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研究論文

Study of bio-ethanol production from cellulosic waste (rice straw)

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This basic research was carried out on bio-ethanol derived from cellulosic waste (rice straw). In view of the fact that rice straw is incorporated into the soil after harvesting the grains to increase mineral soil content, we examined the option of using high pressure pretreatment which does not involve chemical treatment, thus allowing residues from bio-ethanol production to be returned to the soil as a liquid fertilizer. Results from this study showed that i) high-pressure treatment enhanced the saccharification of rice straw and ii) soil containing residues from bio-ethanol production did not inhibit the growth of Japanese mustard spinach planted from seed.

Key words: cellulosic waste (rice straw), high-pressure treatment, soil containing residues, bio-ethanol production

1. Introduction

There is an urge to develop renewable fuels that could replace oil. The present study focused on the use of cellulosic waste (rice straw) as a raw material for fuel production. Rice is extensively cultivated in Japan, and the annual total of inedible rice straw and rice hull generated is estimated at 14 million tons, 70% of which are not being used. In view of this, the possibility of producing bio-ethanol from rice straw that is currently being wasted was examined. Rice straw is generally incorporated into the soil after harvesting and is not being used effectively as raw material for bio-ethanol. Thus, an important goal is to effectively use the cellulose component of rice straw and then return the resulting residues to the soil. The present study is part of a basic research project on the production of bio-ethanol from rice straw.

This paper reports on the development of a method for saccharification pretreatment of rice straw for bio-ethanol production that does not involve the use of chemical treatment, as well as on the outcome of reusing the residues of bio-ethanol production as a liquid fertilizer to grow Japanese mustard spinach planted from seed.

2. Experiment

① Pressure application to rice straw alone

Rice straw was cut into pieces of approximately 3 cm in length, 1 g of which was placed in a plastic bag and sealed. A manual hydraulic pump RIKEN-WP-1B was subsequently used to place the straw under varying pressure conditions (10 MPa, 20 MPa, 30 MPa) at room temperature (25°C) for an hour. The rice straw was removed from the bag, put into a beaker, to which 30 mL phosphate buffer, pH5.5, and 0.6 g cellulase (Onozuka 3S) were added. This was then saccharified at 50°C for 24 hours using a Bio Shaker to measure the glucose level. The glucose level was quantified by an enzymatic method using a glucose kit (Glucose C II test).

② Addition of cellulase and pressure application

1 g rice straw, 80 mL phosphate buffer, pH5.5, and 0.6 g cellulase (Onozuka 3S) were put in a plastic bag and sealed. A manual hydraulic pump was subsequently used to place the straw under varying pressure conditions (10 MPa, 20 MPa) at room temperature (approximately 25°C). Samples placed under differential pressure conditions were then saccharified at 50°C for 24 hours using a Bio Shaker to measure the glucose level.

③ Glucose amount produced by rice straw samples left for varying periods of time

Glucose concentration of the rice straw harvested in 2004 was compared to that harvested in 2009 following steps similar to those in Experiment ② and at a pressure of 20 MPa. Furthermore, non-pressure-treated rice straw harvested in 2004 (control) was compared with rice straw harvested in 2004 and pressure-treated with 0.4 g of commercially available cellulose powder added in terms of the amount of glucose produced.

④ Reuse of treatment residues as liquid fertilizers

Rice straw was cut into pieces of approximately 10 cm in length, sterilized in an autoclave, added with a Trichoderma cellulase preparation (QM9414) and saccharified at room temperature (approximately 25°C). Rice straw had been harvested in 2010. 1 mL cellulase preparation and 30 mL phosphate buffer, pH5.5, were added to every 1 g of rice straw.

The residues from the saccharification treatment were spread over the seedbeds in the vegetable garden used for testing as a liquid fertilizer.

Furthermore, the saccharified sample was added with yeast and underwent alcoholic fermentation at room temperature (approximately 25°C). 0.1g yeast was added to every 1g of the saccharified rice straw. The residues from alcoholic fermentation were spread over the seedbeds in the vegetable garden used for testing as a liquid fertilizer.

Treatment residues were sterilized in an autoclave before they were spread over the soil as liquid fertilizers. The seeds of a leafy vegetable known as Japanese mustard spinach were scattered in the vegetable garden used for testing where the liquid fertilizer had been spread. The growth of the plant was observed to determine the impact of using or not using the liquid fertilizer.

3. Results and Discussion

(1) Glucose produced when pressure was applied to rice straw alone

Results from Experiment ① are summarized in Figure 1.

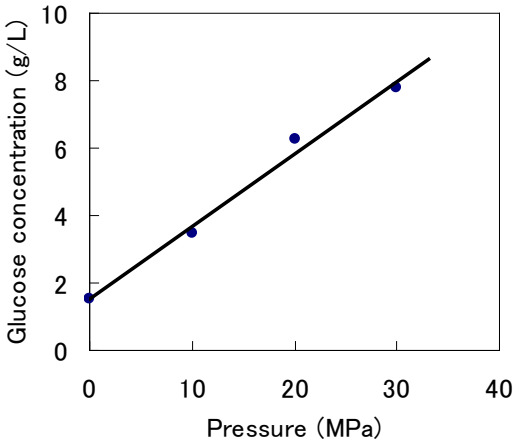

Figure 1: Glucose amount derived from pressure application to rice straw alone

When pressure was applied to rice straw alone before adding cellulase for saccharification, the resulting glucose concentration increased proportionally to the pressure applied, as shown in Figure 1. Furthermore, although pressure conditions higher than 30 MPa were not examined in this experiment, a linear cellulase-induced increase was observed in the rate of saccharification when a relatively high pressure was applied to rice straw alone.

(2) Glucose amount derived from pressure application to rice straw with added cellulase

Results from Experiment ② are summarized in Figure 2.

Two pressure-treated rice straw samples with added cellulase were compared in terms of the amount of glucose produced under differential pressure conditions of 10 MPa and 20 MPa. Although pressure application time was found to be associated with increased glucose production, no association was found between the amount of glucose produced and the amount of pressure applied. Comparison of Experiment ① and Experiment ② at one hour of pressure treatment revealed that the former resulted in a higher production of glucose. These findings suggest that the rate of saccharification induced by cellulase is higher in high-pressure treated rice straw.

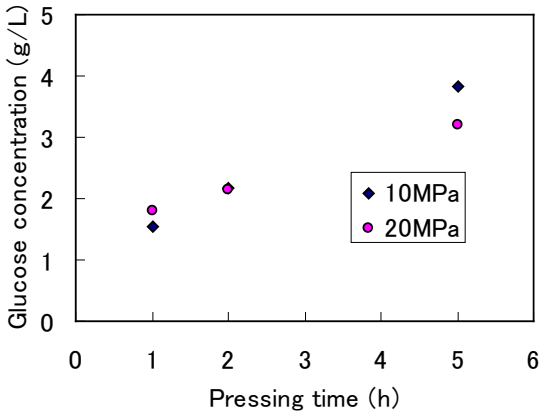

Figure 2: Amount of glucose produced when pressure was applied to rice straw with added cellulase

(3) Comparison of glucose amounts produced by rice straw samples left for varying periods of time

Results from Experiment ③ are summarized in Figure 3.

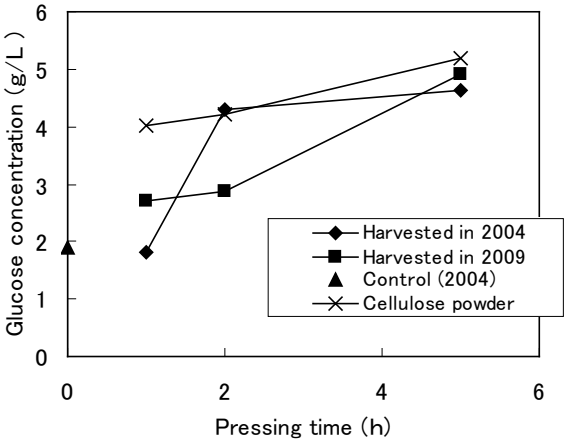

Figure 3: Comparison of rice straw samples left for varying periods of time

Comparison was made between two rice straw samples harvested in 2004: one without high-pressure treatment (control) and the other with high-pressure treatment with added cellulase. Findings from this comparison indicate that pressure application to rice straw with added cellulase allows cellulase to infiltrate into the inside of the fiber structure of rice straw, thus facilitating glucose production. It can therefore be concluded that high-pressure pretreatment is associated with enhanced saccharification.

The comparison of two rice straw samples left for varying

periods of time (one sample harvested in 2004 and the other sample harvested in 2009) in terms of the concentration of glucose produced, revealed that these two samples produce approximately the same glucose amount at five hours of pressure treatment.

On the other hand, the comparison of the experiment performed using cellulose powder and the other carried out with pressure pretreatment revealed that the theoretical amount of glucose can be obtained when a pressure of 20 MPa is applied for a period of five hours. It can therefore be concluded that approximately five hours of pressure pretreatment should suffice.

(4) Reuse of treatment residues as liquid fertilizer

Residues from bio-ethanol production (liquid fertilizer) retain a fibrous structure of the straw, as shown in Figure 4. Table 1 summarizes the components of two types of liquid fertilizers obtained from the residues from saccharification treatment and the residues from fermentation treatment, respectively. The components studied include nitrogen (N), phosphorus (P), potassium (K), carbon (C), and water content.

Table 1: Components of treatment residues (%)

Comp.	Sacchar.T.	Fermen.T.
N	0.6	0.11
P	0.3	0.11
K	1.5	0.22
C	23.0	4.13
Water	33.3	88.1
C/N con.	39.0	37.5

Figure 4: Residues from rice straw (a: saccharification treatment, b: alcohol fermentation treatment)

These components are important minerals for the seedbed soil. Thus, when soil is deprived of such minerals, they must be restored with chemical fertilizers. In view of this, returning the mineral content of residues from bio-ethanol production to the soil is important to keep the soil healthy. Residues from saccharification treatment and residues from fermentation treatment hold different amount of water, which makes the numerical comparison of the two types of residues problematic. However, their carbon/nitrogen content was found to be 39.0% and 37.5%, respectively. It can therefore be assumed that these residues are similar in their mineral content. In other words, no difference was found between saccharification treatment and fermentation treatment in terms of the mineral content of the resulting residues.

Figure 5: Photograph taken after scattering the seeds over the vegetable garden used for testing (a: experiment section 1, b: experiment section 2, c: control section)

Figure 6: Growth of Japanese mustard spinach (a, b, and c are as described in Figure 5, four weeks from seeding)

Japanese mustard spinach to the right and spinach to the left.

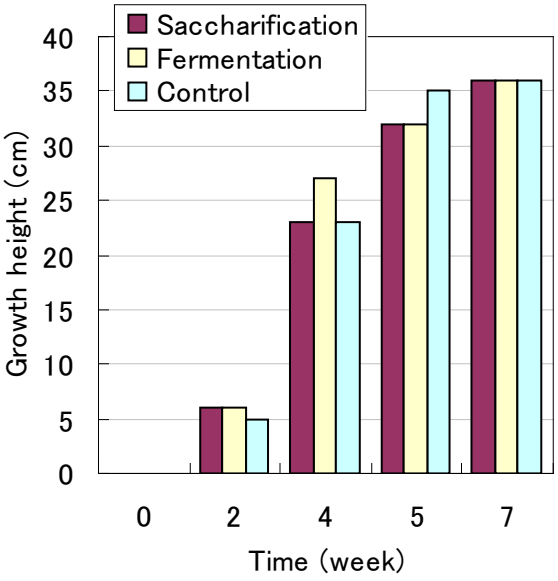

Figure 7: Height vs. time from seeding of Japanese mustard spinach

In view of the fact that rice straw is incorporated into the soil after harvesting grains, we investigated the environmental impact of returning the residues from bio-ethanol production from rice straw to the soil as a liquid fertilizer. Residues from rice straw, which still retained the fibrous structure of the straw (Figure 4), were spread over the soil as a liquid fertilizer. The seeds of Japanese mustard spinach and spinach were directly sown in two experiment sections and a control section in the vegetable garden used for testing (Figure 5). However, due to unfavorable weather conditions and temperatures below 15°C in October, 2010, when the experiment was conducted, spinach, which is known to be sensitive to low temperatures, did not grow well as a whole, as can be seen in Figure 6.

For this reason, the effectiveness of using the liquid fertilizer was determined by observing the growth status of Japanese mustard spinach that is more resistant to temperature changes. The results of this observation are summarized in Figure 7. The control was the Japanese mustard spinach whose seeds had been sown in the seedbed where no liquid fertilizer had been applied. As shown in Figure 7, the growth of Japanese mustard spinach was observed for seven weeks from seeding. No data was taken after week 7 because the Japanese mustard spinach was fully matured and ready to be eaten by that time. At week 7, no significant differences were observed between residues from saccharification treatment and residues fermentation treatment used as liquid fertilizers in terms of their effect on the growth of Japanese mustard spinach. It was thus concluded that the use of these liquid fertilizers does not involve any factors that can inhibit the growth of plants. In other words, returning the residues from bio-ethanol production to the soil does not cause growth inhibition of plants.

Acknowledgment

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草本系セルロース廃棄物(稲藁)からのバイオエタノールの生成に関する研究

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要 旨

草本系セルロース廃棄物、稲藁からのバイオエタノールを生成する基礎研究を行った。本研究は、稲藁は稲穂を採取した後田畑に漉き込まれミネラル成分肥料となっていることを前提として、バイオエタノール生成後の処理残渣を田畑に液肥として還元するために化学処理しない方法での前処理、高圧処理を検討した。その結果、高圧処理した稲藁の糖化が促進された。さらに、処理残渣を田畑に還元したところ、コマツナの播種からの生長に関して生育阻害は観察されなかった。