섬유소 바이오매스로부터 동시 당화 발효공정을 이용한 Acetic acid 생산

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Acetic Acid production from Cellulosic Biomass by Simultaneous Saccharification and Fermentation

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INTRODUCTION

With U.S. production of 4.7 billion lb/year, acetic acid is a widely used commodity chemical. Applications are ubiquitous, from paints to plastics, vinegar to vinyl, inks to insulation. Growth of production has been moderate, averaging 2-3 %/yr. However, calcium-magnesium-acetate (CMA) could bolster acetate production immensely with a potential demand of multi-million tons/yr. There are two large volume potential end-uses for this chemical: non-corrosive road de-icer and a coal combustion additive. Acetic acid accounts for 80% of the mass of CMA and 85-90% of its feedstock costs. Although almost exclusively produced from petrochemical derivatives at the present time, production of acetic acid from homoacetate fermentation is receiving a considerable attention. There is a distinct advantage of the fermentation route that it relies on renewable resources rather than non-renewable (petroleum) resources. Homoacetate organisms such as *Clostridium thermoaceticum* make full use of this advantage by completely converting both glucose and xylose to acid with theoretical weight yield 100% .

There are two major cost factors in this biological process. One is the high downstream processing cost. It is mainly due to the low acetate concentration that develops under the strong inhibition by acetic acid. Substantial amount of research has been undertaken to alleviate this particular problem. One line of research dealt with increasing bacterial tolerance of acetate , while the other looks at novel separation method such as nano-filtration and solvent extraction. The second major cost item is the feedstock. Traditionally, hydrolyzates of corn starch and corn-steep liquor have been used for glucose/nitrogen sources for this process. The purpose of this investigation is to evaluate cellulosic biomass as an alternative feedstock for this process. Conversion of cellulosic biomass to acetic acid involves two different biological processes, the enzymatic hydrolysis of biomass and the fermentation of glucose to acetate. These two processes can be carried out separately (Separate Hydrolysis and Fermentation) or simultaneously (SSF). The two individual elements in the SHF have been investigated extensively. It is the SSF that has not been tested. Our primary interest is therefore in the development of the SSF. To our knowledge, this investigation represents the first attempt at that. As such the SSF was first studied using α-cellulose, a standard substrate. This information was then applied to the development of a process using a more practical feedstock, a pulp mill sludge. The scope of work thus covers the basic factors surrounding the SSF process and the assessment of the overall performance.

MATERIALS/METHODS

Enzymes and Feedstocks

Five commercially available cellulase enzymes were examined for their pH and temperature optima for the applicability in the SSF. Four different cellulosic substrates were analyzed for the composition. They are α-cellulose (Sigma), paper mill sludge-I (primary sludge for a Kraft mill, Mead-Beit Corporation, Columbus, GA), paper mill sludge II, and newsprint waste paper. The α -cellulose and

the sludge-I were chosen for further investigation because of high glucan content. For the digestibility test, a 100mL was supplemented with biomass consistent with 1wt% cellulose, 0.5 mL enzyme, and the remainder 0.1 N buffer solution. Citrate buffer was used for pH 5.0-6.0, and phosphate buffer for pH 6.0-7.0.

Fermentation

The microorganism employed in this work was *Clostridium thermoaceticum* (recently renamed to: *Mooriella thermoacetica*), ATCC 49707. Inoculum cultures were grown at 59°C for 48 hours in undefined Difco Clostridial media. Fermentation media contained (per liter): Yeast Extract, 5g; $(NH_4)_2SO_4$, 1g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 0.04 g; $NiCl_2 \cdot 6H_2O$, 0.00024 g; $ZnSO_4 \cdot 7H2O$, 0.00029 g; Na_2SeO_3, 0.000017 g; Cysteine·HCl·H_2O, 0.25 g. In addition, 0.1 N Phospate/Citrate buffers were utilized, and the oxygen indicator resazurin added in trace amounts. Glucose/biomass was added as indicated. A New Brunswick, Bioflo model-C30 was used as the bioreactor. It was operated with temperature and agitation control and with 400 mL working liquid volume. Oxygen-free environment was maintained by initially sparging 0.3m-filtered CO_2 until resazurin indicator changed from red to colorless and then constantly supplying CO_2 in the headspace of the bioreactor. The pH was controlled with 8N NaOH. Samples were boiled, and stored at 4°C for further analysis. A twelve to twenty-four hour period with no NaOH addition was taken as the fermentation end point.

Analytical

Samples taken from fermentation and hydrolysis experiments were analyzed for sugars and acetic acid by HPLC equipped with a RI detector. A Biorad-HPX-87H column was used at 65°C with 0.005 M H_2SO_4 mobile phase at a flow rate of 0.55 mL/min.

RESULTS AND DISCUSSION

Batch Enzyme Hydrolysis

Of the five enzymes listed in Table 1, ROCKSOFT SUPERACE (RS:) performed best with respect to operable pH range temperature proximity compatible with *C. thermoaceticum*. Under optimum conditions, 68% of α-cellulose and 70% of the mill waste were digestible using RS enzyme. Although Iogen enzyme outperformed RS slightly in reactivity, its temperature optimum (55°C) coincided better with *C. thermoaceticum* (59°C). At pH 5.5, digestibilities of the pulp mill sludge-I and α-cellulose were 61% and 60% using RS enzyme. Pushing pH further to 6.0, the digestibilites were reduced to 49% for α -cellulose and 36% for the sludge.

SSF

On the basis of the data from repeated batch hydrolysis and glucose fermentation runs, our best estimate of the optimum conditions for the SSF were determined to be 55°C and pH 5.5 for the paper mill sludge, and 55°C and pH 6.0 for α -cellulose. This was done in consideration of the yield and productivity of the two biological processes. The discrepancy in pH optimum between the two substrates is unclear at this time. Somehow α-cellulose becomes less digestible at higher pH. For the conversion of pulp mill sludge, the compromise of pH between bacterial the enzymatic processes would have to cope with wider span. Figure 2 depicts the batch SSF data based on α -cellulose with two different enzyme loadings. There is a significant amount of glucose accumulation at the early phase of the SSF. This is due to slow development of microbial growth and reactivity at the beginning of the SSF. We note that the product formation pattern of this organism is predominantly growth-associated. Most of the fermentation process occurs over the span of 40-80 hr. It was also observed the lag phase before the bacterial growth was excessively long that the SSF was not carried out in a normal pattern where the process occurs under glucose-limited condition. Instead, the overall process pattern was close to that of a separate hydrolysis/fermentation (SHF). The overall yield of acetate varies with the enzyme load. The apparent yields for the two runs were 68% and 33%, for 50 and 25

IFPU/g-glucan respectively. It is unclear whether the terminal yield was attained with 25 IFPU/g-glucan during this time span. An enzyme adapted to neutral pH conditions would allow for lower enzyme loadings, as well as increased acetate tolerance at higher operating pH. Enzymes systems with these qualities have been produced on a bench scale, with considerable activity at neutral pH and thermophilic temperature. In order to accomplish a true SSF, a fed-batch operation was attempted. In this run, 1 g of α-cellulose was added every 9 hour along with supplementation of the enzyme such that the overall enzyme input is maintained at the level of 50 IFPU/g-glucan. There were also two additional supplementation of minerals and yeast extract at 120 and 175 h points. The progression of the fed-batch SSF is shown in Figure 3. It is a significant finding that the SSF is operated under a stable condition for 250 hours without any sign of system deterioration especially when it is the first attempt at the SSF on this organism. Under this mode of operation, the initial lag phase was followed by rapid glucose depletion. At the point of glucose depletion, the hydrolysis became the rate-limiting step. Over time, acetate inhibition lowered the activity of both the enzyme and the bacteria (hence a slight curvature in the line). The two sharp drops in the acetate curve at 120 h and 175 h are the result of the dilution caused by minerals and yeast extract supplementation. It is also noticeable that the acetate production accelerates at these two point. It is believed to be the effect of yeast extract addition. As the acetate level increases, the inhibition effect on bacteria also rises. Eventually it reaches a point where the microbial uptake of glucose becomes the rate-limiting step. This is indicated by accumulation of glucose at 180 h point. The total acetate reached ~30 g/L at ther end of the run. Overall yield in fed-batch operation was 60%, somewhat lower than the batch yield. The limitation of the yield has to do with the acetate inhibition. The inhibition affects both the microorganism as well as the enzyme. We have observed the presence of unconverted α-cellulose at the end of the run, a sign of incomplete digestion. Inhibition of enzyme activity by acetate is therefore believed to be a significant factor limiting the yield. We are yet to determine which of the two inhibition factors is more important. A follow-up experiments were conducted using the pulp mill sludge as the feedstock. The results from one of these experiments are shown. A large amount of glucose accumulation is seen over the entire process. It appears that the toxins released from the Sludge are severely liming the glucose uptake. It led to a situation where the microbial uptake glucose (0.13 g/L·hr) is much lower than enzyme hydrolysis rate (0.17 g/L·hr). The intended SSF process was therefore operated under the mode of SHF. Despite the slowness microbial action, overall yield observed in this run was remarkably high at 85%. We have observed that the spent solid residue retained no glucan content at the end of the run indicating a complete digestion. Work is currently in progress in our laboratory to verify the reason why the digestibility has improved in the SSF over that of enzyme hydrolysis.

CONCLUSIONS

We have proven that a fed-batch SSF can be operated under a stable condition for production of acetic acid from α-cellulose. The overall yield of acetate in the SSF using α -cellulose is 60%. The total acetate concentration of 30g/L is attainable in the SSF broth. Acetic acid inhibits not only the fermentation but also the enzymatic hydrolysis. Yield are higher (67%) in the batch SSF of α-cellulose where acetate inhibition on enzyme is not as extensive. In the SSF using a paper mill sludge, bacterial growth is significantly hampered by the toxins released from the sludge. The slowness in microbial action has changed the SSF into a SHF. Despite the sluggishness of the microbial process, the terminal yield obtainable from the paper mill sludge was substantially higher (85%). The toxins from the sludge do not appear to inhibit the enzymatic reaction in the SSF of the sludge.

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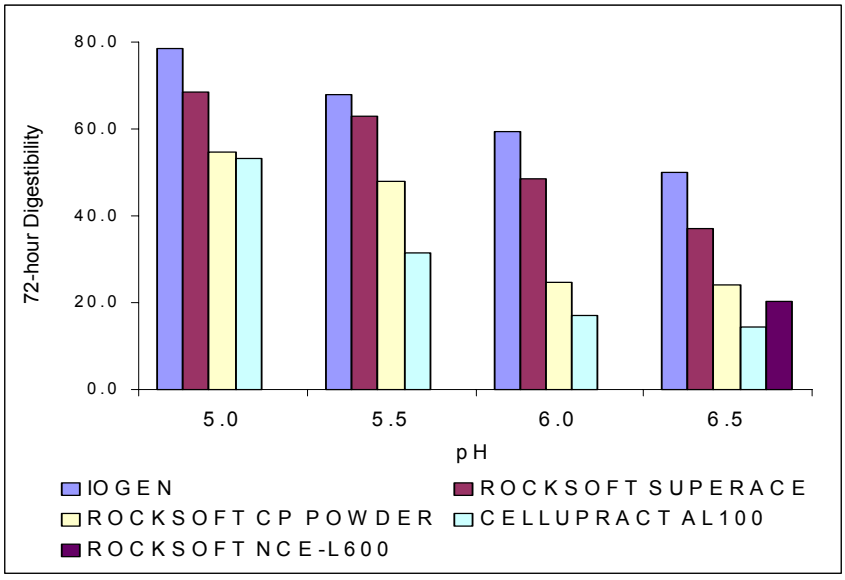

Figure 1. Effect of pH on the enzymatic digestibility of various feedstocks. 1 wt% initial glucan in media.

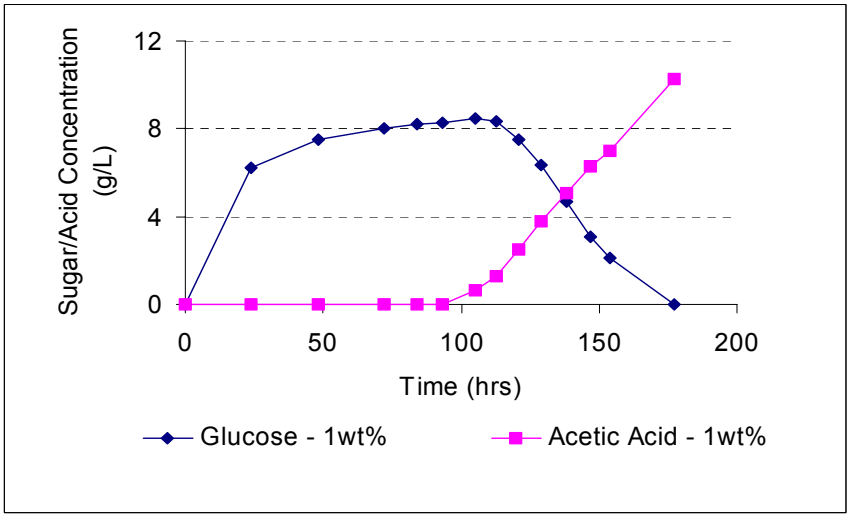

Figure 2. SSF Operation of Paper Mill Sludge at pH 5.5, 55°C