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ETHANOL PRODUCTION BY IMMOBILIZED CELLS OF ZYMOMONAS MOBILIS

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SUMMARY

Columnar reactors containing immobilized cells of Zymomonas mobilis were utilized for the continuous production of ethanol from glucose. Two different immobilization strategies were investigated. In one case, cells were entrapped in borosilicate glass fiber pads, while in the other, cells were immobilized via flocculation. The reactors were operated in both the fixed-bed and expanded-bed manner. Ethanol productivities as high as 132 g/ℓ·h were achieved. Data obtained from studies employing 5.0 and 10.0% glucose concentrations are presented. Problems encountered during the operation of the continuous, immobilized cell reactors are discussed.

INTRODUCTION

In order for the continuous, fermentative production of ethanol as a fuel or a chemical feedstock to be of practical significance, the following criteria must be met: (1) high conversion of substrate to ethanol, (2) high concentration of ethanol in the product stream, and (3) high productivity.

The development of a system which meets the criteria outlined above hinges on overcoming two limitations that appear to be common to conventional ethanol-yielding fermentations — low cell concentration and ethanol toxicity. A number of different strategies have been tested in an effort to overcome these limitations. Cell recycle combined with operation under reduced pressure has been employed in a continuous ethanol fermentation to achieve this objective (Cysewski and Wilke, 1978). In this system, an ethanol productivity of 80.0 g/ℓ·h was obtained. Immobilization of cells onto solid supports has also been used to maintain high biomass concentrations in certain fermentation systems (Ghose and Bandyopadhyay, 1980).

The purpose of this paper is to discuss recent work aimed at developing a continuous ethanol-producing bioreactor employing immobilized cells of

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Zymomonas mobilis. Z. mobilis was chosen on the basis of its high yield of ethanol from glucose and its high specific ethanol productivity (Rogers et al., 1979). The ease with which Z. mobilis can be immobilized was also a major reason for selection of this organism.

MATERIALS AND METHODS

Culture and Media

Zymomonas mobilis ATCC 10988 was maintained on liquid medium containing 5.0% (w/v) glucose and 0.5% yeast extract (Difco). For ethanol production studies in the immobilized cell bioreactors, the same concentration of yeast extract was used; however, the concentration of glucose in this case was either 5.0 or 10.0%.

Immobilization Procedures

Fiber entrapped bioreactor. Approximately 10.0 ml of borosilicate glass fiber pad squares (approximately 2.0 mm x 2.0 mm) was added to a glass column (1.27 cm I.D. x 30.48 cm). The bottom inlet to the column (Fig. 1A) was then connected to a glass test tube (18 x 160 mm) via glass and rubber tubing. Subsequently, the entire apparatus was sterilized by autoclaving at 121°C and 15 psi for 25 min.

Flocculent bioreactor. Approximately 10.0 ml of polystyrene beads (1.0 mm diameter) was added to the bioreactor depicted in Fig. 1B. The total reactor height was 58.42 cm. The bottom glass section (22.86 cm) tapered from 2.54 cm at the top to 1.27 cm (internal diameter) at the base. The remainder of the column had an internal diameter of 2.54 cm. Sampling ports were located where indicated in Fig. 1B. The column was connected to a glass test tube (18 x 160 mm) and sterilized as described above.

Experimental Procedure

A flask containing 100 ml of growth medium (5.0 or 10.0% glucose) was inoculated with 1.0 ml of a working culture of Z. mobilis. The culture was then incubated at 30°C for 48 h in a gyratory shaker (100-rpm shake, 2-in. stroke).

Ten milliliters of the 48-h cell suspension and 10.0 ml of fresh medium were then added to either the fiber entrapped bioreactor or the flocculent bioreactor. The bioreactor was kept at room temperature (approximately 24°C). At 24-h intervals on three successive days, the medium contained in the bioreactor was drained down to the top of the bed (i.e., the styrene beads or borosilicate glass fiber pads just remained submerged). A volume of 10.0 ml

of fresh medium was then added to the column. After 24 h, a continuous flow (1.8 to 10.0 ml/min) of sterile medium was metered through the column using a peristaltic pump. At daily intervals, samples were withdrawn, clarified by centrifugation at 1000 x g for 10 min at 4°C, and the supernatants analyzed for ethanol and glucose.

Analytical Procedures

The methods used to assay for ethanol and glucose were essentially those of Bostick and Burtis (1980) and Tiffany et al. (1972), respectively.

RESULTS AND DISCUSSION

Flocculent Reactor

During the startup phase (i.e., daily batch-feeding) of the flocculent reactor, samples of the polystyrene beads were aseptically removed and observed by phase contrast microscopy. No evidence of significant attachment to the beads was obtained. However, a marked tendency toward bacterial floc formation was observed. Once continuous operation of the bioreactor was initiated, floc formation became more pronounced. The size of the individual floc particles reached macroscopic dimensions (approximately 1.0 mm in diameter), and the density of the particles was such that a volumetric flow rate of 10.0 ml/min tended only to expand the bed and did not wash the floc out of the bioreactor. As was observed in the startup phase of operation, there was no apparent attachment to the styrene beads.

The above pattern of floc formation and lack of attachment to the styrene beads was noted when either 5.0 or 10.0% glucose medium was employed. The concentrations of ethanol produced at 5.0 and 10.0% glucose concentrations are shown in Fig. 2. In both cases, the volumetric flow rate of the medium through the bioreactor was 1.8 ml/min. In this graph, day 0 represents the time at which continuous operation of the bioreactor was initiated. From the figure, it can be seen that no ethanol was detected during the first two days of operation. However, by day 3 a small but significant amount of ethanol was found and the concentration of ethanol in the effluent stream increased dramatically thereafter. The residual glucose profiles (data not given) during the courses of the experiments were as expected (i.e., no glucose removal was detected during the first two days of operation, but the residual glucose levels fell off rapidly during the remainder of the experiment). The rapid increase in ethanol concentrations observed after day 3 was accompanied by a dramatic increase in the amount of flocculent

biomass observed in the bioreactor. The productivities (in g ethanol produced/ℓ reactor volume·h) calculated from the ethanol levels observed on day 6 are given in Table 1. The productivities obtained were 70.8 and 79.9 when the bioreactor was fed 5.0 and 10.0% glucose, respectively. The values for reactor volume used in these productivity calculations were those defined by the geometric boundary of the expanded bed (0.03 and 0.04 ℓ for the 5.0 and 10.0% glucose concentration cases, respectively). It was shown that no measurable change in ethanol concentration occurred between the top of the expanded bed and the reactor vessel liquid outlet port.

Reactor operation at a flow rate of 1.8 ml/min was not continued past day 6 due to problems arising from continued microbial growth. That is, as rapid accumulation of dense flocculent biomass occurred, a flow rate of 1.8 ml/min was unable to maintain the bed in an "expanded" state. Medium channeling and gas (CO_2) bubble accumulation within the bed occurred with intermittent turbulent disruption of the bed by the disengagement of large gas bubbles. This problem was alleviated by increasing the volumetric flow rate to 10.0 ml/min. It should also be mentioned that the 5.6-fold increase in flow rate only resulted in a 3.0-fold and 3.7-fold decrease in the concentration of ethanol in the effluent stream when the column was fed 5.0 and 10.0% glucose, respectively. The increase in flow rate thus led to an increase in reactor productivity – 132.0 g/ℓ·h and 120.0 g/ℓ·h for the reactors being fed 5.0 and 10.0% glucose, respectively (see Table 1).

Fiber Entrapped Bioreactor

During the initial startup phase of the fiber entrapped bioreactor, samples of the medium and bed material were aseptically removed and observed by phase contrast microscopy. In this case, cells were observed "attached to" or caught between the borosilicate fibers of the bed material. The vast majority of the cells found free in the medium occurred either singly or in pairs. Once continuous operation of the bioreactor was initiated, the number of cells associated with the bed material increased rapidly as indicated by microscopic examination and by increase in the fiber pad thickness. (It should be mentioned that some of the increase in thickness is probably due to CO_2 being trapped within the fiber pads). At no time during the course of reactor operation was bacterial flocculation observed.

The concentration of ethanol produced by a bioreactor being fed the 5.0% glucose medium is shown in Fig. 3. Again, it can be seen that there

is a 2-day period during which ethanol was not detected in the exit stream. This period is followed by one characterized by a rapid increase both in the ethanol concentration of the exit stream and the bed-associated biomass. The productivity calculated from the ethanol level observed on day 7 was 68.4 g/ℓ·h (this productivity was calculated at a volumetric flow rate of 1.2 ml/min). The volumetric flow rate was then increased from 1.2 to 3.3 ml/min (i.e., a 2.8-fold increase flow rate), which resulted in a 2.2-fold decrease in the effluent ethanol concentration. The bioreactor productivity therefore increased to 85.5 g/ℓ·h.

CONCLUSIONS

In studies employing immobilized cells of S. cerevisiae, reactor productivities on the order of 25.0 g/ℓ·h were obtained (Ghose and Bandyopadhyay, 1980). Our initial results demonstrate that much higher reactor productivities (120.0 to 132.0 g/ℓ·h) can be attained in bioreactors containing immobilized cells of Z. mobilis. The utilization of immobilized cells allows for the maintenance of high biomass concentrations without requiring a cumbersome cell recycle step. Additionally, a columnar reactor configuration theoretically allows for plug flow of the medium through the column and thus minimizes problems associated with ethanol toxicity.

A number of studies aimed at the development of a continuous ethanol-producing bioreactor are now under way. These studies include: further characterization of the flocculation and attachment phenomena; elucidation of the relationship between film-growth and fermentation; improvement in bioreactor design; utilization of other substrates; and improvement of ethanol yield, substrate conversion, and reactor productivities.

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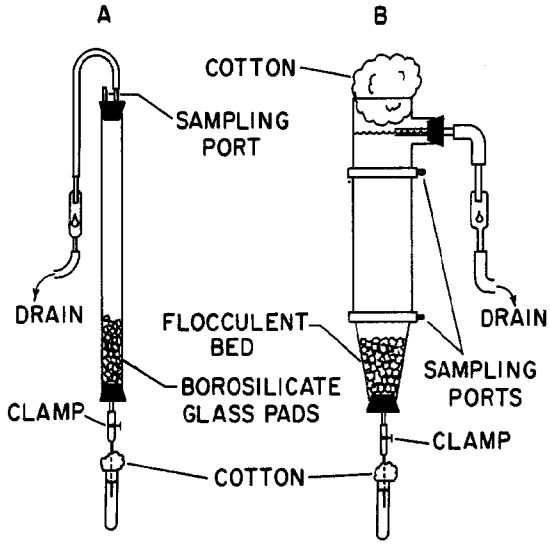

FIGURE 1. BIOREACTORS EMPLOYED IN THIS STUDY. A, FIBER ENTRAPPED BIOREACTOR. B, FLOCCULENT BIOREACTOR.

% GLUCOSE	FLOW RATE (mℓ/min)	% ETHANOL (EFFLUENT)	PRODUCTIVITY (g/ℓ·h)
5	1.8	1.97	70.8
5	10.0	0.66	132.0
10	1.8	2.96	79.9
10	10.0	0.80	120.0

TABLE 1. REACTOR PRODUCTIVITIES OF THE FLOCCULENT BIOREACTORS.

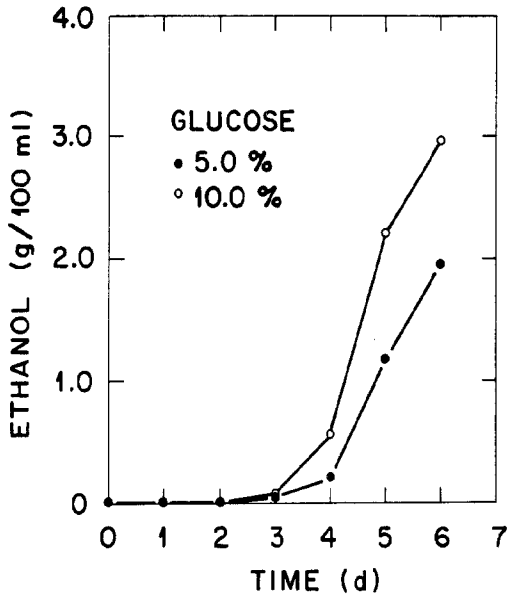

FIGURE 2. ETHANOL PRODUCED IN FLOCCULENT BIOREACTORS WHICH WERE FED 5.0% AND 10.0% GLUCOSE. FLOW-RATE WAS 1.8 mℓ/min.

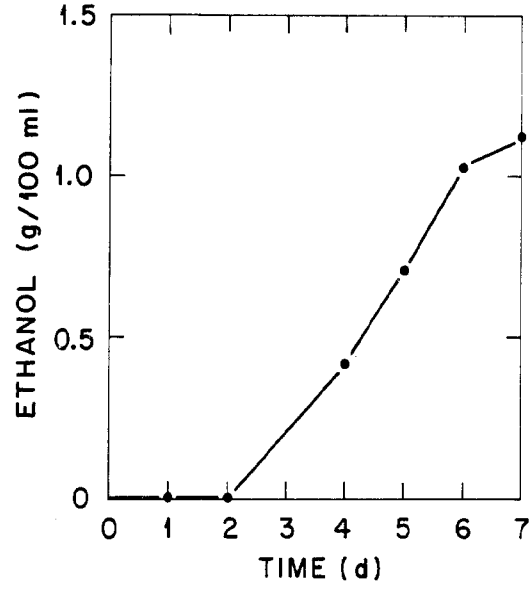

FIGURE 3. ETHANOL PRODUCED IN A FIBER ENTRAPPED BIOREACTOR WHICH WAS FED 5.0% GLUCOSE.