CONTINUOUS PRODUCTION OF ACETIC ACID FROM CO₂ IN REPEATED-BATCH CULTURES USING FLOCCULATED CELLS OF *ACETOBACTERIUM WOODII*

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Key Words: Anaerobic Bacteria, *Acetobacterium woodii*, Flocculation, Bioreactor, CO₂ Fixation, Factorial Design, Repeated-Batch Cultures

The biological utilization of CO₂ in the production of acetic acid in anaerobic fermentations of *Acetobacterium woodii* was studied in a bubble-column bioreactor. To increase the productivity of acetic acid a repeated-batch culture technique was developed by introducing concentrated cells using a synthetic polymeric flocculant. The effects of the operating conditions of gas flow rate, cell density and medium composition on volumetric productivity of acetic acid were examined. At suboptimal operating conditions the production rates of various cultivation schemes were examined. This approach is especially valuable for simplification of the process and utilization of the energy required for the separation of the cells from the liquid content.

Introduction

For global regeneration and environmental conservation, development of technology for CO₂ fixation and utilization has become one of the most important problems in the 1990s. This problem awakes an interest in the engineering design of bioreactors to cultivate chemotrophic bacteria, which are capable of obtaining all the carbon required for biosynthesis from CO₂⁹⁾. Acetogenic anaerobes, which produce acetic acid from CO₂ and H₂, have been extensively used as materials in studies reported in recent publications^{4,6,8,10)}. However, flocculation techniques to achieve concentrated cell population are still poorly examined.

In a previous paper¹¹⁾ we reported the kinetics of acid production from CO₂ and H₂ by *Acetobacterium woodii* in a bubble-column bioreactor using two types of resting anaerobes: suspended cells and immobilized cells. Higher acetate productivity was found in the latter. Optimal cell growth was achieved at 30°C and a ratio of H₂/CO₂ gases between 4:1 and 8:1 (v/v). In the subsequent study¹²⁾, flocculation of *A. woodii* by a variety of flocculants was tested to develop a method for gravity sedimentation of the cells from their media.

The purpose of the present study is twofold: (i) to test the operating characteristics of the productivity of acetic acid by using concentrated cells of *A. woodii*; and (ii) to develop a repeated-batch culture technique for the continuous production of acetic acid from CO₂ and H₂ in a bubble-column bioreactor using suspended high-density cells.

1. Experimental

1.1 Organism and flocculated cells

The strain of *A. woodii* used was ATCC 29683. The basic culture techniques and the medium used are described in our previous work¹¹⁾. Bacterial flocculation was performed by adding commercial polymeric flocculant, ACCOFLOC C470, to a high-density culture obtained from the centrifugation of a late logarithmic culture of *A. woodii* grown heterotrophically in a medium containing fructose as carbon source¹²⁾.

1.2 Operation in bubble-column bioreactor

A schematic diagram of the proposed system for the production of acetic acid from CO₂ and H₂ is shown in **Fig. 1**. A 300-ml bubble-column bioreactor with a porous plate as gas distributor¹¹⁾ was used. The system consists of precultivation by using fructose, cell harvesting by a centrifugal separator, preparation of flocculated cells (by the technique described in the previous section), production of acetic acid, and separation of cells from the liquid content in the bubble-column bioreactor. The bioreactor was utilized in a periodic operation combining gas sparging for 6 hours followed by a pause for 18 hours at 30°C. At 24-hr intervals 50% of the culture supernatant was replaced with the same amount of fresh medium without fructose.

Figure 2 shows the characteristics of sedimentation of the flocculated *A. woodii* cells. The ordinate shows the volume ratio of the culture supernatant (determined by visual measurement) to that of total culture. About 3.2 hours are required to obtain 50% culture supernatant per culture volume, as shown by the dotted line in this figure. To practice daily operations, sedimentation thereafter was carried out for 18 hours in our

* Received December 17, 1993. Correspondence concerning this article should be addressed to T. Suzuki.

Fig. 1 Bubble-column bioreactor system for producing acetic acid from CO_2 and H_2

Fig. 2 Sedimentation test for flocculated cells of *A. woodii* at 30°C and cell concentration of 8.0 kg/m^3

experiments.

Cells of the strain ATCC 29683 is usually in resting state in this autotrophic medium. However, these repeated-batch culture systems enable us to prevent the washout of cells from the continuous-culture bioreactor.

2. Results and Discussion

2.1 Repeated-batch cultivation

Firstly, an operation for continuous production of acetic acid in the bubble-column bioreactor utilizing concentrated cells of *A. woodii* suspended in basal medium was examined. No cell mass growth was observed in this condition. **Figure 3** shows a comparison of the production of acetic acid in the bubble-column bioreactor by concentrated cultures of nonflocculated cells (A) and their flocculated cells (B). The C_p in the figure indicates the amount of acetic acid per unit volume of culture. Broken lines show a pause in gas sparging. It should be noted that acetic acid could be produced even in the pause phase. Dilution of the culture with fresh medium was not carried out during the operation in case (A).

Fig. 3 Acetic acid production in the bubble-column bioreactor by two types of operation: (A) batch; (B) 50vol% of liquid content (supernatant) withdrawn at time denoted in arrows, and same amount of fresh medium fed to the reactor. Operating conditions were: gas flow rate, 2.5 vvm ; gas composition, $\text{H}_2:\text{CO}_2:\text{N}_2 = 12:3:7$; temperature, 30°C ; initial cell concentration, 8.5 kg/m^3

Case (B) shows the cultivation with periodic replacement of the medium. In such case, bentonite (5 kg/m^3) was fed in addition to the flocculant to make heavier floc. This treatment requires no additional flocculants during the repetitive separation of the cells from the culture.

It is apparent that case (B) gives higher productivity of acetic acid. The mean volumetric production rates of acetic acid for the two cases are 0.60 and $0.76 \text{ kg}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ respectively in the gas-sparging phases during three operation cycles. The production rate in case (B) is higher than that in case (A) or batch by a factor of 1.3, showing the possibility of increasing the production rate of acetic acid by proper choice of operating method for the culture. In addition, during the cultivation after 72 hours acetic acid production ceases in case A, while it continues in case B. Although a gradual decrease of productivity due to leakage of cells from the culture is observed in the latter, the lowering of productivity is insignificant. For long-term operation in repeated-batch culture systems, a means to supplement cells is required. Two methods may be considered for solving this problem. One is repeated inoculation of active cells from the preculture vessel; the other is improvement of the medium that holds growing cells.

2.2 Effects of some operating conditions

Some sub-optimal conditions of the operation in the bubble-column bioreactor were examined. The

Fig. 4 Effects of gas flow rate and cell concentration on initial acetate production rate of *A. woodii* in the bubble-column bioreactor at 30°C with gas sparging of H₂-CO₂-N₂ (H₂/CO₂ = 4)

effects of cell density, changing from 0.3 to 17 kg/m³ (1 unit of OD₆₀₀ = 0.34 kg/m³ of dried cells), and the gas flow rate, changing from 1 to 7 vvm (1.4 to 9.8 cm/min), upon the initial volumetric production rate of acetic acid P_{ac} at 30°C are shown in **Fig. 4**. The P_{ac} value increases to a maximum with increasing OD₆₀₀. The maximum productivity of acetic acid was observed at a cell density of about 13.6 kg/m³. Although the effect of cell density on P_{ac} appears clearly even at a lower gas flow rate, it may be considered that the phenomenon depends on the effect of the cell physiology, which derives from the difficulty of preparation of the concentrated cells in this study. As for the effect of gas flow rate, no dependence of the gas flow rate on P_{ac} is observed as the gas flow rate becomes greater than or equal to 4 vvm. Under these conditions, the suboptimal value for productivity was determined as ca. 2.1 kg·m⁻³·d⁻¹. This value provides a CO₂ fixation rate of 3.0 kg·m⁻³·d⁻¹ from the stoichiometry of the net reaction for producing acetic acid from CO₂ and H₂¹⁾.

The CO₂ fixation rate in a unit volume of culture is comparable to that of photosynthesis by the blue-green alga *Anacystis nidulans* in the logarithmic growth phase¹³⁾ at a cell density of about 0.3 to 0.5 kg/m³.

2.3 Effects of medium composition

To further enhance acid productivity, the effect of medium component on cell growth and/or productivity of acetic acid was investigated by fractional factorial design²⁾ experiments in which 18 variations of component chemicals were included in the basal medium. Test tubes containing 20 ml medium solution were incubated

Fig. 5 Effect of initial concentration of yeast extract on the production rate of acetic acid by *A. woodii* (initial cell concentration: OD₆₀₀ = 0.02) under same experimental conditions as in Fig 3.

at 30°C for 24 hours under an atmosphere of H₂/CO₂(4:1, v/v). From this test, yeast extract was found to be the sole dominant component affecting cell growth of *A. woodii*. The effects of other components on cell growth could be ignored under these experimental conditions. In addition, Diekert and Ritter³⁾ reported a nickel requirement for growth of *A. woodii*, but our preliminary experiment showed no such remarkable effect on this strain. In the second step, the volumetric production rate of acetic acid (P_{ac}) utilizing growing cells in the bubble-column bioreactor was examined by varying the yeast extract concentration under the supply of H₂/CO₂ at 30°C. A marked increase in production rate of acetic acid with increasing yeast extract concentration was observed, as shown in **Fig. 5**. It is probably due to the fact that acid production is enhanced by some growth-associated mechanisms. Incorporation of carbon from yeast extract into acetic acid may be ignored under these experimental conditions, as shown by Levy *et al*⁶⁾. Schmidt and Cooney¹⁰⁾ discussed the yield of acetic acid for H₂/CO₂ by varying the yeast extract concentration in the fermentation using resting cells of *Chlostridium* sp.

2.4 Production rate of acetic acid

From the above results, a comparison of the production rate of acetic acid in various cultivation schemes is performed here. A volumetric production rate of acetic acid of 2.1 kg·m⁻³·d⁻¹ was obtained in the batch culture when cultivation was operated under suboptimal conditions: cell density, 13.6 kg-dry cell·m⁻³; gas flow rate, 4.0vvm; gas composition, H₂:CO₂:N₂ = 12:3:7; temperature 30°C. The production rate can be increased to 2.7 kg·m⁻³·d⁻¹ by employing the repeated-batch culture technique. Based on the results shown in **Fig. 5**, if we supplement yeast extract into the basal medium, the production rate in the proposed repeated-batch culture system would be remarkably raised to ca. 21 kg·m⁻³·d⁻¹. Schmidt and Cooney¹⁰⁾ reported production rates of acetic

acid from H_2/CO_2 in the range of 2.4 to 7.2 $kg \cdot m^{-3} \cdot d^{-1}$ in a high-pressure fermentor at a H_2 partial pressure above 3000 kPa using *Clostridium* sp. ATCC 29797 with high yeast extract concentration. Morinaga and Kawada⁸⁾ reported a production rate of 3.5 to 4.5 $kg \cdot m^{-3} \cdot d^{-1}$ in an immobilized-cell type reactor using a newly isolated genus of *Acetobacterium* BR-446. Hence our facile culture technique gives a comparable or higher production rate than those of other workers without employing cell immobilization or pressurization of the reactor. Recent work by Morinaga and Kawada⁸⁾ shows a higher production rate of 71 to 149 $kg \cdot m^{-3} \cdot d^{-1}$, even in comparison with those of the vinegar production processes utilizing immobilized acetic acid bacteria⁷⁾, by using a pressurized (up to 270 kPa) bioreactor with a hollow-fiber membrane. The uses of strain BR-446, the pressurization technique, and the hollow-fiber membrane may be the principal differences from ours. Our current work suggests that effective utilization of high-density cells in two phases, a growth-associated and stationary production phase, and the development of a recovery process for acetic acid from fermentation broth may present an important task for future research in the proposed cultivation scheme for production of acetic acid.

In addition to such problems, the establishment of a recycling system for gases is required from the industrial viewpoint of economy and safety, especially for H_2 gas. Based on the stoichiometry¹⁾ and assuming the yield of acetic acid is 100% for H_2 , the gas consumption rate of H_2 can be estimated to be only 1.3% of H_2 in the feed gases under the following operating conditions in the present repeated-batch system: cell density, 13.6 kg -dry cell- m^{-3} ; gas flow rate, 4.0 vvm; gas composition, $H_2:CO_2:N_2=12:3:7$; temperature, 30°C.

A system for circulating gases has been developed by Ishizaki and Tanaka⁵⁾ for hydrogen-oxidizing bacteria. Development of such a system will be required for the application of our systems in industrial use.

Conclusion

The potential of a process for bioconversion of CO_2

to acetic acid was examined by using *A. woodii* ATCC 29683 as a model acetogenic anaerobe. We proposed a repeated-batch cultivation method for utilizing concentrated cells in a bubble-column bioreactor. This culture technique is easy and convenient, and useful not only for cultivating high-density cells of anaerobes but also as an alternative approach to immobilized bacterial cell processes.

Acknowledgment

This work was supported by a Grant-in Aid for Scientific Research (No. 02650704) of the Japanese Ministry of Education, Science and Culture.

Nomenclature

C_p	= acetic acid concentration	[$kg \cdot m^{-3}$]
P_{ac}	= production rate of acetic acid	[$kg \cdot m^{-3} \cdot d^{-1}$]
t	= fermentation time	[h]
V	= culture volume	[m^3]
V_{sup}	= volume of culture supernatant	[m^3]

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