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Comparison of ethanol and temperature tolerance of *Zymomonas mobilis* strain in glucose and molasses medium

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The effect of temperature and exogenous ethanol was evaluated on the ethanol production potential of *Zymomonas mobilis* MTCC 2428 as an alternative to the classic and widespread use of *Saccharomyces cerevisiae*. At 30°C, this strain produced the maximum amounts of ethanol, i.e. 4.0 and 3.3% (v/v) after utilizing 98 and 83% (w/v) sugar on glucose and molasses medium, respectively. While at higher temperatures (35 & 40°C), the ethanol production by the strain decreased. Moreover, the strain was able to operate in the presence of 8% (v/v) ethanol in glucose medium at 30°C; whereas it tolerated only 6 and 2% (v/v) ethanol at 35 and 40°C, respectively. At the respective temperatures, further higher concentrations of the supplemented ethanol were found to be inhibitory to the isolate.

Keywords: *Zymomonas mobilis*, glucose, molasses, ethanol

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Introduction

The increase in the prices of fuel and possibility of shortfalls has led to an extensive evaluation of alternative sources of energy to meet the global energy demand. Microbial processes have proved useful for the production of alternate energy products from renewable resources. Alcoholic fermentation is one of the most important examples. As a consequence, ethanol is the most promising liquid fuel since it can be readily produced from various agriculture-based renewable materials, like sugarcane juice, molasses, potatoes, corn and barley etc[1].

Currently, yeast *Saccharomyces cerevisiae* is used as the major ethanol producing microorganism worldwide. Despite its extensive use, it has a number of disadvantages, such as high aeration cost, high biomass production and low temperature and ethanol tolerances[2]. Therefore, efforts have been made to improve the existing technologies through the raw materials and alternate strains for ethanol production. *Zymomonas mobilis* has emerged as a potential bacterium for ethanol production. The studies have clearly demonstrated that it has a high specific rate of sugar uptake, high ethanol yield, low biomass production and non-requirement of controlled addition of oxygen to maintain the viability of the cells[3,4]. Hence, it shows the suitability for continuous operation. Despite various efforts undertaken worldwide, *Zymomonas* is not yet ready to compete successfully with the yeast at industrial scale. In the present investigation, studies have been carried to test the effect of both temperature and exogenous ethanol on the fermentation potential of *Z. mobilis* using a glucose medium. The experimentations have also been conducted on molasses medium, being the most commercially used medium for ethanol production.

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Materials and Methods

Procurement and Maintenance of Microbial Culture

Z. mobilis MTCC 2428 was procured from the Institute of Microbial Technology (IMT), Chandigarh, India. The culture was activated on growth medium

21 as per the culture catalogue of IMT. This medium is made up (w/v) of glucose (2.0%), yeast extract (1.0%), potassium dihydrogen orthophosphate (0.1%), ammonium sulphate (0.1%) and magnesium sulphate (0.1%).

Preparation of Inoculum

To prepare the starter culture, 50 mL of the growth medium taken in 250 mL capacity conical flask. The medium was sterilized at 121°C and 15 psi pressure for 20 min, and inoculated with a loopful of the strain. The flasks were incubated at 30°C for 24 h.

Ethanol Fermentation

The fermentation medium containing (w/v) glucose (7.0%), yeast extract (0.7%), potassium dihydrogen orthophosphate (0.1%), ammonium sulphate (0.1%) and magnesium sulphate (0.1%) was used for the production of ethanol. In molasses medium, molasses concentration of 14° Brix was used in place of glucose. The medium was sterilized by autoclaving, inoculated with 24-h-old 10% (v/v) starter culture and incubated at specified temperatures for 48 h.

Ethanol and Temperature Tolerance

The ethanol and temperature tolerance of the strain was tested by supplementing the different concentrations of ethanol (exogenous) into the medium, inoculating and incubating the medium at different temperatures (30°, 35° & 40°C).

Analysis

The total reducing sugars and total sugar were estimated by using dinitrosalicylic reagent method[5] and anthrone reagent method[6], respectively. The ethanol concentration in the fermentation broth was estimated using microprocessor based gas chromatograph equipped with flame ionization detector and porapack Q column[7]. The injector, detector and oven temperature of gas chromatograph were maintained at 200°, 210° and 180°C, respectively.

Results and Discussion

The effect of temperature and exogenous ethanol on ethanol production by *Z. mobilis* strain was monitored using both glucose and molasses medium.

Effect of Temperature on Ethanol Production

Different temperatures (30°, 35° and 40°C) were tested to check the thermal tolerance of bacterium both in glucose and molasses media. The results (Figs 1 &. 2) show that *Z. mobilis* MTCC 2428 produced maximum amount of ethanol at 30°C in both the media and further increase in temperature (35° and 40°C) was inhibitory to its ethanol production ability. The strain produced 4.01 and 3.28% (v/v) at 30°C, and 3.61 and 1.78 % (v/v) ethanol at 40°C in glucose and molasses medium, respectively. The sugar utilization was also found to decrease with increase in the temperature. The strain was able to utilize 97.5% (w/v) glucose at 30°C, whereas, 92.5% glucose was utilized at 40°C. In the case of molasses medium, sugar utilizations of 83.28 and 58.45% (w/v) were observed at 30° and 40°C, respectively. These results show that the tested strain was able to produce ethanol from molasses medium; however, the ethanol production and sugar utilization was better on glucose medium as compared to molasses. Moreover, the strain produced comparable levels of ethanol at 30° and 35°C on glucose medium, which indicated that the strain is more temperature tolerant in glucose medium.

Effect of Initial Ethanol in Glucose Medium on Ethanol Production

Different concentrations (2-10%, v/v) of ethanol were supplemented into the glucose medium and the

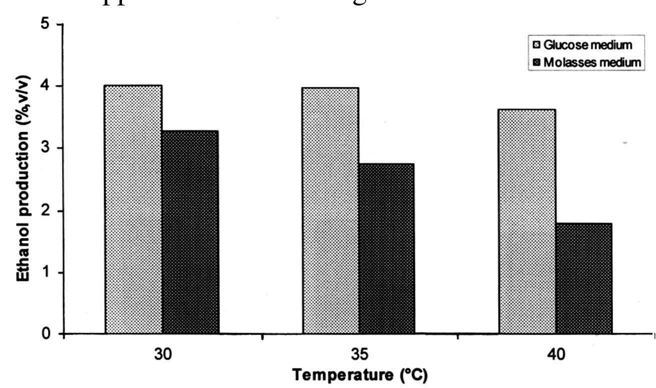

Fig. 1—Effect of temperature on ethanol production by *Z. mobilis* MTCC 2428

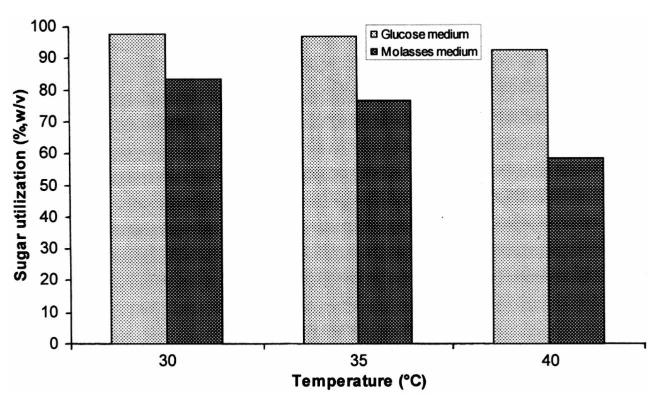

Fig. 2—Effect of temperature on sugar utilization by *Z. mobilis* MTCC 2428

fermentation was carried out at temperatures of 30°, 35° and 40°C. The results (Figs 3 & 4) depict that the maximum amount (3.61%, v/v) of ethanol was produced at 30°C in the presence of 2% (v/v) exogenous ethanol; whereas further increase in exogenous ethanol had decreased the ethanol production and only 1.47% (v/v) ethanol was produced at 8% (v/v) exogenous ethanol concentration. This strain was able to carry out the fermentation in the presence of up to 8, 6 and 2% (v/v) ethanol at 30°, 35° and 40°C, respectively. The higher concentrations of ethanol at these temperatures were found to be inhibitory. The strain produced 1.47% (v/v) ethanol by utilizing 87.5% (w/v) glucose in the presence of 8% (v/v) ethanol at 30°C, whereas it was able to produce 1.43% (v/v) ethanol after utilizing 81.5% (w/v) glucose in the presence of only 2% (v/v) ethanol at 40°C. At 35°C, in the presence of 6% (v/v) ethanol, the strain utilized 62% (w/v) glucose and produced only 0.93% (v/v) ethanol.

Effect of Initial Ethanol in Molasses Medium on Ethanol Production

Different concentrations (1-5%, v/v) of ethanol were supplemented into the molasses medium and the fermentation was carried at 30°, 35° and 40°C of temperatures. The data (Figs 5 & 6) show that the maximum amount (3.01%, v/v) of ethanol was produced at 30°C in the presence of 1% (v/v) supplemented ethanol; whereas further increase in exogenous ethanol had decreased the ethanol production and only 1.43% (v/v) ethanol was produced at 5% (v/v) exogenous ethanol concentration. Moreover, it is clear from the results that it could tolerate 5% (v/v) ethanol, whereas, at 40°C, it was not able to perform good fermentation in media containing more than 1% (v/v) ethanol. Further, the sugar utilization ability of the strain also decreased on increase in exogenous ethanol concentration and temperature. At 30°C, in the presence of 5% (v/v) supplemented ethanol, the strain produced 1.43% (v/v) ethanol while utilizing 67.6% (w/v) sugar; whereas, it was able to produce 0.58% (v/v) ethanol while utilizing 42.25% (w/v) sugar at 35°C, in the presence of 4% (v/v) ethanol. With further increase in temperature, i.e. 40°C, this strain utilized 35.51% (w/v) sugar and produced 0.62% (v/v) ethanol in the presence of 1% (v/v) supplemented ethanol.

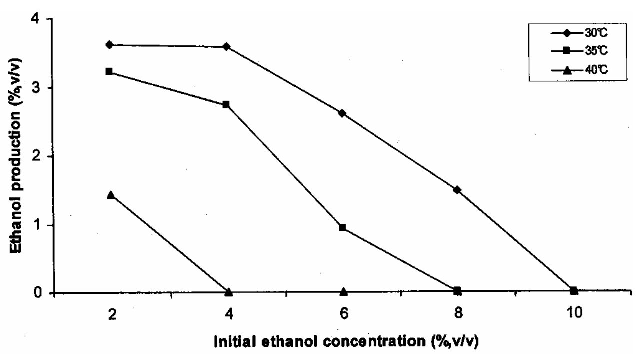

Fig. 3—Effect of initial ethanol concentration in glucose medium on ethanol production by *Z. mobilis* MTCC 2428

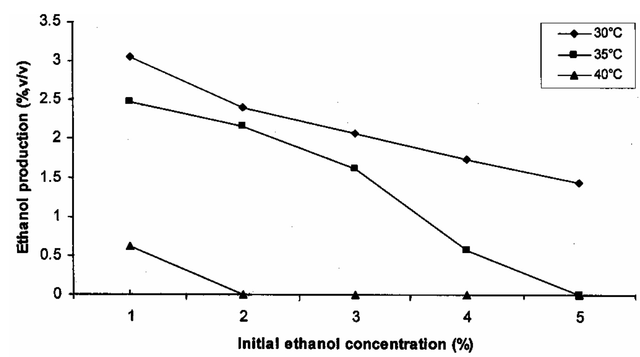

Fig. 5—Effect of initial ethanol concentration in molasses medium on ethanol production by *Z. mobilis* MTCC 2428

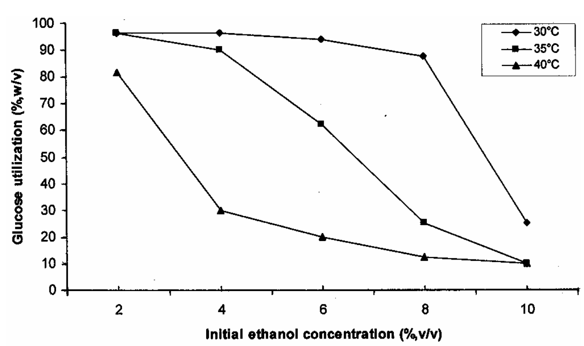

Fig. 4—Effect of initial ethanol concentration in glucose medium on glucose utilization by *Z. mobilis* MTCC 2428

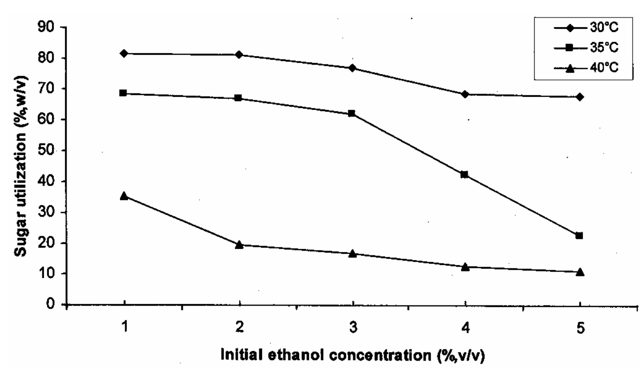

Fig. 6—Effect of initial ethanol concentration in molasses medium on sugar utilization by *Z. mobilis* MTCC 2428

Thus, *Z. mobilis* strain showed maximum ethanol production and sugar utilization at 30°C. Further increase in temperature had shown an inhibitory effect on the ethanol production as well as sugar utilization abilities of the test isolate. It was also observed that the decrease in ethanol production was less between 30°-35°C, in contrast to sharp decrease between 35°-40°C. Similar observations were recorded earlier with other *Z. mobilis* strains[8]. However, the present strain showed better ethanol production potential on molasses medium as compared to our previous studies on other strains[9]. The decrease in membrane phospholipid content may be responsible for the unique thermal sensitivity of cells grown at high temperature (41°C). The leakage of magnesium, nucleotides and proteins from cells grown at 30°C also increased with increasing temperature. The protein loss from the cells was interpreted as a disruption of membrane integrity[10]. The decrease in ethanol production from molasses medium may also be due to high concentration of salts present in the molasses that may have raised the osmotic pressure above acceptable levels, reducing the cell viability and suppressing ethanol production[11]. Similar observations have also been made during sorbitol production with increased molasses concentration[12]. The decrease in the cell viability and final ethanol concentration with the increase in temperature from 30° to 40°C in batch culture has also been found in *Z. mobilis* ATCC 10988[13]. In another study, *Z. mobilis* CP4 has shown optimal ethanol production from sugarcane molasses at 34°C[14].

It is clear from the observations recorded during the course of study that the *Z. mobilis* MTCC 2428 had optimal production of ethanol at 30°C in both glucose and molasses medium. The increase in the temperature and exogenous ethanol concentration had decreased the ethanol production and sugar utilization ability of the microbe in both the media. The present strain was able to utilize molasses medium for ethanol production. However, it performed better on glucose medium as compared to molasses in terms of ethanol production, sugar utilization as well as ethanol and temperature tolerance.

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