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# Continuous Alcoholic Fermentation Process in a Tower Reactor with Recycling of Flocculating Yeast

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## ABSTRACT

The influence of different substrate concentrations on the performance of a continuous system of alcohol prduction by fermentation using a tower reactor with recycling of flocculating yeasts was investigated. All experiments were carried out using a flocculating yeast strain IR-2, isolated from fermented food, and identified as *Saccharomyces cerevisiae*. Cane sugar juice was used as a substrate with sugar concentrations of 160, 170, 180, 190, and 200 g/L. Constant values of dilution rate, 0.20 $h^{-1}$, temperature, 30°C, and pH 3.3, were used. The performance of the reactor was observed to be efficient with high substrate concentrations. Maximum productivities of 18 g/L/h, 99% substrate conversion and ethanol concentrations of 90 g/L were obtained using 200 g/L of sugar in the feedstock. For substrate concentrations of 160 g/L, a maximum yield of 0.45 g of ethanol/g of sugar was observed or 90% of the theoretical value.

**Index Entries:** Tower reactor; flocculating yeast; ethanol.

## INTRODUCTION

Special attention has been given to continuous alcoholic fermentation processes because of the advantages they present with respect to the conventional batch fermentation processes.

Recently, several continuous processes have been developed, such as fermentation with the use of immobilized yeast cells *(1)*, flocculating yeast ceils *(2–7)*, or membrane separation *(8)*.

The continuous production of ethanol from different raw materials, using flocculating yeast, in recycling tower reactors, has been widely studied at the laboratory scale *(3,4,9)*, with the view to optimization and future application at the industrial scale. In this work, the performance of a continuous ethanol production process in a tower reactor with recycled flocculating cells was evaluated. The initial

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sugar concentrations used ranged from 160 to 200 g/L, the dilution rate was 0.20 $h^{-1}$, and the raw material was sugar cane juice (widely employed in Brazilian alcohol and sugar mills). The aim was a higher alcohol production with simultaneous reduction of the production residues (stillage), which is a big problem in many alcohol-producing regions.

## MATERIALS AND METHODS

### Microorganisms

A flocculating yeast strain, *Saccharomyces cerevisiae*, called IR-2, was used, isolated from fermented foodstuffs supplied by the Fermentation Research Institute (Tsukuba-Japan).

### Culture

The *S. cerevisiae* cultures were kept in a refrigerator at 4°C, in an agar malt extract (malt extract: 30.0 g/L; peptone: 5.0 g/L; agar-agar: 15.0 g/L). The cultures were subcultivated every month; the test tubes were then incubated at 28°C for 24 h.

### Medium

The main source of carbon used was the sugar cane juice, containing: sugar concentrations of 160–200 g/L; 1.0 g/L $(NH_4)_2SO4$; 0.20 g/L $KH_2PO_4$; 0.1 g/L $MgSO_4 \cdot 7H_2O$; 0.1 g/L $CaCl_2 \cdot 2H_2O$; 0.5 g/L yeast extract. The medium was sterilized in autoclave at 121°C and 1 atm for 45 min, and after cooling, a 40% formaldehyde diluted 1:6000 and mixtured in a penicilin-via-acid solution was added.

### Inoculum Preparation

The inoculum was prepared in a two-stage growth process. In the first stage, a 1-mL cellular suspension was used to inoculate 50 mL of culture medium in a 125-mL Erlenmeyer flask with sugar cane juice diluted to 10 g/L and additional nutrient salts. Incubation was done at 30°C with shaking at 130 rpm in a rotational shaker for 24 h. Next, the second growth stage was inoculated: the culture cells were transferred to 500-mL Erlenmeyer flasks containing 200 mL of the culture medium, with sugar cane juice diluted to 50 g/L and additional nutrient salts. The incubation conditions were identical to those of the first stage. The reactor was inoculated with the contents of the Erlenmeyer flasks.

### Fermentation Tests

The reactor was inoculated with a concentration of cells of 30 g/L (dry base). The initial phase was of retention and accumulation of yeast cells into the reactor. This was achieved by continuously pumping the sugar cane medium diluted to 50, 100, and 150 g/L sugar concentrations, according to yeast growth. The system remained in operation for 72 h, until a cell concentration of 100 g/L (dry base) was attained. After the accumulation stage, the dilution rate was adjusted to $D = 0.20\ h^{-1}$ and the initial sugar concentration was increased from 160 to 200 g/L. Fermentation was monitored by periodical sampling of the fermenting medium, in order to determine cell concentration and of the effluent for determination of the residual sugar concentration and ethanol. Figure 1 shows a scheme of the reactor used in the experiments.

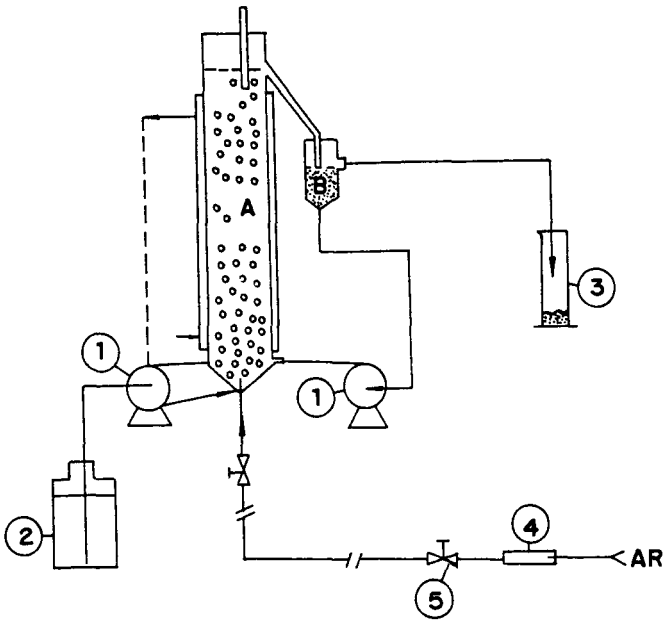


Fig. 1. Schematic diagram of a cell recycling continuous fermentation system using flocculating yeast. A, main reactor 220 mL; B, settler; 1, turbine pump; 2, medium reservoir; 3, product receiver; 4, flow meter; 5, air valve.

## RESULTS AND DISCUSSION

Figures 2–6 show the performance data of the system, for each sugar concentration tested. The initial concentration of cells in the reactor was around 100 g/L for all experiments. Duration was approx 30 d, in which no inhibition of cell growth by the substrate concentrations used was observed. When initial sugar concentrations of 200 g/L were used, cell inhibition with partial deflocculation of the cells took place. This is attributed, likely, to an overload of substrate, defined by critical fermentation rate, with a 20% reduction in the cell concentration. According to Bueno Netto et al. *(10)*, the stability of the system is limited when the substrate load attains a critical load near 39–40 g/L/h, resulting in deflocculation and reduction in biomass.

The performance of the bioreactor for this sugar cane juice medium shows some differences when compared to those reported by Kuriyama et al. *(3)*, who used a simulated medium (brown sugar). However, similar cell densities were found around 90–100 g/L. Concerning the flocculating characteristics of the yeast strain *S. cerevisiae* IR-2, no adverse effects were observed.

Regarding the amount of cells lost in the effluent, average values around 6.0 g/L were encountered. It was also observed that this value might vary a lot, depending on the operational conditions used.

Substrate conversion was approx 99% for all tests. These results are shown in Figs. 2–6. Considering that the tolerated sugar losses in the process are in the range of 1–2 g/L, i.e., minimum conversion rate of 98%, it was possible to obtain virtually complete substrate conversion in all experiments.

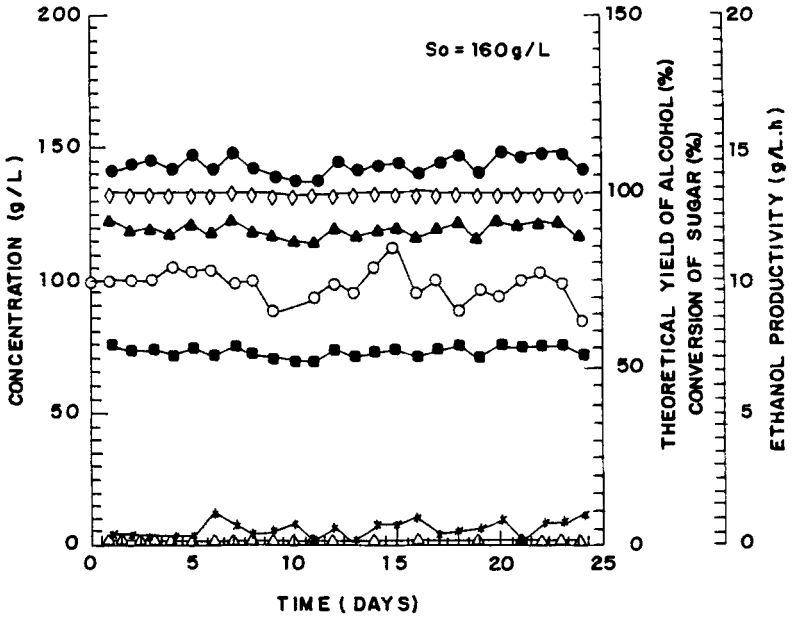


Fig. 2. Performance data in continuous alcohol fermentation with sugar cane juice concentration of 160 g/L. ●, ethanol productivity; ◇, conversion of sugar; ▲, theoretical yield of alcohol; ○, cellular conc. in reactor; ■, ethanol conc.; *, cellular conc. in effluent; △, residual TRS.

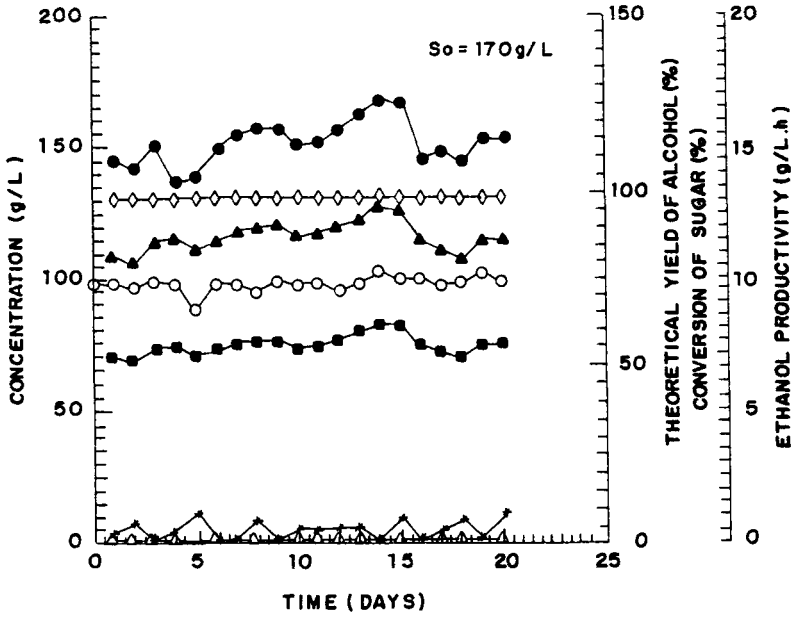


Fig. 3. Performance data in continuous alcohol fermentation with sugar cane juice concentration of 170 g/L. ●, ethanol productivity; ◇, conversion of sugar; ▲, theoretical yield of alcohol; ○, cellular conc. in reactor; ■, ethanol conc.; *, cellular conc. in effluent; △, residual TRS.

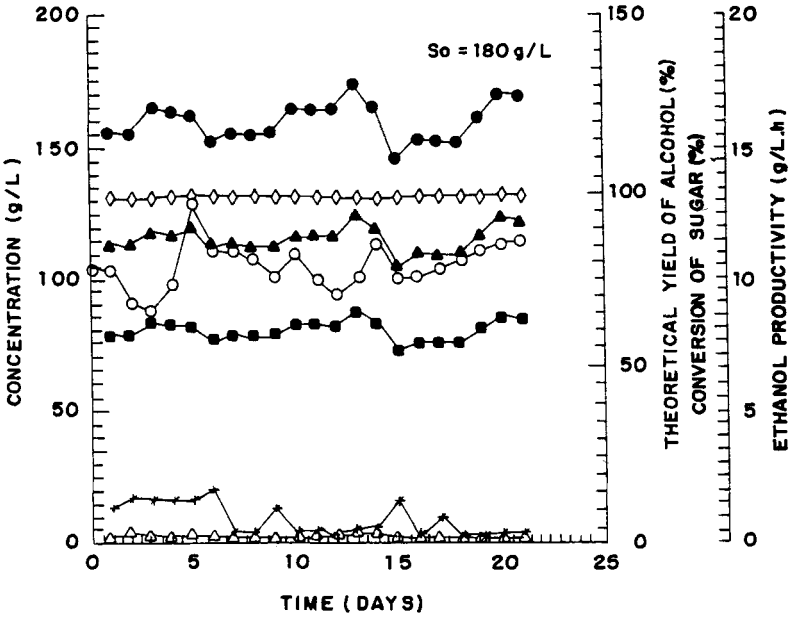


Fig. 4. Performance data in continuous alcohol fermentation with sugar cane juice concentration of 180 g/L. ●, ethanol productivity; ◇, conversion of sugar; ▲, theoretical yield of alcohol; ○, cellular conc. in reactor; ■, ethanol conc.; *, cellular conc. in effluent; △, residual TRS.

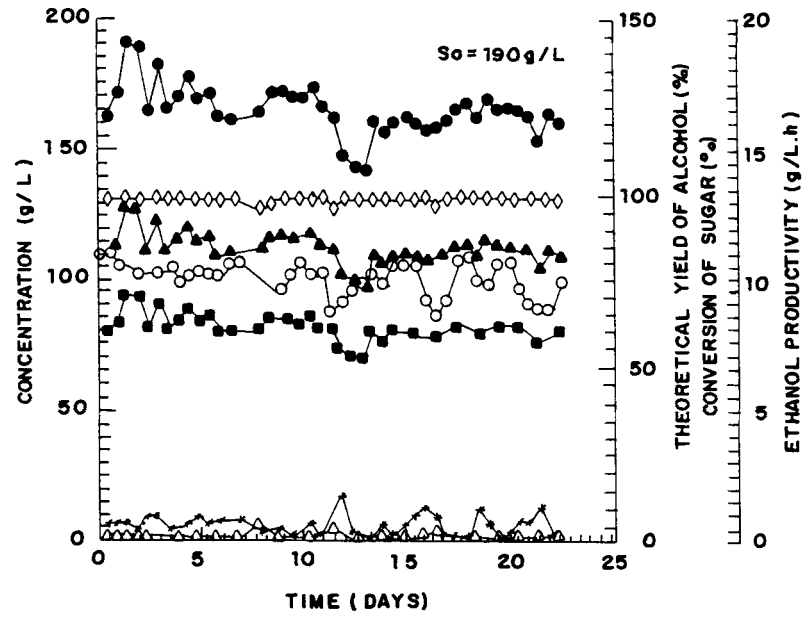


Fig. 5. Performance data in continuous alcohol fermentation with sugar cane juice concentration of 190 g/L. ●, ethanol productivity; ◇, conversion of sugar; ▲, theoretical yield of alcohol; ○, cellular conc. in reactor; ■, ethanol conc.; *, cellular conc. in effluent; △, residual TRS.

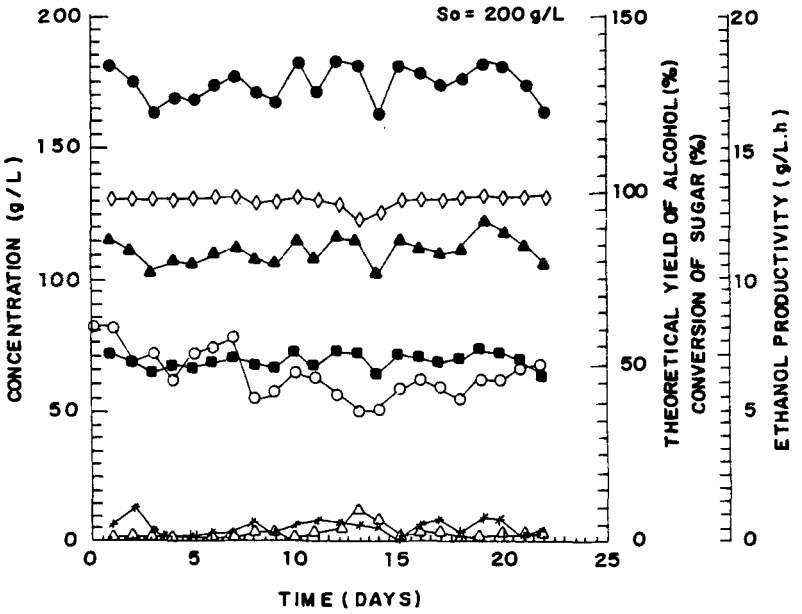


Fig. 6. Performance data in continuous alcohol fermentation with sugar cane juice concentration of 200 g/L. ●, ethanol productivity; ◇, conversion of sugar; ▲, theoretical yield of alcohol; ○, cellular conc. in reactor; ■, ethanol conc.; *, cellular conc. in effluent; △, residual TRS.

Concerning the residual sugar concentration, this tended to increase when an initial concentration of 200 g/L was used. It is interesting to note that even with this increase in residual sugar concentration, no significant variation in the substrate conversion was observed, remaining constant around 99%.

The maximum ethanol concentration attained was 90 g/L with a maximum productivity of 18 g/L/h. The ethanol concentrations and productivity were higher for the fermentations with substrate concentrations of 200 g/L.

Considering the productivity degree of this system and compared to those reported in the literature, it was observed that Kida et al. *(2)* obtained a maximum productivity of 30 g/L/h operating with high dilution rates of 0.5 $h^{-1}$. The concentration of ethanol produced by these authors was 60 g/L, less than the concentrations reported above of 90 and 81 g/L, respectively. Recently, Kuriyama et al. *(4)* obtained productivities of 28–29 g/L/h with 69–70 g/L of ethanol, using flocculating yeast, a tower reactor, or a conventional mixing reactor, resulting in high ethanol production rates and productivities.

The maximum yield obtained in the process was 0.45 g of ethanol/g sugar or 90% of the theoretical value, when substrate concentrations of 160–170 g/L were used. This yield is considered reasonable when compared to those in the literature *(7,10)*.

Table 1 presents the average values of the concentrations of residual total reducing sugars (TRS) and cells in the reactor and effluent, substrate conversion, yield, and productivity, obtained in a 220-mL tower reactor, at steady state, at the different substrate concentrations used. A linear increase in ethanol productivity and concentration and a linear decrease in yield were observed with the increase in initial sugar concentration.

Table 1
Average Values Obtained in Steady State of the Tests in a 220-mL Tower Reactor

| Initial TRS, g/L | Residual TRS, g/L | Cell conc. in the reactor, g/L | Cell conc. in the effluent, g/L | Ethanol conc., g/L | Conversion of sugar, % | Theoretical yield of alcohol, % | Ethanol productivity, g/L/h |
|---|---|---|---|---|---|---|---|
| 160 | 1.0 ± 0.1 | 98.3 ± 6.4 | 6.0 ± 3.3 | 72.5 ± 1.6 | 99.3 ± 0.1 | 88.3 ± 1.9 | 14.4 ± 0.3 |
| 170 | 1.1 ± 0.2 | 99.7 ± 2.6 | 5.7 ± 3.8 | 77.1 ± 3.5 | 99.3 ± 0.1 | 88.3 ± 4.0 | 15.3 ± 0.8 |
| 180 | 1.8 ± 0.6 | 104.3 ± 8.9 | 7.9 ± 6.1 | 79.6 ± 3.7 | 99.1 ± 0.4 | 85.8 ± 3.7 | 15.9 ± 0.7 |
| 190 | 2.0 ± 0.7 | 101.6 ± 6.3 | 6.3 ± 4.1 | 82.4 ± 5.1 | 98.9 ± 0.3 | 84.7 ± 5.2 | 16.5 ± 1.0 |
| 200 | 4.2 ± 3.3 | 80.0 ± 11.7 | 7.1 ± 4.0 | 86.6 ± 3.5 | 97.9 ± 1.6 | 82.6 ± 3.7 | 17.3 ± 0.7 |

## CONCLUSIONS

A continuous tower reactor, using naturally flocculating yeast, enabled the obtention of high productivity degrees (18 g/L/h) and ethanol concentrations for high sugar concentrations without jeopardizing the process performance. These conditions make a future system scale-up possible, envisaging the industrial use of a simple continuous process (just one reactor), with low installation and operational costs, furthermore allowing reductions up to 10% of the total volume of residues normally produced at typical process conditions.

The fact that high concentrations (average of 100 g/L dry base) of naturally immobilized yeast in the reactor were maintained, which are much superior to the concentrations obtained at present in the alcohol industry, provides evidence of the strong flocculating capacity of the cells used, favoring the continuous process proposed.

## ACKNOWLEDGMENTS

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