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Characterization of Commercial Amylases for the Removal of Filter Cake on Petroleum Wells

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Abstract Drilling fluid has many functions, such as carry cuttings from the hole permitting their separation at the surface, cool and clean the bit, reduce friction between the drill pipe and wellbore, maintain the stability of the wellbore, and prevent the inflow of fluids from the wellbore and form a thin, low-permeable filter cake. Filter cake removal is an important step concerning both production and injection in wells, mainly concerning horizontal completion. The drilling fluids are typically comprised of starch, the most important component of the filter cake. A common approach to remove this filter cake is the use of acid solutions. However, these are non-specific reactants. A possible alternative is the use of enzymatic preparations, like amylases, that are able to hydrolyze starch. Wells usually operate in drastic conditions for enzymatic preparations, such as high temperature, high salt concentration, and high pressure. Thus, the main objective of this work was to characterize four enzymatic preparations for filter cake removal under open hole conditions. The results showed that high salt concentrations (204,000 ppm NaCl) in completion fluid decreased amylolytic activity. All enzymatic preparations were able to catalyze starch hydrolysis at all temperatures tested (30, 65, 80, and 95 °C). An increase of amylolytic activity was observed with the increase of pressure (100, 500 and 1,000 psi) for one commercial amylase.

Keywords Amylase · Filter cake removal · Drilling fluid · Pressure · Temperature

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Introduction

Drilling fluids are used to reduce friction between the drill pipe and wellbore, to carry cuttings from the hole and permit their separation at the surface, to cool and clean the bit, to maintain the stability of the wellbore, to prevent the inflow of fluids from the wellbore, and to form a thin, low-permeable filter cake [1]. The most important component of the filter cake is starch [2]. Starch in water-base drilling fluids provides viscosity for friction and gives gel-like properties to the fluid, thus promoting filter cake formation and fluid loss control [3].

The filter cake formed in the pores of the wellbore must be removed because it reduces the flow capacity and can result in significant reduction of the well production [4, 5]. Various methods have been used to remove damage and to increase the flow capacity. The filter cake can be removed using strong mineral acids, organic acids, and oxidizing agents [4, 6]. However, the use of acids or oxidizing agents may not be effective for removing the filter cake in horizontal wells. They are non-specific reactive compounds that can react with treating lines, downhole tubular, and formation components, including hydrocarbons [4]. Moreover, the use of these agents can promote a rapid removal of the previous filter cake, next to the entering of well, which causes an increase on permeability in this area, before the completely deep filter cake removal [5]. An alternative technique using enzymes has been developed to overcome these disadvantages to remove the filter cake and to increase the well production.

Enzymes are specific agents and are also inherently environmentally friendly. Amylases are enzymes that hydrolyze starch molecules to dextrin and progressively smaller molecules composed of glucose units [7]. Some operation conditions of the petroleum well can interfere on enzymatic activity, like high temperature, high salt concentration, and high pressure values.

Thus, the aim of this work was to characterize four enzymatic preparations to hydrolyze starch at completion conditions (high temperature, high salinity, and high pressure)—two commercial amylases and two commercial enzymatic filter-cake breakers used in petroleum industry. The effects of temperature (65, 80, and 95 °C), salt concentration (204,000 ppm of NaCl) and pressure (100, 500, and 1,000 psi) on amylolytic activity were investigated. The enzyme thermostability was also studied. In such a way, it was intended to verify if the commercial amylases chosen and the enzymatic breakers currently used in the oil industry have similar performance in order to replace the latter for lower cost commercial enzymes.

Materials and Methods

Materials

Two commercial α-amylases (A1 and A2) and two commercial enzymatic filter-cake breakers (EB1 and EB2) were kindly supplied by CENPES/Petrobras. The completion fluid supplied by CENPES/Petrobras was a brine solution with a NaCl concentration of 204,000 ppm. Soluble starch P.A. ACS obtained from Vetec Química Fina (RJ, Brazil) was used.

Emulsification Index

The emulsification index (EI) was determined by adding 1 mL of hexadecane to the same amount of sample, vortex-mixing for 2 min, and leaving to stand for 24 h. The sample EI is

given as percentage of height of emulsified layer (centimeter) divided by total height of the liquid column (centimeter) [8, 9].

Electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was used to determine protein molecular mass of the enzymatic preparations using a 12% polyacrylamide gel. After electrophoresis, gel was stained with Coomassie Colloidal Brilliant Blue [10].

Protein Determination

Lowry method was used to determine protein content in the enzymatic products [11]. Bovine serum albumin was used as standard.

Amylolytic Activity Determination

The amylolytic activity was measured by the increase in reducing sugars (glucose) content as a result of starch hydrolysis, spectrophotometrically quantified according to modified dinitrosalicylic acid (DNS) method [12]. An enzyme sample was added to a starch solution (1% *w*/*v*) in a phosphate buffer (0.02 mol L^{-1}, pH 6.9 and NaCl 0.05 mol L^{-1}) and maintained at 30 °C. The enzymatic reaction was stopped after 5 min by the addition of DNS, and the resultant mixture was immediately heated at 100 °C for 10 min. The reducing sugars released were quantified at 540 nm in a Hach DR/4000UV spectrophotometer. One activity unit (U) was defined as the amount of enzyme that releases 1 μmol of reducing end groups per minute under the reaction conditions.

Effect of Temperature and High Salt Concentration on Enzyme Activity

The effect of temperature on enzyme activity was evaluated by measuring the amylolytic activity of the enzymatic preparations (1% *v*/*v*) at different temperatures (30, 50, 65, 80, and 95 °C) in phosphate buffer (0.02 mol L^{-1}) or in completion fluid (204,000 ppm NaCl). The experiments were conducted in duplicate.

Effect of Temperature and High Salt Concentration on Enzyme Stability

All of enzymatic preparations were diluted (1% *v*/*v*) in water or in completion fluid (204,000 ppm NaCl) and were maintained at 65, 80 and 95 °C for 8 h or until the measurement of the residual activity was no longer possible. The experiments were conducted in duplicate.

Determination of Kinetic Parameters

The kinetic parameters (K_m and V_{max}) were determined at 65 and 80 °C. A sample of an enzymatic preparation was incubated in a soluble starch solution (concentration ranging from 1.25 to 8.75 mg mL^{-1}) prepared in 0.02 mol L^{-1} sodium phosphate buffer (pH 6.9 and NaCl 0.05 mol L^{-1}) or in completion fluid (204,000 ppm NaCl). The amount of reducing end groups formed was measured according to DNS method [12]. The amylase

activity was determined as previously described. The experiments were conducted in duplicate.

Effect of Pressure and High Salt Concentration on Enzyme Activity

The influence of the pressure on amylolytic activity was investigated in a Parr Reactor 4843 under different pressures (100, 500, and 1,000 psi) and temperatures (65, 80, and 95 °C), in phosphate buffer (0.02 mol L^{-1}, pH 6.9) and completion fluid (204,000 ppm NaCl). The experiments were conducted in duplicate.

Results and Discussion

Enzymatic Characterization

The commercial α-amylases herein used (A1 and A2) are heat-stable enzymes expressed and produced by a genetically modified strain of *Bacillus*. α-Amylase is an endoamylase that hydrolyzes 1-4-α-glucosidic linkages in amylose and amylopectin, breaking starch into soluble dextrin and oligosaccharides [7, 13]. This results in a rapid reduction of the viscosity of gelatinized starch. Thus, these enzymes have a great potential in filter cake removal applications.

The commercial enzymatic filter cake breakers (EB1 and EB2) are described as products able to remove the filter cake on wellbore walls during completion operations.

The protein concentration of A1 is 53.9 mg mL^{-1}, A2 is 54.1 mg mL^{-1}, EB1 is 58.7 mg mL^{-1} and EB2 is 84.8 mg mL^{-1}.

Protein preparations can sometimes be analyzed by examining the relative band intensities of a specific protein obtained by electrophoresis. According to the literature [7, 14], the amylase molecular weight is dependent on the enzyme source and ranges between 47 to 135 kDa. Although the composition of the enzymatic breakers is till now unknown, the results revealed, as shown in Fig. 1, that all enzymatic preparations have very similar electrophoresis profiles showing an identical protein band with 60 kDa molecular weight, approximately.

The emulsification agents are substances that increase the emulsion stability. In order to characterize the enzymatic preparations and verify their emulsification capacity, the EI was determined. Only commercial enzymatic filter cake breakers, EB1 and EB2, showed emulsification capacity and presented EI of 20.8% and 28.6%, respectively.

Effect of Temperature and High Salt Concentration on Enzyme Activity

Wells usually operate in drastic conditions for enzymatic preparations, such as high temperature and salinity. The effect of temperature (30–95 °C) and high salinity on amylolytic activity was investigated in phosphate buffer and completion fluid.

As shown in Fig. 2, all enzymatic preparations were able to catalyze starch hydrolysis in all temperatures tested. The commercial α-amylase A1 is an industrial thermostable enzyme, and it shows the highest activities in the range of 65–95 °C in phosphate buffer, as expected. Literature reports [14–16] that microbial α-amylase shows the highest activities in the range of 60–80 °C in buffer solutions.

The amylolytic activity presented a sharp decrease in the presence of completion fluid (high salinity, 204,000 ppm NaCl) for all enzymatic preparations in all temperatures

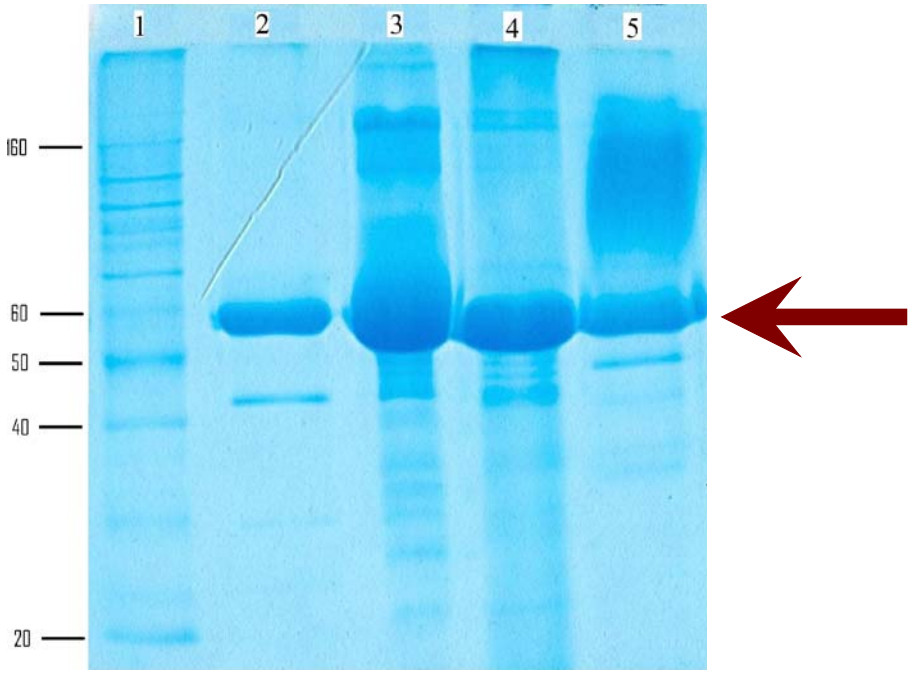

Fig. 1 Electrophoresis: *1* molecular weight standard, *2* A1, *3* EB2, *4* EB1, *5* A2. The *arrow* marks the same molecular weight band

assayed. The commercial α-amylase A1 shows maximum activity value in completion fluid at 95 °C, being 77.1% lower than the value obtained in phosphate buffer. In the petroleum industry, the fluids for drilling and completion steps have to contain high salt concentration, and NaCl is usually used [5].

Effect of Temperature and High Salt Concentration on Enzyme Stability

As wells usually operate at high temperature, the thermal stability of enzymatic preparations, which are used for filter cake removal, is an important parameter. The thermal stability of enzymes is very important because it can demonstrate how long the enzymes retain their activity under different reaction conditions. It is evaluated by measuring the residual activity.

The effect of temperature and high salt concentration was investigated for all enzymatic preparations. The enzymatic preparations were incubated at different temperatures (65, 80 and 95 °C) in water or in the presence of 204,000 ppm of NaCl for 8 h, and then residual activity of amylase was determined. Enzyme activity was expressed as percentage relative activity measured under standard assay conditions. The results are illustrated in Fig. 3.

According to Fig. 3, the enzymatic preparations were stable at 65 °C and maintained 80% of the initial activity value after 8 h in completion fluid.

However, in the experiments carried out at 80 °C in water, after 8 h, the residual enzymatic activity of A1 was 80%, whereas A2 showed only 4%. The enzymatic

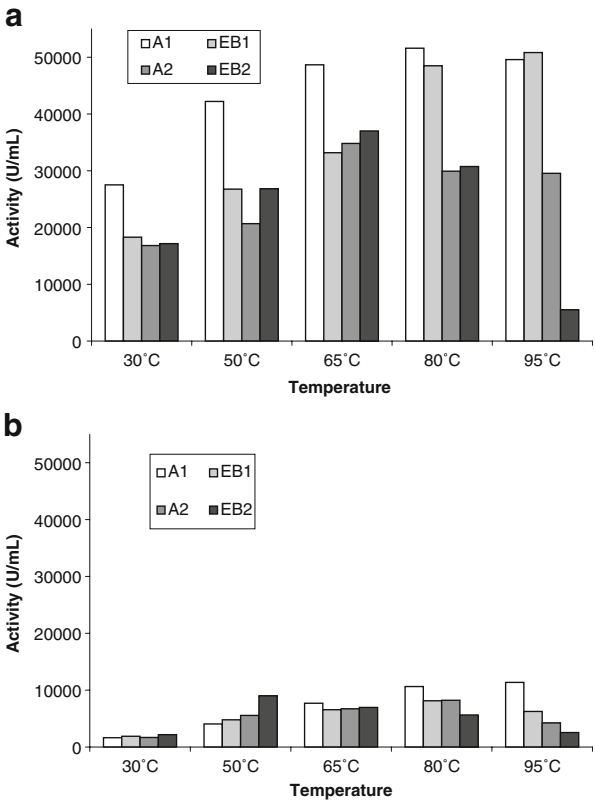

Fig. 2 Effects of temperature on amylolytic activity in phosphate buffer (**a**) and completion fluid (**b**)

breaker EB2 did not present amylolytic activity after 5 h of experiment at this temperature.

In completion fluid at 80 °C, after 8 h, A1 maintained 78% of its initial activity value, but the absolute amylolytic activity value was 77% of the one obtained in water.

After 3 h at 95 °C, A1 retained 45% of its initial activity in control experiment, and the other three enzymatic preparations did not show activity after 3 h. In completion fluid, the residual activity of A1 was only 23%, whereas the other three enzymatic preparations lost almost all activity after 2 h of experiment.

Determination of Kinetic Parameters

The stability results show that the enzymatic preparations studied are able to maintain their activity at 65 and 80 °C. Thus, the hydrolytic behavior of these amylases was evaluated at 65 and 80 °C in phosphate buffer and in completion fluid. The initial reaction rates of amylases were determined at different starch concentrations raging from 1.25 to 8.75 mg mL^{-1} (pH 6.9). The values of kinetic parameters (K_m and V_{max}) were determined using Lineweaver–Burk plot. K_m and V_{max} are significant parameters in enzymatic kinetic. Higher V_{max} and lower K_m values indicate the efficiency of one enzyme for a certain reaction.

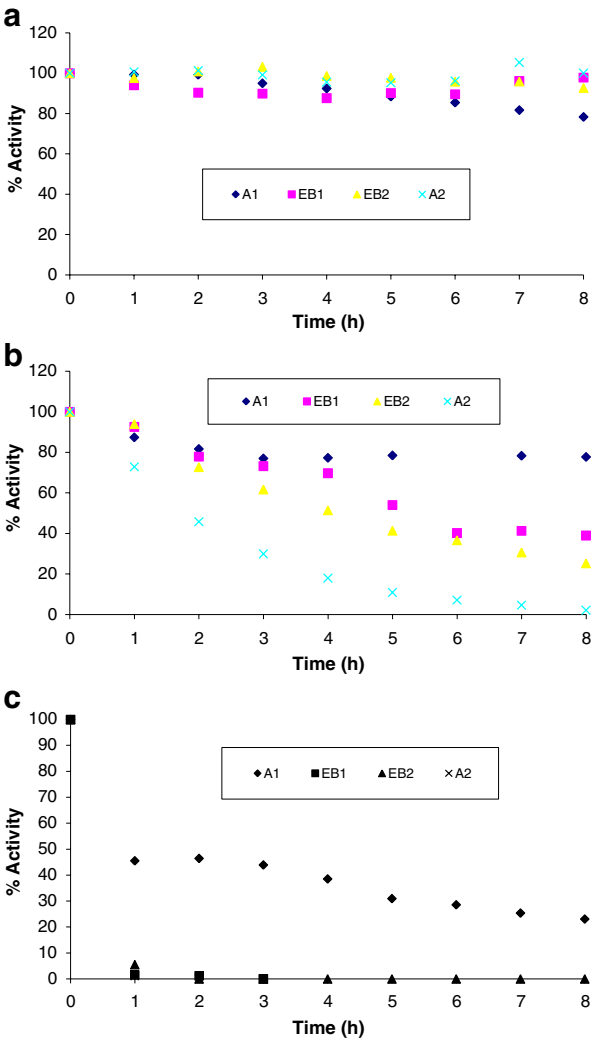

Fig. 3 Effect of temperature on stability of the enzymatic preparation in completion fluid. **a** 65 °C, **b** 80 °C, and **c** 95 °C. *Diamond A1*, *square EB1*, *triangle EB2*, *ex symbol A2*

The results are presented in Table 1.

The results indicate that commercial amylases behave according to Michaelis–Menten kinetics in phosphate buffer, as reported in literature [10, 13, 16, 17] and as can be seen in Figs. 4 and 5, which show the results obtained in the reactions carried out with commercial amylase A1, at 65 °C, in phosphate buffer. The increase of temperature leads to a higher initial reaction rate, as expected. Enzymatic preparations presented similar behavior in completion fluid for both temperatures.

Effect of Pressure and High Salt Concentration on Enzyme Activity

Pressure is an important parameter to be considered when filter cake removal is done in the presence of enzymes because it has been recognized as a potential denaturant

Table 1 Kinetic parameters (K_m and V_{max}) for commercial amylases (A1 and A2) and enzymatic breakers (EB1 and EB2) at 65 and 80 °C, in phosphate buffer and completion fluid.

Enzymatic preparation	Temperature (°C)	Medium	K_m (mg mL^{-1})	V_{max} (μmol mL^{-1}min^{-1})	R^2
A1	65	Buffer	3.16	12.78	0.99
		Completion fluid	4.18	1.42	0.99
	80	Buffer	5.67	16.08	0.98
		Completion fluid	1.45	2.71	0.99
EB1	65	Buffer	1.32	2.92	0.99
		Completion fluid	0.88	0.53	0.99
	80	Buffer	1.79	3.25	0.98
		Completion fluid	1.15	0.87	0.99
A2	65	Buffer	1.26	1.45	0.98
		Completion fluid	8.50	0.93	0.99
	80	Buffer	1.24	2.41	0.98
		Completion fluid	5.70	0.91	0.99
EB2	65	Buffer	1.68	3.31	0.99
		Completion fluid	6.54	0.94	0.99
	80	Buffer	3.42	4.23	0.99
		Completion fluid	2.75	0.41	0.99

agent for proteins. The pressure range in which proteins suffer denaturation differs depending on its structural characteristics. However, there are also examples of pressure stabilization effect on proteins [18, 19].

Therefore, the effect of pressure on amylolytic activity was investigated for A1, considering that this commercial amylase presented the best results in the experiments herein.

Three different pressures were studied (100, 500 and 1,000 psi) at different temperatures (65, 80, and 95 °C) in phosphate buffer (0.02 mol L^{-1}, pH 6.9) and completion fluid (204,000 ppm NaCl). The results (Fig. 6) show an increase of amylolytic activity with the increase of pressure for all temperatures tested in phosphate buffer and in completion fluid.

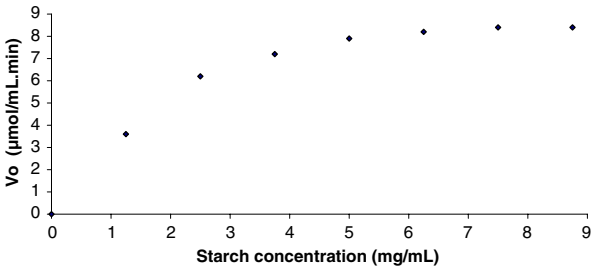

Fig. 4 Effect of starch concentration on initial reaction rate (reactions carried out with commercial amylase A1, at 65 °C, in phosphate buffer)

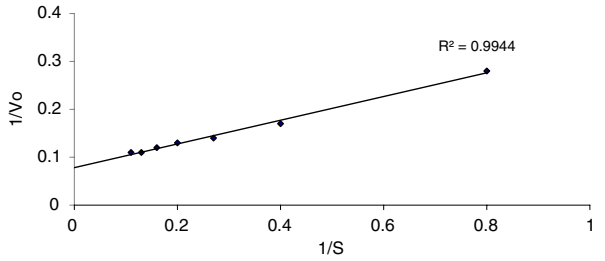

Fig. 5 Lineweaver–Burk plot (reactions carried out with commercial amylase A1, at 65 °C, in phosphate buffer)

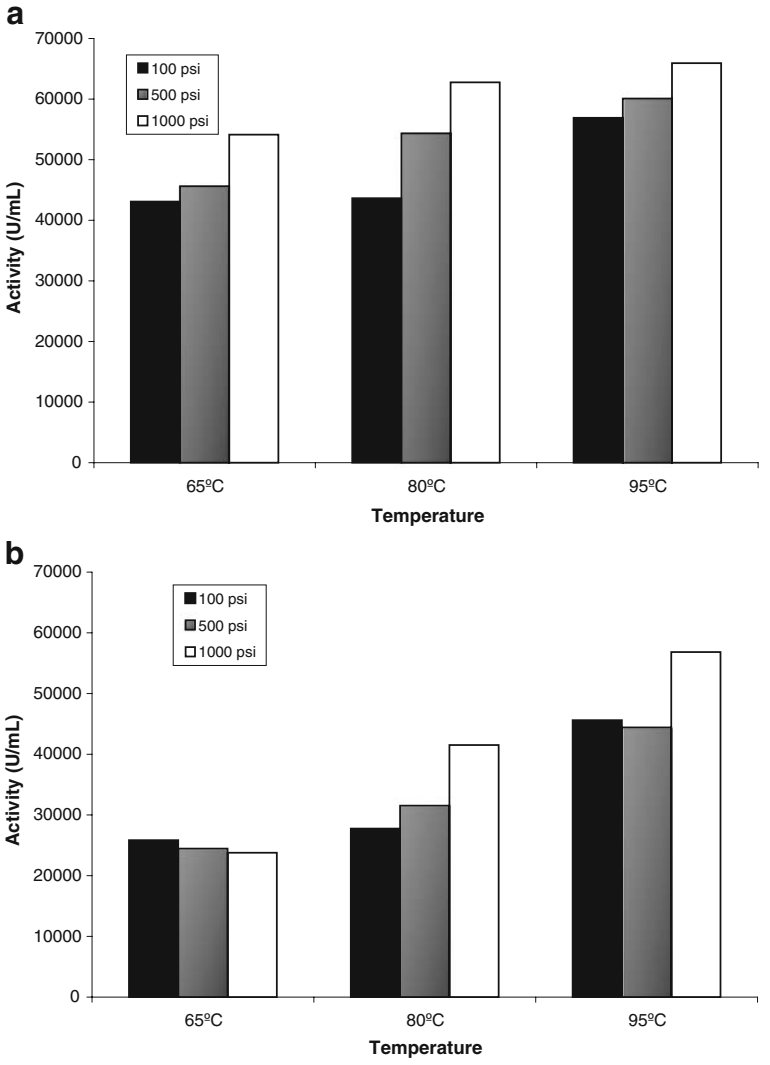

Fig. 6 Effect of pressure on commercial amylase A1 activity at 65, 80, and 95 °C in phosphate buffer (**a**) and completion fluid (**b**)

Conclusions

All enzymes were able to catalyze starch hydrolysis under hard completion fluid conditions (high salt concentration and high temperature). The tested enzymatic preparations presented lower amylolytic activity values in the presence of completion fluid. The commercial α-amylase A1 was stable at 65 and 80 °C during 8 h, and it lost about 50% of initial amylolytic activity at 95 °C after 4 h of incubation. Enzyme activity of A1 increased with the increase of pressure (100, 500 and 1,000 psi) at 65, 80, and 95 °C. The commercial α-amylase A1 showed the best performance among all studied enzymatic preparations, indicating its potential to hydrolyze starch in completion conditions.

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