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Short communication

Parametric Studies on Batch Alcohol Fermentation Using *Saccharomyces Yeast* Extracted from Toddy

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Abstract—The present study deals with the development of a *Saccharomyces cerevisiae* yeast strain from toddy and the study of important process parameters which will facilitate the fermentation of sugar to alcohol using the developed strain. Evaluation of the yeast strain was performed in a batch fermenter of 1 L capacity under varying pH, temperature, sugar concentration, inoculum time and medium constituents. The optimum pH and temperature were found to be 4.25 and 30°C. The present yeast strain was found to be efficient to ferment 200 g/L sugar solution without any difficulty and 90 g/L ethanol was formed with an ethanol yield based on sugar utilized of 0.462. The fermentation time was reduced from 96 to 63 hours by increasing the inoculum time from 12 to 48 hours. From the study of the nutrient composition it was established that beef extract can be substituted for costly yeast extract. A higher concentration and yield of ethanol were obtained using a yeast strain developed from toddy compared to bakers' yeast *Saccharomyces cerevisiae*.

Key Words : Alcohol, Fermentation, Ethanol, *Saccharomyces cerevisiae*, Toddy

INTRODUCTION

Due to rapid depletion of world petroleum reserves and it's rising prices day by day, new sources of hydrocarbons must be found to supply our chemical and energy needs (Sitton and Gaddy, 1980; Lee *et al*., 1983). In this context alcohol fermentation offers promising alternative as it can be produced from various sources of raw materials. In view of increasing importance of alcohol as an alternative source for chemicals & liquid fuel, a great deal of research interest in ethanol fermentation has been generated in the last two decades (Vega *et al*., 1987; Deway *et al*., 1984; Mancilha *et al*., 1984; Converti *et al*., 1985). The overwhelming advantage of fermentation is that the raw materials are renewable, but at present, the major draw back of alcohol fermentation is that the cost of production is high due to several factors.

Keeping this in view, improvements in ethanol fermentation have been focused on taking up of renewed interest in research works in several areas such as use of improved mutant strains, yeast strain development from cheaper sources, use of cheaper source of raw materials (renewable source), optimum reactor design, better nutrients for optimum cell growth *etc*. (Mancilha *et al*., 1984; Converti *et al*., 1985; Gregory *et al*., 1984; Shiyun *et al*., 1987; Torres *et al*., 1986; Tobias *et al*., 1983). Toddy is produced abundantly in India and is easily available. So, it may be used as a cheaper source for the development of a suitable yeast strain for ethanol fermentation.

The present study is therefore aimed to develop a suitable biocatalyst from toddy and to determine the favorable conditions under which the yeast will function well, giving a high yield of ethanol.

MATERIALS AND METHODS

Organism and culture media

The *Saccharomyces yeast* was extracted from toddy using series dilution technique in our laboratory. The yeast was maintained on agar slants containing 1% glucose, 0.5% peptone, 0.3% beef extract, 3% malt extract, and 2% agar-agar. After keeping at room temperature for 5 days the culture was stored at 4°C.

Preparation of inocula for fermentation

The medium was prepared with 10 g sucrose, 0.2 g beef extract, 0.04 g magnesium sulfate, 0.2 g ammonium sulfate, and 0.5 g KH_2PO_4. The pH of the solution was adjusted to pH 4.25 with sulfuric acid and then sterilized in an autoclave for 15 min at 15 psi pressure. After the broth was cooled to room

temperature, colonies of the yeast were introduced in to it. Then the culture was kept for growth in an incubator at 30°C and the speed of the agitator was maintained at 110 rpm.

Equipment and the experimental procedure

A fermenter of one liter capacity, equipped with an agitator, pH, and temperature control systems was used as the batch fermenter in this study. The fermenter was cleaned and steam sterilized at 15 psi for 15 minutes. Then the sterilized medium containing the inoculum was transferred to the fermenter. The temperature of fermentation was maintained within 0.5°C. The pH of the fermentation broth was regulated within pH 0.1 unit by the peristaltic pump which injected a fine stream of sulfuric acid or sodium hydroxide. The fermentations were carried out at atmospheric pressure. The agitator speed was maintained constant through out the experiment at 200 rpm.

Analytical procedures

The concentrations of ethanol and sugar were monitored spectrophotometrically. Ethanol was determined by measuring OD at 600 nm after standard distillation. The sugar was assayed by OD measurement at 550 nm using DNS reagent.

RESULTS AND DISCUSSION

Effect of pH

The pH has a significant influence on fermentation due to its effect on yeast growth, fermentation rate and by product formation. Therefore maintenance of pH is of paramount importance in fermentation processes. In the present study, the efficiency of the yeast strain was evaluated in the pH range 3.75 to 5.5. The results are shown in Figs. 1 and 2. From

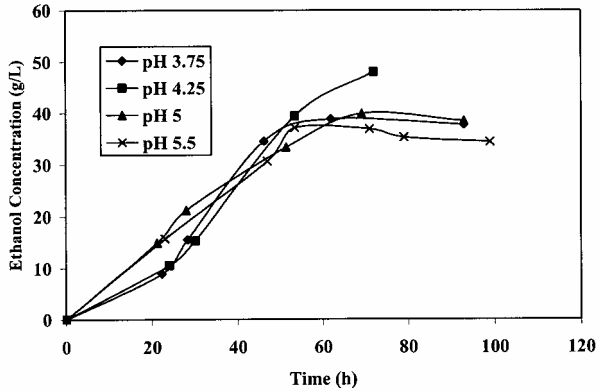

Fig. 1. Influence of pH on ethanol concentration (sugar 100 g/L, 30°C, inoculum time 24 h).

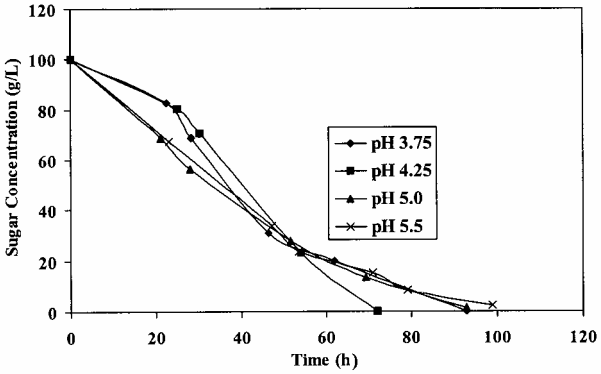

Fig. 2. Effect of pH on sugar conversion (sugar 100 g/L, 30°C, inoculum time 24 h).

Fig. 1 it is observed that ethanol concentration was increased steadily with time with all pH values though the rate of production varied considerably. The maximum ethanol concentration of 48 g/L was achieved with pH 4.25 followed by 40 g/L ethanol with pH 5.0. The lower activity of the yeast strain at pH 3.75 is because the pH is too low to activate the enzymes to react. The rate of ethanol production initially was higher at higher pH but maximum ethanol concentration achieved was less than those obtained with pH 4.25.

Figure 2 shows that the sugar concentration was reduced steadily with time for all pH values. However percentage conversion was decreased with increase of pH from 4.25 to 5.5. The time taken for maximum sugar conversion was 72 h for pH 4.25, where as the fermentation time for other pH values was found to be more. From yield calculation data as shown in Table 1 it is indicated that maximum ethanol yield of 0.48 g ethanol/g sugar consumed was obtained with both pH 4.25 and 3.75 which is higher than the yields obtained with pH values 5.0 and 5.5. The lower ethanol yield and sugar conversion obtained with higher pH values was possibly due to the formation of undesired products like glycerol, organic acids *etc*. at the expense of ethanol. Therefore from the pH study pH 4.25 was found to be the optimum pH value for ethanol fermentation using yeast extracted from toddy.

Table 1. Effect of pH on ethanol yield.

pH	Ethanol Yield (g ethanol/g sugar consumed)
3.75	0.48
4.25	0.48
5.00	0.46
5.50	0.45

Effect of temperature

The temperature has a marked influence on the production of biomass and ethanol. Usually, the rate

of alcoholic fermentation increases with temperature to an optimum between 30°C and 40°C using conventional yeast. However, both optimum and temperature tolerance for growth and fermentation are strongly strain dependent (Rousseau *et al*., 1992). Therefore, fermentation experiments were conducted under varying temperature in the range 30-38°C to see the effect of the newly developed strain towards ethanol production. The results are shown in Figs. 3 and 4.

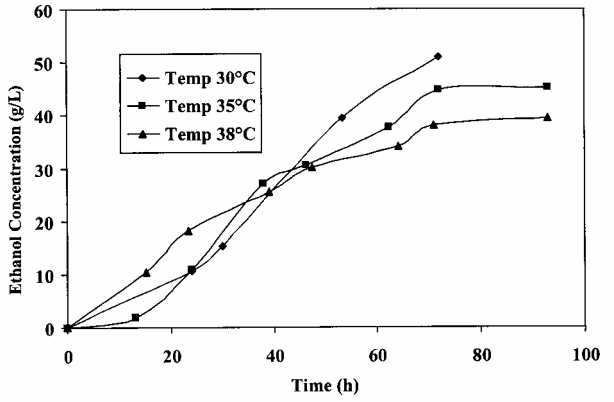

Fig. 3. Effect of temperature on ethanol concentration (pH 4.25, sugar 100 g/L, inoculum time 24 h).

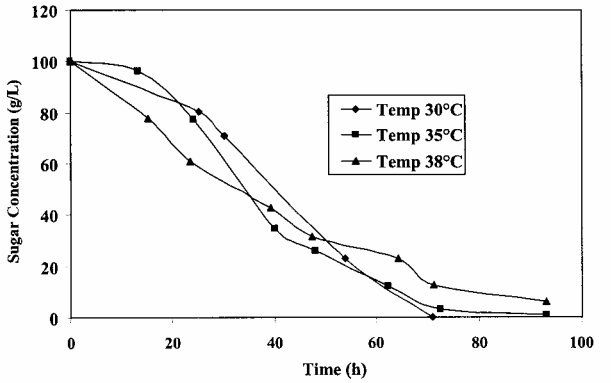

Fig. 4. Effect of temperature on sugar conversion (pH 4.25, sugar 100 g/L, inoculum time 24 h).

Figure 3 shows that the ethanol concentration increased steadily with time. Though, the rate of production was initially found to be higher at the high temperatures 35°C and 38°C but the optimum ethanol concentration of 48 g/L achieved at 30°C. The sugar concentration as shown in Fig. 4, initially reduced rapidly for higher temperatures and then the rate of substrate consumption became slow comparatively. The percentage conversion of sugar was found to be 100%, 97%, and 87.3% at 30°C, 35°C, and 38°C, respectively. From Table 2 it is indicated that a very high yield of 0.48 was obtained at 30°C which is higher than those obtained with other temperatures studied. The lower efficiency of the yeast towards ethanol formation may be attributed to the loss of enzyme activity at higher temperatures. So 30°C was found to be optimum with respect to optimum ethanol concentration and rate of reaction.

Table 2. Effect of temperature on ethanol yield.

Temperature (°C)	Ethanol Yield (g ethanol/g sugar consumed)
30	0.48
35	0.46
38	0.42

Effect of sugar concentration

An interesting research field in alcoholic fermentation is the study of yeast strains able to utilize sugar solutions more concentrated than those generally fermented in usual practice (Converti *et al*., 1985) and hence it is important to establish the limits of ethanol tolerance of the yeast strain (Shiyun *et al*., 1987). Therefore the fermentations were conducted with sugar concentrations range from 50 to 250 g/L with an intention to obtain high yield of ethanol in reasonable time.

The results shown in Figs. 5 and 6 indicate that ethanol concentration increased with increase in substrate concentration but there was wide variation in

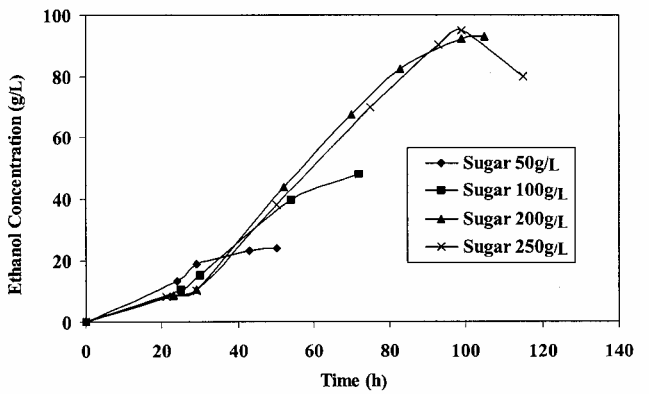

Fig. 5. Effect of sugar concentration on ethanol production (pH 4.25, 30°C, inoculum time 24 h).

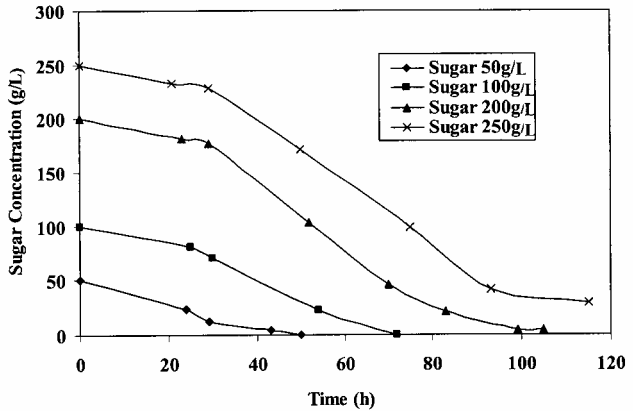

Fig. 6. Effect of sugar concentration on sugar conversion (pH 4.25, 30°C, inoculum time 24 h).

time taken for complete fermentation. Maximum ethanol concentrations of 23.9 g/L, 48 g/L, and 93 g/L were obtained in 50 h, 72 h, and 105 h with 50 g/L, 100 g/L, and 200 g/L sugar solutions. The corresponding conversion of substrate was 100%, 100% and 96%. As was observed for the lower concentration of sugar, the production of ethanol was growth associated only for a short period of time and hence required less fermentation time. There was no difficulty in fermenting solutions containing up to 200 g/L sugar.

However when a still higher concentration of sugar, *i.e.*, 250 g/L was fermented, ethanol concentration and sugar conversion were observed to be lower than those obtained with 200 g/L sugar solution. The inhibitory effect of high sugar concentrations for alcoholic fermentation may be due to plasmolysis of yeast cells as reported. Further it was observed that ethanol became inhibitory when its concentration reached about 95 g/L.

So, it was found that the new yeast is tolerant of sugar concentrations to at least 200 g/L with an ethanol yield of 0.47 as shown in Table 3 which is comparable to the yield obtained with 100 g/L sugar solution. Complete and efficient fermentation of such highly concentrated sugar solutions is beneficial from energy consumption point of view for distillation of the alcohol.

Table 3. Effect of sugar concentration on ethanol yield.

Sucrose (g/L)	Ethanol Yield (g ethanol/g sugar consumed)
50	0.49
100	0.48
200	0.47
250	0.43

Variation in inoculum time

In fermentation reactions, inoculum time is very important in obtaining maximum ethanol production with minimum time. After the particular cell density is reached the growth phase slows and the life cycle of the yeast deviates from the growth path and produces ethanol. If the cell density is less, more time will be taken for complete fermentation. In the present study, the inoculum for fermentation was grown in incubator for different periods of time 12 h, 24 h, and 48 h and 10 vol% of inoculum was added to the fermenter. Table 4 indicates that the variation in inoculum time did not have any remarkable influence on the yield of ethanol.

However, inoculum time had a considerable effect on the overall time of the fermentation. It was observed from Figs. 7 and 8 that the rate of ethanol production was increased with increase in growth time and therefore required less time for achieving maximum sugar conversion. The high rate of ethanol production and substrate uptake with increase in inoculum time is due to the high cell mass concentration achieved in 48 h and hence time of fermentation was reduced from 96 to 63 h to achieve the maximum ethanol concentration.

Table 4. Effect of inoculum time on ethanol yield.

Inoculum Time (h)	Ethanol Yield (g ethanol/g sugar consumed)
12	0.476
24	0.480
48	0.478

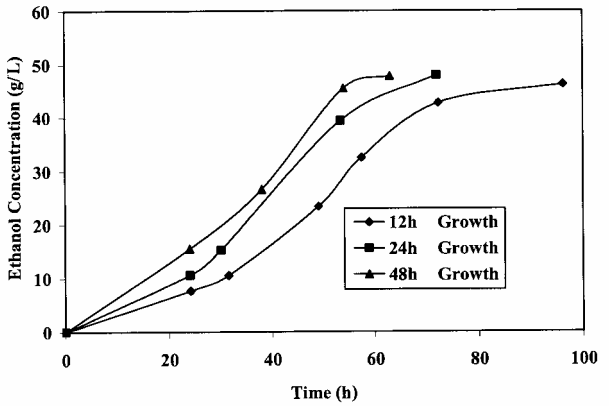

Fig. 7. Effect of growth time on ethanol production (pH 4.25, 30°C, sugar 100 g/L).

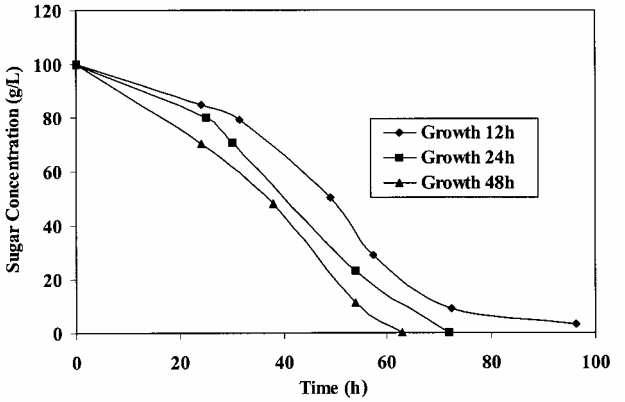

Fig. 8. Effect of growth time on sugar conversion (pH 4.25, 30°C, sugar 100 g/L).

Variation in medium constituents

The competitive position and potential profits from fermentation products are closely tied to the costs of the various components of the production medium. Inoculum medium is usually less expensive. In contrast, a single high cost medium component for the production medium can virtually dictate the selling price of the fermentation product. So attempts should be made to find an alternate low cost re-

placement for such a medium component (Casida, 1993). In this context, fermentations were carried out using beef and yeast extracts keeping the other nutrients same.

It was observed that there is no significant change in the performance of the new yeast strain towards alcoholic fermentation using yeast and beef extract as medium constituents as shown in Figs. 9 and 10. In fact, a slightly higher concentrations and yields of ethanol were obtained with beef extract within the same fermentation time. Therefore, it is evident that beef extract which is cheaper than yeast extract can be used as medium component in alcoholic fermentation.

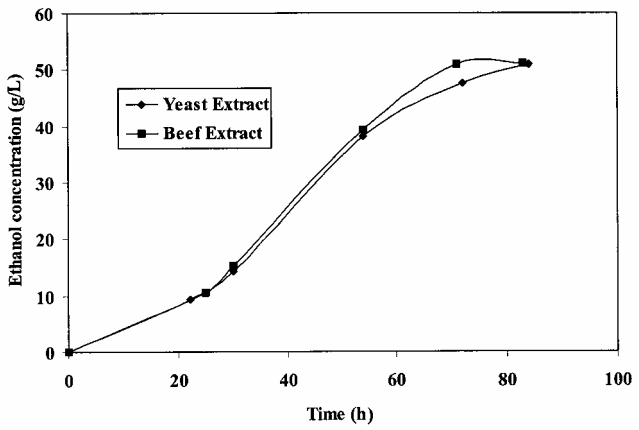

Fig. 9. Effect of medium component on ethanol production (sugar 100 g/L, pH 4.25, 30°C, inoculum time 24 h).

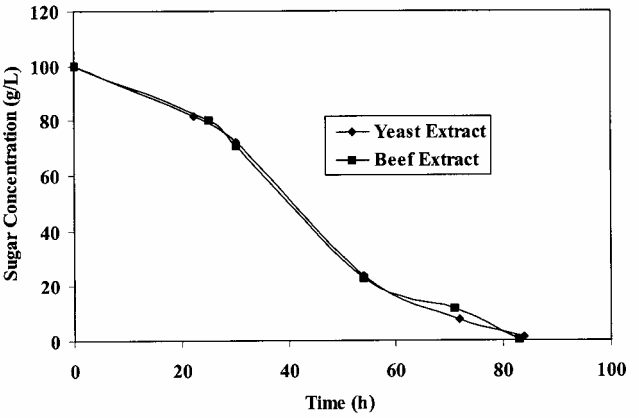

Fig. 10. Effect of medium constituent on sugar conversion (pH 4.25, 30°C, sugar 100 g/L, inoculum time 24 h).

Comparison with bakers' yeast

Alcoholic fermentation was carried out to find the suitability of the new yeast over the performance of the bakers' yeast. Table 5 compares the productivity and efficiency of *Saccharomyces cerevisiae* yeast strain obtained from toddy and bakers' yeast. It was observed that about 6 wt% more ethanol was produced by the new yeast compared to the bakers' yeast. The yield of ethanol was also higher than the yield obtained with the conventional yeast strain.

Table 5. Comparison of *Saccharomyces cerevisiae* developed from toddy and bakers' yeast.

Yeast	Initial Sugar Conc. (g/L)	Fermentation Time (h)	Ethanol Conc. (g/L)	Ethanol Yield (g ethanol/g sugar consumed)
Baker's yeast	100	72	45.3	0.44
Yeast strain (from toddy)	100	72	48.0	0.48

CONCLUSION

The *saccharomyces yeast* developed from toddy has shown substantial alcohol fermentation activity. It was established in this work that pH 4.25 and 30°C are the optimum pH and temperature. The new yeast strain was able to ferment the sugar solution containing at least 200 g/L sugar and 93 g/L ethanol was obtained with an ethanol yield based on sugar utilized of 0.47. Inhibition of product was observed above 200 g/L sugar solution. High rate of fermentation was achieved with increase in growth time from 12 to 48 h and hence fermentation time was reduced from 96 to 63 h to obtain optimum ethanol concentration of 48 g/L. The costly addition of yeast extract could be avoided and good growth and ethanol production were obtained using beef extract as medium component. The yeast strain showed better results in terms of both ethanol concentration and ethanol yield compared to a bakers' yeast strain.

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