PRODUCTION OF ACETONE AND BUTANOL BY BATCH AND CONTINUOUS CULTURE OF CLOSTRIDIUM ACETOBUTYLICUM UNDER NITROGEN LIMITATION

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SUMMARY

Acetone and butanol can be produced by batch and continuous culture of Clostridium acetobutylicum on a nitrogen limited synthetic medium. When cells are grown on a high glucose concentration, conversion yields close to 0.3 g solvents/g glucose are obtained at pH 5.0. The fermentation kinetics are similar in batch and continuous cultures.

INTRODUCTION

The kinetics and regulation of acetone/butanol production by Clostridium acetobutylicum is receiving increasing attention (Prescott and Dunn, 1959; Spivey, 1978; Petitdemange et al., 1976; Gottschal and Morris, 1982). Both on synthetic and complex media two distinct acids and solvents production phases are observed. The medium composition and pH has been shown to strongly influence the fermentation selectivity (Monot et al., 1982; Bahl et al., 1982a). A minimal level of glucose and potassium was found a prerequisite for solvent production; on the other hand an excess of ammonium acetate was reported to promote acid production. However, because of the ever changing substrates and products concentrations, it is difficult to precisely assess the influence of environmental conditions on the regulation of C. acetobutylicum metabolism in a batch fermentation.

Continuous cultures at steady state, on the contrary, provide an environment with constant composition, thus allow a more definite analysis of the effect of different nutrients and metabolites on the microorganism physiology and activity. Continuous production of acetone and butanol has been recently reported in single stage chemostat at sufficiently low dilution rates. Highest productivities in continuous cultures have been obtained on complex media with high glucose and nitrogen concentrations (Wang, 1981). Significant levels of solvents were also reported in phosphate limited chemostate (Bahl et al., 1982b). In continuous cultures with ammonium limitations, on the other hand, only weak or no solvent production was observed (Gottschal and Morris, 1981; Andreisch et al., 1982).

The objective of this study is to investigate in greater detail, both in batch and continuous culture, the influence of nitrogen limitations on the metabolism of C. acetobutylicum.
MATERIALS AND METHODS

Microorganism
The organism used was *Clostridium acetobutylicum* strain ATCC 824. Spores of the culture were stored at 4°C in RCM medium ("Reinforced Clostridia Medium" - Oxoid).

Medium
The culture medium was a synthetic medium (Monot et al., 1982) containing the following components, per liter of distilled water: Glucose, 45.5 g; Ammonium acetate, 1.0 g; KH₂PO₄, 0.5 g; K₂HPO₄·3H₂O, 0.5 g; MgSO₄·7H₂O, 0.2 g; Fe·(SO₄)₂·7H₂O, 0.07 g; p-aminobenzoic acid, 1 mg; Biotin, 0.61 mg.

Fermentor
A 2 liter Biolafitte fermentor was used in this study. The agitation speed was maintained at about 200 rpm and the temperature at 35°C. The pH of the medium was controlled at 5.00 by automatic addition of 5 N NaOH.

Batch fermentation
A 10% growing culture taken at the end of the growth phase was used as inoculum. Pre-cultures were also made on the described medium. The fermentor medium was kept anaerobic by a flow of purified nitrogen before and after inoculation. The nitrogen flow was stopped once the culture was observed to be growing, since bacteria provided sufficient quantities of hydrogen and carbon dioxide to realize their own anaerobiosis. 15 ml samples were taken in ice.

Continuous fermentation
The fermentation was first carried out batchwise with the same initial concentrations of ingredients. Continuous feeding was started after 17 hours, i.e at the end of the growth phase. The dilution rate was first fixed at 0.038 hr⁻¹ and then stepwise increased up to 0.196 hr⁻¹. At each dilution rate an approximate steady state was reached after about 4 days and maintained during 2 days before changing the flow rate. The feed medium was kept free of oxygen by frequent sparging with N₂. The volume was kept constant at 1 liter by means of peristaltic pumps.

Methods of analysis
Cell concentration was estimated by optical density and by cell dry weight measurement using a predetermined correlation between optical density at 660 nm and cell dry weight.

Other analysis were made on supernatants of samples previously centrifuged at 12,000 rpm for 10 minutes. Residual glucose was determined on a Technicon autoanalyser with an hexokinase reagent. Ammoniacal nitrogen determination was based on the production of a coloured complex between ammonium, sodium salicylate and chlorine, in an alkaline medium. This analysis was also made on a Technicon autoanalyser.

Concentrations of solvents (ethanol, acetone, butanol) and acids (acetic and butyric) were determined by injecting acidified supernatants into a Intersmat IGC 121FL gas chromatograph equipped with a flame ionization detector. Separation took place in a glass column, 2 meters long by 2 millimeters in diameter, and packed with PORAPAK Q, 80/100 mesh. N₂ was used as carrier gas. Injector and detector temperatures were 220°C and column temperature was programmed from 160°C to 200°C. The analysis of chromatographic data were carried out by a Intersmat ICR 1 B integrator.