Clostridium thermoaceticum 균주를 이용한 자일로오스로부터 아세트산 생산

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Acetic Acid Production from Xylose by *Clostridium thermoaceticum*

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INTRODUCTION

Acetic acid is an important feedstock for many chemicals including vinyl acetate polymer, cellulose acetate, terephthalic acid/dimethyl terephthalate, acetic acid esters acetic anhydride and calcium magnesium acetate. At present these products are made from petroleum-derived acetic acid (1). Fermentation is potentially a cost-effective alternative for acetic acid production. Production of acetic acid via fermentation using renewable biomass feedstock has been studied extensively since late 1970s (2-4). The cellulose and hemicellulose in lignocellulosic biomass are the two most abundant renewable sources of carbon for fermentation to industrially useful chemicals. Efficient utilization of these constituents is vital to the development of economically viable bioconversion processes. Homoacetate anaerobic organisms such as Clostridium thermoaceticum convert glucose and xylose to acetic acid with a theoretical weight yield of 100% (5-7). Fermentation of glucose to acetic acid by the modified Clostridium thermoaceticum strain (ATCC 49707) has been extensively studied (10,11). However, its ability to ferment xylose into acetic acid is unknown. In this paper, we report on the characteristics of acetic acid production by this organism using xylose as the carbon source.

MATERIALS AND METHODS

A modified/mutant strain of Clostridium thermoaceticum registered as ATCC 49707 (12) and renamed Moorella thermoacetica was used in this study. The culture was grown in Difco Reinforced Clostridial medium at 59° C. It was maintained in the active state by transferring it alternately between this medium and this medium containing 3% sodium acetate. To acclimatize this strain to using xylose as principal sugar source, it was transferred to the fermentation medium described below for 48 hours for three generations alternating with growth in Clostridial broth. This adapted strain was stored at 4° C for future fermentation runs using xylose as the principal sugar source. The fermentation medium, similar to that used by Ljungdahl et al (2) contained in g/L: $(NH_4)_2SO_4$ [1.0]; $MgSO_4.7H_2O$ [0.25]; $Fe(NH_4)_2(SO_4)_2.6H_2O$ [0.04]; $NiCl_2.6H_2O$ [0.00024]; $ZnSO_4.7H_2O$ [0.00029]; Na_2SeO_3 [0.000017]; cysteine.$HCl.H_2O$ [0.25]; yeast extract [5] or corn steep liquor [variable]; KH_2PO_4 [7.5]; K_2HPO_4 [4.4]; NaOH [0.415]; $NaHCO_3$ [5]; Xylose [variable] with resazurin to detect trace amounts of oxygen.)

All fermentation experiments were conducted in a New Brunswick Bioflo model C-30 bioreactor at a temperature of 59° C with a working volume of 400 mL of the fermentation medium described above. Anaerobic environment was achieved by sparging filtered CO_2 until the oxygen indicator resazurin changed from pink to colorless and then was maintained by supplying CO_2 in the headspace of the reactor. The pH was maintained at 6.7-6.9 with 8-N NaOH so that there would be no appreciable change in working liquid volume. Fermentation was initiated by transferring 7 mL of 24 hour xylose adapted inoculum to the reactor medium. Xylose concentration and amount of corn steep liquor added were varied in batch and fed batch experiments. The yield of acetic acid to xylose was based on consumed xylose.
The fermentation samples were analyzed for sugar and acetic acid by HPLC (Water Associates) equipped with an RI detector. BioRad's HPX-87H column was used at 65° C with 0.005-M H_2SO_4 as mobile phase

at a flow rate of 0.6 mL/min. For mixed sugars, the substrate profiles were analyzed using BioRad HPX-87-P column operated at 85° C with de-ionized water as mobile phase and flow rate set at 0.55 mL/min. The cell density of the fermentation media was measured by a Turbidimeter (Hach Model 2100N) (16). The Nephelometric Turbidity Units (NTU) data was calibrated with four different Formazin standards prior to use.

RESULTS AND DISCUSSION

The original strain of Clostridium thermoaceticum ATCC 49707 was maintained through growth in medium with glucose as the only carbon source. Upon transfer to a medium containing a mixture of glucose and xylose, it consumed xylose first before consuming glucose. However, xylose uptake was very slow. With subsequent transfers in xylose medium, the rate of utilization increased. This culture was stored at 4°C and used in all fermentation experiments. Fig. 1 shows typical batch fermentation profiles of cell growth, xylose utilization and acetic acid production through fermentation at pH 6.9 and 59°C by Moorella thermoacetica (ATCC 49707). A lag phase was observed for the first 20 hours after which an exponential growth phase occurred for about 60 hours as depicted in Fig.2. Almost all of the xylose consumption and acetic acid production occurred during the log phase indicating a growth-associated acid production. Cell numbers decreased after the log phase of growth indicating that there was an autolytic decay of cells. Batch fermentation experiments were conducted over a range of initial xylose concentrations to find the optimal initial sugar concentration for acetic acid production. The data indicated that a concentration of 15 g/L resulted in a maximum yield of acetic acid at 0.84 (g acetic acid / g xylose consumed). The maximum concentration of product was 15.2 g/L which occurred with a 20 g/L xylose concentration with a yield of 76%. With increases in xylose concentration, the amount of unconsumed xylose in the medium increased which decreased the yield. The effects of initial xylose are summarized in Fig 2. Subsequent fermentation experiments were conducted using an initial xylose concentration of 15-20 g/L. One obstacle to the successfully commercializing this bioconversion process is the high cost of nutrients, such as yeast extract, required by this strain. Corn steep liquor (8) has been identified as an inexpensive nitrogen-rich nutrient sources (4,9). Corn steep liquor (CSL), a by-product of wet milling of corn, is a rich source of amino acids, minerals and vitamins. It also contains other nitrogen compounds useful for microbial growth (13). CSL has been used as medium for industrial production of penicillin (14). Using this nutrient source, instead of yeast extract, can reduce the fermentation cost significantly. Several experiments were performed to estimate the amount of CSL that would be required to obtain a yield of acetic acid comparable to that obtained using yeast extract. The results in Fig. 3 show that the fermentation profile with CSL is similar to that with yeast extract. When the concentration of CSL was 25 g/L and initial xylose loading was 20 g/L, the final concentration and yield of acetic acid were 16.57 g/L and 0.84 (g acetic acid / g xylose consumed) respectively. The profiles with varying CSL also are presented in Table 1. In a medium containing a mixture of glucose, xylose, galactose, fructose, arabinose and mannose, this strain consumes fructose first and then xylose. However, the rate of fructose consumption was faster than the rate of xylose consumption. Glucose was the third sugar to be utilized as carbon source. When xylose, fructose and glucose were completely consumed, the organism appeared to utilize arabinose, mannose and galactose in that order, but at an extremely slow rate. Considering that the yield is higher at low sugar and high nitrogen source, a fed-batch mode of operation was perceived as a way to enhance yield by maintaining the optimal conditions in the reactor. Therefore three sets of experiments were performed where the xylose concentration was maintained at 15-20 g/L level. The results are given in Fig.4. In experiment 1, adding 8 g of xylose led slightly increased cell viability after the initial log phase. After 126 hours of fermentation, however, there was no further uptake of xylose. In experiment 2, the same trend was observed despite adding 8 g of CSL to the 400 mL medium. It is suspected that cell death could have resulted due to lack of mineral supplementation. In experiment 3, mineral solution and cysteine-HCl were therefore added in addition to 8 g of CSL and 6 g of xylose. This strategy worked only up to 95 hours fermentation, a slight improvement over the previous set of experiments where growth ceased after 80 hours. The organism produced acetic acid at the same rate as in the initial log phase of growth. However, further addition of sugar or nutrients did not increase the growth rate, sugar consumption, nor acid yield. The organism could not be revitalized after the initial 80-90 hour

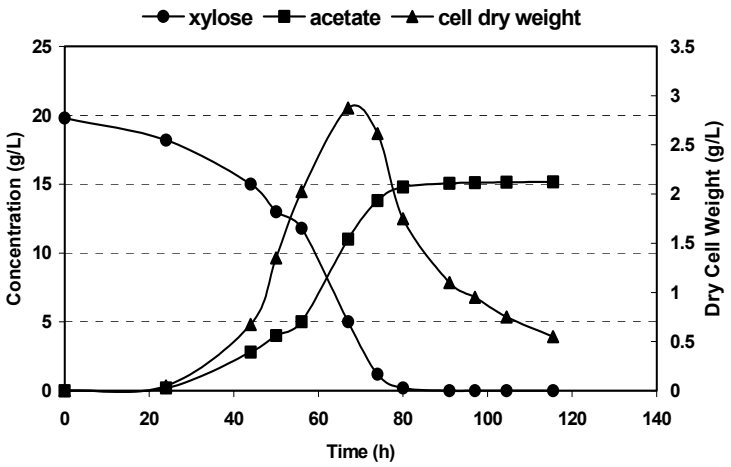

Fig. 1. Batch fermentation profile at pH6.8, 59 C

period. Accumulation of an intermediate product was also detected in experiment 3. This substance was identified to be D-xylulose using a pure standard in HPLC. It is an intermediate in the xylose metabolism of most bacterial systems formed by the action of xylose isomerase on xylose.

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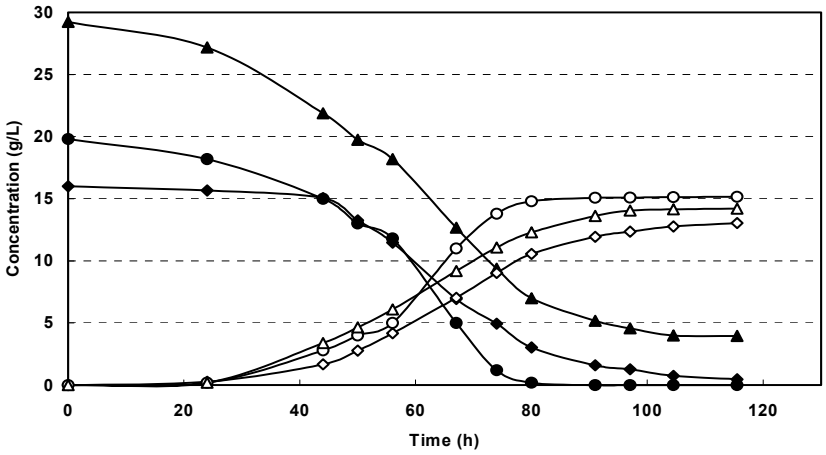

Fig. 2. Effect of initial xylose loading on the acetic acid yield

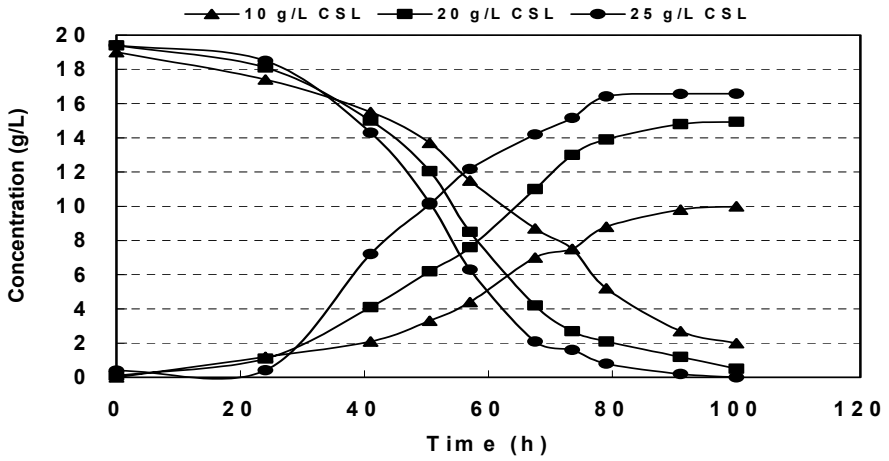

Fig.3. Effect of concentration of CSL on xylose utilization.

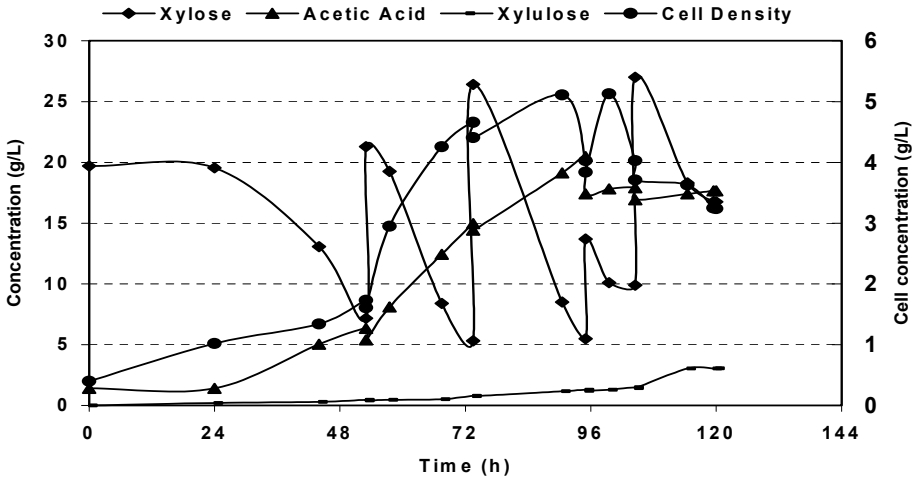

Fig.4. Fermentation profile under fed-batch operation.