Effects of Mn and Fe (II) Salts on Enzyme Saccharification of Rice Straw

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ABSTRACT

Mn and Fe (II) salts were investigated as catalysts for the enzymatic hydrolysis of rice straw. The hydrolysis efficiency of liquefied RS was characterized by sugar yield. The results indicated that adding metal ions of Mn and Fe (II) could improve sugar yield. The yield of Mn group was higher than that of blank group but lower than that of Fe (II) group during the same enzymolysis time. The single-factor experiments were suggested and the enzyme loading of Mn group decreased from 25 mg/g of blank group to 20 mg/g respectively, while Fe (II) group was consistent with the blank group. The proper enzymolysis temperature of Mn and Fe (II) group lowered from 50°C of blank group to 40 and 35°C, respectively. It was concluded that adding Mn and Fe (II) in saccharification process can disorganize the crystalline structure of cellulose, increase contact area of enzyme and substrate as well as, improve enzyme activity, which finally enhances the efficiency of enzymolysis and reduces energy consumption.

Key words: Rice straw, Mn salt, Fe (II) salt, enzyme saccharification.

INTRODUCTION

Agricultural residues such as rice straw, a kind of renewable and abundant resources, which includes cellulose and hemicellulose were given as an important raw material for producing biofuels (Ibrahim et al., 2011; Kang et al., 2013). The conversion process includes mainly of four steps, that is, pretreatment, enzymatic hydrolysis, fermentation and distillation.

Enzymatic hydrolysis has been recognized as an indispensable procedure because hydrolysis can not only influence the ultimate production, but also control the reaction rate of the entire process. Because of the interior tight crystal structure and exterior persistence lignin (Zeng and Xiaohong, 2013), enzyme is unable to exert its fullest potential, which leads to low efficiency of enzyme hydrolysis and being adverse to subsequent energy conversion. Nevertheless, high concentration of reducing sugar is the basis of energy conversion. Therefore, how to reach the goal of high sugar yield with low cost has been a major priority in this research.

It has been reported by some researches that metal ions (Fe³⁺ and Fe²⁺) pretreatment can remove lignin effectively, eliminate structural and compositional obstacles to hydrolysis, increase the sugar yield of hydrolysis of cellulose and hemicelluloses (Zhao et al., 2011; Nguyen, 2002). Also, it has been suggested by other studies that adding metal ions such as Mg²⁺ and Fe³⁺ in saccharification process can enhance enzyme activity (Wang et al., 2013; Bin and Hongzhang, 2010). The results from the research made by Bi ((2013) on the effects of Mn and Fe (II) on ethanol yield by simultaneous and saccharification (SSF) shows that both salts increases ethanol yield and enzyme activity. The impact of metal ions on cellulose hydrolysis is therefore an important topic to discuss. Indeed, numerous studies have identified the positive role of metal ions on improving sugar yield, but few reports optimize parameters of adding metal ions on enzymatic hydrolysis. Furthermore, under the same experimental condition, the differences of the effects of different metal salts on enzymatic hydrolysis of liquefied rice straw were seldom compared.

On the basis of laboratory research, this study chose...
liquefied rice straw as substrate; optimized the hydrolysis process and investigated the effects of salt concentration, enzyme loading, temperature and time on the enzymatic hydrolysis. Meanwhile, the role of inorganic salts on the mechanism of converting cellulose into reducing sugar was also put into discussion. The results from the study may provide an insight on the conversion of cellulose into reducing sugar more efficiently.

MATERIALS AND METHODS

The air-dried rice straw (RS) without rotting collected from Yongchuan District, Chongqing; cellulose (Tviride) purchased by Shanghai Sinopharm chemical reagent Co., Ltd, enzyme activity as described by supplier is 15000 u/g; the Mn and Fe (II) salts used in this study are MnSO₄, FeSO₄·7H₂O, respectively. All chemicals for this experiment are analytical reagent.

Methods

Pre-treatment of rice straw

RS was cut to 1 to 2 cm and grinded by ball mill and then made to passed through a 80 mesh sieve (0.18 mm). The milled material was dried in oven at 105°C for 24 h to a constant weight and stored under room temperature for further analysis.

In the liquefied pretreatment (Wang et al., 2012; Rixin et al., 2011), the oven-dried RS 20 g and glycol 120 g were placed into a three-neck flask equipped with a reflux condenser, a thermometer and a motor-driven stirrer, refluxed in an oil bath at 160°C with continuous stirring, 3% sulfuric acid added as the catalyst at the setting temperature and then reacted for 60 min. After the reaction accomplishment, the flask was taken out of the oil bath and cooled down to stop the reaction and the liquefied products were filtrated with methanol and hot water until the liquid was neutral. The solids were oven-dried and used as substrate.

Enzyme saccharification of RS

The enzyme saccharification was carried out in a 100 ml Erlenmeyer flask. The substrate and sodium citrate buffer (0.05 M, pH4.8) were added at 1:10 ratio. The samples with a certain amount of enzyme were incubated on a thermostat water bath at 80 rpm. Mn and Fe (II) were individually put to saccharification reaction mixtures prior to the initiation of reaction. The flasks were taken at predetermined intervals and put in boiling water for 5 min to terminate the reaction and centrifuged at 7000 rpm for 10 min. The supernatant was estimated for the sugar yield after being appropriately diluted.

The experiments were designed into three groups: blank group, Mn and Fe (II) groups. The effects of dosage of metal salts and enzyme, enzymolysis temperature and time were investigated by single-factor experiments.

Analysis methods

In weight difference method, 72% sulfuric acid and coal proximate analyzer exerted their function to evaluate the weight of cellulose, hemicellulose, lignin, ashes and moisture, respectively. SEM was used to investigate the RS morphology before and after pretreatment. The dinitrosalicylic acid (DNS) method was used to measure the glucose concentration and the sugar yield was calculated according to Equation (1):

\[ \eta = \frac{nCV}{m} \]  

Where \( \eta \) is sugar yield per unit mass of substrate (mg/g), \( n \) is dilution ratio, \( C \) is sugar concentration (mg/ml), \( m \) is the weight of substrate (g), and \( V \) is reaction volume (ml).

RESULTS AND DISCUSSION

Effect of liquefied pretreatment on the structure of RS

Pretreatment is one of the most dominating steps during the process of effective enzymatic saccharification (Li et al., 2009). The effect of pretreatment has been described as a disruption of cell wall, as well as, solubilizing partial cellulose and hemicellulose. Pretreatment is also able to decrease the degree of polymerization and crystallinity. The components of RS before and after pretreatment are investigated by single-factor experiments.

Table 1. The components of RS before and after pretreatment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
<th>Lignin (%)</th>
<th>Ashes (%)</th>
<th>Moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS</td>
<td>33.4</td>
<td>29.7</td>
<td>17.1</td>
<td>14.1</td>
<td>5.7</td>
</tr>
<tr>
<td>Substrate</td>
<td>54.3</td>
<td>17.4</td>
<td>11.6</td>
<td>11.4</td>
<td>5.3</td>
</tr>
</tbody>
</table>

Pretreatment broke the...
crystal structure, removed the package of lignin, depolymerized and dissolved hemicellulose. The pretreatment made the separation of cellulose, hemicellulose and lignin, as well as, the degradation of partial hemicellulose and lignin, which at last, resulted in fully exposure of cellulose.

Figure 1A and B shows the results of SEM before and after pretreatment, respectively. The complete and clear inner and outer surface structures no longer existed; replaced with loose fragments, which was hardly distinguished from its fragmental interior and exterior structure (Zeng, 2012). It indicates that liquefied pretreatment degrades lignin, decreases the crystallinity and increases the porosity and also provides abundant contact area for subsequent enzyme saccharification.

Influencing factors of enzyme saccharification

Effect of dosage of Mn and Fe (II) salts on enzymatic saccharification

In the optimal enzyme loading (25 mg/g) of blank group, 6, 7, 8, 9, 10 mg/g Mn salts and 1, 2, 3, 4, 5 mg/g Fe (II) was added into the Erlenmeyer flask, separately. The enzyme saccharification was performed at 50°C on a thermostat water bath at 80 rpm for 72 h. The relationship between enzyme loading and sugar yield is shown in Figure 4. As seen from it, when enzyme loading is 25 mg/g, the sugar yield of blank and Fe (II) group reached highest, that is, 348 and 403 mg/g, separately. However, the largest sugar yield of 349 mg/g for Mn group is achieved with 20 mg/g enzyme. A common feature is found in all groups, namely, sugar yield rises firstly and then drops down with the augment of enzyme loading. It might be explained that small amount of enzyme leads to incomplete degradation of cellulose and sugar yield reduction while excessive loading result in supersaturation. In the initial phase of enzymolysis, the hydrolysis rate of substrate increased as well as, the cumulative rate of cellulose, thereby inhibiting the further effect of enzyme and lowering the final sugar yield.

From Figures 2 and 3, the sugar yield goes down as metal salts continue to go up. It means that moderate metal salt can disorganize the crystalline structure of cellulose, increase contact area of enzyme and substrate and improve enzyme activity. The enzyme activity is inhibited and sugar yield declines while the dosage exceeds the suitable ranges. The conclusion that metal salt is an enzyme activator and inhibitor accords with what Nguyen (2002) concluded in his study.

Effect of enzyme loading on enzymatic saccharification

15, 20, 25, 30, 35 mg/g enzyme was added into the Erlenmeyer flask with the following plus of 9 mg/g Mn salt and 4 mg/g Fe (II) salt, respectively. The enzyme saccharification was performed at 50°C on a thermostat water bath at 80 rpm for 72 h. The relationship between enzyme loading and sugar yield is shown in Figure 4. As seen from it, when enzyme loading is 25 mg/g, the sugar yield of blank and Fe (II) group reached highest, that is, 348 and 403 mg/g, separately. However, the largest sugar yield of 349 mg/g for Mn group is achieved with 20 mg/g enzyme. A common feature is found in all groups, namely, sugar yield rises firstly and then drops down with the augment of enzyme loading. It might be explained that small amount of enzyme leads to incomplete degradation of cellulose and sugar yield reduction while excessive loading result in supersaturation. In the initial phase of enzymolysis, the hydrolysis rate of substrate increased as well as, the cumulative rate of cellulose, thereby inhibiting the further effect of enzyme and lowering the final sugar yield.
significantly improve sugar yield. It is probably due to the increase of enzyme activity and sufficient action of enzyme and substrate. On the contrary, adding Mn can increase the amount of sugar in inception phase, but the highest yield is
similar to that of the blank group, which describes that Mn neither stimulated nor inhibited cellulase during the experiment. Compared with blank and Fe (II) groups, adding Mn salt can reduce enzyme loading, which is similar to the trend of enzyme loading in ethanol preparing process as reported by Bi (2013).

**Effect of temperature on enzymatic saccharification**

For Mn group, 9 mg/g Mn salt and 20 mg/g enzyme were added into the reaction system. And for Fe (II) group, 4 mg/g Fe (II) salt, 25 mg/g enzyme was added. The 25 mg/g enzyme was added into the blank group. The enzymatic saccharification was performed on a thermostat water bath at 80 rpm for 72 h. Reactive temperature was respectively controlled in 35, 40, 45, 50 and 55°C. As shown in Figure 5, the highest sugar yield, 348 mg/g of blank group reaches at 50°C, while 446 mg/g of Fe (II) group at 35°C and 412 mg/g of Mn group at 40°C. In the suitable temperature range, sugar yield increases along with the rise of temperature, when temperature goes up and becomes too high, the yield declined, which agreed with the previous report that high temperature leads to decrease of enzyme activity (HaiPeng et al., 2014). Figure 5 reveals that the temperature of Mn and Fe (II) groups was lower than that of the blank group. This finding confirms a possibility that adding metal salts can reduce the active energy, meanwhile, different metal salts have different effects on the enzyme activity; thereby the growing rate of sugar yield is different.

**Effect of enzymolysis time on enzymatic saccharification**

In Mn group, 9 mg/g Mn salt and 20 mg/g enzyme was added and reaction was finished at 40°C. For Fe (II) group 4 mg/g Fe (II) salt and 25 mg/g enzyme was added and reaction was completed at 35°C. The 25 mg/g enzyme was added in the blank group and reaction was accomplished at 50°C. Enzymolysis time was under control at 48, 60, 72, 84, 96, 108 h, respectively. The enzymatic saccharification was performed on a thermostat water bath at 80 rpm. Figure 6 shows the relationship between time and sugar yield.

From Figure 6, the sugar yield of the blank group gradually levels off after 96 h and reaches 443 mg/g at this time. For Mn group, the sugar yield is slow-paced after 84 h, and is 435 mg/g at 84 h. The sugar yield of Fe (II) group gradually stabilizes after 72 h and is 446 mg/g at this moment.

The sugar yield of all groups increases with increasing time and reaches similar level about 108 h. However, sugar yield of Mn group was higher than that of the blank group, but lower than that of Fe (II) group within the same enzymolysis time.

One reason for this phenomenon is that with increase of time, the linkages of cellulose and hemicellulose becomes relatively loose, which promotes the accessibility of enzyme to cellulose and improves enzymatic activity. When the action time given is enough to a certain value, the by-product of enzymatic saccharification will inhibit the whole reaction which results in low reaction rate.
Conclusions

The effects of Mn and Fe (II) salts on sugar yield of RS saccharification which was pretreated by liquefying were investigated by single-factor experiments. The following conclusions could be drawn.
The experiments suggested that the enzyme loading of Mn group decreased from 25 mg/g of blank group to 20 mg/g, while Fe (II) group was consistent with the blank group. The proper enzymolysis temperature of Mn and Fe (II) group lowered from 50°C of blank group to 40 and 35°C, respectively. Within the same enzymolysis time, Mn group was higher than that of the blank group, but lower than that of the Fe (II) group, which provided a good basis for subsequent energy conversion.

Adding Mn not only reduced enzyme dosage and enzymolysis time but also lowered temperature. Adding Fe (II) could also lower the temperature, save time and improve sugar yield, which was better than that of Mn salt. In saccharification process, adding Mn and Fe (II) in saccharification process can disorganize the crystalline structure of cellulose, increase the contact area of the enzyme and substrate, improve enzyme activity to enhance the efficiency of enzymolysis and reduce energy consumption.

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