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

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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.

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

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alkylpolyglycosides are capable of increasing enzyme stability in liquid-detergent formulations (Von Rybinski and Hill, 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 and Eliasson, 1990; Gudmundsson, 1992; Hoshino and Tanaka, 2003; Hui et al., 1983; Kim and Robinson, 1979; Lundqvist et al., 2002a,b,c; 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 non-ionic 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 summarises 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 (Fig. 1). The wads measured roughly 2 cm in diameter and weighed between 0.80 and 0.85 g (fibres 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 70 °C 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 12 h in an oven at 60 °C; (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 summarises the composition of the dry starch. Moisture was determined by drying at 110 °C 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 550 °C 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 160 °C to dryness. Then 4 mL of HNO_3 were added, kept 1 h at 80 °C, 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-Alameda et al. (2007) that simulates a CIP system (Fig. 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 (40–60 °C), 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):