pH INFLUENCE ON ETHANOL PRODUCTION
AND RETAINED BIOMASS IN A PASSIVELY
IMMOLIZED Zymomonas mobilis SYSTEM

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SUMMARY

A broad pH range of 4.5-7.5 for maximum ethanol productivity and ethanol yield was observed with a passively immobilized Z. mobilis system. Total retained biomass (as suspended flocs and entrapped cells) was >50 g/l for medium pH values between 4.0-8.0. The entrapped cells to suspended flocs ratio was highest at pH 4.0, whereas at pH above 5.2 it was close to 1.0. The observed enhancement of cell immobilization on the packing support at low pH seemed to be related to formation of bacterial filaments.

INTRODUCTION

A variety of systems has been proposed for high productivity continuous ethanol production with Zymomonas mobilis (Rogers et al., 1982; Baratti and Bu'Lock, 1986). Among them those based upon passive immobilization of cells (adhesion to solid supports and flocculation) have received less attention because of their doubtful stability against pH and temperature changes, variation of media, high flow rate or stop of flow during a period of time (Margaritis and Merchant, 1984; Klein and Kressdorf, 1986). High ethanol productivities have been attained using adsorbed (Arcuri, 1982; Krug and Daugulis, 1983; Amin et al., 1987) and flocculated cells of Z. mobilis even in mineral media (Toran-Diaz et al., 1984; Baratti et al., 1986), however little work has been done to evaluate the influence of the above factors on the performance of passively immobilized cell systems.

In our laboratory the use of a mixed system with immobilized Z. mobilis in a fibrous matrix and suspended flocs has shown a satisfactory performance with high ethanol productivity (nearly complete glucose
utilization) and high stability against long-term operation and temperature changes (Borrego et al., 1987). The mixture of immobilized and flocculated cells has also been stated as a good catalyst for ethanol production by others authors (Jain et al., 1985). The aim of this work was to study the effect of pH on ethanol production, total retained biomass within the reactors and stability of the system at low pH using a passively immobilized system with Z. mobilis for continuous ethanol production.

MATERIALS AND METHODS

Microbiology. Z. mobilis ZM4 (kindly supplied by Dr. J. C. Baratti, University of Provence, Marseille, France) was used and maintained on agar slants containing (g/l): glucose, 20; yeast extract, 10; KPO4H2O, 2; (NH4)2SO4, 1; MgSO4?7H2O, 0.5 and agar, 20; pH was adjusted to 5.0. Fermentation medium contained the same nutrients except that glucose concentration was 100 g/l; pH was adjusted from 3.5 to 8.0 (depending on the experiment) using H2SO4 or KOH. Glucose was always sterilized separately and aseptically mixed.

Immobilization procedure in a fibrous support (cellulose 50% and polyester 50%), bioreactor design and analytical methods were described in detail in a previous paper (Borrego et al., 1987).

Experimental Procedure. Two different sets of experiments were performed. The first one consisting of seven identical reactors operated at the same time by using a multichannel peristaltic pump. Continuous operation with medium at pH 5.2 was initiated at D=0.25 h-1 increasing dilution rate stepwise when steady glucose and ethanol effluent concentrations occurred until D=1 h-1. When steady state conditions were reached at this dilution rate, pH of the input feeding was changed from 5.2 to 3.5, 4.0, 4.5, 6.5, 7.5 and 8.0, respectively, so that each reactor was run at a different pH. One reactor was left as the control with fermentation medium at pH 5.2. Samples of the effluent were analyzed for ethanol and unconverted glucose during five days. At the end of the run, the total retained biomass within each reactor (as entrapped cells and suspended flocs) was obtained as dry weight of cells.

In a second experiment, a reactor was run until D=1 h-1 in the same manner. When steady state was reached pH of the input feeding was changed to 3.5, kept during four days at this value, returned to 5.2 and maintained until new steady state conditions were achieved.

EXPERIMENTAL RESULTS

Table 1 illustrates the effect of pH on the selected steady fermentation parameters. The response of the system to the pH changes was related to the degree of inhibition at the new pH value. Thus, the system took 90 hours to reach the new steady state when pH was changed from 5.2 to 3.5 (glucose conversion: 1%) and only 24 hours when it was changed from 5.2