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# Continuous Production of Butanol with Immobilized Cells of *Clostridium acetobutylicum*

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## Abstract

Spores of *Clostridium acetobutylicum* were immobilized in calcium alginate. An active gel preparation was obtained after outgrowth of the spores to vegetative cells within the gel matrix. A 100 mL column containing the immobilized cells was used for continuous production. At steady-state conditions the productivity of butanol was 67 g/L reactor volume/day.

**Index Entries:** Butanol, production from immobilized *Clostridium acetobutylicum;* immobilized cells, of *Clostridium acetobutylicum;* cells, of *Clostridium acetobutylicum,* immobilized; *Clostridium acetobutylicum*, immobilized cells of; fermentation, of butanol from *Clostridium acetobutylicum*.

## Introduction

The classical fermentation for butanol and acetone production is a batch process (*1*). It is characterized by a rather low overall productivity (7-9 g butanol/L/day) and a low final concentration of solvents (max 19 g/L total solvents) (*2*, *3*). Biologically, it is a two-phase system in which solvents are produced in the latter nongrowth stage. Attempts have also been made to accomplish continuous production in a number of serially connected fermentors (*4*). This approach was apparently less successful because of contamination problems.

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The aim of this work has been to elucidate the biological capacity of the butanol-producing organisms when used in a totally different system: immobilized nongrowing but viable cells. Using this technique it has been found that:

1. Continuous production in an one step process is possible.
2. Higher productivity can be obtained.
3. The immobilized cells are less sensitive to oxygen than are free cells in a suspension.
4. A simpler substrate can be used.
5. The production system facilitates the subsequent product recovery since the medium leaving the reactor is free from cells, thus allowing alternative separation methods.
6. The problem with waste cell-mass is reduced.

## Experimental and Results

Spores of the organism *Clostridium acetobutylicum* ATCC 824 were immobilized in a calcium alginate gel as described earlier (*5*). The gel beads were also treated with glutaraldehyde (1 g/L, overnight at + 4°C) to improve their mechanical strength. After washing the gel, hot (95°C) growth medium (*5*) was added in order to heat-activate the spores and the gel beads were then incubated at 35°C for outgrowth of vegetative cells within the gel matrix. When the organism had entered the solvent production phase, growth was interrupted by washing off the nutrients. The so-obtained gel preparation was then used in further experiments.

A column (100-mL volume) containing the alginate beads (100 g of a spore-alginate solution was used, which yielded approx. 50 g of alginate gel, wet weight) was connected to a laboratory fermenter in such a way that the bulk liquid (2500 mL) in the fermenter could be pumped through the column and back to the fermenter at a high flow rate. In this way, a homogeneous distribution of substrate and products in the column was achieved. For continuous production, fresh medium was added to the fermenter where the liquid level also was controlled. For production, a nongrowth substrate was used that contained glucose (30 g/L), butyric acid (3 g/L), and inorganic salts. The pH was kept constant at 4.5 and the temperature was 35°C.

Using a flow rate of of 137 mL/h ($D$ = 0.055 $h^{-1}$ calculated on total bulk liquid) a steady-state butanol concentration of 2.05 g/L was obtained. Acetone was also produced, the ratio of butanol to acetone being 5.5:1 (w:w). During these conditions both glucose (6.75 g/L) and butyric acid (1.11 g/L) was consumed. Data from a 5-day continuous experiment are presented in Fig. 1. The results are expressed as grams of butanol produced per liter of reactor volume per day; that is, the actual production volume—in this case, the 100-mL column. The steady-state productivity was found to be 67 g butanol/L reactor volume/day.

## Conclusions

The data presented here show that butanol-producing organisms possess a vast biological potential when maintained under appropriate production conditions. More-

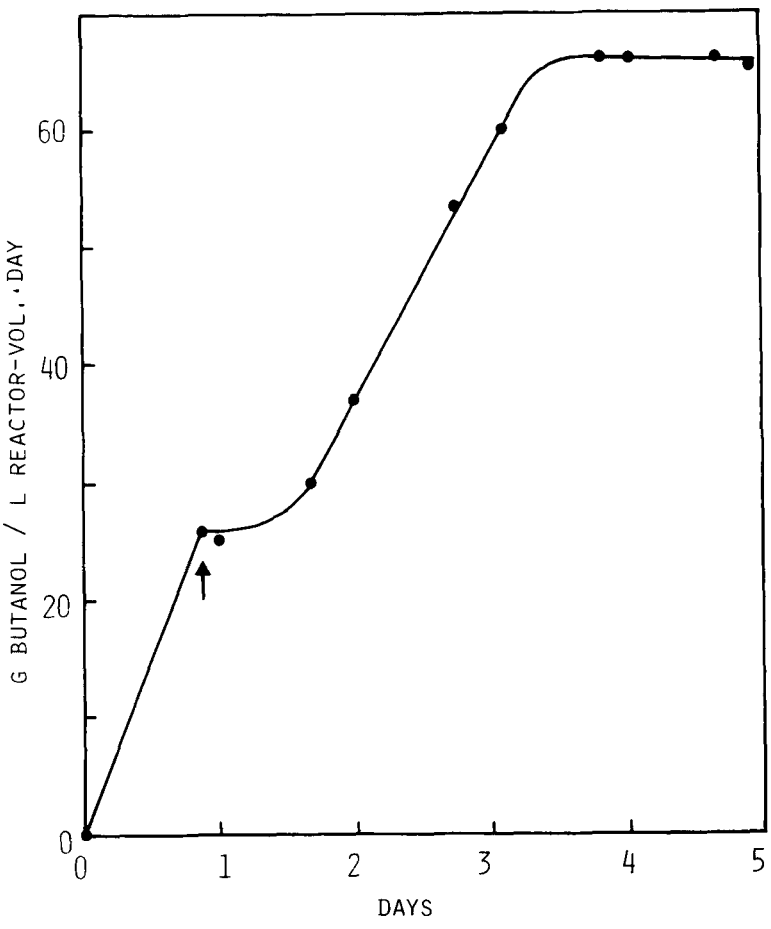


Fig. 1. Continuous butanol production by immobilized *C. acetobutylicum*. The arrow indicates the start of the medium flow.

over, the technique used has made it possible to obtain butanol production in a simple one-step continuous process. There is certainly scope for further improvements using this technique, e.g., with respect to the yield coefficient and recovery procedures.

## References

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