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Analysis of Key Factors Affecting Ethanol Production by *Saccharomyces cerevisiae* IFST-072011

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Abstract: Ethanol production by *Saccharomyces cerevisiae* is affected not only by fermentation conditions (temperature, pH and sugar concentration) but also by the intrinsic factors e.g., culture medium, dissolved O_2, immobilization and other micronutrients. In order to investigate the influence of key factors on ethanol production by *S. cerevisiae,* a laboratory strain *S. cerevisiae* IFST-072011 was used in this study. Several fermentation runs were carried out varying temperature, pH, sugar concentration, aeration, immobilization and supplementation of metal ions employed. Experimental data on several fermentation runs showed that reducing sugar concentration ranged between 5-6%, temperature of 30°C and pH between 5.0 and 6.0 were optimum for maximum yield of ethanol by *S. cerevisiae* IFST-072011.The strain produced 86.9 g L^{-1} ethanol by free cells using the initial reducing sugar concentration 5.50% at 48 h under shaking condition. Maximum yield of ethanol 94.8 g L^{-1} was produced by immobilized cells using the reducing sugar concentration 5.50% at 48 h. Ethanol production was higher by immobilized cells in shaking conditions than free cells with same culture conditions. Influence of boron, chromium, copper and magnesium was investigated on ethanol production. Only chromium was found to show slight stimulatory effect on ethanol production.

Key words: Ethanol, molasses, optimization, *Saccharomyces cerevisiae*, immobilization

INTRODUCTION

Saccharomyces cerevisiae is extremely used in fermentation to convert sugars to ethanol for the production of beverage, industrial solvents and biofuels (Boboye and Dayo-Owoyemi, 2009). As a result of the increase in price of crude petroleum and ethylene used for alcohol production, world attention has turned to the production of alcohol by fermentation (Nadir *et al*., 2009). Ethanol production from biomass also important for global demand for reducing greenhouse gases emissions from fossil fuels (Ibeto *et al*., 2011). Ethanol combustion emits relatively low volatile organic compounds, carbon monoxide and nitrogen oxides (Akin-Osanaiye *et al*., 2008). Several advantages are offered by renewable energy such as being indigenous, increasing security of supply and reducing dependency on oil import (Jegannathan *et al*., 2011) and can contribute to a cleaner environment (Chaudhary and Qazi, 2006).

Saccharomyces cerevisiae is considered as the world's premier industrial microorganisms being the best studied and exploited microorganism in terms of both old and new biotechnologies (Noor *et al*., 2003). *Saccharomyces cerevisiae* is being used for long for industrial production of ethanol due to its ability to produce high concentrations of ethanol from hexoses and to tolerate high concentration of ethanol and other inhibitory compounds (Somda *et al*., 2011a). Bioethanol that can be produced from renewable biomass, such as molasses, starch or lignocellulosic materials, is the most promising and sustainable alternative energy resources (Somda *et al*., 2011b). Molasses, a byproduct of sugar industry, is the most widely used raw material for the production of ethanol. Molasses does not compete with human food and provide a great value addition to the byproduct fermentation (Tahir *et al*., 2010). Stress tolerance such as tolerance to high temperatures and high ethanol concentrations are important properties of microorganisms of interest to industry (Somda *et al*., 2011c). The ability of yeast to produce ethanol depends on many factors such as strains, growth factors and fermentation conditions (Khongsay *et al*., 2010).

In the present study, key factors affecting ethanol productivity of *S. cerevisiae* IFST-072011 in molasses were analyzed and optimized.

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MATERIALS AND METHODS

Organism and culture maintenance: *Saccharomyces cerevisiae* IFST-072011 was obtained from culture collection pool of Industrial Microbiology Research Section, Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh. The strain was cultured and maintained on Yeast Malt Agar, medium obtained from Hi-Media (India) and used as working organism for the experiment.

Molasses pretreatment: In this study molasses were used as carbon source for ethanol fermentation by the yeast strain and were obtained from local market. The molasses were pretreated with concentrated sulfuric acid to remove the sludge e.g., colloids, particles, sand etc. and also to kill organisms. 1 kg molasses is diluted with 0.5 L distilled water and 0.001% concentrated Sulfuric acid were added. It was then heated to the boiling and allowed to cool down for experimental use.

Sugar estimation: The concentration of reducing substances (sugar) of fermentation media was measured by DNS method (Miller, 1959).

Alcohol estimation: Ethanol concentration in the fermentation broth was measured by Redox titration (Micro-diffusion) method (Conway, 1939).

Fermentation procedure: Two hundred fifty milliliter of sterile fermentation medium containing g L^{-1}: pre-treated molasses 250, yeast extract 3 and urea 0.1 was prepared in 500 mL Erlenmeyer flasks, the initial pH of the medium was adjusted 6.0. The medium was inoculated with 1000 μL of 24 h culture (10^8 CFU mL^{-1}). The fermentation runs were carried out at varying temperature, pH and agitation (rpm).

RESULTS

Effect of reducing sugar concentration on ethanol yield: The highest yield of ethanol (86.90 g L^{-1}) was obtained with initial reducing sugar concentration 5.5%. The increase or decrease of initial reducing sugar concentration from 5.5% w/v results a decrease of ethanol yield. Figure 1 shows that ethanol yield increases sharply when initial reducing sugar concentration increase from 4.3 to 5.5%w/v but declines slightly to 79 g L^{-1} with 6% reducing sugar concentration and ethanol yield remains unchanged during fermentation with 6-10% reducing sugar concentration.

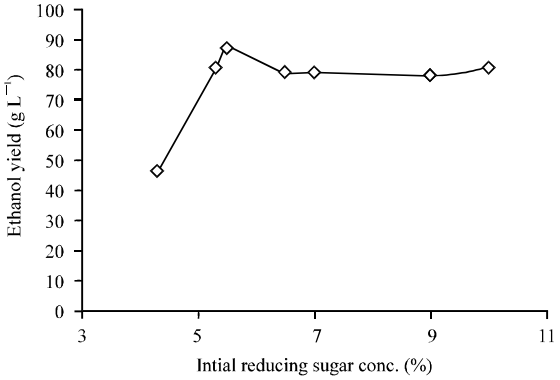

Fig. 1: Effect of reducing sugar concentration on ethanol production by *S. cerevisiae* IFST-072011 (fermentation conditions: Temp.= 3 0°C, initial pH = 6.0, incubation for 48 h).

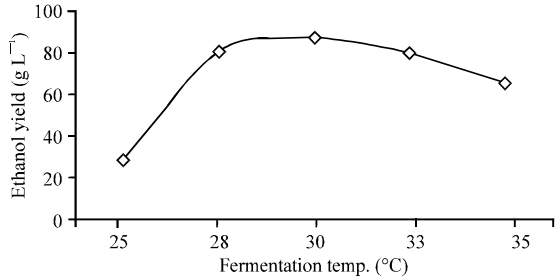

Fig. 2: Effect of fermentation temperature on ethanol production by *S. cerevisiae* IFST-072011 (fermentation conditions: initial reducing sugar concentration = 5.5 w/v, initial pH = 6.0, for incubation 48 h)

Effect of temperature on ethanol yield: Temperature showed marked influence on ethanol production by the strain using molasses as carbon source. Ethanol production gradually increased during fermentation temperature of 25 to 30°C and then sharply decreased with higher fermentation temperatures (Fig. 2). At 25°C ethanol production was 28.84 g L^{-1}, at 28°C, 80.42 g L^{-1}, at 30°C, 86.9 g L^{-1} and at 35°C it was 65.33 g L^{-1}. The yield of ethanol at various temperatures indicates that the fermentation temperature 30°C is the optimum temperature for production of ethanol by the strain, *S. cerevisiae* strain IFST-072011.

Effect of pH on ethanol yield: Initial pH of fermentation broth had influence on ethanol production by the strain using molasses as carbon source. Ethanol yield by *S. cerevisiae* strain IFST-072011 was higher at pH 6.0 (80.42 g L^{-1}) than 5.4 (48.82 g L^{-1}) and 6.5 (61.34 g L^{-1}) (Fig. 3).

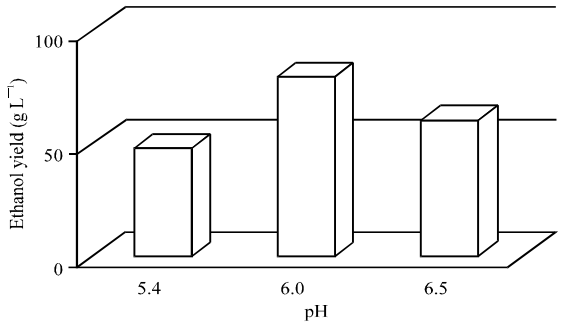

Fig. 3: Effect of initial pH on ethanol production by *S. cerevisiae* IFST-072011 (fermentation) condition: initial reducing sugar concentration = 5.5 w/v, initial pH = 6.0, Incubation for 48 h

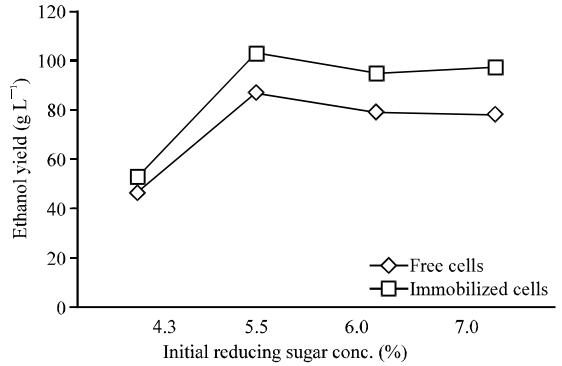

Fig. 4: Effect of immobilization on ethanol production by *S. cerevisiae* IFST-072011 with varying reducing sugar concentration at 30°C and initial pH 6.0 after 48 h

Effect of immobilization ethanol production: Immobilization was done according to Mariam *et al.* (2009) and it showed to increase ethanol production of the *S. cerevisiae* strain IFST-072011. At the fermentation temperature and initial pH of maximum ethanol yield (30°C and 6.0), free cells of *S. cerevisiae* IFST-072011 was able to yield 86.90 g L^{-1} ethanol after 48 h while immobilized cells of the yeast strain produced 102.70 g L^{-1} ethanol at the same fermentation condition. At every runs of fermentation with varying initial reducing sugar concentration, immobilized cells produce more ethanol than free cells of *S. cerevisiae* IFST-072011 (Fig. 4).

Effect of metal supplementation on ethanol yield: Supplementation of metal salts in fermentation broth as source of metal ions was carried out to investigate the effect of metal ions on ethanol production by the experimental yeast strain. Four metal salt namely copper sulphate ($CuSO_4$), potassium-di-chromate ($K_2Cr_2O_7$),

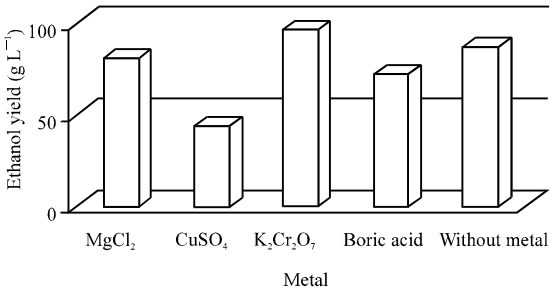

Fig. 5: Effect of metal ions on ethanol production by *S. cerevisiae* IFST-072011 with 5.5% reducing sugar at 30°C and pH 6.0 after 48 h

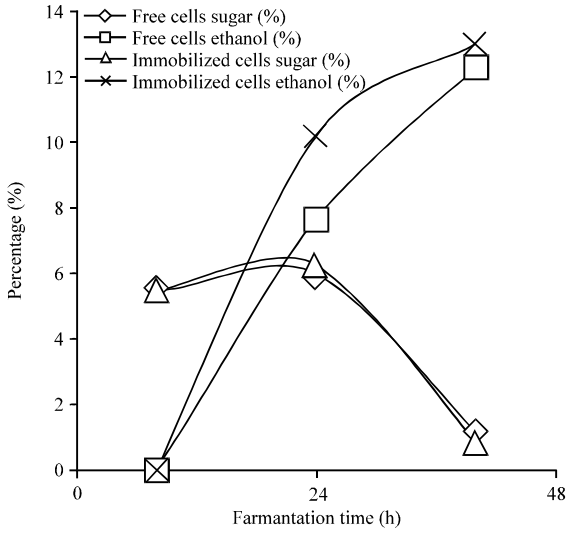

Fig. 6: Ethanol production by free and immobilized cells of *S. cerevisiae* IFST-072011 under optimum condition (Temp.: 30°C, pH: 60, initial reducing sugar: 5.5%, rpm: 115 for 48 h

magnesium chloride ($MgCl_2$) and boric acid were supplemented in fermentation broth. $CuSO_4$ showed a marked decreased of ethanol production by the yeast strain with same fermentation condition. Although $K_2Cr_2O_7$ and boric acid were found slight stimulatory and inhibitory effect respectively (Fig. 5) but requires further research for any conclusion in this regard.

Comparison of sugar conversion and ethanol production: Analysing all the data, optimum conditions for ethanol production of *S. cerevisiae* IFST-072011 was 30°C temperature, pH 6.0 and initial reducing sugar concentration 5.5% at 115 RPM for 48 hours and at this condition 12.2% (97.17 g L^{-1}) ethanol produced. Immobilization of cells increases production up to 13% (102.7 g L^{-1}). Figure 6 shows ethanol production and

sugar utilization pattern of free cells and immobilized cells of *S. cerevisiae* IFST-072011 throughout fermentation. In case of immobilized cells, more sugar in used than free cells, hence increases the ethanol production. Though ethanol production is close after 48 h of fermentation, after 24 h, immobilized cells produced much higher ethanol than free cells. This fact acknowledges the advantages of immobilization.

DISCUSSION

To determine the effect of reducing sugar on ethanol production by the strain, a no of experiments were conducted at 30°C and pH 6.0 for 48 h with different initial reducing sugar concentration. Ethanol production increases from 4.3-5.5% reducing sugar concentration and then decreases slightly with increasing reducing sugar concentration which is in accordance with Tahir *et al.* (2010). 46.215 g L^{-1} ethanol produced with 4.3% reducing sugar and 80.42 g L^{-1} with 5.5% reducing sugar. Ethanol production then decreases slightly to 80.42 g L^{-1} at 10% reducing sugar concentration. In case of 7 and 10% initial reducing sugar concentration, a significant amount of sugar remained unutilized (2.6% in case of 7 and 5.12% in case of 10% initial reducing sugar concentration). In every experiment, reducing sugar increased with after 24 h than the initial and then declines. This phenomenon may be due to the enzymatic activity of yeast for conversion of sucrose to glucose and fructose at high temperature. Similar results obtained in the study of Al-Judaibi (2011).

To determine the effect of temperature on ethanol production of the isolate, three fermentation experiments was carried out at 28, 30 and 37°C with initial reducing sugar concentration 5.5% at pH 6.0. Ethanol production was higher at 30°C as 80.42 g L^{-1} ethanol produced at this temperature. 28.84 g L^{-1} ethanol produced at 28°C and 34.44 g L^{-1} ethanol produced at 37°C. These results contradict with the study of Yah *et al.* (2010) who found optimum temperature of ethanol production to be 25°C. As *S. cerevisiae* IFST-072011 can produce ethanol at higher temperature, it will be more suitable for industrial production of ethanol.

The optimal pH range for yeast growth can vary from pH 4.0 to 6.0 depending on temperature, the presence of oxygen and the strain of yeast. Optimum pH value is very important for the activity of plasma membrane-bound proteins, including enzymes and transport proteins (Narendranath and Power, 2005). To determine the effect of pH on ethanol production of the isolate, two fermentation experiments was carried out at pH 5.4 and 6.0 at 30°C with initial reducing sugar concentration 5.5%. 48.82 g L^{-1} ethanol produced at pH 5.4 and 80.4 g L^{-1} ethanol produced at pH 6.0. Hence, pH 6.0 was selected as optimum pH for the isolate. This result also in contradiction with of the study of Buzas *et al.* (1989) who reported optimum pH for ethanol production to be 4.5, though their isolate have similar productivity in the range of pH 4.5-6.2.

Agitation showed marked influence on ethanol production of isolate IFST-072011 as under shaking condition ethanol production was increased. Ethanol production was higher at 115 rpm (79 g L^{-1}) than 130 rpm (72.68 g L^{-1}) at 30°C and pH 6.0 with initial reducing sugar 5.5%. This result is in accordance with the study of Rodmui *et al.* (2008).

Immobilization offers various advantages in industrial applications of yeast. The most significant advantages of immobilized yeast cell systems are the ability to operate with high productivity at dilution rates exceeding the maximum specific growth rate, the increase of ethanol yield and cellular stability and the decrease of process expenses due to the cell recovery and reutilization (Lin and Tanaka, 2006). Immobilization of cells showed significantly affect of ethanol production, as ethanol production increased with immobilized cells. At 30° and pH 6.0 using the reducing sugar concentration 5.50%, maximum ethanol production was 86.90 g L^{-1} by free cells whereas 102.70 g L^{-1} ethanol produced by immobilized cells in the same condition at 48 hrs shaking condition (115 rpm). But when reducing sugar concentration was increased to 6.0%, keeping other conditions unchanged, 94.8 g L^{-1} ethanol was produced by immobilized cells (Fig. 5). A study by Vucurovic *et al.* (2009) supports this finding, where they concluded that immobilized *S. cerevisiae* produced higher concentration of ethanol than free cells and within 24 h, immobilized *S. cerevisiae* strain consumed all the available sugar.

Additions of very minute amount of metals also affected ethanol production. Of all the four metals used in this study, $CuSO_4$ dramatically decreased ethanol production in the fermentation media but $K_2Cr_2O_7$ increased ethanol production. Only 43.53 g L^{-1} ethanol was produced by applying $CuSO_4$, while in the presence of $K_2Cr_2O_7$, 96.38 g L^{-1} ethanol was produced at 48 h using the reducing sugar concentration 5.50%, pH 6.0 and 30°C temperature. These results are in accordance with the study of Palukurty *et al.* (2008), who also reported increase of ethanol production due to addition of trace amounts of metal to fermentation media.

From the present results, it can be concluded that a successful fermentation process depends on sugar concentration of the medium and nutritional parameters. The maximum production of ethanol was obtained after

48 h of incubation at 115 rpm with 5.50% reducing Sugar, pH 6.0, 30°C temperature. At this condition 97.17 g L^{-1} ethanol produced by free cells and 102.70 g L^{-1} ethanol produced by immobilized cells of *S. cerevisiae* IFST-072011. Immobilized cells were better in terms of ethanol production than free cells. Some metals such as Boron, Chromium etc. had stimulatory effect on ethanol production. This strain can be used for industrial production of ethanol from molasses.

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