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Ethanol production by immobilized whole cells of *Zymomonas mobilis* in a continuous flow expanded bed bioreactor and a continuous flow stirred tank bioreactor

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Continuous ethanol fermentation by immobilized whole cells of *Zymomonas mobilis* was investigated in an expanded bed bioreactor and in a continuous stirred tank reactor at glucose concentrations of 100, 150 and 200 g L^{-1}. The effect of different dilution rates on ethanol production by immobilized whole cells of *Zymomonas mobilis* was studied in both reactors. The maximum ethanol productivity attained was 21 g L^{-1} h^{-1} at a dilution rate of 0.36 h^{-1} with 150 g glucose L^{-1} in the continuous expanded bed bioreactor. The conversion of glucose to ethanol was independent of the glucose concentration in both reactors.

Keywords: continuous flow reactor; ethanol; expanded bed reactor; immobilization; *Zymomonas mobilis*

Introduction

The fermentative production of ethanol from carbohydrates has been accomplished traditionally by yeasts. However, *Zymomonas mobilis* converts sugar to ethanol more quickly and efficiently [2, 5]. Compared to yeasts the bacteria showed higher ethanol yield and much higher specific ethanol productivity. Much of the current research in the fermentative production of ethanol is directed towards the development of continuous flow bioreactor systems using immobilized whole cells. In addition to achieving the process advantages of continuous fermentation, it is hoped that the ethanol productivity can be increased by overcoming two major limitations of conventional ethanol-producing fermentation processes, *viz*, low cell concentrations in the bioreactor and the inhibitory effect of ethanol on cells. The expanded bed bioreactor has potential as a continuous fermentation system [1]. This communication deals with the fermentation process with immobilized whole cells of *Zymomonas mobilis* in a continuous mode. The performance of the expanded bed bioreactor has been compared with a mechanically stirred tank bioreactor.

Materials and methods

Organism and culture maintenance

Zymomonas mobilis MTCC 92 (ATCC 10988, NCIM 2428) was grown on an agar medium containing (in g L^{-1}): glucose: 20, yeast extract 5, $MgSO_4 \cdot 7H_2O$ 0.5, NaH_2PO_4 1 and $(NH_4)_2SO_4$ 1. The initial pH of the medium was adjusted to 6.2–6.5. The organism was subcultured at regular intervals of 15 days and preserved at 4 ± 1°C.

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Cultivation medium and culture conditions

The cultivation media contained (in g L^{-1}): glucose 50, 100 or 150, yeast extract 5, $(NH_4)_2SO_4$ 1, NaH_2PO_4 1, $MgSO_4$ 0.5 and $CaCl_2 \cdot 2H_2O$ 1. The initial pH of the medium was adjusted to 5.5 to 5.6 with 1 M NaOH and the temperature was maintained at 30°C. For higher glucose concentrations, glucose and other medium constituents were autoclaved separately and added to the culture flask aseptically.

Immobilization of Zymomonas mobilis

Cells in exponential phase were centrifuged at 10000 rpm for 20 min [4]. A solution of 4% pectin in water was prepared, adjusting the pH to 8.5 with 0.7 N NH_4OH. To 10 g of 4% pectin solution (pH 8.5) containing 0.1 g dry weight equivalent of cells of *Z. mobilis*, 4% of iron oxide was added to increase the specific density. The mixture was then used to prepare beads following the technique given by Navarro *et al* [3]. The cell and pectin mixture was pumped through a hypodermic needle (0.5–1 mm i.d.) into a mixture of 0.2 M $CaCl_2 \cdot 2H_2O$ and $Na_2B_4O_7 \cdot 10H_2O$ with gentle stirring at 30°C. The beads were stabilized by curing them in a calcium chloride and borax mixture for 24 h at 4°C. Fermentation medium containing 20 g glucose L^{-1} was used to incubate beads containing *Z. mobilis* for 24 h at 30°C. After that the beads were separated and washed with distilled water to eliminate free and weakly attached cells from the immobilized catalysts.

Estimation of glucose, ethanol and free cell mass

Glucose, ethanol and free cell concentrations were determined by methods described earlier [6].

Bioreactor systems

Expanded bed bioreactor: A schematic diagram of the continuous expanded bed bioreactor system is shown in Figure 1. The 350-ml expanded bed bioreactor column was made of glass. The system included a recycle device for the beads coming out of the bed. The column was sur-

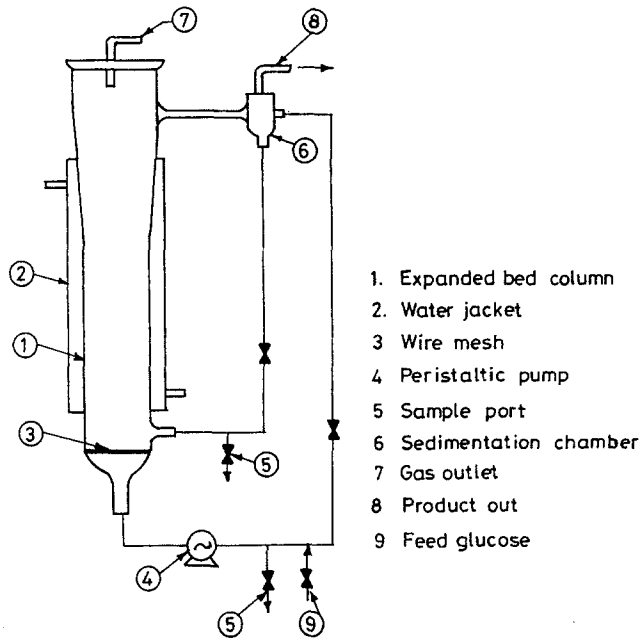

Figure 1 Schematic diagram of the continuous expanded bed bioreactor system.

rounded by an annular water jacket through which water at 30°C was continuously circulated. The system was also equipped with pH control. A peristaltic pump (Ismatech MSC-WM5, Glattburg, Zürich, Switzerland) circulated the liquid medium and also expanded the bed inside the reactor. The expanded bed bioreactor was sterilized empty and was then filled with medium and 0.1 g (on a dry weight basis) of immobilized cell mass. The circulation pump was switched on to start the operation. Dilution rates were varied from 0.08 to 1.44 h^{-1}. Dilution rate was calculated by dividing the flow rate of liquid medium by the total working volume of the reactor. Sterile feed medium was pumped continuously to the column by another peristaltic pump (Ismatech MSC-WM5) covering a dilution range of 0.08–1.44 h^{-1}. Samples were taken from the effluent at regular intervals and ethanol, glucose and free cell concentration were determined as described earlier [6]. After the dilution rate was set, the glucose and ethanol concentrations were monitored until no change was observed over a 6–8 h span. This was taken to be the steady state condition. Ethanol productivity was calculated from the ethanol concentration in the effluent multiplied by the feed rate and divided by the liquid volume in the reactor. The yield coefficient was calculated by dividing the ethanol produced by glucose consumed.

Stirred tank reactor: A mechanically stirred tank reactor (total volume 2 L, model Biostat M, B Braun, Melsungen AG, Germany) was used with immobilized cells to produce ethanol in a continuous mode. In order to achieve adequate mixing of the immobilized beads the impeller was operated at 160 rpm. The working volume was 1 L. An immobilized cell mass of 0.16 g (dry weight equivalent) was loaded in the reactor and the temperature was maintained at 30°C.

Results and discussion

Medium was continuously fed into the system at a rate based on the desired dilution rate. The ethanol productivity was affected by the dilution rate as well as by the sugar concentration in the feed. The effect of dilution rates ranging from 0.08 to 1.44 h^{-1} on ethanol fermentation kinetics was studied separately for sugar concentrations of 100, 150 and 200 g L^{-1} in both bioreactors. In the expanded bed bioreactor, the highest ethanol concentration obtained was 68.7 g L^{-1} (92.9% glucose conversion) at a dilution rate of

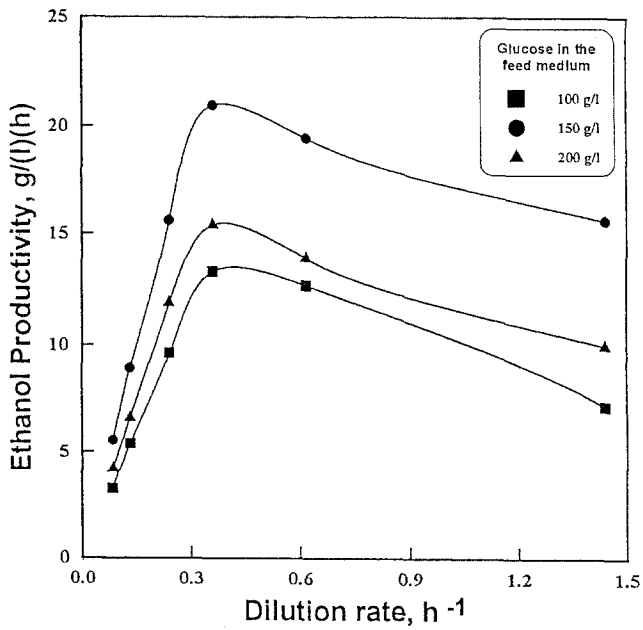

Figure 2 Effect of dilution rate on ethanol productivity for different glucose concentrations in the continuous expanded bed bioreactor.

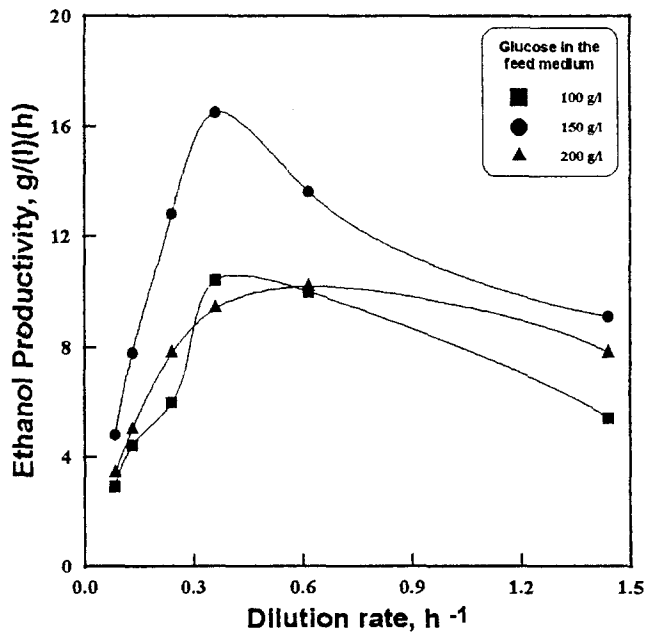

Figure 3 Effect of dilution rate on ethanol productivity for different glucose concentrations in the continuous mechanically agitated tank reactor.

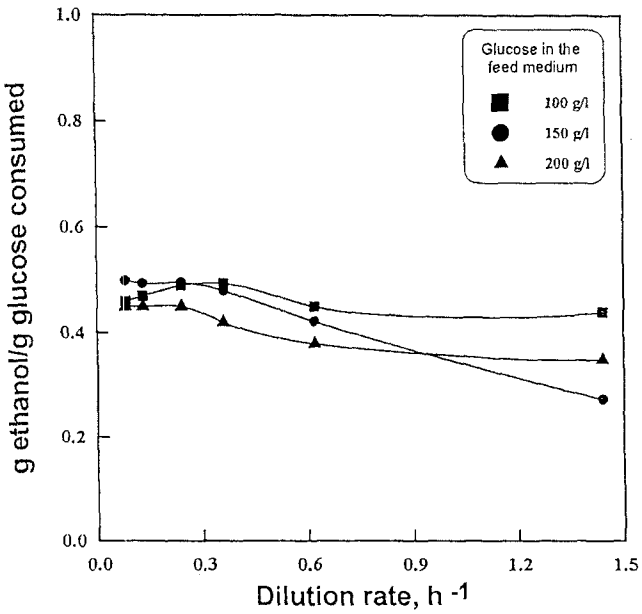

Figure 4 Relationship between yield coefficient (g of ethanol g^{-1} of glucose) and dilution rate for the continuous expanded bed bioreactor.

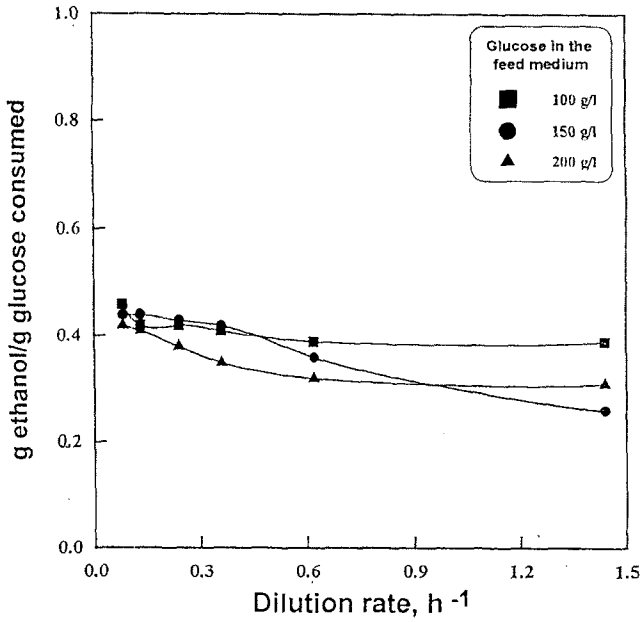

Figure 5 Relationship between yield coefficient (g of ethanol g^{-1} of glucose) and dilution rate for the continuous mechanically agitated tank reactor.

0.08 h^{-1} with 150 g of glucose L^{-1} in the feed. Similarly, for the mechanically agitated tank bioreactor, the highest ethanol concentration of 59 g L^{-1} (89.9% glucose conversion) was observed at a dilution rate of 0.08 h^{-1} using 150 g of glucose L^{-1} in the feed. In the expanded bed bioreactor, the percentage of glucose conversion and the ethanol concentration were higher at any dilution rate than those obtained in the mechanically agitated tank bioreactor (data not shown). This was true for any glucose level in the feed. Lesser production of ethanol using the mechanically agitated tank bioreactor was probably due to greater shear

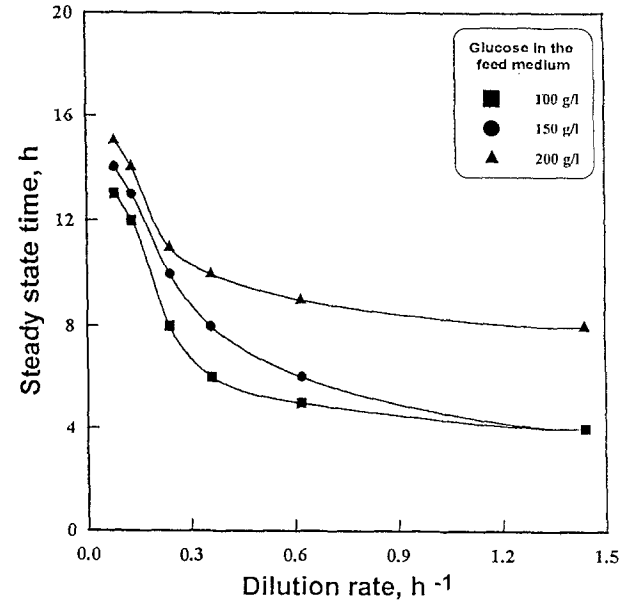

Figure 6 Effect of dilution rate on steady state time (h) for different glucose concentrations in the continuous expanded bed bioreactor.

stress on the immobilized whole cells in pectin beads that resulted in disintegration of beads.

Ethanol productivities were also calculated for each condition at steady state and are shown in Figures 2 and 3. In the expanded bed bioreactor, higher ethanol productivity was achieved at a dilution rate of 0.36 h^{-1} for all concentrations of glucose in the feed (glucose concentration in the feed between 100 g L^{-1} and 200 g L^{-1}, Figure 2). Above and below this rate ethanol productivity was reduced sharply. On the other hand, using the mechanically agitated tank bioreactor, the dilution rate of 0.36 h^{-1} was more suitable

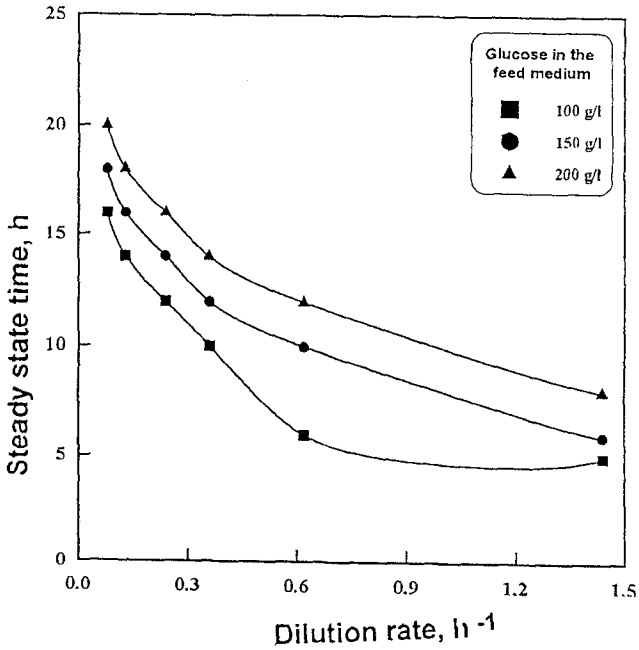

Figure 7 Effect of dilution rate on steady state time (h) for different glucose concentrations in the continuous mechanically agitated tank reactor.

for 100 and 150 g of glucose L^{-1} in the feed. In this case also ethanol productivity decreased above and below a dilution rate of 0.36 h^{-1}. However, for 200 g of glucose L^{-1} in the feed, ethanol productivity continuously increased from a dilution rate of 0.08 h^{-1} to 0.36 h^{-1} and then the productivity remained almost constant up to a dilution rate of 0.8 h^{-1} which was followed by slow decrease in productivity (Figure 3). Higher ethanol productivity was observed at a dilution rate of 0.36 h^{-1} for both the continuous flow expanded bed bioreactor and the continuous flow mechanically agitated tank bioreactor: 20.95 g L^{-1} h^{-1} for the continuous flow expanded bed bioreactor and 16.6 g L^{-1} h^{-1} for the continuous flow mechanically agitated tank bioreactor.

The g ethanol per g glucose consumed was also calculated for each condition at steady state (Figures 4 and 5). The conversion of glucose to ethanol was almost 90% of the theoretical value and it showed a marginal variation in the dilution rates between 0.08 h^{-1} and 0.36 h^{-1} in case of the expanded bed bioreactor considering a particular glucose concentration in the feed. In either reactor g ethanol per g glucose consumed remained almost constant for a glucose feed of 100 and 150 g of glucose L^{-1} in dilution rates between 0.08 and 0.36 h^{-1}. For a feed of 150 g of glucose L^{-1}, above 0.36 h^{-1} dilution rate, g ethanol per g glucose consumed decreased to approximately 0.3 g ethanol produced per g of glucose consumed (Figures 4 and 5). For 200 g of glucose L^{-1} in the feed, g ethanol per g glucose consumed gradually decreased as the dilution rate increased in the expanded bed bioreactor, whereas this value was decreased at dilution rates between 0.08 and 0.62 h^{-1} and remained constant beyond a dilution rate of 0.62 h^{-1} for the mechanically agitated tank bioreactor. For 100 g of glucose L^{-1} in the feed, the yield was almost constant at dilution rates above 0.62 h^{-1} for the mechanically agitated tank bioreactor. Figures 6 and 7 show the relationship between steady state time and dilution rate. The continuous flow expanded bed bioreactor took less time to reach steady state than the continuous flow mechanically agitated tank bioreactor. In both reactors the time to reach the steady state increased with the glucose concentration in the feed.

In summary, the expanded bed bioreactor results better than the mechanically agitated tank bioreactor. The maximum ethanol productivity achieved in the expanded bed bioreactor was 20.95 g L^{-1} h^{-1} for a dilution rate of 0.36 h^{-1} with 150 g of glucose L^{-1}.

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