THE EFFECTS OF GAS SPARGING ON GROWTH AND GLUCOSE UTILIZATION OF ZYMOMONAS MOBILIS AT LOW GLUCOSE CONCENTRATIONS

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SUMMARY

In a batch culture of Zymomonas mobilis the specific growth and specific glucose uptake rates and the molar growth yields were similar under CO2 and N2 sparging. The use of N2 or N2 with nucleation agents to strip dissolved CO2 from the medium did not improve growth rates or yields or enhance glucose consumption rates. Carbon dioxide produced no direct feedback inhibition on metabolic rates. The catabolic enzymes of Z. mobilis are not allosterically controlled by CO2.

INTRODUCTION

Zymomonas mobilis is an obligately fermentative Gram negative rod. This organism possesses a split TCA cycle and an apparently intact electron transport chain, but it does not perform oxidative phosphorylation (Swings and DeLey, 1977). In the presence of oxygen, both growth rates and ethanol yields are reduced. The reduction in ethanol yields is due to a shift in end products from ethanol plus carbon dioxide to ethanol plus carbon dioxide and acetate. The ethanol intermediate acetaldehyde also accumulates aerobically (Belaich and Senez, 1965). Since oxidative phosphorylation does not occur in Z. mobilis, the observed decrease in growth rate aerobically is not due to the Pasteur effect involving the TCA and electron transport chain. Neither is the production of oxygen radicals the cause of decreased growth aerobically since both superoxide dismutase (SOD) and catalase are constitutive. Furthermore, SOD is produced at elevated levels by Z. mobilis in the presence of oxygen. The decreased growth rates in oxygen are probably due to the toxicity of the elevated levels of acetaldehyde in aerobic conditions (Bringer et al., 1984).

The effects of CO2 on metabolic rates are not fully reported in the literature. Removal of dissolved CO2 by sweeping the medium with N2 or by using protective agents has been attempted. Soy flour has been used to increase ethanol productivity rates in continuous fermentations (Ju et al., 1983). Diatomaceous earth, a nucleation agent, and nitrogen were used to increase initial

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glucose uptake rates (Burrill et al., 1983). Osman and Ingram (1985) reported that ethanol levels above 20 g/L resulted in decreased fermentation rates and a dose related loss of plasma membrane integrity. Both Ju et al. and Burrill et al. used media with initial high glucose levels which resulted in final ethanol concentrations above that which causes membrane permeabilization. Thus, these studies were not able to separate CO$_2$ from ethanol effects on metabolic rates. In batch cultures foaming caused by abundant CO$_2$ release is noted even in media containing 20 g/L glucose. At glucose levels of 100 g/L foaming becomes a serious problem.

The objective of this study was to investigate the effects of CO$_2$ on growth and glucose utilization rates of _Z. mobilis_ in batch culture. To isolate CO$_2$ effects from ethanol effects, a complex medium limited in glucose was selected to prevent the final ethanol level from rising above 10 g/L. Varying CO$_2$ or N$_2$ sparge rates, as well as, nucleation agents were employed to investigate the inhibitory effects of CO$_2$.

**MATERIALS AND METHODS**

The strain used for all experiments was _Z. mobilis_ ATCC 10988 obtained from the ATCC. The culture was maintained in a complex medium (GPY) containing (g/L): glucose, 20; yeast extract (Difco), 10; peptone (Difco), 10. Stock cultures were grown for 24 hours at 30°C and then stored at 4°C. Transfers were made every three months.

All experiments were conducted using complex medium (GPY). When needed, 0.01% bentonite and 0.2% sea sand were added prior to autoclaving. Seed cultures were grown for 16 to 20 hours (log phase) at 30°C without shaking in the same medium used for the growth study. Growth experiments were conducted in a 1 L custom built working volume fermenter. Anaerobic conditions were achieved by degassing the medium prior to autoclaving and then promptly removing the fermenter from the autoclave and sparging with either nitrogen or carbon dioxide gas. The gases were deoxygenated by passing them over 350°C copper turnings. No reducing agents were employed. Sparging rates were 50 or 250 ml/L/minute. All experiments were conducted at 30°C.

Growth was measured by optical density at 540 nanometers with a B & L Spectronic 20 spectrophotometer and converted to cell mass using a dry-weight calibration curve. Generation times were calculated according to Stainer, et al., 1986. Confidence levels were calculated from the means of all growth rates and biomass produced using the Student "t" distribution and n-1 degrees of freedom tests. Glucose was determined by the anthrone method (Hanson and Phillips, 1981). The specific substrate utilization rates were calculated from the slope of the reciprocal substrate levels _versus_ the integration of biomass with time during log phase growth. Confidence levels were calculated from the specific uptake rate plots.

**RESULTS AND DISCUSSION**

Results are summarized in Table 1. Under anaerobic conditions with N$_2$ or CO$_2$, the specific growth rates were not significantly different at the 99% confidence level. The aerobic culture had a significantly decreased growth rate at the same confidence level. The growth rates shown here are two- to four- fold higher than those reported for growth in 100 g/L glucose by King et al. (1983), Rogers et al. (1979) and Lee et al. (1979). Biomass production is also