KINETICS OF PRODUCTION OF ACETIC ACID FROM CARBON DIOXIDE BY *ACETOBACTERIUM WOODII* IN BUBBLE-COLUMN BIOREACTOR

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Introduction

An anaerobic bacterium, *Acetobacterium woodii*, can produce acetic acid from CO_2 and H_2. The utilization of such bacteria is of enormous value, not only for the development of C1 chemistry but also for the fixation of CO_2 in the atmosphere. Since the discovery of *A. woodii*[1], its physiology has been studied extensively. Except in a few cases[2,5,6], little attention has been paid to its industrial usage. Goldberg and Cooney[2] found that the rate of mass transfer of H_2 and CO_2 from gas to liquid phase was a limiting factor in cell growth and acid production in a mixed culture of anaerobes. Levy *et al.*[5] studied the production of acetic and butyric acids from CO_2/H_2 or CO/H_2 in mixed culture of anaerobes using high-pressure (to 70 psig) flasks, and suggested the feasibility of their application in the production of organic chemicals from gasifier gases composed of a mixture of CO, CO_2, and H_2. Recently, Morinaga and Kawada[6] developed a membrane-filtration type reactor for producing acetic acid from CO_2/H_2 by a newly isolated genus of *Acetobacterium* (BR-446) at an acid productivity of 149 $g \cdot L^{-1} \cdot d^{-1}$. Although this is the highest value among the references, the culture characteristics are not yet fully understood.

The present research was aimed at the elucidation of the kinetics of acid production of suspended and immobilized cells of *A. woodii* using a bubble-column bioreactor.

1. Experimental

1.1 Organism and culture medium

The strain of *A. woodii* used was ATCC 29683. Anaerobic culture techniques[3] were used throughout this investigation. The medium for autotrophic growth was prepared by modifying the composition of constituents described by Kerby *et al.*[4] The medium (final pH of 7.3) contained (in mg per liter): NH_4Cl, 1000; NaCl, 470; $MgSO_4 \cdot 7H_2O$, 360; $CaCl_2 \cdot 2H_2O$, 100; $NaHCO_3$, 3000; KH_2PO_4, 4000; K_2HPO_4, 4000; $MnSO_4 \cdot 4H_2O$, 11.2; $FeSO_4 \cdot 7H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 3.6; $ZnSO_4 \cdot 7H_2O$, 3.6; $CuSO_4$, 1.2; $AlK(SO_4)_2 \cdot 12H_2O$, 0.4; H_3BO_4, 0.4; $NaMoO_4 \cdot 2H_2O$, 0.4; yeast extract (Difco), 1000; resazurin, 1.0; L-cystein $HCl \cdot H_2O$, 300; biotin, 0.04; folic acid, 0.04; pyridoxine HCl, 0.2; thiamine HCl, 0.1; riboflavin, 0.1; nicotinic acid, 0.1; calcium pantothenate, 0.1; vitamin B_{12}, 0.002; *p*-aminobenzoic acid, 0.1; thioctic acid, 0.1; nitrilotriacetic acid, 10.

1.2 Culture techniques

Precultures were grown heterotrophically in medium containing fructose (5 g/L). It was shaken in 30-ml test tubes containing 20 ml medium solution at 30°C for 24 hours under an atmosphere of N_2/CO_2 (70 : 30, v/v).

Growth characteristics and suboptimal conditions of temperature and gaseous atmosphere for *A. woodii* were determined by using test tubes in the same way as precultures. A 300-ml bubble-column bioreactor, which has a porous plate as gas distributor for effective utilization of CO_2 and H_2, was newly developed. It is an 18.0-cm high and 6.0-cm diameter glass vessel with a water-jacket. Suspended and immobilized cells were used in the cultivation. The experimental apparatus is shown schematically in **Fig. 1**. The immobilized cells were prepared by entrapping bacterial cells in calcium arginate (2.0 wt%). Growth was monitored by measuring optical density at 600 nm. Acetate was assayed enzymatically (acetyl-CoA synthetase; F-Kit Acetic Acid (Cat. No. 148261, Boehringer Mannheim GmbH).

2. Results and Discussion

The effects of culture temperature and atmospheric H_2/CO_2 ratio upon the autotrophic growth of *A. woodii* are shown in **Fig. 2**. The fermentation conditions for achieving optimal cell growth are found to be a temperature of 30°C and a gas ratio of

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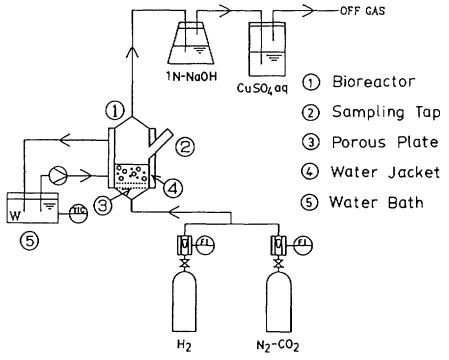

Fig. 1. Experimental apparatus

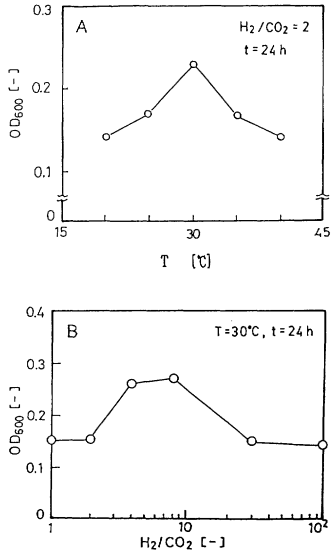

Fig. 2. Effects of culture temperature (A) and gas ratio of H_2/CO_2 atmosphere (B) on the growth of *A. woodii*. The initial OD_{600} values were fixed at 0.03 for (A) and 0.02 for (B) respectively.

H_2/CO_2 in the range 4:1 to 8:1 (v/v). From these observations, subsequent experiments were performed at these suboptimal conditions. It is interesting to note that the optimal gas ratio is considerably lower than that predicted from the gas solubilities of CO_2 and H_2 into the medium solution and the stoichiometry of H_2 oxidation and CO_2 reduction to acetic acid. This may result from a relatively low dissolved H_2 concentration for the optimum acetic acid production rate and/or the activity of the hydrogenase of *A. woodii* as pointed out by Morinaga and Kawada[6].

Table 1 shows the specific rates of cell growth and acetic acid production of *A. woodii* in shake flasks. Both the growth and acid production of *A. woodii* with H_2–CO_2 are lower than those with fructose. Our experiments also showed the difficulty of yielding a sufficient amount of cell masses of *A. woodii* by autotrophic culture techniques. For this reason, although the specific acid production rate of autotroph, q_p, in stationary phase is much lower than that in the logarithmic growth phase, the use of a high-density culture of non-growing cells is chosen in our works for the effective production of acetic acid from CO_2 and H_2 in the bubble-column bioreactor. The steps are as follows: (1) Preculture to yield a large amount of cells is carried out, using a medium containing fructose; (2) High-density culture is obtained from the centrifugation of these heterotrophs in late logarithmic growth; (3) Cell suspensions or gel-immobilized cells are inoculated to the bubble-column bioreactor. Such an approach has not been tried previously.

Table 1. Characteristics of growth and productivity of acetic acid by *A. woodii* in shake flasks at 30°C.

Substrate	μ [d^{-1}]	q_p [d^{-1}]	
		log. growth phase	stationary phase
Fructose	7.34	6.34	—
CO_2+H_2	6.90	2.65	0.34

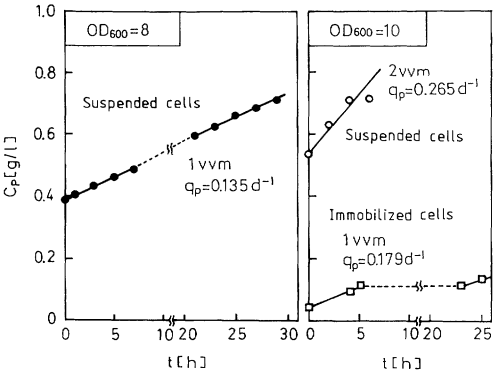

Fig. 3. Acetic acid production in the bubble-column bioreactor using both suspended and immobilized cells at 30°C (H_2–CO_2–N_2 ($H_2/CO_2=4$))

The productivity of acetic acid by high-density cultivation of resting cells in the bubble-column bioreactor is shown in **Fig. 3**. C_p in the figure indicates the amount of acetic acid per unit volume of culture. Broken lines show a pause in gas bubbling. Apparently the pauses had no effect on the productivity of acetic acid. Suspended cells were used in two runs in which cell density (1 unit of $OD_{600}=0.34$ g/L of dried cells) and gas flow rate were varied, while immobilized cells were used in another run. Comparison of the results for suspended cells and immobilized cells shows the advantage of suspended cells regarding the productivity of acetic acid. For immobilized cells, a serious lowering of mechanical strength of the gel was observed during the cultivation. The specific rate of acetic acid production at 0.265 d^{-1} is calculated from the curve of suspended cells at a gas flow rate of 2 vvm.

As suggested by the results of **Fig. 3**, the dependence of the specific rate of acetic acid on both gas flow rate and initial cell concentration is found under various experimental conditions (data are not shown). Although optimization of cell density and gas flow rate is not yet established in this work, we can expect higher productivity of acetic acid by carrying out additional work on these systems.

Conclusion

The production of acetic acid from H_2 and CO_2 by *A. woodii* was examined in a bubble-column bioreactor using two types of resting anaerobes: suspended and immobilized cells. Higher acetate productivity was found in the culture of suspended cells.

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Nomenclature

C_p	=	acetic acid concentration	[g/L]
OD_{600}	=	optical density at 600 nm	[—]
q_p	=	specific production rate of acetic acid	[d^{-1}]
T	=	culture temperature	[°C]
t	=	fermentation time	[h]
μ	=	specific growth rate in the logarithmic growth phase	[d^{-1}]

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