PRODUCTION OF ETHANOL AND n-BUTANOL FROM HEXOSE/PENTOSE MIXTURES USING CONSECUTIVE FERMENTATIONS WITH SACCHAROMYCES CEREVISIAE AND CLOSTRIDIUM ACETOBUTYRICUM

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SUMMARY

Saccharomyces cerevisiae and Clostridium acetobutylicum have been used to consecutively ferment molasses (5% solids) containing added pentose sugars (30 g/l). Butanol concentrations of 6.6 g/l and 3.7 g/l have been achieved from L-arabinose and D-xylose, respectively, in the presence of 22 g/l of ethanol.

INTRODUCTION

The use of wood hydrolysates as a substrate for ethanol production by yeast is being widely investigated. Unfortunately, such hydrolysates contain, in addition to hexose sugars, pentose sugars such as D-xylose and L-arabinose which Saccharomyces spp. cannot ferment to ethanol. This results in unutilised sugar which could present an effluent treatment problem. For this reason, it would be advantageous if the pentoses could be converted to some useful product. Schneider et al (1981) have recently reported that the yeast Parachysolens tannophilus can ferment D-xylose to ethanol, but this process has not yet been proven commercially. One possible way of utilising both the pentose and hexose components of wood hydrolysates would be to produce n-butanol using Clostridium acetobutylicum, an organism which is known to ferment pentose sugars (Compere and Griffith, 1979). However, this may not represent the desired use of the hydrolysates. The present work, therefore, was carried out to ascertain whether a hexose/pentose mixture can be fermented to a mixture of ethanol and n-butanol using Saccharomyces cerevisiae and Cl. acetobutylicum. Possible options included a mixed culture or consecutive fermentations prior to product recovery, but the basic aim was for the yeast to convert the hexoses to ethanol, and the bacterium to convert the pentoses to n-butanol.
METHODS

Materials. Molasses (approx. 50% solids), obtained from New Zealand Sugar Co. Ltd. (Auckland, New Zealand), was diluted to either 5% or 10% solids and adjusted to the appropriate pH value prior to use. Sulphuric acid casein whey filtrate was obtained from New Zealand Dairy Research Institute as previously described (Maddox, 1980). Yeast extract (Oxoid Ltd., London, U.K.) was added to both media to a final concentration of 5 g/l. D-xylose, L-arabinose, acetone, ethanol, n-butanol and n-butyric acid were obtained from B.D.H. Chemicals Ltd., (Palmerston North, New Zealand).

Organisms. Clostridium acetobutylicum, N.C.I.B. 2951, was obtained from the National Collection of Industrial Bacteria, Aberdeen, Scotland. Cultures were maintained by growth on Cooked Meat Medium (Oxoid, Ltd.), containing glucose (20 g/l), at 30°C for 2 days followed by storage at 4°C. For inoculum preparation, 1 ml of this stored culture was transferred to 20 ml of Cooked Meat Medium, supplemented with 20 g/l of D-xylose, L-arabinose or lactose (depending on the sugar source in the subsequent experiment), contained in 25 ml screw-capped bottles. After heat-shocking at 70°C for 90 sec. the culture was incubated at 30°C for 24 hours prior to using 5 ml as inoculum.

Saccharomyces cerevisiae, Y16, was maintained on slopes of Potato Dextrose Agar (Oxoid, Ltd.). Inocula were prepared by inoculating 100 ml of molasses (10% solids, pH 4.5), in a 250 ml Erlenmeyer flask, and incubating at 30°C in still-culture for 24 hours. 5 ml of this culture were used as an inoculum.

Cultivation conditions. For experiments involving yeast alone, 100 ml of molasses (10% solids, pH 4.5) were dispensed in a 250 ml Erlenmeyer flask. After inoculation, fermentation proceeded at 30°C in still-culture. For experiments involving Cl. acetobutylicum alone, 100 ml of whey filtrate, pH 6.5, were dispensed in a 120 ml screw-capped bottle. After inoculation, fermentation proceeded at 30°C in still-culture. For consecutive fermentations with the yeast and Cl. acetobutylicum, 100 ml of molasses (5% solids, pH 4.5), supplemented with 30 g/l of either D-xylose or L-arabinose, were dispensed in 120 ml screw-capped bottles. Fermentation then proceeded at 30°C in still-culture as described below. Although it has been shown that increased production rates and yields of n-butanol are obtained when a positive head-space pressure is maintained during the fermentation (Maddox et al., 1981), the present experiments were performed at atmospheric pressure because of the difficulty of accurately controlling pressure on the 100 ml scale. This small scale was used in order to carry out fermentations simultaneously using identical inocula.

Analyses. These were performed as previously described (Maddox, 1980).

RESULTS AND DISCUSSION

The aim of this work was that Sac. cerevisiae should convert the hexose sugars in the mixture to ethanol, while Cl. acetobutylicum converted the pentoses to n-butanol. Hence it appeared logical that the yeast should be allowed to act first, else the bacterium would ferment