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ScienceDirect

Energy Procedia 100 (2016) 155 – 159

Energy Procedia

3rd International Conference on Power and Energy Systems Engineering, CPESE 2016, 8-12 September 2016, Kitakyushu, Japan

Production of ionic liquid-tolerant cellulase produced by microbial consortium and its application in biofuel production

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Abstract

To produce biofuels and specialty chemicals via biorefining process, conversion of lignocellulosic biomass to sugars requires both efficient pretreatment and hydrolysis enzymes. Ionic liquids (ILs) are effective solvent for lignocellulosic biomass pretreatment, however the adverse effect of IL to cellulase has been demonstrated. Here, a lignocellulose degrading microbial consortium was newly bred from saline soil in rice paddle field in Thailand by successive subcultivations. To study the IL-tolerant property, activities of partially purified cellulase produced from CT-1 were evaluated in the present of an IL, 1-ethyl-3-methylimidazolium acetate ([C2mim][OAc]). At 1 M of [C2mim][OAc], the cellulase activity was remained more than 85.09% of non-IL condition. For application in biogas production, the rice straw residues mixing with activated sludge wastewater prior to anaerobic digestion was investigated by using this CT-1 consortium as inoculums in batch bioreactors. The experimental results showed that the maximum biogas yield (170.92 mL/g-VS) was obtained. These properties demonstrated that this microbial consortium is potential to be applied for lignocellulose conversion to fuel and other industrial chemicals.

Peer-review under responsibility of the organizing committee of CPESE 2016
Keywords: Biogas production, ionic liquid, cellulase, microbial consortium, linocellulosic biomass

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Peer-review under responsibility of the organizing committee of CPESE 2016
doi:10.1016/j.egypro.2016.10.158

1. Introduction

Rice straw residue is lignocellulosic biomass that is normally left on the rice field and burned to dispose after harvesting season. In Thailand, about 29.15 million metric tons of rice straws in 2013 was produced [1]. Based on this statistic data, the theoretical emission per year of uncontrolled greenhouse gas from the rice straw residues is about 5,000,000 ton, however there is no official record for the use of this feedstock for biofuel production [2]. Therefore, it is interesting to promote its utilization and also other agricultural wastes to produce biofuels. The utilization of rice straw residues is not only providing benefits to energy security but also to the environment as well.

Anaerobic digestion of agricultural residues is a process that has been continuously developed from lab study to industrial scale application because its ability to degrade organic matter into valuable biogas and into a nutrient-rich digestate with agronomic qualities [1]. Lignocellulosic biomass is hydrolyzed to sugars by lignocellulolytic enzymes produced by various microorganisms. Fermentable sugars then could be converted to biogas through fermentation process. However, one of the main problem of biogas production is the difficulty in hydrolysis step because of the recalcitrant structures of biomass [3]. Ionic liquid (IL) pretreatment gained attention because it is green solvent that effectively solubilizes cellulose, and can be recycled for many rounds of pretreatment [3]. IL-pretreatment has many advantages, including high monomeric sugar yields, short pretreatment times, low production of cellulase-inhibitor [4, 5]. Unfortunately, inhibitory effects of ILs to cellulase enzymes have been observed [4, 6]. One of the strategies to overcome inhibitory effects of ILs on cellulase activities is using of the IL-tolerant cellulase. Here, we aimed to produce IL-tolerant cellulase from newly-bred microbial consortium, CT-1, for application in biogas production. The lignocellulolytic activities of CT-1 were analyzed in buffer containing IL, 1-ethyl-3-methylimidazolium acetate ([C2mim][OAc]) to observe the tolerance effect of purified cellulase. Furthermore, CT-1 was also applied in biogas production in anaerobic batch reactor, and biogas yield was observed to find correlation with cellulase activities.

2. Materials and methods

2.1. Isolation of microbial consortium

The microbial consortia, CT-1, was obtained from saline soil in rice paddle field in Nakhonrachsrima province, Thailand. One gram of soil was dissolved in 50 ml of basal medium (containing 0.2% (w/v) rice straw, 0.1% $NaNO_3$, 0.1% K_2HPO_4, 0.1% KCl, 0.05% $MgSO_4$, 0.05% yeast extract). Each culture was incubated at 50°C under aerobic conditions for 7 days. Then, 1 ml of the culture was transferred into 50 ml of fresh medium. The procedure was repeated by successive subcultivations for 5 times to obtain a stable community capable of degrading rice straw.

2.2. Preparation of cellulase enzyme and study of cellulase activity

100 µl of CT-1 was inoculated in 10 ml basal medium. The mixture was incubated at 50°C for 7 day in shaker incubator and used as a starter inoculum. Then 500 ml of fresh media was inoculated with starting inoculum. The cultures was centrifuged at 6,000 rpm for 15 min. The supernatants were collected as the crude enzyme. The crude enzyme was precipitated by the addition of $(NH_4)_2SO_4$ and was collected by centrifugation. The pellets were resolubilized in 50 mM sodium phosphate buffer, pH 7 and was desalted by using dialysis membrane. The samples were concentrated and subjected to a Sephacryl S-100 chromatography column at a flow rate of 0.2 ml/min. Each eluted fraction was collected separately to test for enzyme activity [6].

To measure enzyme activities, 2% w/v carboxymethyl cellulose (CMC) were added and incubated at 50 °C for 1 h, then the amount of reducing sugar was measured by using DNS assay [7]. To evaluate the IL-tolerant properties of cellulase, 0.5 M and 1.0 M of [C2mim][OAc] (Sigma-Aldrich) were added. The optimum temperature and pH of the concentrated cellulase were determined, by varying reaction temperatures from 30 to 70 ^oC, and pH from 2 to 10. To test the effect of pH, each reaction was performed in appropriate buffer (containing 2% w/v of CMC) that adjusted pH to tested condition (2.0-6.0, sodium acetate; 6.0-8.0 sodium phosphate; 8.0-10.0 Tris-HCl). The reactions were set up at 50 ^oC. To test the effect of temperature, each reaction was performed in 50 mM sodium phosphate buffer at pH of 6.0. All experiments were performed with three replicates.