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IMMOBILIZATION OF YEAST CELLS ON VARIOUS SUPPORTS FOR ETHANOL PRODUCTION

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ABSTRACT

An immobilization technique has been developed for a packed bed fermenter which is being considered as one stage of a process for the production of fuel-grade ethanol from sugar solutions. Relatively inexpensive beech wood chips have been successfully used as the support material and relatively high cell loadings of 188 mg DW cells/g DW support have been achieved for a test system of Saccharomyces cerevisiae cultures.

No washout of adsorbed cells occurs below a superficial liquid velocity of 8.9×10^{-2} cm/s which can be increased to 9.7×10^{-2} cm/s by including up to 1% Hercofloc solution in the reactor medium during the immobilization procedure. The immobilization procedure is practically unaffected by pH and temperature in the range 3.5 to 5.0 and 22°C to 37°C respectively.

Typical ethanol productivity of 21.8g/l·hr has been obtained with wood-chip-adsorbed cells, which compares well with optimal values of 18 to 32g/l·hr obtained using free-suspension cultures in stirred-tank fermenters with cell recycle.

KEYWORDS

Immobilized yeast cells; packed-bed reactor; cell loading; critical flow velocity; wood chips; ceramic.

INTRODUCTION

Over the past few years, the growing concern about the energy crisis, which was caused by the shortage of petroleum and the resulting increase in the price of gasoline, has created a surge of interest in alternative energy sources, especially renewable ones (Sitton and colleagues, 1979; Wick and Popper, 1977; Ghose and Tyagi, 1979; Cysewski and Wilke, 1976; Larsson and Mosbach, 1979). The production of ethanol with improved biotechnology has been viewed with particular interest since it can be used directly as fuel in existing motor vehicles. However, significant improvements in ethanol production technology are necessary in order to substantially reduce the overall base cost of production.

Continuous flow, stirred reactors which lose microorganisms in the effluent are limited in throughput by cell growth rates. Immobilized cell reactors are not

limited (to the same extent) by cell washout and relatively high cell densities and throughput rates are possible. Consequently, this type of reactor is expected to give a higher volumetric fermentation capacity with possibly superior performance economics.

Little quantitative information is available regarding the key factors (particle size, pH, temperature and the critical flow velocity capable of creating hydrodynamic forces sufficient to detach the cells from the support) which influence the immobilization procedure and, hence, the operation of an immobilized yeast cell reactor for ethanol production. The purpose of this paper is to report the results of a quantitative investigation of those factors which influence the degree of cell loading (mg DW cell/g DW support) obtained during the immobilization step. Primarily, we have studied the immobilization of S. cerevisiae by adsorption on beech wood chips.

MATERIALS AND METHODS

Experimental Equipment

The packed-bed reactor used for the immobilization studies consisted of a jacketed glass column 0.8m in height and 4.7×10^{-2}m i.d. Sample withdrawal ports were located along the height of the column at 11.5×10^{-2}m intervals.

During immobilization, a concentrated cell suspension (10 to 15 kg DW/m^3) was circulated from a surge tank through the packing in upflow. The surge tank was equipped with a temperature indicator-controller by means of which the contents were maintained at the desired test temperature. The column reactor together with the support material and connecting tubes was pre-sterilized in an autoclave at 121°C for 30 min.

Microorganism

Saccharomyces cerevisiae NRRL Y-132 was obtained from the NRRL, USDA (Peoria, Illinois). The culture was maintained on malt extract-yeast extract-glucose-peptone (MYGP) slants. Prior to experimental use, cells were grown aerobically for 24 hrs in a shaker incubator at 30°C in 250ml flasks containing 100ml of medium containing 1% glucose, 0.5% peptone, 0.3% yeast extract and 0.3% malt extract (pH 5.0). The concentration of cells was increased to 10 to 15g DW/l by batch centrifugation.

Supports

(a) Wood chips (beech) were obtained from Abitibi Paper Company Ltd., Sheridan Park, Ontario.
(b) Ceramic (Diatomite) was obtained from Eagle Pitcher Industries Inc., Cincinnati, Ohio.

The beech wood was used in the form of chips of various sizes. The chips were initially heat treated in boiling water for 4 hours followed by soaking in 10% (v/v) ethanol for 2 hours and in boiling water for another 1 hour to remove water-and alcohol-soluble compounds from the wood.

Analytical

The concentration of suspended cells was measured either by turbidimetry or by a gravimetric method. The optical density of the cell suspension was measured at 600 nm in a spectrophotometer (Turner model 330). During fermentation, ethanol concentration was determined by gas chromatography (Hewlett-Packard F&M Scientific 700). A 1.22m by 6.25 x 10^{-3}m o.d. column packed with Porapack Q (100-120 mesh) was used with a flame ionization detector. Both the injector and the detector were kept at 170°C and the column oven operated isothermally at 150°C. Helium was used as the carrier gas at a flow rate of 30ml/min.

Immobilization Procedure

During immobilization, the concentrated suspension of cells was recirculated through the packed column for about 12 hours at a superficial velocity of about 1.7 x 10^{-2}cm/s. The column was then allowed to stand for 12 hours. In order to determine the cell loading (mg DW cells/g DW support), the circuit and support were washed by passing 0.1% glucose solution through the column (once-through mode) in order to remove the non-adsorbed cells. The liquid velocity was then increased to 1.0cm/s in order to strip off the cells from the support. The dry weight of cells stripped off the support was then measured. The above procedure was repeated for various particle sizes and for different pH's and temperatures. The time course required for the immobilization was determined by monitoring the optical density of the cell suspension in the feed tank with time during the recirculation of the cell suspension through the column. Maximum cell loading was considered to have been achieved when the optical density of the cell suspension in the surge tank reached a constant minimum value for a period of at least 2 hours.

Critical Sloughing-off Velocity

Following cell immobilization by adsorption, cell-free medium was passed through the column in a once-through, upflow mode at progressively increasing superficial velocity. The optical density of the column effluent was monitored. The liquid superficial velocity at which an appreciable rise in the effluent cell concentration was first observed was taken as being the minimal value capable of creating hydrodynamic forces sufficient to detach the cells from the support, i.e., the critical sloughing-off velocity.

RESULTS AND DISCUSSION

Cell Loading

The effects of pH, temperature and particle size on cell loading onto wood chips are shown in Table 1.

TABLE 1 Effect of pH, Temperature and Particle Size on Cell Loading (Wood Chips)

Equivalent Particle[1] Size, m x 10^3	pH	Temperature °C	mg DW cells / Specific Surface area of Support mg/m	mg DW cells / g DW Support
2.12	4.5	28	2.06	65
	4.5	37	2.10	66.7
1.28	4.0	30	4.17	163.2
	5.0	30	4.07	161.0
	5.0	22	3.98	157.9
1.22	4.0	30	4.25	188

It is evident that there is little effect of pH and temperature on the amount of cells adsorbed in the range 4.0 to 5.0 and 22°C to 37°C, respectively. However, as the size of the support [1] is decreased from 2.12 x 10^{-3}m to 1.2 x 10^{-3}m, the cell loading increases significantly from about 70mg DW/g DW support to about 190mg DW/g DW support. This increase in cell loading is due to increased availability of surface for immobilization as shown by the figures for the mg DW cells adsorbed per unit specific surface area of support. The cell loading onto the ceramic support is shown in Table 2.

TABLE 2 Effect of Temperature on Cell Loading (Ceramic Support)

Particle Size m x 10^3	pH	Temperature	mg DW cells / g support
2	4.5	28	14.2
	4.5	30	16.2
	4.5	37	14.8

[1] The equivalent particle size is defined as the diameter of a sphere with the same surface area as the chip. It is given by $d_p = w[d_c l_c + 0.5\, d_c^2]^{0.5}$ (Satterfield, 1970), where w is the shape factor, d_c is the diameter of the chip and l_c is the length of the chip.

From Table 2, the data for cell loading show that there is little effect of temperature in the range 28°C to 37°C. Comparing the results of Tables 1 and 2, cell loading onto wood chips is seen to be significantly higher than for the ceramic support. This is in agreement with the findings of Durand and Navarro (1978) who also found that the cell loading varies markedly for different supports.

Effect of Liquid Flow Rate

Figure 1 shows a typical time course of immobilization.

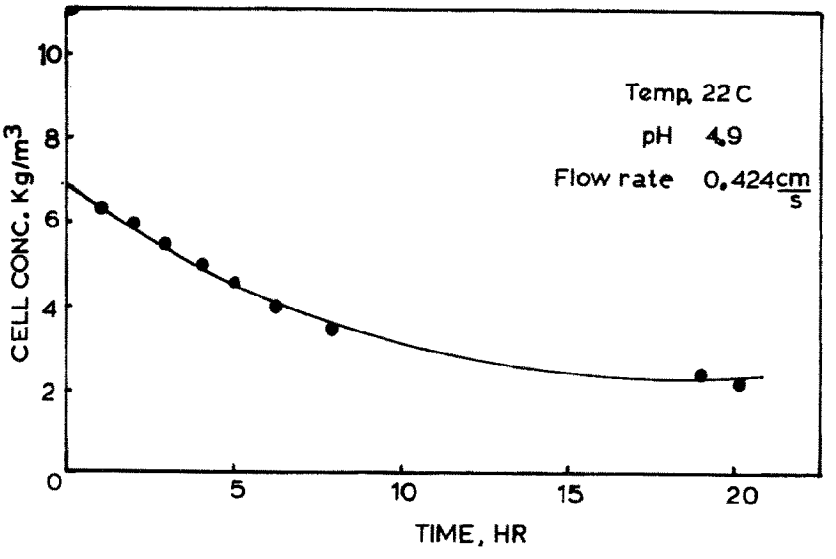

Figure 1. Decrease in free-suspension cell concentration in surge tank during immobilization procedure.

As can be seen from Fig. 1, the immobilization is time dependent. Generally, a period of 10 to 12 hrs is required for the adsorption to become fully established.

Figure 2 shows that the adsorption of yeast cells onto wood chips is a reversible process. As the superficial flow rate of the cell suspension is increased from 0.086 cm/s to 0.424 cm/s, all the cells are practically stripped off the support in a period of less than 2 hours, mainly in this case because sufficient time was not allowed for the immobilization to become fully established. When the flow velocity was then decreased to 0.086 cm/s, and maintained at this velocity for 10hrs, the cells become readsorbed and only a smaller percentage of cells become stripped-off the support when the flow velocity was once again increased to 0.424 cm/s.

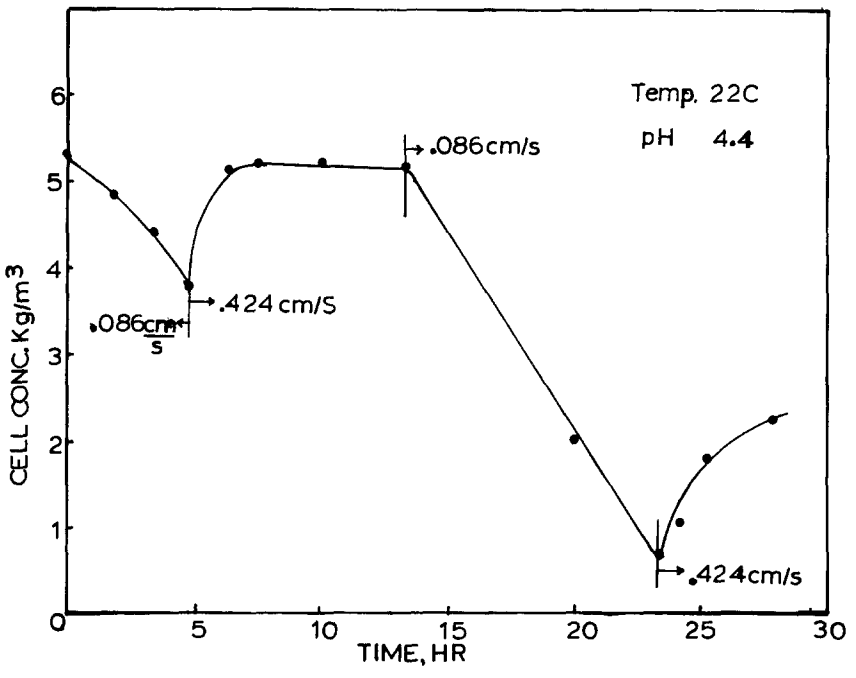

Figure 2. Effects of liquid flow velocity on cell retention in packed bed. Free-suspension cell concentration measured in surge tank during recirculation mode operation. Increase in velocity (at 5 and 23 hours) to above the critical sloughing-off velocity strips cells from support. Cell desorption is reversible; velocity reduction to normal operating range (at 13 hours) results in re-adsorption.

It is important to determine the critical sloughing-off velocity in a packed column of adsorbed cells since liquid velocities greater than the critical velocity will result in a significant amount of cells being washed off the support. Values of the critical sloughing-off velocities are shown in Table 3.

TABLE 3 Critical Sloughing-off Velocities

Support	pH	Critical Liquid Superficial Velocity cm/s x 10^2
Wood chips	3.5	8.83
	4.0	8.83
Ceramic	4.0	9.17

It is to be noted that these velocities are significantly greater than the terminal setting velocity of the cells which is on the order of 1.0×10^{-6}m/s.

By recircling up to 1% Hercofloc™ (a flocculant) solution through the packed column after immobilization, the critical sloughing-off velocity was found to increase, as shown in Table 4.

TABLE 4 Effect of Hercofloc Recirculation Through the Packed-Bed Reactor (Wood Chips)

Hercofloc Concentration (w/v)%	Critical Liquid Superficial Velocity cm/s x 10^2
0.0	8.83
0.2	9.42
0.5	9.30
1.0	9.72

Ethanol Production

Preliminary tests have been conducted using the immobilized yeast cell, packed bed reactor to produce fuel-grade ethanol from a glucose medium. A typical preliminary ethanol productivity of 21.8g/l·hr has been obtained with an effluent ethanol concentration of 60.24g/l using wood chips as the support.

CONCLUSIONS

An immobilization technique has been developed for a packed bed fermenter which is being considered as one stage of a process for the production of fuel-grade ethanol from sugar solutions. Relatively inexpensive wood chips have been successfully used as the support material and relatively high cell loadings have been achieved for a test system of glucose/yeast cultures.

Typical preliminary ethanol productivity of 21.8 g/l·hr has been obtained which compares favourably well with optimal values of 18 to 32 g/l·hr obtained for free-suspension cultures in stirred-tank fermenters with cell recycle (Ghose and Tyagi, 1979; Cysewski and Wilke, 1976). No washout of cells occurs below a superficial liquid velocity of 8.9×10^{-2}cm/s which can be increased to 9.7×10^{-2}cm/s by recirculating up to 1% Hercofloc solution in the packed-bed reactor during the immobilization procedure. The immobilization procedure is practically unaffected by pH and temperature in the range 4 to 5 and 22°C to 37°C, respectively.

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