ETHANOL PRODUCTION USING ZYMOMONAS MOBILIS IMMOBILIZED ON AN ION EXCHANGE RESIN

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SUMMARY

Ten ion exchange resins as well as activated carbon and ceramic chips were examined for their ability to adsorb cells of Zymomonas mobilis. A cationic macroreticular resin was shown to be the most efficient adsorbant and was used to immobilize cells of Z. mobilis in a column bioreactor. The bioreactor was operated with a feed glucose concentration of 100 g/L at a dilution rate of 11.2 h⁻¹ and a productivity based on void volume of 377 g ethanol/L-h was obtained with 80% substrate utilization. It was observed that the cell concentration in the bioreactor increased during continuous operation and that the form of Z. mobilis changed from single cells to filamentous forms of the bacterium. Plugging problems occurred after 200 h of operation as a result of excessive filamentous cell growth.

INTRODUCTION

One area of recent interest has been the design and operation of more efficient fermentation systems for the production of ethanol. Bioreactor designs have included systems involving vacuum fermentation, cell recycle and various processes to immobilize cells within bioreactors (Rogers et al., 1982). Traditionally the microorganisms used in these systems have been yeasts. Recently, however, many studies have also included consideration of the bacterium Zymomonas mobilis, which has been demonstrated to have some significant advantages over yeasts (Swings and De Ley, 1977; Rogers et al., 1979). Rogers et al. (1982) presents a summary of various bioreactor systems and the ethanol productivities obtained. The highest productivity quoted is 200 g ethanol/L-h for a cell recycle system using Z. mobilis. Other researchers have obtained similar high productivities with various systems (Arcuri, 1982; Strandberg et al., 1982; Bland et al., 1982).

Daugulis et al. (1981) demonstrated that Saccharomyces cerevisiae could be immobilized on the surface of ion exchange resins and used in a continuous bioreactor for ethanol production. Such a system eliminates the need for cell recycle equipment and maintains a very high cell concentration even at high dilution rates (1.44 h⁻¹). This system is also free of many of the mass transport limitations which can arise in systems where cells are immobilized within gel matrices such as calcium alginate.
The present study is an investigation of the adsorption of Z. mobilis on ion exchange resins and the use of these adsorbed cells for the continuous production of ethanol.

**MATERIALS AND METHODS**

Zymomonas mobilis (ATCC 29191) and the liquid medium formulation of Rogers et al. (1979) were used in this study.

**Adsorption Experiments**

Polycarbonate columns with an inner diameter of 2.57 cm and a length of 31 cm were used in this experiment. Ten ion exchange resins (16-50 mesh), as well as activated carbon (20-40 mesh) and ceramic chips (20-50 mesh) were examined for their ability to adsorb cells of Z. mobilis. Resin characteristics are shown in Table 1. The packing materials were added to the columns and the resulting void fractions were found to range between 27% and 37%. These void fractions represent the void space between particles and do not include the minute volumes of the pores for those materials having a microporous structure. Because adsorbing cells partially fill these micropores, and because the bulk flow of liquid occurs between the particles, subsequent calculations are based on these values of initial effective interparticle void space. The packing materials were sterilized and conditioned in situ by pumping 10% HCl through the column, followed by sterile distilled water. A 600 ml suspension of Z. mobilis containing 2.0 g dry weight cells/L was recirculated through each of the columns until the entering and exiting cell concentrations were the same. The cell concentrations in the suspension before and after recirculation were determined, and a mass balance was used to calculate the amount of cells adsorbed. Additional experiments were conducted to investigate the effect of culture age on the extent of adsorption. The culture ages reported in Table 1 are the times between inoculation of the suspension with a 10% (v/v) culture and use in the experiment. In all cases fermentation activity in the culture had ceased prior to use in the experiment. All experiments were conducted at room temperature.

**Continuous Production of Ethanol**

One of the columns used in the adsorption studies was filled with ion exchange resin IRA 938 and sterilized and conditioned in the same manner as for the adsorption tests. Cells of Z. mobilis were allowed to adsorb onto the resins by recirculating a 1 L suspension of Z. mobilis containing 2.0 g/L of cells. A 100 g/L glucose medium with the formulation as reported by Rogers et al. (1979) but containing four times the KH₂PO₄ (to improve the buffering capacity) was sterilized and used as feed to the bioreactor. This medium was pumped through the column initially at a dilution rate based on void volume of 2.2 h⁻¹. The CO₂ production rate was monitored to give an indication of ethanol concentration. Liquid samples of the effluent were taken periodically and analyzed for glucose using the DNS method (Miller, 1959), for ethanol using a flame ionization gas chromatograph, and for cell concentration by turbidity measurements. Samples of the effluent were also examined under a light microscope to monitor any changes in the morphology of the Z. mobilis cells. When the effluent ethanol concentration had stabilized, the flowrate to the column was increased. This procedure was repeated several times until a dilution rate of 11.2 h⁻¹ had been reached. At this dilution rate only 80% of the glucose was