Biodegradation of Grass by a Rot Fungus Isolated from Cattle Faeces

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Abstract. In this study, the capability of a rot fungus isolated from cattle rumen was investigated as a means of biodegrading field grass. \textit{Coprinopsis cinerea}, which is classified as an obligate aerobic organism, was isolated under semi-aerobic conditions from cattle rumen. The grass was inoculated with the isolated fungus under aerobic conditions for 30 days. In a microscopic examination of the fungi-treated grass some cavities could be seen. It was found that the amounts of cellulose, hemicellulose and total solids in the fungi-treated grass were lower than those of the untreated grass by 8\%, 9\%, and 13\% respectively. The lower contents of cellulose, hemicellulose and total solids in the fungi-treated grass indicated the biodegradation activity of the isolated \textit{C. cinerea}. Data from batch biogas production suggested that the initial conversion rate of the fungi-treated grass to methane was more than double that of the untreated grass.

Keywords: \textit{Coprinopsis cinerea}, biopretreatment, hydrolysis, biogas, pretreatment, biomass

1. Introduction

Biological pretreatment of lignocellulosic materials has been shown to enhance biogas production [1]. The pretreatment can change the chemical structure of the lignocellulosic biomass and make it more accessible to hydrolysis [2]. The biological treatment is considered to be more environmentally friendly and less energy-consuming than mechanical and chemical pretreatments [3].

A number of fungal and bacterial strains have been shown to effectively degrade lignocellulosic biomass. Among fungal strains, white-rot fungi have been shown to be potential biomass degraders. A white-rot fungus \textit{Pleurotus florida} which produces lignin-degrading enzymes was reported to help the hydrolysis of raw corn straw [4]. \textit{P. ostreatus} was found to degrade phenolic compounds in olive-mill wastewater and consequently to enhance stability of the biogas production system [5]. It has been shown that ethanol production from biomass under aerobic conditions can be increased by use of a white-rot fungus \textit{Chalara parvispora} and a soft-rot fungus \textit{Trametes versicolor} when compared with fermentation using only \textit{Saccharomyces cerevisiae} [6]. However, there has been little work on the effects of biomass pretreatment by \textit{Coprinopsis cinerea}, a litter-decaying fungus.

In this study, the capability of \textit{C. cinerea} isolated from cattle rumen in degrading field grass was investigated. Changes in the physical structures of the field grass after 30 days of aerobic treatment were examined and the biogas productions of the untreated and the fungi-pretreated grass were compared.

2. Materials and Method

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2.1. Materials

Buffalo grass (*Brachiaria mutica*) was used as the carbon source. The grass was dried at 30-35°C until the moisture content dropped to less than 10% [7]. The materials were chopped by a chopper (20 mm) and ground into 2-3 mm powder by a blender. The raw materials were then stored in a black bag at room temperature (30-35°C) prior to use.

Mesophilic anaerobic sludge was used as the inoculum for anaerobic biogas production. This sludge was obtained from Ngaung-Khaem Water Quality Control Plant, a domestic wastewater treatment plant in Bangkok, Thailand.

*C. cinerea* isolated from cattle rumen was used as the inoculum in the aerobic treatment of the buffalo grass. Molecular identification was made using the 18s rDNA sequences database. The identification showed that the white mycelia fungus was *Coprinopsis cinerea* (99% similarity). The nucleotide sequence was as follows.

\[
\text{GCCCGTCACCTTTATTTCTCCACCTGTGCACACACTGTAGGCCTGGATACCTCTCGTCGCAAGGCGGATGCGTGGCTTGCTGTCGCTTTCGAAAGAAGGCCGGCTTGCCATGAA}$

\[
\text{TTTCCAGGTCATGTCGCTTATTTCAACAACCCCAAACCTGAGATATGGAATGTCATCTC}$

\[
\text{AAGGCCCTGTTCATATAAACCTATACCATATACAACTTTCAGCAACGGATCTCTTGGCTCTCG}$

\[
\text{CATCGATGAAAAACGCACACCGGAAATGCGATAAGTAATGTGAATTGCAAAATTCAGTGA}$

\[
\text{ATCATGGAATCTTTGGAACGCACCTTGCGCTCCTTGGTATTCCAAGGACATGCTGT}$

\[
\text{TTGAGTGTCATTAAATCTCAACCTCACCACCTTGTGTGTGTGCAGG}$

2.2. Aerobic pretreatment of the buffalo grass by the isolated fungus

Five grams (dry weight) of the grass powder (2-3 mm long) were transferred into a 250 ml Erlenmeyer flask and moisturized with 15 ml of tap water. Then the grass was autoclaved for 15 min at 121°C to ensure sterilization. The flask was then inoculated with 10 disks (each with 1 cm in diameter) of the fungal culture and incubated at 28°C for 30 days.

After the specified pretreatment period, the grass was washed with 37.5 ml distilled water and filtered under vacuum. The solid fractions were then dried at 85°C [8] and analyzed for dry weight and cellulose, hemicellulose and lignin contents. The liquid fractions were analyzed for pH, reducing sugar, volatile fatty acids (VFA), soluble chemical oxygen demand (sCOD), total nitrogen (TN) and total phosphate (TP).

2.3. Anaerobic biogas production

One hundred milliliter serum bottles, each with a rubber stopper, were used as batch reactors. The bottles contained 1 g volatile solids (VS) of buffalo grass (with or without biopretreatment) and anaerobic sludge at a ratio of 1 g VS/gVS. The batch reactor had 5% total solids (TS). The reactor bottles were first flushed for 1 min with 99.995% argon to ensure anaerobic conditions and then incubated under mesophilic temperatures between 30°C and 35°C for 1 month. The generated biogas was collected using either 25 ml- or 50 ml-hospital needle syringes [9]. The amounts of biogas production were reported per gram VS under standard conditions (STP: 0°C and 1 atmosphere). The biogas samples were taken daily with a syringe through the septum from the headspace of the reactors for the measurement of biogas composition.

For each run, a control blank with only the inoculum was run in parallel. After each specified incubation period, the samples were collected for analysis of pH, volatile fatty acid (VFA), VS and TS, soluble chemical oxygen demand (sCOD) and cellulose, hemicellulose and lignin content.

2.4. Analysis

Biogas composition was determined by a gas chromatograph equipped with a thermal conductivity detector (GC-2014, SHIMADZU, Japan) and a unibeads C column. The argon flowrate was 25 ml/min.

Measurements of cellulose, hemicellulose and lignin contents were performed by the detergent method [10]. The hemicellulose content was calculated from the difference between neutral detergent fibre (NDF) and acid detergent fibre (ADF). The lignin content was the difference between ADF and permanganate lignin (PML). After the PML analysis, the cellulose content was estimated from the weight loss of the sample when held at 550°C for 3 hrs.
Reducing sugar was measured by the dinitrosalicylic acid (DNS) method [11]. Total nitrogen (mg/l) and total phosphate (mg/l) were measured using HACH Test’N test tubes [12]. VFA concentration was determined by a gas chromatograph (GC-2010, SHIMADA, Japan) equipped with a flame ionization detector. The concentrations of TS, VS and VSS were determined by the Standard Methods [13], and the sCOD was determined using the closed-tube method [14]. The pH was measured by a pH meter (Schott, Lab 850, Germany).

The microscopy images of the untreated grass and the fungi-pretreated grass were obtained using a MEIJI TECHNO ML 2000 (Japan) microscope.

3. Results and Discussion

3.1. Changes in physical structures and chemical compositions of the field grass

Rot fungi are known to secrete cellulase from their hyphae [15]. This leads to the formation of microscopic cavities inside cellulosic materials [16]. Fig. 1 shows a comparison of the microscopy images of the untreated grass and of the fungi-pretreated grass after 30 days under aerobic conditions. It can be seen that some microscopic cavities are present in the fungi-treated grass. The pretreatment should therefore increase the specific area of the cell wall saccharides. The increase in the specific area should in turn accelerate enzymatic hydrolysis as suggested by Gharpuray et al. [17].

![Fig. 1: Microscopy images of the untreated grass (left) and of the fungi-pretreated grass (right) after 30 days under aerobic conditions.](image)

Table 1 compares the chemical compositions of the insoluble parts and the soluble parts of the untreated grass and the fungi-treated grass. The values shown in Table 1 are based on one gram of grass substrate (dry weight). The amount of dry weight or total solid content of the fungi-treated grass was lower than that of the untreated grass by 13%. Similarly, the cellulose and the hemicellulose contents of the fungi-treated grass were lower than those of the untreated grass by 8% and 9%, respectively. The lower contents of the total solids, cellulose and hemicellulose in the fungi-treated grass indicated the biodegradation activity of the isolated *C. cinerea*.

The pH of the soluble part of the untreated grass was acidic, which corresponded to the high content of VFA. The acidic pH and the high content of VFA in the untreated grass suggested the presence of hydrolysis and acidogenesis activities of bacteria which may naturally present in the grass substrate. In contrast, the pH of the soluble part of the fungi-treated grass was slightly alkaline. The content of soluble COD compounds and specifically the amount of glucose in the fungi-treated grass was much lower than those of the treated grass. The data suggested that *C. cinerea* utilized glucose and some other soluble compounds for their growth. In the literature, it has been reported that white-rot fungi uptake and utilize glucose for their growth [18].

3.2. First-stage of biogas production from the untreated and the fungi-pretreated grass

In contrast to anaerobic degradation which is usually coupled with anaerobic biogas production using co-cultures or tri-cultures, aerobic degradation by fungi is a separate step. The aerobic degradation can be easily terminated in order to start anaerobic digestion by limiting the air supply to the biogas production system.
Fig. 2a shows the methane content in the biogas produced from the untreated and the fungi-treated grass. The methane content in the biogas produced from the fungi-treated grass reached a maximum of 60% within 4 days, while that of the untreated grass increased slowly and reached a maximum of 55% on day 10. Fig. 2b compares the cumulative methane production from the untreated and the fungi-treated grass. The methane production rate associated with the digestion of the fungi-treated grass was constant at 112 ml/l-day during the first fourteen days. In contrast, the methane production rates associated with the digestion of the untreated grass can be divided into two phases. During the first seven days, the methane production rate was 48 ml/l-day, which was lower than half of that of the fungi-treated grass. During the second seven days, the methane production rates became comparable. The results suggested that biotreatment helped to accelerate the hydrolysis at the initial stage. The fungal pretreatment therefore should be useful for biogas production because hydrolysis is known to be the rate-limiting step of the biomass-to-biogas conversion process [19].

A previous study by Romano et al. [20] illustrated that the addition of enzyme products containing cellulase, hemicellulase, and β-glucosidase to anaerobic digestion systems had positive effects on the solubilization of wheat grass when used alone to treat grass. However, no significant differences in biogas and methane yields, and volatile solids reduction resulted when the enzyme products were tested in the anaerobic digestion systems. The results in this study suggested that the enhanced solubilization of grass lignocelluloses may help accelerate the biogas production process, even though it may not help increase the biogas and methane yields.

### Table 1: Chemical composition of the untreated and the fungi-treated grass

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Untreated grass</th>
<th>Fungi-treated grass</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insoluble part</strong></td>
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<td></td>
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<tr>
<td>dry weight (g)</td>
<td>0.83</td>
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<tr>
<td>cellulose (g)</td>
<td>0.29</td>
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<td>hemicellulose (g)</td>
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<td>lignin (g)</td>
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<tr>
<td><strong>Soluble part</strong></td>
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<td></td>
</tr>
<tr>
<td>sCOD (mg)</td>
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<td>82.70</td>
</tr>
<tr>
<td>TN (mg)</td>
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<td>6.27</td>
</tr>
<tr>
<td>TP-PO₄³⁻ (mg)</td>
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</tr>
<tr>
<td>reducing sugar (mg)</td>
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</tr>
<tr>
<td>pH</td>
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<td>7.52</td>
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<tr>
<td>VFA (mg)</td>
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<td></td>
</tr>
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<td>acetate</td>
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</tr>
<tr>
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</tr>
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<tr>
<td>valerate</td>
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</table>

Fig. 2: Comparative methane composition in biogas produced from anaerobic digestion (a) and cumulative methane production (b) of the untreated and the fungi-treated grass
4. Conclusion

Biopretreatment of field grass by *C. cinerea* was found to increase the initial methane production rate during the first seven days. The overall conversion rate of the fungi-treated grass to methane was around double that of the untreated grass. Some cavities can be observed in the fungi-treated grass at a microscopic level. The biopretreatment may increase the specific area of the cell wall saccharides, which in turn should accelerate enzymatic hydrolysis. However, the chemical composition data suggested that *C. cinerea* utilized glucose and some other soluble compounds for their growth. Effects of the biopretreatment on biogas and methane yields should be further investigated.

5. Acknowledgements

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6. References


