Effective use of Enzyme Zymase for Enhancement of Ethanol Production Couple with Parametric Effect

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Introduction

Natural energy sources such as oil and coal have been consumed at much high rates in recent decades. Due to its impact on the environment and the fact that it may eventually be exhausted, the heavy reliance on these fuels is bound to end. Therefore, alternative fuel resources such as ethanol are becoming more and more important. Bioethanol is one of the most important renewable fuels, helping to reduce the negative environmental impact of the global use of fossil fuels [1]. The traditional ethanol industry produces ethanol from variety of biomass-based waste, which is considered an important option to solve this problem [2]. When organic substrates are fermented, such as agro-industrial residues like sugarcane molasses [3], wheat stillage [4], fish residues [5], pineapple waste [6], carbon sources can be converted into ethanol, a popular well known bio-fuel. Several well-known microorganism for ethanol production were focused, such as Saccharomyces cerevisiae, Leuconostoc oenos and Zymomonas mobilis, which are capable of converting sugary material into valuable fuel products [7, 8]. During sugar cane refining, the molasses generated as a by-product has been identified as a potential sugar source that can be converted to value chemical fuels. This is because sugarcane is rich in sucrose and its substrate does not require pretreatment before fermentation. In conventional batch fermentation process, Saccharomyces cerevisiae is used to produce distilled ethanol. However, growth inhibition may be the result of product or substrate concentration which limit its productivity [9]. Ethanol production can be improved by different technologies. These include continuous culture, continuous fermentation of cell recycle, vacuum distillation of cell recycle [10] and immobilization of yeast cells [11]. The maximum yield, theoretical

Abstract. Pakistan has large resources of sugarcane crops coupled with significant amount of molasses generated annually. In this study, the effects of enzyme zymase concentration (2-5 g/l), pH (5.5-6), aeration rate (0.1-0.2 vvm/l) and agitation speed (250-300rpm) were investigated for ethanol production. Saccharomyces cerevisiae was used as a biocatalyst. The maximum ethanol production of 82g/l was obtained at 0.2vvm/l, pH5.5, 300rpm and 35°C at 4g/l of enzyme. This study suggests a novel method of utilizing the enzyme separately for improving ethanol production.

Keywords. Sugarcane molasses, Enzyme zymase, pH, Aeration rate, Agitation speed, ethanol production.
yield (YE/S), volumetric productivity (Qv) and total sugar consumption of ethanol were reported 19.15 g/l, 46.23%, 2.39 g l⁻¹ h⁻¹ and 96%, respectively [12].

The most useful microorganism for the production of ethanol by alcoholic fermentation is *Saccharomyces cerevisiae* [13]. This leads us to define new strategies for intensive production to maximize Qv and yield. The use of continuous reactors to produce ethanol has been studied to improve economics and performance of fermentation process [14]. Although, high Qv can be achieved if high cell retention is employed [15]. The general strategy for cell retention involves fixing the microbial cells in a fermenter or separating the membrane from the product stream and then recycling it back to the fermenter [16]. Higher ethanol concentrations were obtained in the case of non-sterilized molasses (47.4-50.6 kgm⁻³ at 35°C and 44.2-48.2 kgm⁻³ at 40°C) when compared to sterilized molasses, where ethanol concentration reported from 35.6 to 46.6 kgm⁻³ at 35°C and 30.8-44.2 kgm⁻³ at 40°C [16]. The increase in ethanol productivity and to reduce the intensity of work, factors such as bioreactor volume, energy consumption and cellular immobilization of ethanol production have been studied evaluated [17-19]. Various researchers have proposed the idea of immobilizing yeasts and bacteria during fermentation from various agricultural biomass residues. It was included that the completion of process for continued use of the same, and not the organic and inorganic recipients, between the organic matter such as calcium Alginate, polyacrylamide; polyvinyl alcohol [18] and inorganic materials such as alumina [20], silica [21] and zeolite [18, 22]. In raw biomass containing glycosyl radicals readily available for fermentable sugars may be considered ideal substrate for ethanol production. However, pretreatment step of converting the starch and cellulose materials into fermentable sugars is required. Recent studies on alcoholic fermentation have shown that sugarcane and sugar beet can provide high yields of ethanol and renewable substrates. The current work was focused on investigation of optimum dose of enzyme zymase coupled with different parametric effects for ethanol production.

2. Materials and Methods
A viscous by product obtained by refining sugar cane or sugar beets known as molasses or black treacle was collected from Al Abbas Distillery, Sindh. The amount of sugar in molasses depends on method of extraction and the age of the plant. The characterization of molasses is shown in Table 1. The enzyme zymase was previously produced by [23] and extracted from yeast *klyeromyces Marxians* was used. During fermentation process, enzyme loses their activity by different operational parameter. For this purpose, enzyme *zymase* was used separately within the 24 hours of fermentation process. The enzyme zymase was added in order to maintain activity for utilization with sugary material.

| Component | brix | Sucrose | Purity | TSI | TS | RS | NFS | FS |
|-----------|------|---------|--------|-----|----|----|-----|----|
| Average   | 90   | 35.4    | 39.60  | 51.9| 50.1| 14.1| 3.2 | 48 |

The ethanol production can be inhibited by the different process parameters coupled with the activity of the microorganism. The different process parameters were studied and investigated in order to obtain the optimized dose of zymase to improve ethanol production at a fixed temperature of 35°C. The experimental setup for ethanol production can be seen in Figure 1.
3. Results and Discussion

3.1 Effects of enzyme zymase on ethanol production

The effects of zymase concentration on biomass production are shown in Figure 2(a). It can be observed that as the time proceeds, cell concentration changes. Maximum biomass concentration was observed with 3g/l of zymase concentration. The minimum biomass production occurred at 2g/land 5g/l because the substrate needed to fill the active side of the enzyme was utilized and thus resulted in inhibiting the growth. With 3 g/l, the production of ethanol was observed maximum. The zymase concentration on sugar utilization can be seen in Figure 2(b). It can be observed that sugar concentration decreases as the time proceed; likewise the parametric condition had important effect. The maximum sugar utilized when 3g/l and 5g/l zymase was used. Figure 2(c) highlighted the effects of various dose of zymase on cell growth. It can be observed that with the use of enzyme from 2-5 g/l altered ethanol production.

![Figure 2 (a). Cell growth at various doses of enzyme](image)
Figure 2 (b). Cell growth at various doses of enzyme

Figure 2 (c). Ethanol production at various doses of enzyme

3.2 Operational parameter on ethanol production
3.2.1 Effects of aeration rate
Figure 3 highlighted the effects of different aeration rates with 3 g/l of enzyme. Aeration rates of 0.1vvm/l to 0.2vvm/l were tested for ethanol production. Proper aeration rate can be provided, which can result in increase in microbial activity, as the microbes would be able to digest more fermentable sugar present in molasses. This could be beneficial for industry as the percentage of un-fermentable sugar can be utilized, otherwise rejected as waste in the spent wash. Effective utilization of the enzyme zymase dosage could also make the sugar consumable.
3.2.2 Effects of pH

Figure 4 presents the effects of pH on substrate utilization and cell biomass on ethanol production. It can be seen; maximum ethanol was produced at 5.5 pH. The microbial activity is challenged when pH is changed. pH supports microbial growth and convert sugars. Changes in pH can also affect enzyme activity. The most favorable pH, the point at which the enzyme is most active is considered optimum pH. Extremely high or very low pH usually results in the loss of complete activity of enzymes. pH is also considered a factor in enzyme stability. As with activity, there is also a region of optimal pH stability for each enzyme. During fermentation process, range of optimal pH is required for microbial activity to ensure maximum ethanol production. This study was carried out to investigate the optimal dose of enzyme for the conversion of un-fermentable sugar into fermentable sugar. The main objective of the enzyme is to hydrolyze the un-fermentable sugar and change it into convertible sugar for ethanol production.

3.2.3 Effects of agitation speed

Figure 5 highlights the effect of different agitation speeds on ethanol production. It was observed that the controlled condition for maximum ethanol occurred at 300rpm. Homogenizing the temperature needs some external source which could result in higher microbial growth to be inhibited with respect to the agitation speed. The agitator is provided to overcome this problem as the homogenize temperature can alter the ethanol production, which can be either increased or decreased. In order to enhance the cell growth, we used different agitator speeds leading to an increase in ethanol production. Different ranges of agitation speeds were used to identify the optimized range for maximum ethanol production.
Figure 4. Effects of pH on substrate utilization, cell growth and ethanol production

Figure 5. Effect of Agitation intensity on substrate utilization, cell growth and ethanol production
4. Conclusion
Present study was conducted to integrate ethanol production by converting the un-fermentable sugar into fermentable sugar. The enzyme zymase was used as biocatalyst to improve ethanol production. Various operational and nutritional parameters were investigated and analyzed to determine the optimum ethanol production. The operational parameters included the aeration rate, pH and agitation speed together with the nutritional parameters were studied. The maximum ethanol production of 82g/l was obtained at 0.2vvm/l, pH5.5, 300rpm and 5g/l of enzyme at a fixed temperature of 35oC. This study suggests a novel method of utilizing the enzyme separately for improving ethanol production.

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