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SURFACTANT ADDITION ON ENZYMATIC HYDROLYSIS OF CORNSTARCH FOR BIOBUTANOL PRODUCTION

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ABSTRACT

The increasing demand for energy and the depletion of fossil fuel reserves have encouraged the development of renewable biofuels. Biobutanol is considered a promising alternative fuel because its physicochemical properties are more similar to gasoline than ethanol. In this study, biobutanol production from cornstarch was investigated through enzymatic hydrolysis followed by fermentation using Clostridium acetobutylicum. The hydrolysis performance was evaluated based on reducing sugar production, while the effects of enzyme concentration, surfactant addition, and hydrolysis time were examined. Tween-80 was used as a non-ionic surfactant to enhance the enzymatic hydrolysis process. The results showed that the addition of low concentrations of Tween-80 increased reducing sugar production, whereas excessive surfactant concentrations reduced hydrolysis efficiency. The optimum enzyme concentration for the liquefaction process was found to be 0.7% (v/v). Furthermore, increasing inoculum concentration initially enhanced biobutanol production; however, excessive inoculum concentrations led to decreased butanol production due to nutrient limitation and fermentation inhibition. These findings demonstrate the importance of optimizing hydrolysis and fermentation conditions to improve biobutanol production from cornstarch.

KEYWORDS: Cornstarch, surfactant, enzymatic hydrolysis, biobutanol

INTRODUCTION

The increasing global demand for energy, combined with the gradual depletion of fossil fuel reserves, has become one of the major challenges in recent decades [1]. Extensive exploitation of petroleum, natural gas, and coal has not only reduced the availability of these non-renewable resources but also caused fluctuations in fuel prices worldwide. In addition, the combustion of fossil fuels releases large amounts of carbon dioxide, nitrogen oxides, and sulphur oxides, which contribute to global warming, climate change, and environmental pollution. These concerns have encouraged researchers to develop renewable and environmentally friendly alternative fuels, including biofuels [2], [3].

Among the various types of biofuels, biobutanol has attracted significant attention because its properties are relatively similar to those of conventional gasoline. Compared with ethanol, butanol has a higher energy density, lower vapor pressure, and lower hygroscopicity, making it more suitable for transportation fuel applications. In addition, biobutanol can be blended more easily with gasoline and transported using existing fuel infrastructure. Biobutanol is commonly produced through acetone–butanol–ethanol (ABE) fermentation using microorganisms such as Clostridium acetobutylicum. Various carbohydrate-rich materials, including sugar- and starch-based feedstocks, can be utilized as raw materials for this process [4], [5].

Cornstarch is considered a promising feedstock for biobutanol production because of its high starch content and wide availability. However, starch cannot be directly utilized by microorganisms during fermentation and must first be converted into simpler sugars, mainly glucose, through a hydrolysis process [6]. Enzymatic hydrolysis is widely preferred over chemical hydrolysis because it operates under milder conditions, is more selective, and minimizes unwanted side reactions. Despite these advantages, enzymatic hydrolysis often suffers from relatively slow reaction rates, which can reduce the overall efficiency of the process.

To overcome this limitation, several strategies have been explored to improve the performance of enzymatic hydrolysis, including the addition of surfactants. Non-ionic surfactants such as Tween-80 have been reported to enhance enzymatic hydrolysis in various polysaccharide systems. Surfactants can improve enzyme stability and influence the interaction between enzymes and substrates, thereby facilitating enzyme adsorption and desorption during the hydrolysis process. Previous studies have shown that Tween-80 could enhance the enzymatic hydrolysis of chitosan and lignocellulosic materials by reducing non-productive enzyme interactions and improving substrate accessibility [7], [8], [9].

Based on these considerations, this study investigates the enzymatic hydrolysis of cornstarch using α-amylase in the presence and absence of Tween-80 surfactant. The effects of enzyme concentration, surfactant concentration, and hydrolysis time on reducing sugar production were evaluated. Furthermore, the influence of inoculum concentration during the fermentation process using Clostridium acetobutylicum on biobutanol production was also investigated.

2. MATERIALS AND METHODS

2.1 Materials

Corn starch was obtained from Tepung Jagung Bintang, Malaysia. α-Amylase was used as the enzyme for the hydrolysis process, while glucoamylase was utilized for the saccharification process. Clostridium acetobutylicum was used as the fermentation inoculum. The α-amylase, glucoamylase, and Clostridium acetobutylicum were purchased from Sigma-Aldrich. Phosphate buffer (pH 7) was used for the preparation of the α-amylase solution, whereas acetate buffer (pH 4.5) was used for the preparation of the glucoamylase solution. Tween-80, sodium carbonate, glacial acetic acid, sodium acetate, and sodium phosphate were purchased from Merck.

2.2 Corn starch enzymatic hydrolysis

A 30 g/L cornstarch solution was prepared by dissolving cornstarch powder in 0.1 M acetate buffer solution. The mixture was heated at 50°C for 30 min to obtain gelatinized starch. Tween-80 surfactant was then added to the starch solution at concentrations of 0, 1, 2, 3, 4, and 5% (v/v).

Subsequently, α-amylase enzyme was added at concentrations of 0.1, 0.3, 0.5, 0.7, and 0.9% (v/v) for the liquefaction process. The optimum condition obtained from the liquefaction stage was further used for the saccharification process. After liquefaction, the pH of the slurry was adjusted to 4.5 to deactivate the α-amylase and provide the optimum condition for glucoamylase activity. Glucoamylase was then added at a concentration of 0.1% (v/v), and the saccharification process was carried out for various reaction times.

2.3 Fermentation

Fresh Clostridium acetobutylicum inoculum was cultivated in nutrient broth medium prior to fermentation. The hydrolysate obtained from the saccharification process was filtered to separate the liquid filtrate from the residual solids. The filtrate was subsequently inoculated with Clostridium acetobutylicum and fermented under anaerobic conditions at 37°C with agitation at 200 rpm for 4 days.

2.4 Analytical methods

The reducing sugar concentration was determined using the DNS (3,5-dinitrosalicylic acid) method. DNS reagent was mixed with the sample solution at a ratio of 1:1 in a glass bottle. The mixture was heated in boiling water (100°C) for 5 min to terminate the reaction and subsequently cooled in ice water for 5 min. The absorbance was measured at 550 nm using a UV–Visible spectrophotometer. Glucose solutions with concentrations ranging from 0 to 10 g/L were used as standard solutions, and the reducing sugar concentration was expressed in g/L. The concentration of butanol produced after fermentation was analyzed using High Performance Liquid Chromatography (HPLC).

3. RESULTS AND DISCUSSION

3.1 Effect of enzyme concentration on the liquefaction process of cornstarch

Enzymatic hydrolysis causes the cleavage of polysaccharide chains into smaller carbohydrate molecules. Each cleavage of glycosidic bonds in the polysaccharide structure produces reducing sugars and shorter-chain oligosaccharides [10]. Therefore, the formation of reducing sugars can be used as an indicator of enzyme activity during the liquefaction process. In this study, the performance of enzymatic liquefaction was evaluated based on the reducing sugar concentration produced during hydrolysis.

Figure 1 shows the effect of α-amylase concentration on reducing sugar formation. The reducing sugar concentration increased with increasing enzyme concentration up to a certain point. This result indicates that higher enzyme concentrations provided more active sites for starch hydrolysis, leading to increased cleavage of glycosidic bonds in cornstarch. However, further increases in enzyme concentration resulted in a decrease in reducing sugar production. Based on Figure 1, the optimum enzyme concentration for the liquefaction process was found to be 0.7% (v/v).

The enzyme used in this study was α-amylase, which catalyzes the random and non-selective hydrolysis of α-(1→4) glycosidic bonds in amylose and amylopectin molecules. The hydrolysis process mainly produces maltose, maltotriose, and short-chain oligosaccharides rather than glucose as the primary products. In addition, the action of α-amylase is limited in hydrolyzing the branched regions of amylopectin, resulting in the formation of dextrins. Similar findings were reported by Rahnama et al. (2014), who stated that increasing enzyme concentration could enhance the hydrolysis rate; however, excessive enzyme addition did not significantly improve hydrolysis efficiency due to substrate limitation and possible enzyme saturation effects [11].

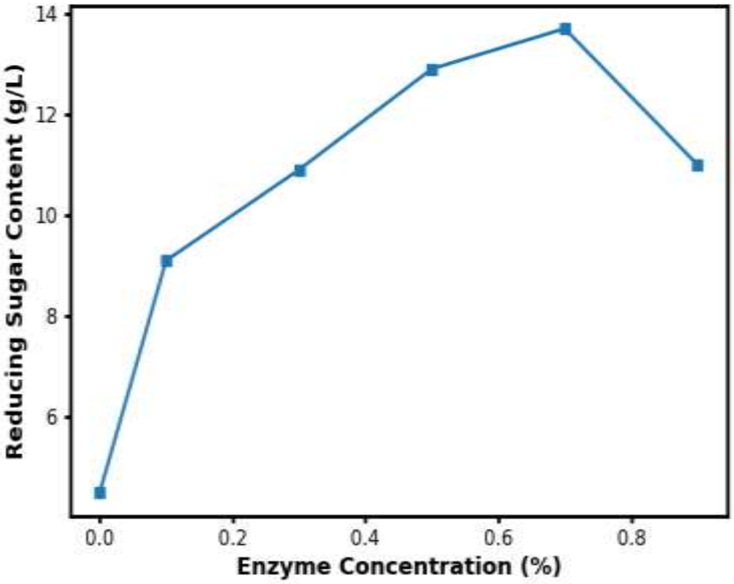

Figure 1: Effect of α-amylase concentration on reducing sugar production during the liquefaction process of cornstarch

3.2 Effect of Surfactant Addition on the Liquefaction Process of Cornstarch

Chen et al. (2014) reported that the positive effect of surfactant addition during enzymatic hydrolysis may be attributed to several mechanisms [12]. Surfactants can modify the structure of the substrate, making it more accessible to enzymes, as well as stabilize enzyme molecules and reduce enzyme denaturation during the hydrolysis process. In the present study, the effect of Tween-80 addition on reducing sugar production during the liquefaction process is presented in Figure 2.

The results showed that the addition of Tween-80 enhanced reducing sugar formation compared with the system without surfactant addition. At low surfactant concentrations, the hydrolysis rate increased due to improved enzyme–substrate interactions. Surfactants may facilitate enzyme adsorption and desorption on the starch surface, thereby improving the accessibility of α-amylase to the glycosidic bonds in cornstarch. Similar observations were reported by Yang et al. (2011), who found that non-ionic surfactants could enhance enzymatic hydrolysis efficiency by protecting enzyme activity and reducing non-productive interactions [13].

However, Figure 2 also shows that reducing sugar production decreased when the surfactant concentration exceeded 3% (v/v). According to Zhou et al. (2015) and Chen et al. (2014), enzymatic hydrolysis strongly depends on the adsorption and desorption behavior of enzymes on the substrate surface [12], [14]. At excessive surfactant concentrations, interactions between surfactant molecules and enzymes become more dominant, which may reduce effective enzyme adsorption onto the substrate and consequently decrease hydrolysis efficiency.

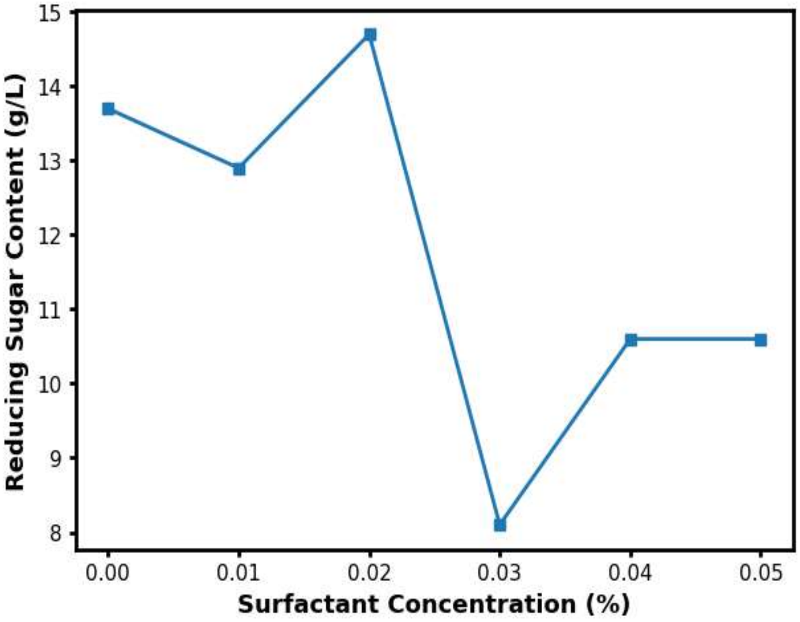

Figure 2: The effects of surfactant concentration on the content of reducing sugar

In addition, Parnthong et al. (2018) reported that surfactant concentration is closely related to the critical micelle concentration (CMC). Above the CMC, surfactant molecules tend to form micelles that can interfere with enzyme activity and substrate accessibility [15]. Since Tween-80 possesses a relatively high CMC, excessive surfactant addition may promote micelle formation, thereby reducing reducing sugar production through the micellar effect. Similar findings were also reported by Rokhati et al. (2018), who observed that low surfactant concentrations were more effective in enhancing enzymatic hydrolysis, whereas excessive surfactant concentrations weakened enzyme adsorption onto substrate functional groups.

3.3 Effect of Hydrolysis Time on the Liquefaction Process of Cornstarch

The effect of hydrolysis time on reducing sugar production during the enzymatic hydrolysis of cornstarch is shown in Figure 3. At the initial stage of hydrolysis, reducing sugar formation increased rapidly, indicating active cleavage of glycosidic bonds by the enzyme. As the reaction time increased, the reducing sugar concentration gradually increased and reached its optimum value after 48 h of hydrolysis. However, extending the hydrolysis time beyond 48 h resulted in a decrease in reducing sugar production.

The decrease in reducing sugar concentration after prolonged hydrolysis may be attributed to product inhibition during the enzymatic reaction. The accumulation of hydrolysis products can inhibit enzyme activity and reduce further substrate conversion [16]. In addition, prolonged reaction times may decrease enzyme stability and catalytic efficiency, thereby limiting hydrolysis performance.

Saccharification of starch into glucose is commonly carried out using glucoamylase-producing microorganisms such as Aspergillus niger. Tomasik and Horton (2012) reported that glucoamylase produced by A. niger is capable of achieving high starch-to-glucose conversion under suitable operating conditions [17]. Similarly, Zhang et al. (2011) found that a hydrolysis time of approximately 48 h was sufficient to achieve effective saccharification, whereas extending the reaction time did not significantly increase sugar production [18].

The increase in reducing sugar concentration with increasing hydrolysis time indicates continuous substrate conversion during the enzymatic reaction. According to Permanasari et al. (2018), longer hydrolysis times provide greater opportunities for enzyme–substrate interactions, leading to increased product formation [19]. However, as the reaction approaches equilibrium, the hydrolysis rate gradually decreases in accordance with Michaelis–Menten enzyme kinetics. At this stage, the enzyme remains active, but the overall conversion rate becomes limited due to reduced substrate availability and product accumulation.

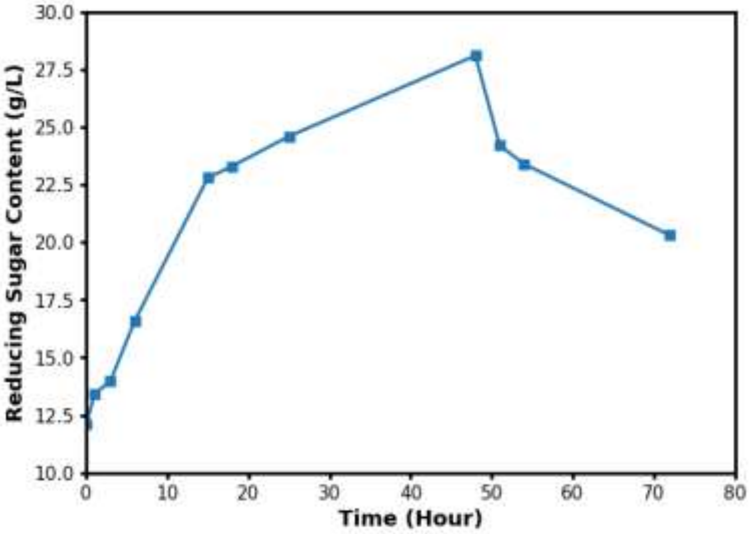

Figure 3: Effect of hydrolysis time on reducing sugar production during the enzymatic hydrolysis of cornstarch

3.4 Effect of Bacterial Concentration on the Fermentation Process

Figure 4 shows the effect of inoculum concentration on biobutanol production during the fermentation process. Increasing the bacterial concentration initially increased biobutanol production, indicating that a higher number of active microbial cells enhanced substrate utilization and solvent production. However, when the inoculum concentration was further increased to 15%, butanol production decreased.

Microbial growth generally consists of four phases, namely the lag phase, exponential phase, stationary phase, and death phase [20]. During the lag phase, microorganisms adapt to the surrounding environmental conditions before entering the exponential growth phase, where rapid cell division and active metabolism occur. The growth rate during this phase is strongly influenced by nutrient availability and environmental conditions. As fermentation progresses, the culture eventually reaches the stationary phase, where the number of growing cells becomes equal to the number of dying cells due to nutrient depletion and accumulation of metabolic products.

The decrease in butanol production at higher inoculum concentrations was likely caused by insufficient nutrient availability to support excessive microbial growth. Increased microbial populations may accelerate nutrient consumption, leading to competition among cells and reduced metabolic efficiency. In addition, butanol itself exhibits toxic effects toward Clostridium acetobutylicum, which can inhibit cell growth and solvent production at elevated concentrations [21]. Consequently, excessive inoculum concentrations may negatively affect the fermentation performance and reduce biobutanol yield.

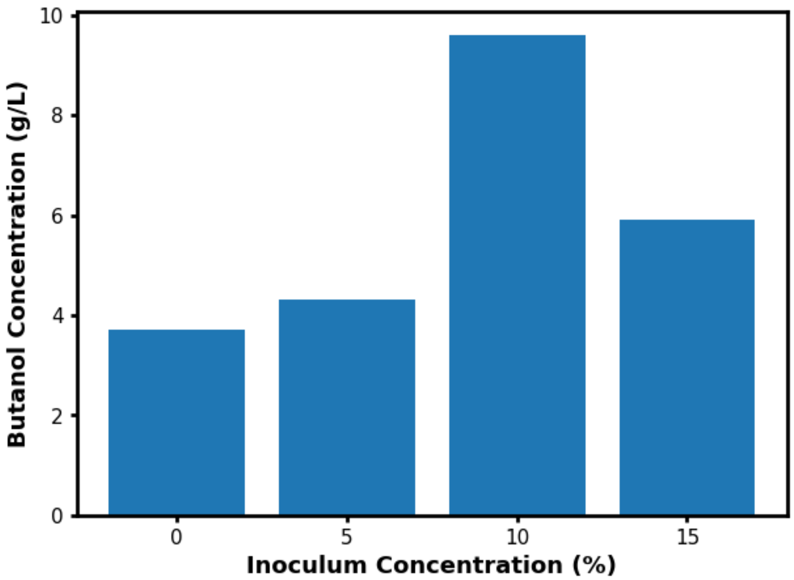

Figure 4: Effect of inoculum concentration on biobutanol production during the fermentation process

4. CONCLUSIONS

The results of this study demonstrated that increasing the α-amylase concentration up to 0.7% (v/v) enhanced reducing sugar production during the liquefaction process of cornstarch. However, further increases in enzyme concentration resulted in a decline in reducing sugar formation, indicating a reduction in hydrolysis efficiency at excessive enzyme concentrations. The addition of Tween-80 at low concentrations improved reducing sugar production, whereas excessive surfactant addition reduced hydrolysis performance due to unfavorable enzyme–surfactant interactions.

The fermentation study also showed that increasing inoculum concentration enhanced biobutanol production up to an optimum level. However, excessive inoculum concentration resulted in decreased butanol production, likely due to nutrient limitation and the inhibitory effect of butanol on Clostridium acetobutylicum. Overall, the study demonstrates that appropriate optimization of enzyme concentration, surfactant concentration, and inoculum concentration plays an important role in improving biobutanol production from cornstarch through enzymatic hydrolysis and fermentation processes.

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