Available online at www.sciencedirect.com

ScienceDirect

Energy Procedia 141 (2017) 180–183

Energy Procedia

www.elsevier.com/locate/procedia

4th International Conference on Power and Energy Systems Engineering, CPESE 2017, 25-29 September 2017, Berlin, Germany

Effect of Cellulase-producing Microbial Consortium on Biogas Production from Lignocellulosic Biomass

Prapakorn Tantayotai[a], Peerapong Pornwongthong[b], Chotika Muenmuang[c], Theerawut Phusantisampan[d], Malinee Sriariyanun[e,] *

[a]*Department of Microbiology, Faculty of Science, Srinakahrinwirot University, Bangkok 10110, Thailand*
[b]*Department of Agro-Industrial, Food, and Environment Technology, Faculty of Applied Science, KMUTNB, Bangkok 10800, Thailand*
[c]*Department of Chemical Engineering, Faculty of Engineering, KMUTNB, Bangkok 10800, Thailand*
[d]*Department of Biotechnolgy, Faculty of Applied Science, KMUTNB, Bangkok 10800, Thailand*
[e]*Department of Mechanical and Process Engineering, TGGS, KMUTNB, Bangkok 10800, Thailand*

Abstract

The product of degraded lignocellulose is sugar which can be utilized by microbial consortium for biogas production. However, the hydrolysis of lignocellulose to produce sugar is addressed to be the rate-limiting step due to the complexity of lignocellulose that is barricade for enzyme accessibility. The aim of this work is to study the effect of a lignocellulose degrading microbial consortium to enhance the biogas production from rice straw. Microbial consortium were isolated from natural samples, including horse manure and decomposed wood. The cellulase activities of each microbial consortium derived from horse manure and decomposed wood were characterized to be endo-β-glucanase (0.417 and 0.434 U/mg), exo-β-glucanase (0.116 and 0.184 U/mg) and β-glucosidase (1.069 and 3.184 U/mg), respectively. The batch experiments for biogas production were performed to investigate the effect of each microbial consortia. The results showed that both microbial consortium enhanced the biogas production because the biogas yield increased to 109.60 and 161.49 ml/g-VS when adding microbial consortium derived from horse manure and decomposed wood, respectively. This work is considered to be a contribution to the research on lignocellulosic biomass degradation by complex microbial community with potential for further biotechnological applications, especially the degradation of lignocellulose-based feedstocks.

Peer-review under responsibility of the scientific committee of the 4th International Conference on Power and Energy Systems Engineering.

Keywords: Lignocellulosic biomass ; Biogas ; Cellulase ; Microbial consortium ; Hydrolysis ; Rice straw

1. Introduction

Lignocellulosic biomass has been used as an alternative and sustainable resources for biofuel production. Lignocellulosic biomass materials, including rice straw, were produced and discarded as waste after harvesting season. In 2009, 55.61 million tons of agricultural residues were left unused on fields in Thailand. These much amounts of biomass were estimated to has potential to generate energy for 732,534 TJ [1]. Therefore, it is interesting to promote the use of lignocellulosic biomass to produce biofuels.

* Corresponding author. Tel.: +66-81-398-0439;.
E-mail address: macintous@gmail.com

1876-6102
Peer-review under responsibility of the scientific committee of the 4th International Conference on Power and Energy Systems Engineering.
10.1016/j.egypro.2017.11.034

One of biofuel that readily integrates to infrastructures of industries and households is biogas. Biogas is naturally produced via biomass decomposition in anaerobic environment. Conversion of lignocellulosic biomass to biofuel requires three steps including pretreatment, hydrolysis and fermentation [1, 2]. Pretreatment has a role to loosen the bundles of lignocellulose fibrils and remove hydrolysis inhibitors. Lignocellulose fibrils are hydrolysed by lignocellulase enzymes and converted to small sugar molecules. Then, sugars are converted to different forms of biofuels via fermentation. Due to multiple steps of the process, it is still not easy to establish an economically feasible process for biofuel production from lignocellulose. To simplify this biorefining process, the integration of pretreatment, hydrolysis and fermentation has been developed with state-of art [3].

Here, we aimed to study the effect of cellulase-producing microbial consortium on the improvement of biogas production from rice straw. Two microbial consortium obtained from horse manure and decomposed wood were newly-bread from successive subcultivations in cellulose-enriched media. The cellulase activities of these two microbial consortium were characterized to be endoglucanase, exoglucanase and β-glucosidase. These two microbial consortium were added in batch anaerobic digesters to observe the effect on biogas production from rice straw.

2. Materials and methods

2.1. Construction of microbial consortium

The microbial consortium were bred from horse manure and decomposed wood collected from Ayuthaya province, Central part of Thailand. One gram of sample 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) [4]. Each culture was incubated at 50°C 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 five times to obtain a stable community capable of degrading rice straw [5].

2.2. Study of cellulase activities

100 µl of microbial consortium were inoculated in 10 ml basal medium to prepare starter inoculum and incubated at 50°C for 7 day in shaker incubator. The starter inoculum was subsequently inoculated in 500 ml of fresh media and incubated at 50°C for 3 day. The supernatant fraction was collected as by crude enzyme centrifugation at 6,000 rpm for 15 min. The crude enzyme was partially purified by addition of $(NH_4)_2SO_4$ to 80% saturation and the precipitated enzyme was collected by centrifugation at 6,000 rpm for 20 min. The pellets were resolubilized in 50 mM sodium phosphate buffer, pH 7 and was desalted by using dialysis membrane with 10 kDa MWCO. The partially purified enzyme was concentrated using spin column with 10 kDa MWCO [4].

To measure enzyme activities, 2% w/v of various types of cellulose substrates (carboxymethyl cellulose (CMC), avicel, and cellobiose) were mixed in phosphate buffer and incubated at 50 °C for 1 h. The amounts of reducing sugars and glucoses were measured by using DNS assay [6] and Glucose Oxidase assay kit (Megazyme, USA), respectively. Enzyme activity of cellulase was defined as the amount of enzyme that released 1.0 µmol of reducing sugars (calculated as glucose) per minute at experimental condition.

2.3. Anaerobic digester set up

The batch anaerobic digesters were set up to observe the effect of microbial consortium on the biogas production. The seed anaerobic sludge was obtained from an anaerobic digester plant in a local municipal wastewater treatment plant. The inoculum was prepared as our previous study [7]. Batch biogas production experiments were performed at 38 ^{o}C in sealed flasks with 500 ml of working volume (containing 3% (TS) of anaerobic wastewater sludge, 3% w/w of rice straw, 3% w/w of microbial consortium, 10 ml of Nutrient broth media and 15 ml of bicarbonate buffer (pH 6)). The amount of biogas produced was recorded using water displacement method. Each batch experimental test was carried out with 45-day period.

2.4. Analysis of microbial consortium diversity

Total genomic DNA of microbial consortium was extracted as in our previous study [8]. Quantitative real-time PCR (qPCR) was performed to analyze microbial community of microbial consortium using the LightCycler® 480 (Roche, Switzerland). Total 5 primer sets were used to identify methanogens in microbial consortium (Methanobacteriales, Methanosarcinales, Methanomicrobiales and Methanococcales) (Table 1) [9,10]. qPCR mixture was prepared using innuMIX qPCR Mastermix SyGreen (Analytikjena, Germany) based on manufacturer's