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IMPROVED STABILITY OF THE CONTINUOUS PRODUCTION OF ACETONE-BUTANOL BY CLOSTRIDIUM ACETOBUTYLICUM IN A TWO-STAGE PROCESS

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ABSTRACT

A stable continuous culture has been maintained for 30 days at a high 20 g/l solvent concentration. This substantial increase in the stability of the continuous culture of **Clostridium acetobutylicum** at the maximal solvent level was achieved by using a two-stage process with a dilution rate of 0.1 h^{-1} in the first fermentor and 0.04 h^{-1} in the second fermentor. The two-stage continuous fermentation allows an optimal growth of cells and induction of solvent metabolism in the first stage, and a maximal production yield of solvents in the second stage.

INTRODUCTION

To increase the solvent productivity of the acetone-butanol fermentation continuous cultures of **Clostridium acetobutylicum** have been used (LEUNG and WANG, 1981; MONOT and ENGASSER, 1983; FICK et al., 1985). Productivity improvements have been reported with single stage continuous cultures at sufficiently low dilution rate . LEUNG and WANG (1981), for instance, attained a solvent productivity of 2.5 g/l at solvent concentrations of 12 g/l in a complex medium with 15 g/l yeast extract. Highest productivities have been recently obtained by coupling the continuous fermentation to an ultrafiltration process for cell recycling (AFSCHAR et al., 1985; FERRAS et al., 1986; PIERROT et al., 1986; SCHOLTE and GOTTSCHALK, 1986). Productivities higher than 6 g/l have thus been maintained for several weeks at solvent concentration near 12 g/l.

To be industrially competitive a continuous process must yield the high final concentration, around 20 g/l, obtainable with the batch process. Yet at such high solvent levels continuous cultures are very unstable, and show rapid degeneration because of the strong toxic effect of butanol. It is only at solvent level not exceeding 10 g/l butanol that stable continuous cultures have been reported. FICK et al., (1985) maintained a stable continuous culture for two months at a productivity of 0.75 g/l.h and at a 13 g/l solvent level.

The butanol inhibitory effect can be decreased by operating the continuous culture in a two-stage fermentor. Using this technology the two acidogenic and solventogenic phases of the microbial process are separately performed in two consecutive fermentors (BAHL et al.,1982; AFSCHAR et al., 1985). Only cells in the second stage are exposed to the highest solvent concentration. A two-stage process can also be expected to increase the longevity of the continuous process at high solvent concentrations, as a result of the continuous feeding of fresh cells to the second stage. The aim of the present investigation is to evaluate the longevity of an optimized two-stage continuous culture that yields a high solvent concentration.

MATERIALS AND METHODS

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

Medium: The culture medium is a complex medium containing the following components, per liter of distilled water: glucose, 60 or 70 g; ammonium acetate, 2.2 g; KH_2PO_4 1 g; $K_2HPO_4.3H_2O$, 1 g; $MgSO_4.7H_2O$, 0.2g; yeast extract 3g.

Fermentation: A 2 liters and a 6 liters Biolafitte fermentor were used. The agitation speed was maintained at about 200 rpm and the temperature at 35°C. The pH of the medium was controlled by automatic addition of 3N NaOH. Both fermentors are first run batchwise for 8 hours. A 10% growing culture taken at the end of the growth phase and at the beginning of the solvent phase is used as inoculum. Precultures are also made on the described medium. . The fermentor medium is kept anaerobic by a flow of purified nitrogen before inoculation and until reaching a sufficient fermentation activity. The two fermentors are then connected and continuous feeding started. The volume is kept constant by means of peristaltic pumps. Absence of contamination is regularly checked by microscope observation.

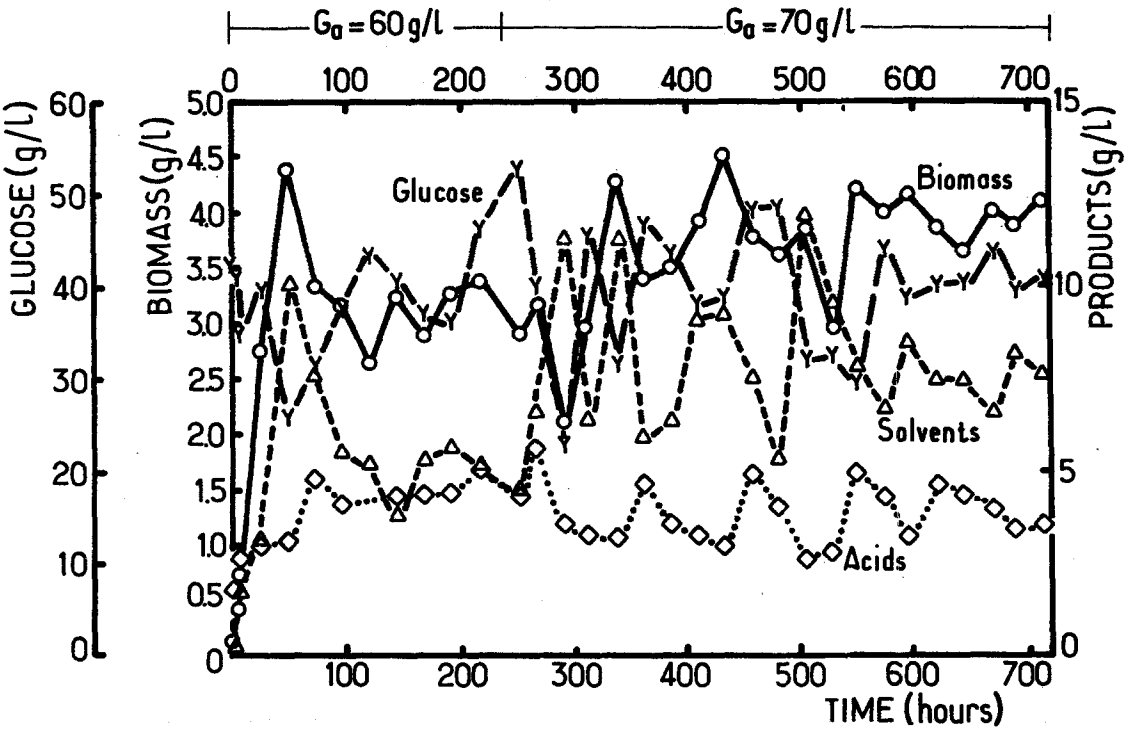
Analysis: Cell concentration is estimated by optical density and by dry weight using a predetermined correlation between optical density at 660 nm and cell dry weight. Residual glucose is determined on a Technicon autoanalyser with an hexokinase reagent. Concentrations of solvents (ethanol, acetone, butanol) and acids (acetic and butyric) are determined by injecting acidified supernatants into an Intersmat IGC 121 FL gas chromatograph equipped with a flame ionization detector. Separation takes place in a glass column, 2 meters long by 2 millimeters in diameter, and packed with PORAPAK Q, 80/100 mesh. Nitrogen is used as a gas carrier. Injector and detector temperatures are 220°C and column temperature is 180°C.

RESULTS AND DISCUSSION

When operating a two-stage continuous fermentation several parameters relating to the medium composition and to the dilution rates have to be optimized. In a preliminary study (GODIN, 1987) it was found that with **Clostridium acetobutylicum** ATCC 824 a pH of 4.5 is optimal for both stages. Moreover, with a feed glucose concentration of 60 g/l a dilution rate of 0.1 h^{-1} in the first fermentor and 0.04 h^{-1} in the second fermentor give the best conversion yield of sugars into solvents.

Figures 1a and 1b show under these conditions the time course of the two-stage fermentation, with the concentration of cells, residual glucose acids and solvents in the two fermentors. During the 250 first hours a 60 g/l glucose concentration was fed to the first fermentor. To test for possible glucose limitations the sugar level in the feed is thereafter increased to 70 g/l.

Time fluctuations of the concentrations are observed in the first fermentor, with an average concentration around 3.5 g/l for biomass, 3.5 g/l for acetic and butyric acids, and 6.5 g/l for acetone, butanol and ethanol. Much more stable concentrations are obtained in the second fermentor. At a 60 g/l glucose feed an average 4 g/l biomass, 3 g/l acids and 18 g/l solvents are achieved. Increasing the glucose feed to 70 g/l yields higher sugar conversions: 4.5 g/l biomass and 20 g/l total solvents (13 g/l butanol, 5.5 g/l acetone and 1.5 g/l ethanol). The acid level, on the contrary, decreases to about 2 g/l, which shows a reconsumption in the second stage of the acids produced in the first fermentor.



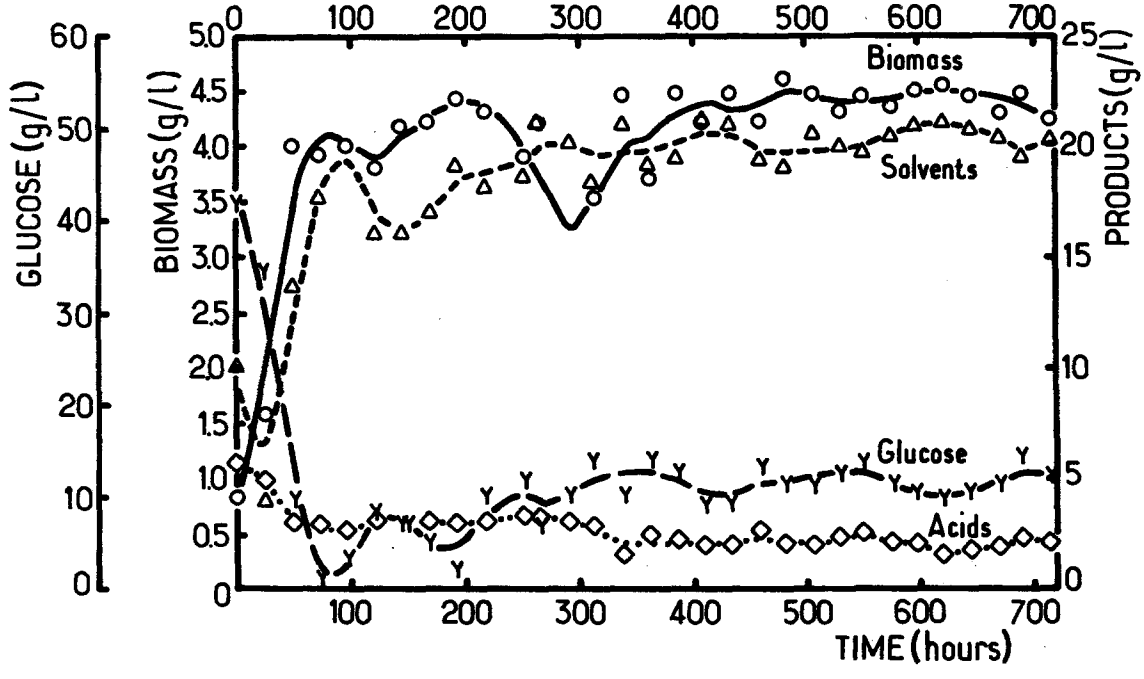


Figure 1a and 1b.

Two-stage continuous culture of **Clostridium acetobutylicum.**

a) Kinetics in the first stage with a dilution rate of 0.1 h^{-1}

b) Kinetics in the second stage with a dilution rate of 0.04 h^{-1}

Most interestingly this continuous fermentation has been maintained stable for 30 days without any observed degeneration of the culture at the high 20 g/l solvent concentration. This result confirms the expected stabilizing capacity of a two-stage process.

Comparing the composition of the two stages, one sees that cells are mainly produced in the first stage. Yet a small cell growth is also maintained in the second stage, which may also improve the stability of the process. Acids are partly reconsumed in the second stage. This contributes to the high overall conversion yield of 33 % of glucose into solvents. It can also be noticed that under these optimal conditions a significant proportion, about half, of the solvents are already produced in the first stage. This result suggests that the actual function of the first stage is not limited to the growth of new cells. It is also in the first stage that the metabolic transition from acidogenesis to solventogenesis has to be induced, so that cells entering the second stage are in the proper metabolic state for optimal solvent production.

The overall productivity of the fermentation is 0.57 g/l.h solvents, which is slightly higher than the productivity of a single-stage continuous culture under the same overall dilution rate. Additional gains in productivity may still be expected by coupling the second stage to an ultrafiltration device so as to reach high cell densities by cell recycling.

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