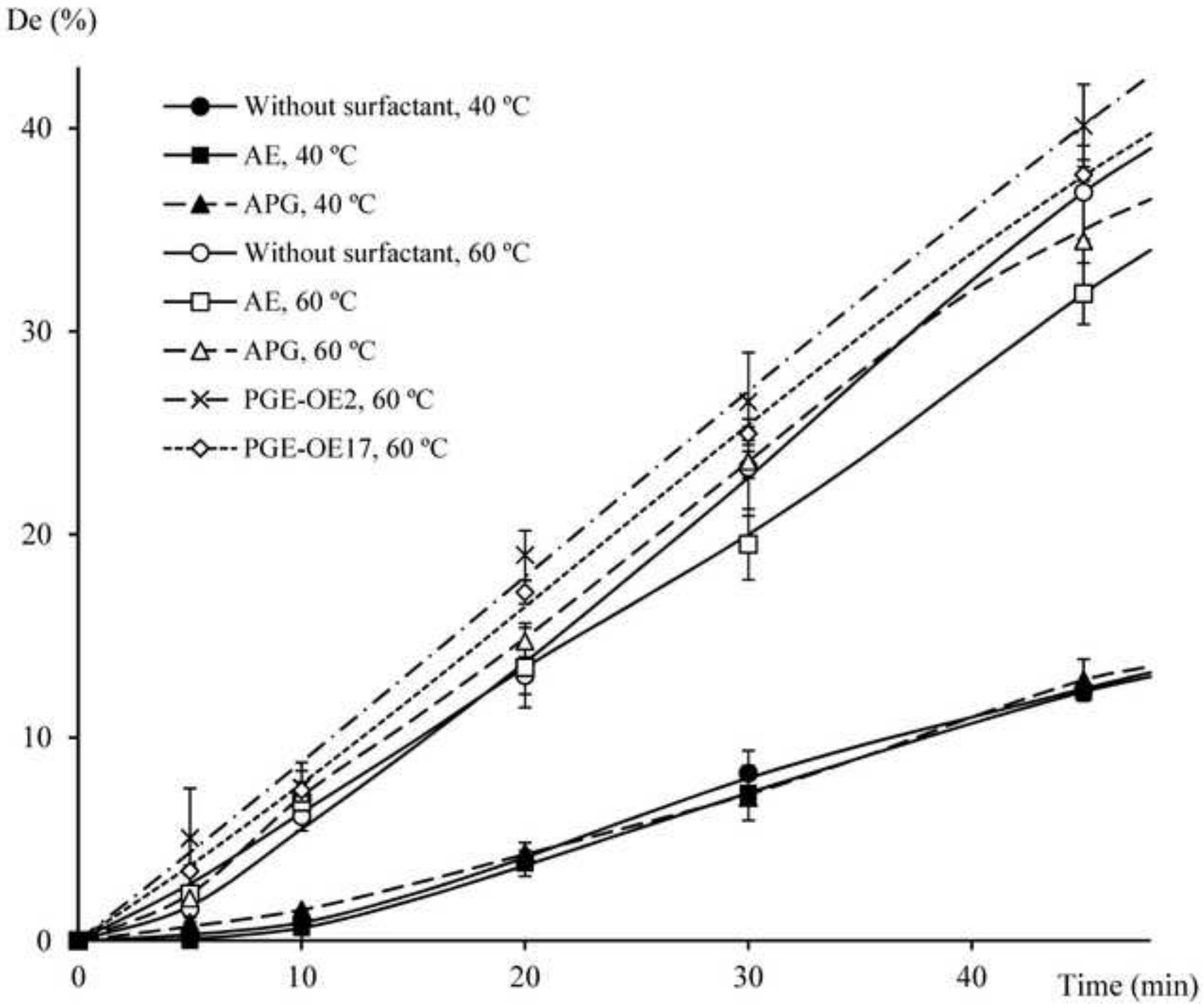

De (%)
Without surfactant, 40 ℃
AE, 40 ℃
APG, 40 ℃
Without surfactant, 60 ℃
AE, 60 ℃
APG, 60 ℃
PGE-OE2, 60 ℃
PGE-OE17, 60 ℃
0
10
20
30
40
Time (min)

Figure7

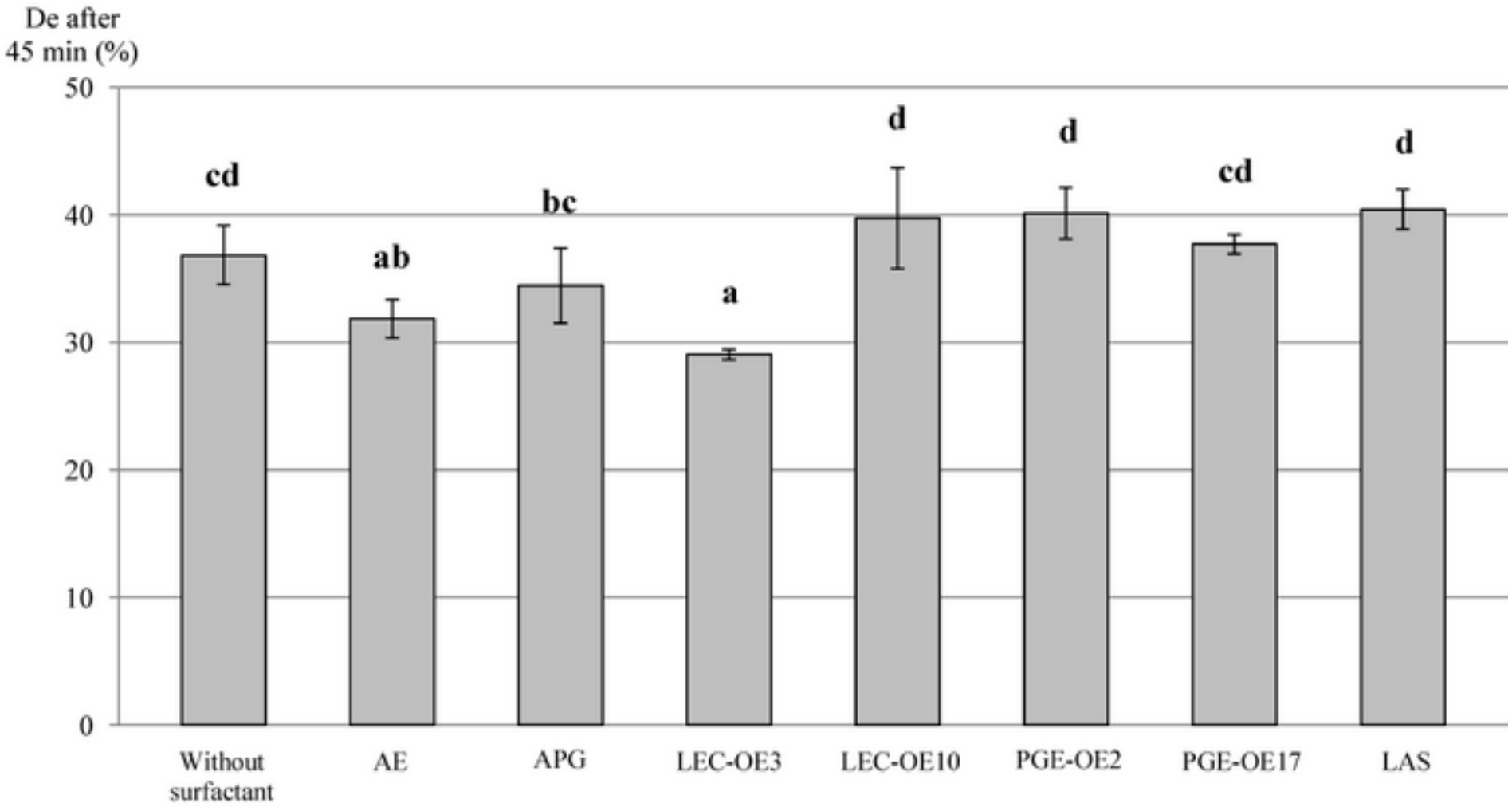

De after
45 min (%)
50
40
30
20
10
0
cd
ab
bc
a
d
d
cd
d
Without surfactant
AE
APG
LEC-OE3
LEC-OE10
PGE-OE2
PGE-OE17
LAS

Figure8

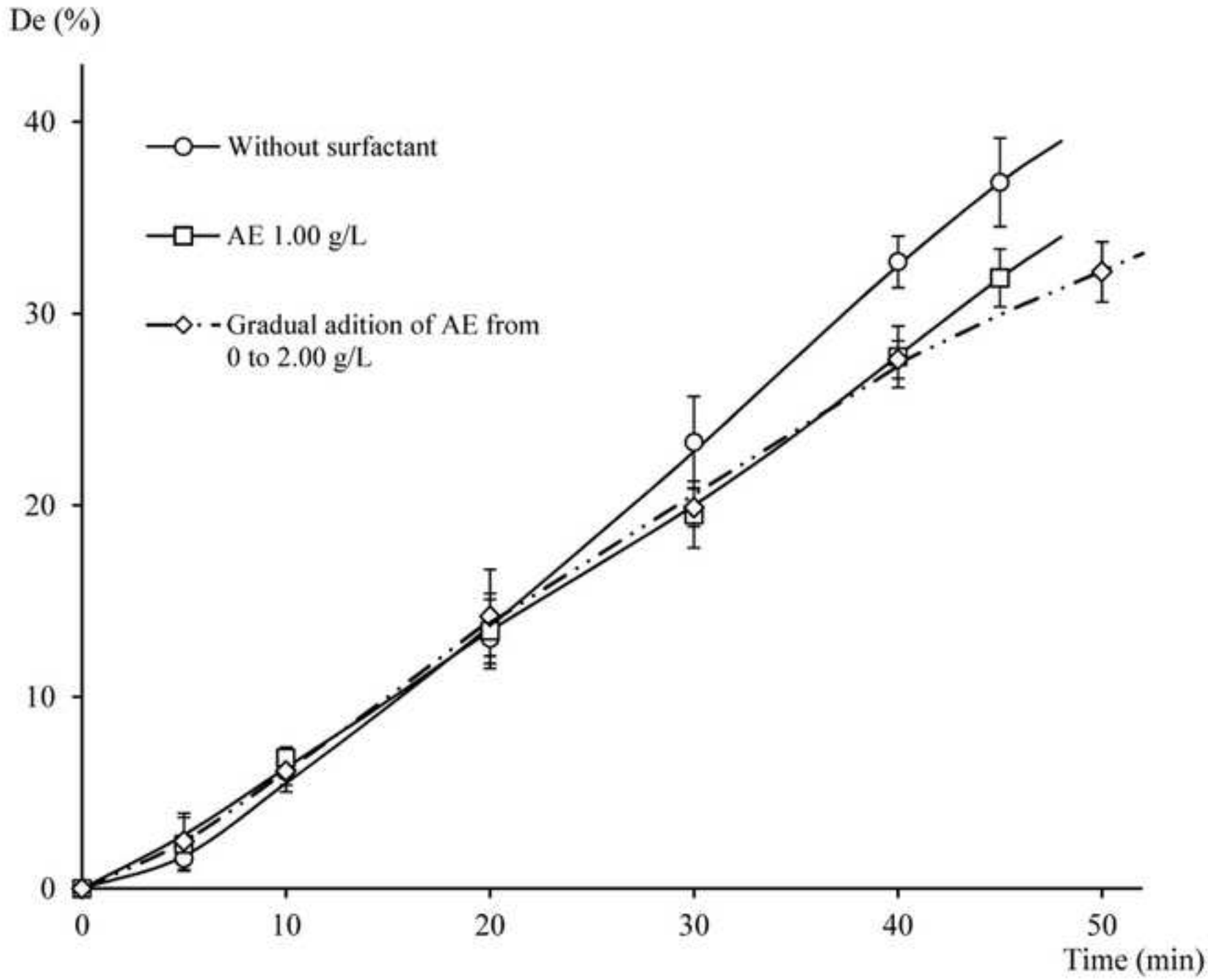
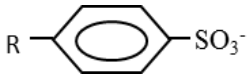
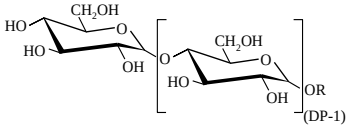
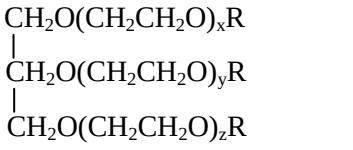

De (%)
40
30
20
10
0
Without surfactant
AE 1.00 g/L
Gradual adition of AE from
0 to 2.00 g/L
0
10
20
30
40
50
Time (min)

Table 1.

Classification	Surfactant	Trade name	Structural formula	Chemical composition	HLB	Water content (% w/w)	CMC (g/L)	MW (g/mol)	DID List mumber/ Aerobic degradation[b]/ Anaerobic degradation[b]
Anionic	LAS (linear alkyl benzene sulfonate)	LAS		R= C_{10}-C_{13}	-	54.6 (Jurado-Alameda et al., 2012)	1.018 (37ºC) (Martínez Gallegos, 2005)	342 (Jurado-Alameda et al., 2012)	A(1)/Readily biodegradable(Lechugaet al., 2014)/ Not biodegradable
Anionic (at pH=7)	LEC-OE3 (Polyoxyethylene(3) lauryl ether carboxylic acid)	Akypo RLM 25	R-O-(CH_2CH_2O)$_n$-CH_2COOH	R= C_{12}-C_{14} [a] n=2.5 [a]	-	7.0 [a]	0.033 (25 ºC) (Jurado et al., 2012)	356[a]	A(18)/ Readily biodegradable(Jurado etal., 2012)/ The ingredient has not been tested
Anionic (at pH=7)	LEC-OE10 (Polyoxyethylene(10) lauryl ether carboxylic acid	Akypo RLM 100		R= C_{12}-C_{14} [a] n=10[a]	-	8.0-12.0 [a]	0.071 (25 ºC) (Jurado et al., 2012)	686 (Martínez-Gallegos et al., 2011)	A(18)/ Readily biodegradable(Jurado et al., 2012)/ The ingredient has not been tested
Non-ionic (fatty ethoxylated alcohol)	AE (Polyoxyethylene(11) alkyl(C_{12-14}) ethers)	Findet 1214N/23	**Error! Objects cannot be created from editing field codes.**	R= C_{12}(70%), C_{14} (30%) n=9.9 (Bravo Rodriguez et al., 2005)	14.4(Martínez-Gallegoset al., 2011)	0.3 (Bravo Rodriguez, et al., 2005)	0.021 (37 ºC) (Martínez-Gallegos et al., 2011)	629(Bravo Rodriguez et al., 2005)	A(29)/Readily biodegradable(Jurado et al., 2013)/ The ingredient has not been tested
Non-ionic	APG (alkyl polyglycoside)	Glucopon 650 EC		R=C_8-C_{14} (Bravo Rodriguez et al., 2005) DP =1.3	11.9(Bravo Rodríguez et al., 2008)	50.4 (Bravo Rodriguez, et al., 2005)	0.073 (37 ºC) (Bravo Rodríguez et al., 2008)	397(Bravo Rodriguez et al., 2005)	A(49)/ Readily biodegradable(Jurado etal., 2011a)/ Biodegradable
Non-ionic	PGE–OE2 (Polyoxyethylene(2) mono- and di-glycerides)	Levenol C-421		x+y+z=2 R= H or R'-CO (Coconut chain) [a]	11.3[a]	4.9(Jurado et al., 2011b)	0.0193 (40 ºC)	298	A(43)/Readily biodegradable / Biodegradable
Non-ionic	PGE–OE17 (Polyoxyethylene(17) mono- and di-glycerides)	Levenol C-201		x+y+z=17 R= H or R'-CO (Coconut chain) [a]	13.0[a]	3.3(Jurado et al., 2011b)	0.0343 (40 ºC)	1129	A(44)/Readily biodegradable / Biodegradable

[a] Data supplied by the manufacturer.

[b] Degradation according to OECD guidelines (Detergent Ingredients Database (DID-list)

Table 2.

Composition	Concentration
Protein (g/100 g)	0.37
Fat(g/100 g)	0.42
Carbohydrates (g/100 g)	90.37
Moisture (g/100 g)	7.84
Ashes (g/100 g)	0.99
Na (mg/100 g)	46.55
Ca (mg/100 g)	38.96
K (mg/100 g)	290.36
Mg (mg/100 g)	32.55