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Ethanol Production by a Flocculant Yeast Strain in a CSTR Type Fermentor with Cell Recycling

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Abstract

Tests were performed in a continuous stirred tank reactor (CSTR), with and without cell recycling, to produce ethanol. The reactor without cell recycling produced the kinetic model of ethanol production, whereas the reactor with cell recycling allowed for a study of process stability.

The Levenspiel kinetic model was adopted; however, in the case of fermentation with cell recycling, the coefficient of cell death was added. It was observed that cellular viability varied greatly throughout the fermenting process and that microaeration is of fundamental importance in maintaining the stability of the process.

Index Entries: Ethanol; flocculant yeast.

Introduction

The fermenting process of ethanol production has its roots dating back to Brazil's colonial period. This explains its choice, during the worldwide oil crisis of the 1970s, as a motor fuel deriving from a renewable source.

Brazil today produces approx 12 million m^3 of ethanol per year, with the industry employing around 4 million workers per year. Most of the ethanol produced is used as motor fuel or an additive in gasoline to improve its octane level.

Several changes to the kinetic model proposed by Monod *(1)* have been cited in the literature. In the specific case of alcohol fermentation, the models of cell growth inhibition by the substrate or the product have been suggested. Among these models, those that show more generic relations for inhibition by the product are the ones proposed by Levenspiel *(2)* and Luong *(3)*.

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On the other hand, it seems that aeration exerts a beneficial effect on cell growth and on ethanol productivity *(4–6)*. Therefore, this study was developed to propose an alternative process for ethanol production using a flocculant yeast strain, in order to save electricity by substituting the centrifuge for a settler and facilitating cell separation from the fermented broth.

The study began with a search for a kinetic model that would describe the continuous process of ethanol production, using a laboratory-produced culture for improved control. Subsequently, the validity of this kinetic model for ethanol production, using treated sugar-cane syrup diluted and supplemented only with ammonia sulfate, was confirmed. Process stability and improvements through microaeration were also studied.

Materials and Methods

Organism

The organism used was the naturally flocculent strain *Saccharomyces cerevisiae NRRL* Y-265. The strain was kept on a Saboraud agar medium and was subcultured monthly at 30°C for 5 d.

Medium

Four different media were used, two for the experiments without the settler and two for the experiments with the settler. In the experiments without the settler, the growth medium (medium A) contained per liter, 60 g sucrose, 6 g peptone, 5 g KH_2PO_4, 1 g $MgSO_4 \cdot 7H_2O$, 5 g $(NH_4)_2SO_4$, 5 g $(NH_4)_2HPO_4$, 1 g yeast extract, and tap water at a final pH of 5.5. The ethanol production medium (medium B) contained 100–200 g/L glucose, 5 g/L KH_2PO_4, 1 g/L $MgSO_4 \cdot 7H_2O$, 10–20 g/L $(NH_4)_2SO_4$, 10–20 g/L yeast extract, and tap water at a final pH of 5.5.

The experiments with the settler first used growth medium A, so a second growth medium (medium C) was also used containing per liter: sugar cane syrup (about 50 g sucrose), 5 g KH_2PO_4, 12 g $(NH_4)_2SO_4$, 10 g $(NH_4)_2HPO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g $CaCl_2 \cdot 2H_2O$, 1 g yeast extract, and tap water at a final pH of 4.5. The production medium (medium D) contained sugar cane syrup (150–260 g/L sucrose) and 1 g/L $(NH_4)_2SO_4$ at a final pH of 4.5. The pH of all media were corrected with 1.0 *M* NaOH or 1.0 *M* HCl.

Seed Culture (Inoculum)

The inoculum was prepared in two stages of successive cultures in 500 mL Erlenmeyers.

In the first stage (preinoculum), six loopsful of the stock test tube were transferred for each 70 mL of growth medium A contained in three 500-mL Erlenmeyers and then maintained at 30°C for 48 h on a shaker at 300 rpm.

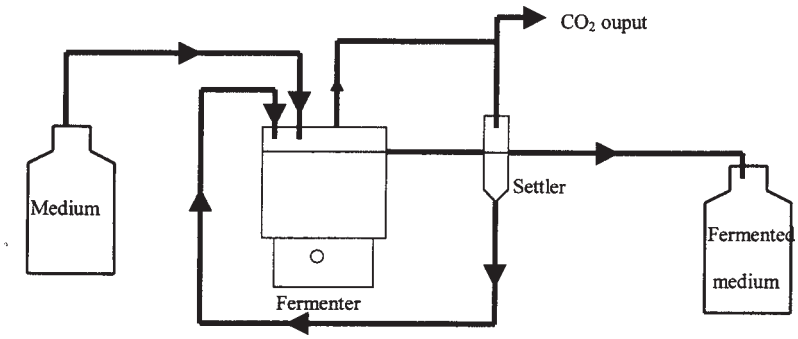

Fig. 1. Schematic diagram of the fermentation system.

In the second stage, actual inoculum, the previous product was transferred to the three 500-mL Erlenmeyers of the inoculation system, each jar containing 70 mL of medium C, which was then also kept at a temperature of 30°C for 48 h on a shaker at 300 rpm.

The inoculant obtained was then transferred to the sterilized 3-L volume fermenter to begin the growth phase in a fed-batch process.

Continuous Ethanol Production

Because the continuous production of ethanol was a sequential stage to growth, the fermenter's mixing and controls were already adjusted (temperature 30°C, air injection 0.05 vvm, medium feed 140 mL/h, agitation about 200 rpm). It was, therefore, necessary simply to wait for the fermenter to fill up and the medium to overflow into the settler when the cell recycling pump was turned on and the flow adjusted. A diagram of the system with cell recycling is shown in Fig. 1. The system consisted of a stirred tank-type reactor (CSTR) with a total volume of 3 L and real volume maintained at 1.4 L, with stirring done by a magnetic stirrer. The settler was jacketed and had a total volume of 500 mL, with a real volume of 300 mL. It was 50 mm in diameter, with a conical bottom from which the settled cells were removed for recycling to the fermenter through a peristaltic pump. The settler's temperature was kept at 10°C.

Biomass

Biomass was determined by filtering a 10-mL sample onto a 0.45-µm Millipore membrane and drying it at 105°C until it reached a constant weight.

Viability of Cells

Ten milliliters of the sample were centrifuged and the precipitate was suspended again for the cell viability analysis. The cells were deflocculated with a 5% ethylenediamine tetra-acetic acid (EDTA) solution and treated with a solution of methylene blue, as proposed by Lee et al. *(7)*, for a viable cell count under a microscope.

Glucose

The Somogyi method *(8)* was used to assay glucose. Media containing sucrose were previously hydrolyzed by 0.9 *N* HCl. The hydrolysate was neutralized with a solution of 2 *N* NaOH, using phenolphthalein as an indicator.

Ethanol

Ethanol was assayed by gas chromatography under the following conditions: The packing material used was Cromossorb W coated 15% Carbowax 20 with a 2.2-m length and 1/8 in. inner diameter; and carrier gas, nitrogen, and *n*-butanol as the internal standard. The temperature of the column, detector, and vaporizer were 120, 170, and 150°C, respectively.

N-butanol (Merck, Rahway, NJ) was used in a 40 g/L concentration weighed on an analytical scale. Synthetic air and hydrogen flows were 20 mL/4 s and 20 mL/28 s, respectively, both maintained at a line pressure of 30 psi. The calculations were made by a processing integrator. The samples taken from the process were diluted to a proportion of 1:10 and added to a quota of *n*-butanol solution in a 1:1 proportion before being injected into the chromatograph. The volume injected was 1 µL.

Results and Discussion

Fermentation Without Cell Recycling

The continuous fermentations without cell recycling were made to define the kinetic model and characterize alcohol fermentation using flocculant yeast in a continuous stirred tank reactor. Six experiments were carried out with different initial concentrations of glucose (Cso), 100, 110, 127, 146, 164, and 192 g/L. These experiments are illustrated in Figs. 2–4. A considerable deviation from Monod's kinetics can be observed when one compares the residual concentrations of glucose at low dilution rates for the different experiments. It can be seen that the concentration of residual glucose is very low for 100 g/L Cso and that there is a gradual increase when one increases the initial concentration of glucose. Since the concentration of glucose is far below the inhibiting concentration, inhibition can be attributed to the product, in this case to ethanol. The method employed to make the necessary adjustment was the nonlinear regression. Of the several models of inhibition by ethanol, the Monod model modified by Levenspiel *(2)* proved to be the most suitable *(9)*:

$$\mu = \mu_{max}\left(1 - \frac{C_p}{C_p^*}\right)^n \cdot \frac{C_s}{K_s + C_s}, \quad (1)$$

$$C_x = Y_{x/s}(C_{so} - C_s) \quad (2)$$

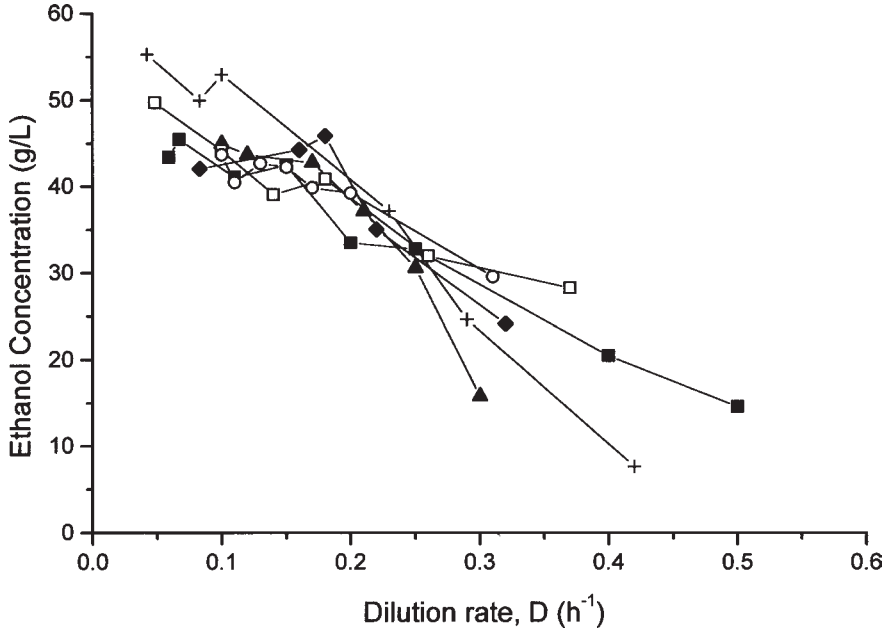

Fig. 2. Variations of cell concentrations for different dilution rate and initial concentrations of glucose (Cso). —■—, Cso 100 g/L; —▲—, Cso 110 g/L; —◆—, Cso 127 g/L; — + —, Cso 146 g/L; —□—, Cso 164 g/L; —○—, Cso 192 g/L.

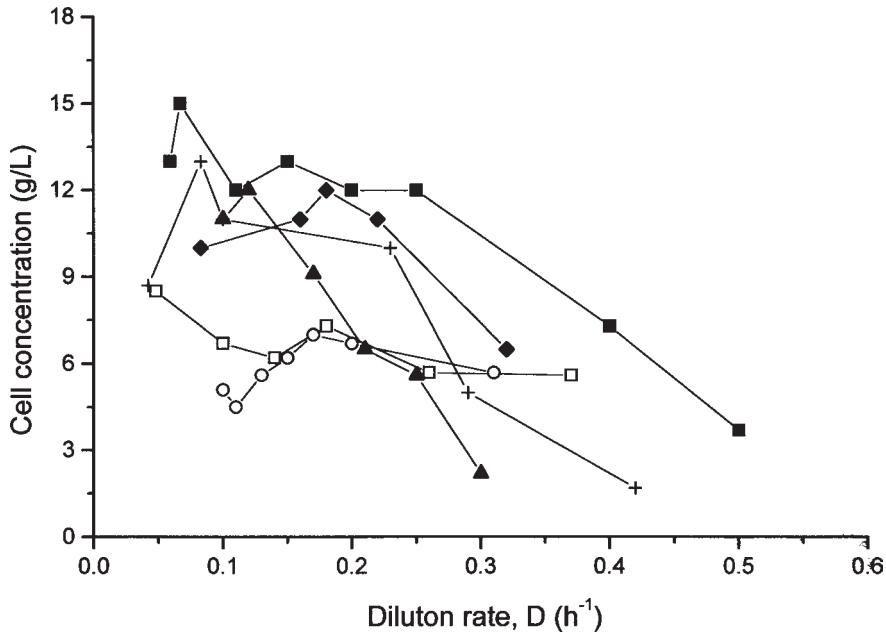

Fig. 3. Variations of ethanol for different dilution rates and initial concentrations of glucose (Cso). —■—, Cso 100 g/L; —▲—, Cso 110 g/L; —◆—, Cso 127 g/L; — + —, Cso 146 g/L; —□—, Cso 164 g/L; —○—, Cso 192 g/L.

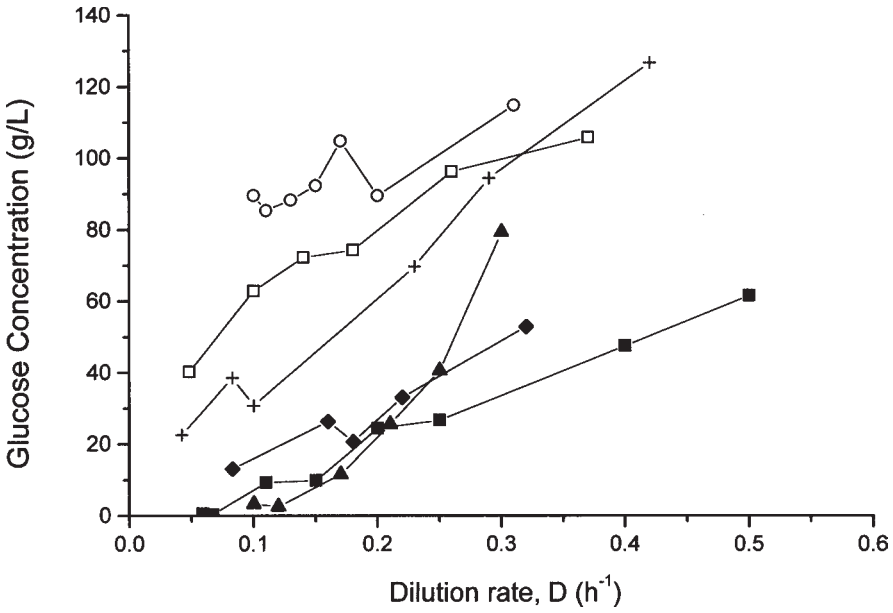

Fig. 4. Variations of glucose for different dilution rates and initial concentrations of glucose (Cso). —■—, Cso 100 g/L; —▲—, Cso 110 g/L; —◆—, Cso 127 g/L; — + —, Cso 146 g/L; —□—, Cso 164 g/L; —○—, Cso 192 g/L.

$$C_p = 0.43(C_{so} - C_s), \tag{3}$$

where $\mu_{max} = 0.6/h$, $C_p^* = 80$ g/L, $n = 1.8$, and $eK_s = 0.57$ g/L.

where 0.43 is the $Y_{P/S}$ obtained from the angular coefficient of the graph of specific rates of ethanol production ($-\sigma$) based on the specific rate of substrate consumption (ν), as shown in Fig. 5.

From Fig. 6, one can see that cell yield varies with the initial concentration of glucose, which makes its use necessary to calculate the medium's glucose concentration in the numerical integration of the differential equation to determine the cell concentration.

Fermentation with Cell Recycling

Stability Study Tests

Several experiments were carried out to study the stability of the process. All the tests were made with high hydraulic residence times and high concentrations of substrate to determine whether the inhibiting effect of both ethanol and alcohol-related treatment were factors that would influence stability. Figure 7 groups the experiments carried out with hydraulic residence times of 10–15 h with different cell residence times.

An analysis of the graphs of the experiments shown in Fig. 7 shows that although the system is not unstable, it operates with a significant fluctuation of cell, ethanol, and substrate concentrations. Tests with

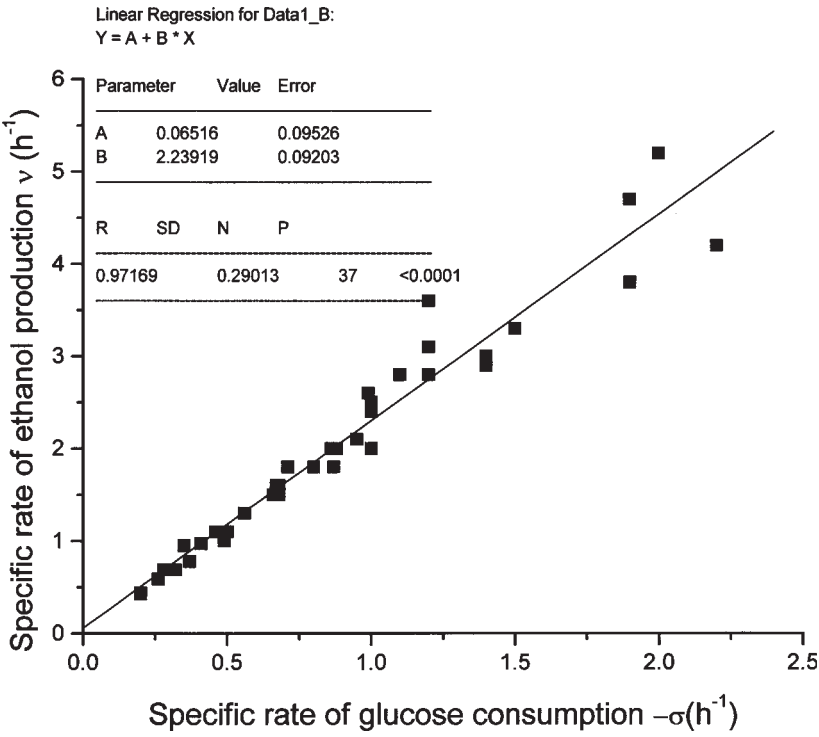

Fig. 5. Relationship between specific rate of ethanol production and specific rate of glucose consumption for calculation coefficient $Y_{P/S}$. ■, Data; ——, linear fit of data 1_B.

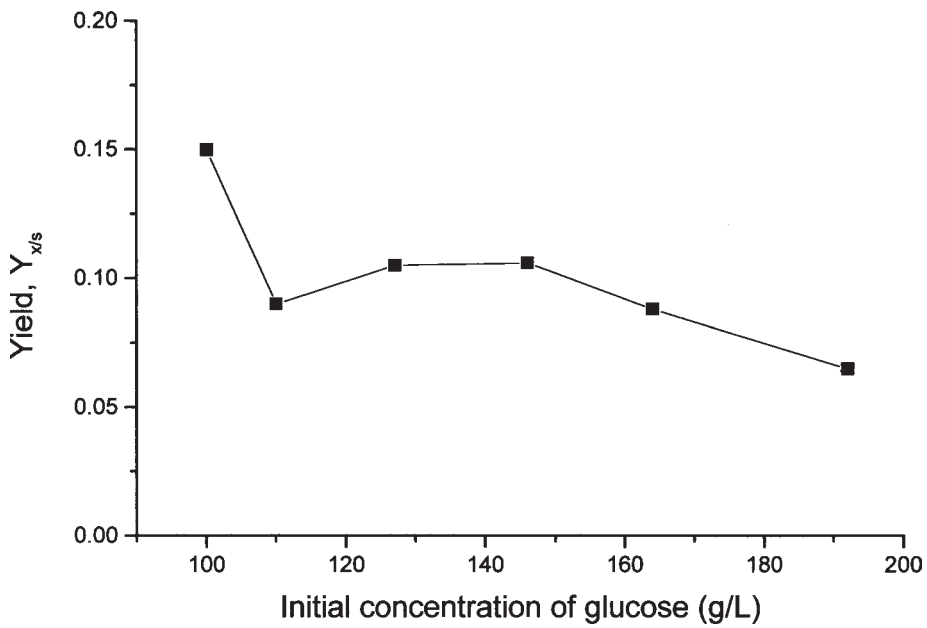

Fig. 6. Cell yield in view of the initial concentration of glucose.

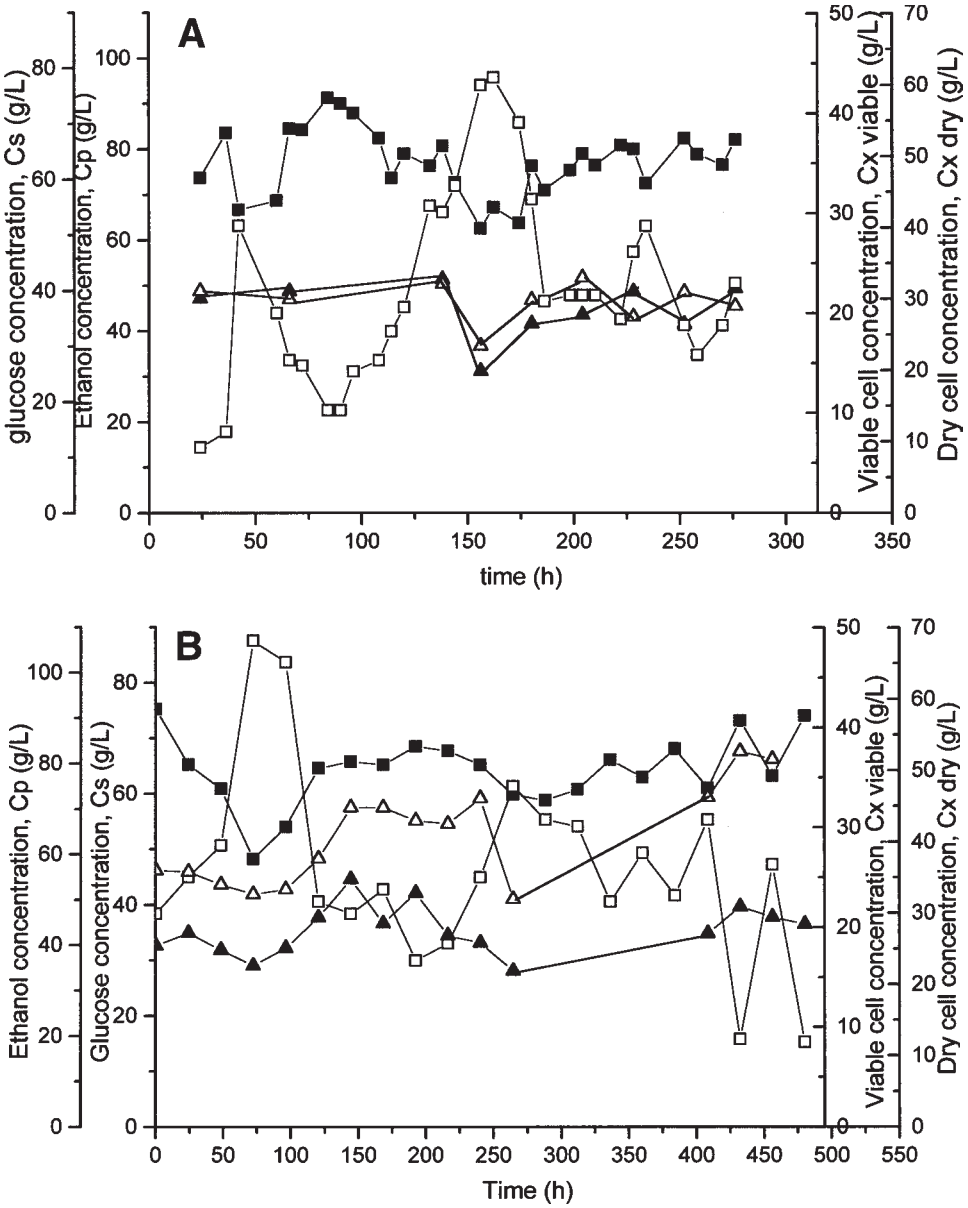

Fig. 7. Typical fermentation without microaeration and cell recycling. —▲—, Cx viable; —△—, Cx dry; —■—, Cp; —□—, Cs.

microaeration were therefore proposed as an attempt to stabilize the concentration of ethanol produced, considering that it is produced for 7 mo/yr in Brazil (Fig. 8). Figure 8 shows the result of tests with microaeration (0.05 vvm or 70 mL of air/min), where the concentrations remain stable. Of note is the influence of microaeration, particularly in the maintenance of viable cell concentrations, which remain stable at approx 20 g/L despite

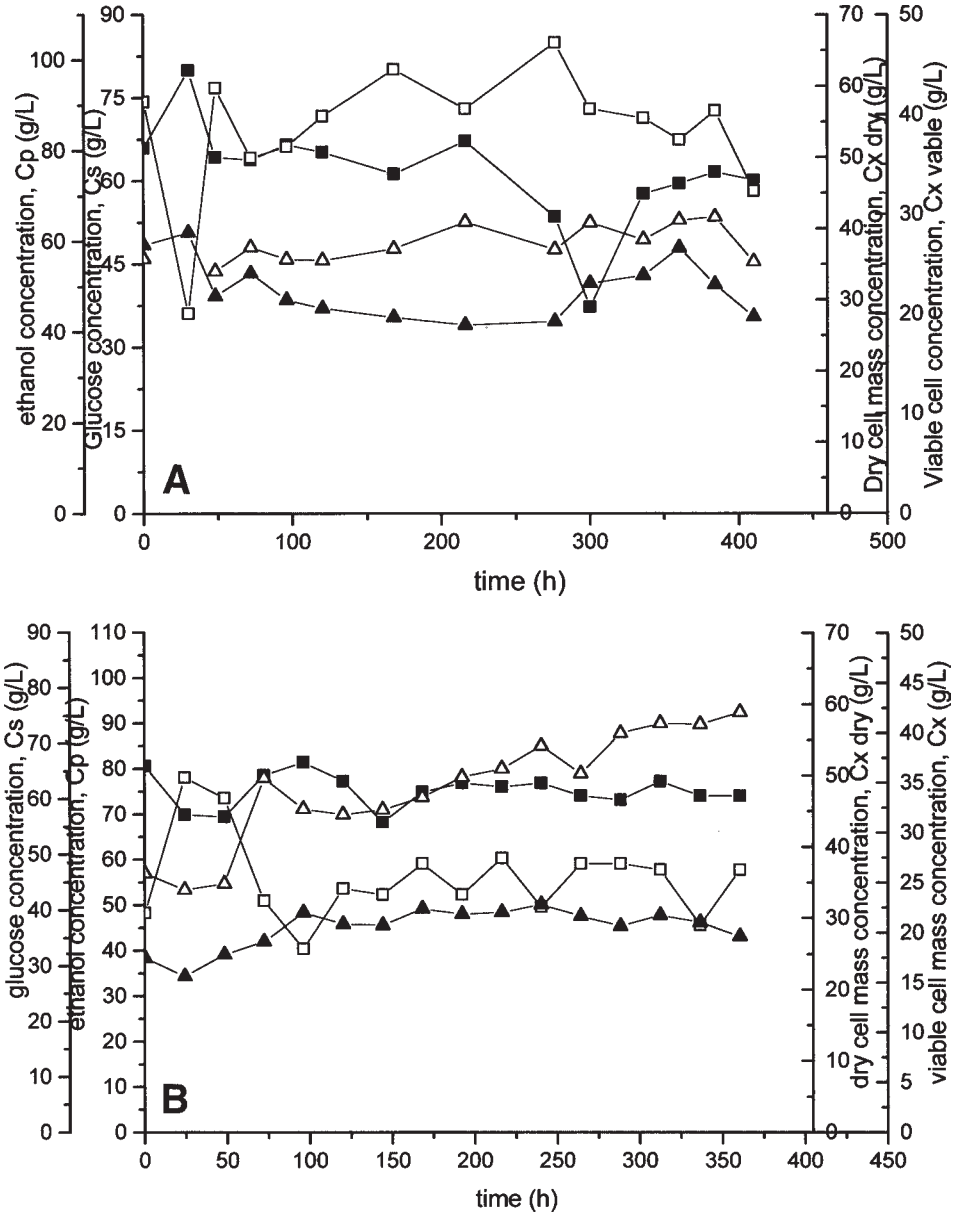

Fig. 8. Typical fermentation in reactor type CSTR with cell recycling and without **(A)** and with **(B)** microaeration with hydraulic residence time of 10 h. —▲—, Cx viable; —△—, Cx dry; —■—, Cp; —□—, Cs.

an increase in total cell concentration. Therefore, analyzing viable cell concentrations is extremely important in studying the process, since analyzing the global cell concentration by means of the dry mass, assuming a 100% viability, does not correspond to the hypotheses established at the time the problem was set forth.

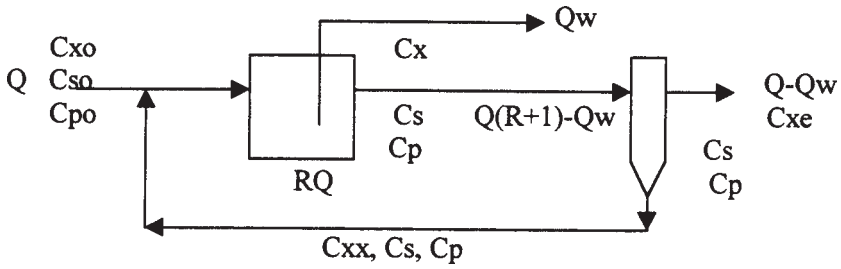

Fig. 9. Schematic drawing for mass balance.

Analysis of the process with cell recycling was performed on the balance of the system's mass (considering the process as drawn in Fig. 9).

Assuming the system is homogeneous, perfectly stirred, isothermal, and without any reaction in the settler and: C_{xo}, concentration of viable cells in the feeding system; C_{so}, initial substrate concentration; C_{po}, concentration of the product in the feeding system; C_x, concentration of viable cells in the reactor; C_s, concentration of substrate in the reactor; C_p, concentration of the product in the reactor; C_{xe}, concentration of viable cells in the exit of the settler; C_{xx}, concentration of purged viable cells; R, recycling rate; Q, feed flow; and Q_w, purge flow, the global balance of mass for the cells is

$$QCxo + v_x V = (Q - Q_w)C_{xe} + Q_w C_x + accumulation + (-v_d)V \quad (4)$$

$$(\mu - K_d) = \frac{(Q_w C_x + (Q - Q_w)C_{xe})}{C_x V} \quad (5)$$

assuming

$$\theta_c = \frac{mass \text{ of cells in the reactor}}{\text{output rate of cells}}, \quad (6)$$

$$\theta_c = \frac{1}{\mu - K_d} = \frac{VC_x}{(Q - Q_w)C_{xe} + QC_x}, \quad (7)$$

when C_{xe} is low, $\theta_c \cong \frac{V}{Q_w}$, so the substrate mass balance in steady state:

$$QC_{so} = QC_s + (-v_s)V \quad (8)$$

and remembering that $(-v_s) = \frac{v_x}{Y_g}$ and assuming $\theta_h = \left(\frac{V}{Q}\right)$,

$$\frac{C_{so} - C_s}{C_x} = \frac{\mu C_x}{Y_g} = \frac{\theta_h}{\theta_c}.\frac{1}{Y_g} + \frac{K_d \theta_c}{Y_g}, \quad (9)$$

so, plotting $\frac{\theta_c}{\theta_h} \cdot \frac{C_{so} - C_s}{C_x}$ x θ_c one can find K_d and Y_g. The resulting values were $K_d = 0.0054/h$ e $Yg = 0.014$. Thus, the kinetic model for the process with cell recycling was established as *(10)*:

$$\mu = 0.6\left(1 - \frac{C_p}{90}\right)^{1.8} \cdot \frac{C_s}{0.57 + C_s} - 0.0054 \qquad (10)$$

Conclusions

1. Levenspiel's kinetic model offers a good explanation of the process of alcohol fermentation using NRRL Y 265 flocculant yeast.
2. The use of flocculant cells and their recycling may constitute a good choice for modifying the classical process of alcohol fermentation.
3. The process of alcohol fermentation using flocculant yeast, with or without cell recycling, proved to be quite stable.
4. Addition of the coefficient of cell death to the kinetic model in the system with cell recycling indicates that cell death is a factor that must be considered in continuous fermentation because of its long periods of operation (7 mo) if the hydraulic residence time is high.
5. The culture medium based on sugar cane syrup and the form of operation (microaeration) may alter the microorganism's tolerance to the inhibitor (ethanol), which can be observed by the modification of the C_p^* in the kinetic model.
6. The viable cell analysis was of fundamental importance in establishing the kinetic model of the fermentation process with cell recycling.
7. Microaeration is extremely important to maintain the stability of the process.

Acknowledgments

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