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OPERATIONAL STABILITY OF IMMOBILIZED *S. CEREVISIAE* IN A PACKED BED REACTOR FOR VARIOUS FERMENTATION MEDIA AND COMPARISON OF FREE AND IMMOBILIZED MICROORGANISM SYSTEMS

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ASTRACT

Using various fermentation media, ethyl alcohol production in a packed bed reactor with *S. cerevisiae*, immobilized in calcium alginate gel, was investigated. For the rich medium, productivity changed between 17 and 21 g/l. hr, during a period of 52 days. When a poor medium was used, during a period of 10 days productivity decreased to 72 % of the initial value and, when molasses solution was used, the productivity decreased to 33 % of the initial value. The productivity of the immobilized system turned out to be 7 to 40 times of the free system, depending on the residence time.

KEYWORDS

Ethyl alcohol, *S. cerevisiae*, Packed bed, Calcium alginate.

INTRODUCTION

The primary advantage of using a packed bed reactor containing immobilized microorganisms is the high cell concentration per unit reactor volume and consequently high productivity. It is not surprising, thus, that in recent years many researchers investigated production of ethyl alcohol using immobilized microorganisms (Gencer, 1983; Ghose, 1982; Han, 1982; Linko, 1981). These researchers utilized several support materials and fermentation media, and tried to determine the optimum operating conditions. In this paper, the results obtained using *S.cerevisiae* immobilized in calcium alginate gel are presented. A packed bed column was used as the reactor. Operational stability

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of the system for different fermentation media were determined and compared with other studies. The deactivation and reaction rate constants for *S.cerevisiae* were calculated in these media. A comparison between free and immobilized microorganism systems was performed.

THE DECSRIPTION OF THE SYSTEM

S.cerevisiae immobilized in calcium alginate gel can be considered as a porous catalyst. The sucrose molecules diffuse into the gel and upon contacting the yeast cells give ethanol and carbondioxide after a few intermediate steps. The products thus formed also leave the gel by diffusion. This system can deactivate by time, and if the system is properly defined, this deactivation can be predicted. The following assumptions were made in this study in order to define the system mathematically:

1. The calcium alginate particles are spherical and porous,

2. The yeast cells are homogeneously distributed in the gel,

3. Both the substrate and the products can enter and leave the gel freely, without any accumulation,

4. Change in the particle dimensions are neglible,

5. The yeast cells are enzyme stores. Deactivation occurs with time due to the denaturation of the enzyme's active regions and the loss of the yeast cells,

6. Deactivation rate is independent of substrate and product concentrations,

7. Conversion reaction of sucrose to ethyl alcohol and the deactivation are of the first degree.

For a plug flow in a reactor containing catalyst particles, the performance equation is given as (Levenspiel, 1982):

$$\frac{WC_{so}}{F_{so}} = -\int_{C_{so}}^{C_s} \frac{dC_s}{-r_s} \tag{1}$$

According to the assumptions 6 and 7, we can write,

$$-r_s = ke^{-k_d t} C_s \tag{2}$$

Using this reaction rate, we can obtain,

$$\frac{WC_{so}}{F_{so}} = -\int_{C_{so}}^{C_s} \frac{dC_s}{ke^{-k_d t} C_s} \tag{3}$$

Taking the integral, the following expression can be obtained:

$$\ln \ln (C_{so}/C_s) = \ln \left(k \frac{WC_{so}}{F_{so}}\right) - k_d t \tag{4}$$

By plotting the left hand side of Equation (4) as a function of time, k and k_d can be determined from the intercept and the slope of the straight line, respectively.

MATERIALS AND METHOD

3.1. Microorganism and Culture Conditions:

S.cerevisiae NRRL–Y–567, obtained from the Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois, U.S.A. was used in this study. The medium in which the microorganisms growed consisted of 2.00 g/l K_2HPO_4, 3.35 g/l $(NH_4)_2$ SO_4, 3.76 g/l NaH_2PO_4, 0.52 g/l $MgSO_4.7H_2O$, 0.018 g/l $CaCl_2.4H_2O$, 6 g/l yeast extract, and 0.2 ml/l antifoam agent. The pH and temperature were kept at 4 and 30 °C, respectively. On the other hand, the rich medium used to produce ethyl alcohol consisted of 6 g/l yeast extract, 1.63 g/l $(NH_4)_2SO_4$ and 5.53 g/l $CaCl_2$, while the poor medium only contained 5.53 g/l $CaCl_2$, and the half-poor medium hald 1 g/l yeast extract, 1 g/l K_2HPO_4 and 5.53 g/l $CaCl_2$. All these media contained 100 g/l sucrose.

3.2. Apparatus

For the packed column, a cylindrical ,jacketed glass column (40x2.8 cm i.d) was used with perforated glass discs at the top and the bottom. Water was circulated in the jacket to keep the temperature constant. Additional equipment consisted of substrate solution feed and collection pumps, substrate and product reservoirs, and a thermostat controlled bath. A detailed schematic diagram is shown in Figure 1.

3.3. Method of Analysis:

The ethanol and sucrose concentrations were determined spectrohotometrically. Dichromate method (Snell, 1957) and enzymatic method (Bergmeyer, 1965) were used for ethanol and sucrose determinations, respectively.

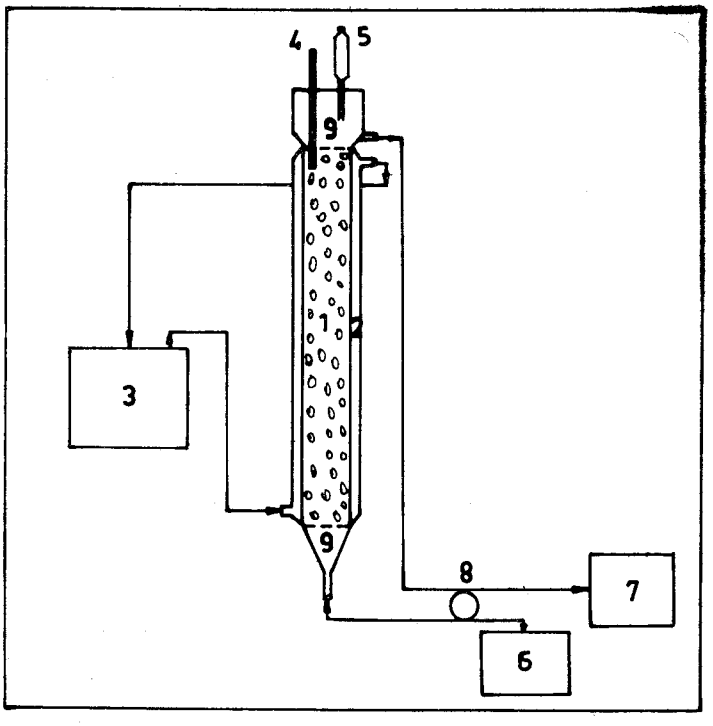

Figure 1. Detailed configuration of a packed bed reactor (40x2.8 cm i.d.) for immobilized yeast. 1. İmmobilized cells, 2. Water jacket, 3. Thermostat, 4. Thermometer, 5. Fermentation buble, 6. Feed Reservoir, 7. Product reservoir, 8. Pump, 9. Perforated discs.

RESULTS

4.1. Effect of the medium composition on the system's stability:

The effect of the medium composition on the system's stability is shown in Figures 2 and 3. The first one shows the results for the rich medium. The variation of productivity during a period of 52 days was considered to be unimportant. No mutation or contamination was observed in this period. Figure 3 compares the variation of productivity with time for the rich, poor, half-poor media and the molasses solution. With the poor medium, the productivity decreased 72 % during 10 days and this result is close to Han and Choi's, 1982, who found 70 % decrease under similar conditions. The malasses solution showed 33 % decrease in 10 days, which again is in accord with Linko, 1981.

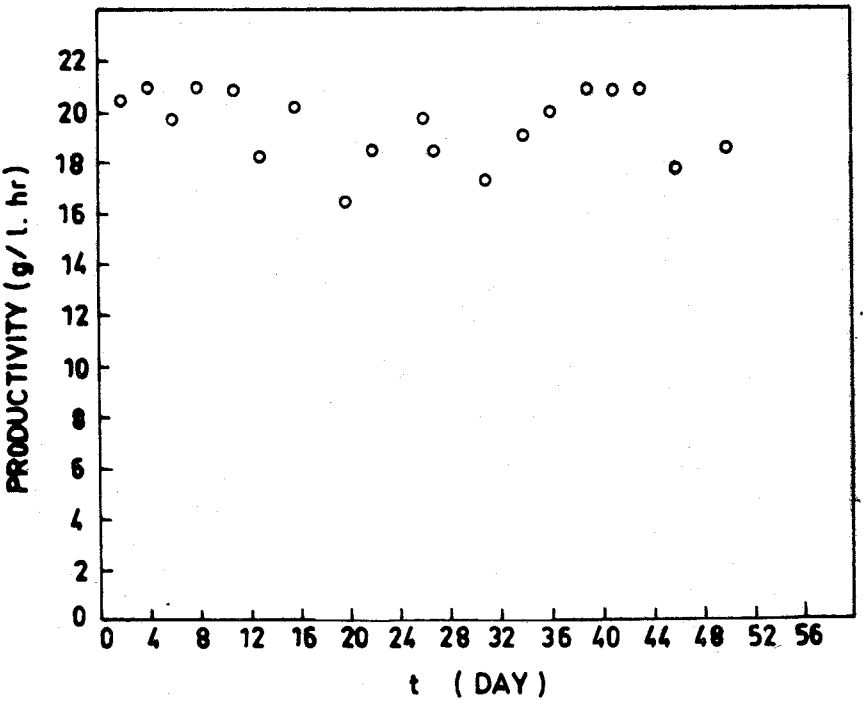

Figure 2. The change of productivity with time for the rich medium (C_{so}=100, g/l, T=30°C, pH=5, d_o=3.5, mm, τ=2.38 hr, W=12.5 g., ε=0.35).

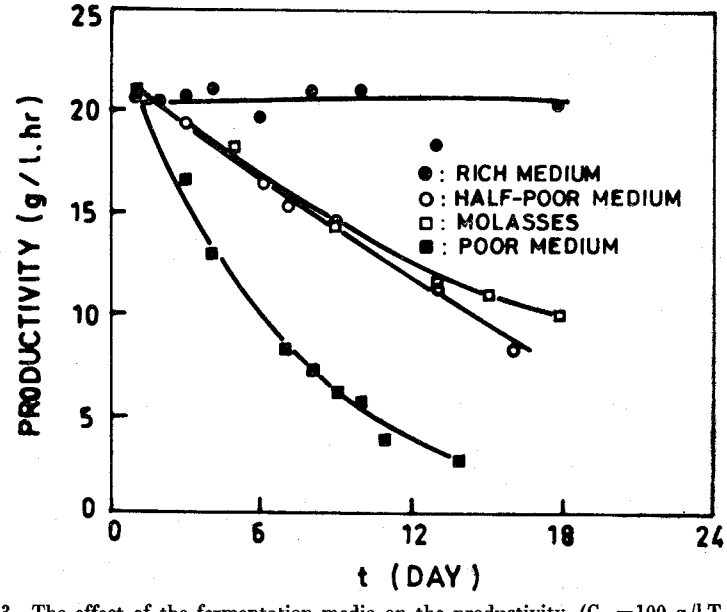

Figure 3. The effect of the fermentation media on the productivity. (C_{so}=100 g/l, T =30°C, pH=5, τ=2.38, hr., d_o=3.5 mm, W=12.5 g, ε=0.35).

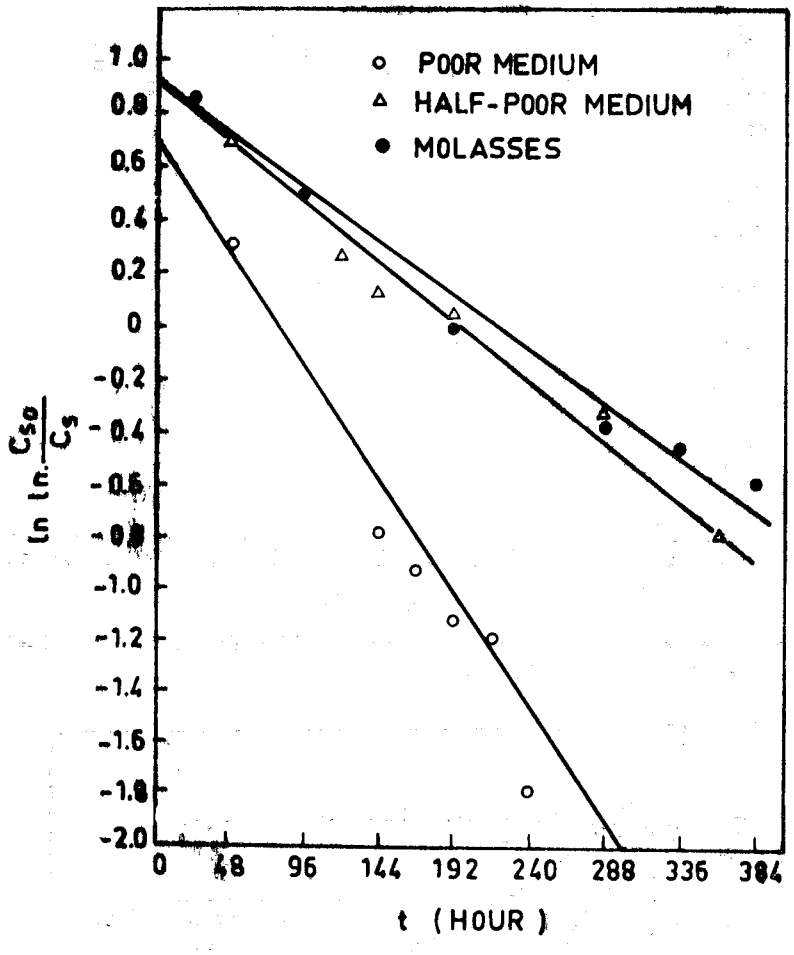

Figure 4. Calculation of the reaction and deactivation rate constants. (C_{so}=100, g/l, τ=2.38 hr., W=12.5 g, T=30°C, pH=5, d_o=3.5 mm, ε=0.35).

4.2. Calculation of the deactivation and reaction rate constant:

Table 1 shows the adectivation and reaction rate constants and the half-life values for different medium compositions It can be seen that k_d decreases as the medium gets richer. The increase of the half-life, which indicates the usability period, is also apparent. The rich medium also provides smaller decativation rates, higher reaction rates and longer periods of stable operation.

Table 1. The values of activity, half-life, reaction deactivation rate constants for various fermentation media (C_{so}=100 g/l, T=30°C, pH=5, d_o=3.5 mm, τ=2.38 hr., W=12.5 g,ε=0.35).

	$k_d(hr^{-1})$	$t_{1/2}$(hr)	$k\left(\frac{cm^3}{hr.g.d.c}\right)$	a
Poor Medium	$9.33x10^{-3}$	74.3	4.68	e-0.00933t
Half-Poor Medium	$4.58x10^{-3}$	151.3	5.70	e-0.00458t
Molasses	$4.13x10^{-3}$	167.8	5.76	e-0.00413t

4.3. Comparison of the free and the immobilized systems:

Table 2 shows the productivity values and the ethanol concentrations for the free and the immobilized microorganisms. It can be observed that the productivity of the column system is much higher (30 to 40 times for τ = 0.67 hr) than that of free system.

Table 2. Comparison of free and immobilized microorganisms. (T=30°C, pH=5, W=8.75 g,ε=0.35)

C_{so}	Free S. Cerevisiae		τ = 0.67 hr.		İmmobilized S. τ = 2.38 hr.		τ = 3.70 hr.	
	P_m(g/l)	Pr.(g/l.hr.)	P_e(g/l)	Pr.(g/l.hr.)	P_e(g/l)	Pr.(g/l.hr.)	P_e(g/l)	Pr.(g/l.hr.)
50	23.00	0.958	21.00	29.00	–	—	—	—
75	25.00	1.042	28.00	41.00	—	—	—	—
100	40.00	1.677	31.00	46.26	40.00	16.80	44	11.89
125	31.25	1.303	31.40	46.86	41.62	17.49	52.5	14.19

DISCUSSION AND CONCLUSIONS

The composition of the fermentation medium is an important factor for the production of ethyl alcohol by immobilized microorganisms. Figure 3 and Table 1 illustrates that serious deactivation takes place with the poor medium. When molasses solution is used, deactivation is again observable. Thus, addition of vitamins and salts was necessary for high productivity. Another way to relieve this problem is periodical regenaration. The results obtained clearly demonstrates the superiority of the immobilized system over the free microorganism. This is due to the fact that the microorganism concentration can be made very high in the column system. High flow rates are also possible since no entrainment takes place.

Finally, it can be stated that when kinetic parameters are to be investigate in the column system, the poor medium should be used so that only the enzymatic activities are involved.

NOMENCLATURE AND ABBREVIATIONS

C_s = Cubstrate concentration (g/l)
F_s = Feed flow rate (ml/min)
k = Reaction rate constant $\left(\frac{cm^3}{gr.h.d.c.}\right)$
k_d = Deactivation rate constant (hr^{-1})
P_e = Product concetration in the exit stream (g/l)
$t_{1/2}$ = Half-life (hr)
ε = Void fraction
τ = Residence time (hr)
Pr. = Productivity

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