Effects of Metal Salt Catalysts on Yeast Cell Growth in Ethanol Conversion

Chung-Yun Hse[1] and Yin Lin[2]
[1]USDA Forest Service, Southern Research Station, Pineville, LA. USA
[2]School of Bioscience and Bioengineering, South China University of Technology, Guangzhou, China

Abstract

The effects of the addition of metal salts and metal salt-catalyzed hydrolyzates on yeast cell growth in ethanol fermentation were investigated. Four yeast strains (*Saccharomyces cerevisiae* WT1, *Saccharomyces cerevisiae* MT81*, Candida sp.* 1779*, and Klumaromyces fragilis*), four metal salts ($CuCl_2$**,** $FeCl_3$**,** $AgNO_3$**,** and I_2), two metal salt-catalyzed hydrolyzates ($CuCl_2$**,** and $FeCl_3$), and a H_2SO_4-catalyzed hydrolyzate were evaluated in this study. *Candida sp.* 1779 was selected as the most suitable yeast strain to evaluate the effects of catalyst and hydrolyzate on this ethanol fermentation experiment. The addition of $FeCl_3$ and I_2 did not have any effects on the cell growth of the yeast. It is noted that addition of 0.1 g/L or less concentration of $CuCl_2$ also showed no effect on yeast growth; but further increase of the concentration resulted in substantial decrease in yeast growth. In the case of $AgNO_3$ addition, the inhibitory effect on the yeast growth covered the entire range of the concentration. Compared to the yeast cell growth of H_2SO_4-catalyzed hydrolyzate, $FeCl_3$-catalyzed hydrolyzate had very similar early log-phase yeast cell growth; but the $CuCl_2$-catalyzed hydrolyzate resulted in a much lower cell growth. These results strongly suggest that the $FeCl_3$-catalyzed hydrolyzate is the superior catalyst among the metal salt catalysts in this study. The much less corrosiveness of the $FeCl_3$catalyst system, as compared to the H_2SO_4 system, makes it a strong candidate for further research and development.

Introduction

The use of biomass as a chemical raw material, or as an energy source, has been the focus of interest over the past three decades owing to fuel shortages in the early 1970s and to dwindling petroleum supplies predicted in the future. Liquefaction, pyrolysis, gasification, and hydrolysis of cellulose have all been proposed as methods for utilizing renewable biomass as an energy or chemical source. In the near term, wood hydrolysis and conversion to ethanol for fuel is the method recognized as one of the most attractive and practical technologies for better utilization of woody biomass.

The hydrolysis of wood can be carried out either with cellulolytic enzymes or mineral acids. Enzymatic hydrolysis occurs under mild conditions with good sugar yields but the process is not economical because of high enzyme costs and slow conversion rates (Badger, 2002). Acid hydrolysis in concentrated mineral acids is based on the concentrated acid decrystallization of cellulose followed by dilute acid hydrolysis of sugars. Good sugar yields can be obtained (Kusama, 1960); however, the process is slow and cost effective acid recovery systems have been difficult to develop. In addition, equipment corrosion and the huge amount of slurry resulting from neutralizing the used sulfuric acid are serious problems. For the dilute acid process, a fast rate for hydrolysis allows for continuous processing. Dilute acid solutions are easier to prepare and need not be recycled; corrosion of metallic parts during the process is also less of a problem. A significant advantage is that the moisture content of wood materials is not necessarily controlled, allowing the processing of wet raw material. Shortcomings include high temperature and high pressure reaction conditions. Under such severe reaction conditions, the degradation of sugars can be significant.

The development of a variety of hydrolysis reactors, such as the bed-shrinking flow-through reactor (Torget et. al., 2000), plug flow reactor (Church and Wooldridge, 1981; Thompson and Grethlein, 1979), and screw-fed, continuous, single-stage reactor (Rugg and Brenner, 1980) have greatly improved the economic effectiveness of the dilute acid process by shortening the reaction time and increasing the yield of glucose. It appears that the prevailing reaction temperatures for the dilute acid hydrolysis process range from 170 to 230^{o}C. Little research has been done at higher temperatures mainly due to the difficulty in controlling the glucose degradation and the probable high equipment costs. Several recent studies, however, have shown that cellulose can be converted at a high rate into water-soluble products at residence time of less than few seconds in near-supercritical and supercritical water without an acid catalyst (Kabyemela et. al., 1998; Ehara and Saka, 2002; Sasaki et. al. 2002). Related research on sugar conversions at the Southern Research Station Pineville Laboratory have focused on the development of a pre-treatment technology and a non-acid catalyst wood hydrolysis system at moderately high temperature (250 to 325^{o}C). Preliminary results to date have shown that addition of metal salts such as ferric chloride and copper chloride can hydrolize cellulose to glucose without the addition of sulfuric acid as a catalyst (Hse, 2005). Since wood hydrolysis is an important pre-treatment for the energy conversion of the woody biomass, it suggests that the effects of metal salt catalysts on the ethanol fermentation become the focus point of future research. Therefore, the objectives of this work were to obtain the data on the effects of metal salt addition and metal salt-catalyzed hydrolyzate addition on yeast cell growth in ethanol fermentation. This information is part of the integral data base needed for the determination of the technical feasibility and the potential application of the wood hydrolysis process with an addition of metal salt as a catalyst without using a sulfuric acid catalyst.

Materials and Methods

Hydrolysis of cellobiose in Batch Reactor

Cellobiose was used as the model compound for the hydrolysis experiment. Three catalysts, H_2SO_4, $CuCl_2$, and $FeCl_3$, were included in the experiment. Hydrolysis reactions were conducted in a batch reactor heated by an oil bath. The temperature of the oil bath was 250°C. Two grams of cellobiose, 68 ml of distilled water and catalysts (in concentrations of 0.01-0.3% wt based on distilled water) were premixed in a 75 ml reactor tube, followed by sealing the reactor and then mixing thoroughly in an ultrasonic bath for 10-15 minutes at room temperature. To initiate the hydrolysis, the suspended sample solution in the batch reactor was immersed into the oil bath. At the end of the predetermined reaction period, the reactor was removed from the oil bath and immediately immersed into an ice-cold water bath to terminate further reaction. The reaction mixture was then filtered to separate the soluble and residual products. The water in the soluble product was removed with a rotary evaporator under reduced pressure. The concentrated hydrolyzate was stored for the fermentation experiment.

Fermentation of Hydrolyzates

Selection of yeast strains

Four kinds of yeast strains, *Saccharomyces cerevisiae* WT1, *Saccharomyces cerevisiae* MT81, *Candida sp.* 1779, *and Klumaromyces fragilis* were incubated in the cell growth medium containing 20.0 g/l hydrolyzate (H_2SO_4-catalyzed hydrolysis products), 2.0 g/l KH_2PO_4, 1.0 g/l Mg $SO_4 \cdot 7H_2O$, and 10.0 g/l yeast extract (pH 5.5) in shaking Erlenmeyer flasks (180 rpm) at 30°C. Progress of cell growth was monitored by the absorbance reading at 600 nm in a common laboratory spectrophotometer (1 cm light path).

Effect of catalyst on the cell growth

The best yeast strain selected from the experiment above was used to evaluate the effect of the catalysts on cell growth. The cell growth medium consisted of 20.0 g/l hydrolyzate (H_2SO_4-catalyzed hydrolysis products), 2.0 g/l KH_2PO_4, 1.0 g/l Mg $SO_4 \cdot 7H_2O$, 10.0 g/l yeast extracts, and various concentrations (0. 0.1, 0.25, 0.50, 0.75, 1.0 g/l) of $CuCl_2$, $FeCl_3$, $AgNO_3$, and I_2, respectively. The medium was then sterilized at 121°C for 15 minutes. The selected yeast strain was inoculated onto the media and the cell growth was monitored.

Effect of hydrolyzate on cell growth

The effects of the three hydrolyzates (i.e., H_2SO_4, $CuCl_2$, and $FeCl_3$ catalyzed) obtained from the above experiment on yeast cell growth were evaluated. The cell growth medium consisted of 30.0 g/l hydrolyzate, 2.0 g/l KH_2PO_4, 1.0 g/l Mg $SO_4 \cdot 7H_2O$, and 20.0 g/l yeast extracts. The growth medium was then sterilized at 121°C for 15 minutes. The selected yeast strain was inoculated onto the medium in shaking Erlenmeyer flasks (180 rpm) at 30°C, and the cell growth was monitored. The cell biomass was harvested by centrifugation at 2,300 × g for 10 minutes, washed with ice-cold de-ionized water, and calculated in terms of biomass dry weight mg/mL glucose.

Results and Discussion

Yeast strains

The relationships of cell growth and cultivation time of four yeast strains are summarized in Fig. 1.

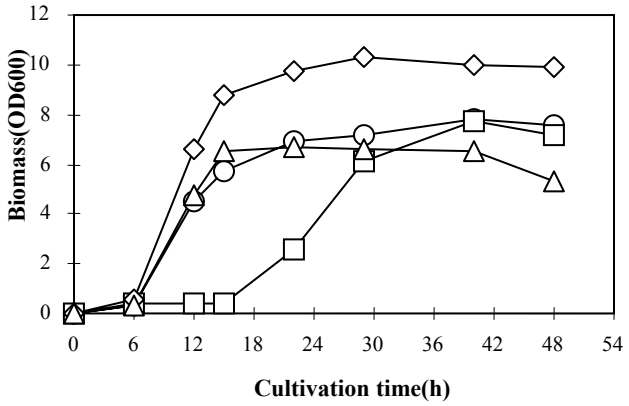

Fig. 1. Relationships of cell growth and cultivation time of four yeast strains.
Δ-- *S. cereviisiae* WT1, -□- *S. cereviisiae* MT-81, -◊-*Candida sp.*, -○-*K. fragilis.*

It is noted that, all yeast strains, with exception of *S cereviisiae* MT-81, grew exponentially over time after cultivation time of 6 h. It is further noted that *Candida sp* had the highest cell growth and the *S cereviisiae* MT-81 the least. Thus, *Candida sp.* 1779 was selected as the most suitable yeast strain to evaluate the effects of catalyst and hydrolyzate on the ethanol fermentation experiment.

Effect of catalyst on cell growth of Candida sp.

The effects of catalysts and catalyst concentrations on yeast cell growth are summarized in Table 1.

Table 1. Effect of catalyst and catalyst concentration on cell growth of *Candida sp*. 1779.

Catalyst concentration (g/L)	Biomass (OD_{600})			
	$CuCl_2$	$FeCl_3$	$AgNO_3$	I_2
0.00	3.42	3.42	3.42	3.42
0.10	4.20	3.90	0.58	3.78
0.25	0.13	3.80	0.51	3.50
0.50	0.15	3.60	0.80	3.41
0.75	0.30	3.35	0.90	3.24
1.00	0.28	3.22	0.87	3.08

The mean OD_{600} values for both $FeCl_3$ and I_2 showed little difference over the concentration of up to 1.00 g/L indicating that addition of $FeCl_3$ and I_2 did not have any effect on the growth of the yeast.

It is noted that the addition of 0.1 g/L or less concentration of $CuCl_2$ also showed no effect on yeast growth, but further increase of the concentration resulted in substantial decrease in yeast growth.

In the case of $AgNO_3$ addition, the results show its inhibitory effect on the yeast's cell growth covers the entire range of concentrations. Even though $AgNO_3$ was one of the desired catalyst to convert sugar in high yield, its negative effect on yeast cell growth turned its to be not a desirable catalyst.

Effects of hydrolyzates on yeast cell growth

The relationships of yeast cell growth and cultivation time as affected by the addition of hydrolyzates from hydrolysis with three catalyst systems (H_2SO_4, $CuCl_2$, and $FeCl_3$) are summarized in Fig. 2.

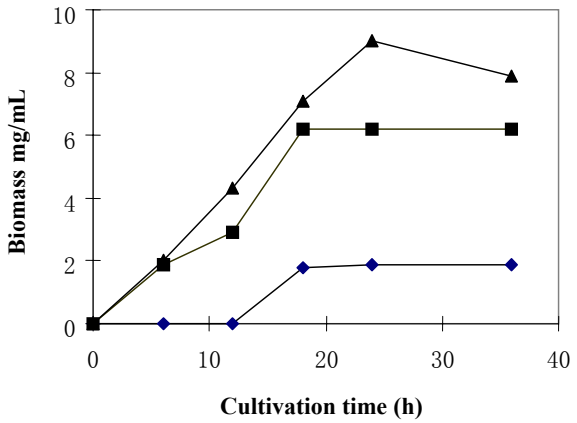

Fig. 2. Effects of hydrolyzates catalyzed with three catalysts on the cell growth.
---▲—H_2SO_4,--■—$FeCl_3$ --◆---$CuCl_2$

The results show that the yeast cell growth was best with H_2SO_4 catalyzed hydrolyzate. Since conventional acid wood hydrolysis mostly uses H_2SO_4 as a catalyst, the H_2SO_4-catalyzed hydrolyzate was treated as a control in this study. Compared to the yeast cell growth of H_2SO_4- catalyzed hydrolyzate, $FeCl_3$ -catalyzed hydrolyzate had very similar early log-phase yeast cell growth, but $CuCl_2$-catalyzed hydrolyzate resulted in a much lower cell growth. The results strongly suggest that $FeCl_3$-catalyzed hydrolyzate is the superior catalyst among the metal salt catalysts in the study. The much less corrosiveness of the $FeCl_3$catalyst system, as compared to the H_2SO_4 system, makes it a strong candidate for further research development.

Conclusions

The effects of additions of metal salts and metal salt-catalyzed hydrolyzates on yeast cell growth in ethanol fermentation were investigated.

Candida sp. 1779 was selected as the most suitable yeast strains to evaluate the effects of the catalysts and hydrolyzates on this ethanol fermentation experiment.

The addition of $FeCl_3$ and I_2 did not have any effect on the growth of the yeast. It is noted that the addition of 0.1 g/L or less concentration of $CuCl_2$ also showed no effect on yeast growth; but further increase of the concentration resulted in substantial decrease in yeast growth. In the case of $AgNO_3$ addition, the inhibitory effect on the yeast growth covered the entire range of the concentrations.

Compared to the yeast cell growth of H_2SO_4-catalyzed hydrolyzate, $FeCl_3$-catalyzed hydrolyzate had very similar early log-phase yeast cell growth; but $CuCl_2$-catalyzed hydrolyzate resulted in a much lower cell growth. The results strongly suggest that $FeCl_3$-catalyzed hydrolyzate is the superior catalyst among the metal salt catalysts in the study. The much less corrosiveness of the $FeCl_3$catalyst system, as compared to the H_2SO_4 system, makes it a strong candidate for further research.

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