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Hydrogen producer isolated from agricultural wastewater and molasses

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Abstract

Biohydrogen is one of the alternative energy that has a potential to be convenient future green energy. The supremacy of using H_2 is that the only product of O_2 and H_2 reaction is water without carbon dioxide. H_2 can be produced by biological dark-fermentative hydrogen-producing bacteria. *Bacillus* is one of efficient hydrogen producer belonged to the division of Firmicutes which is related to highly-effective hydrogen producer *Clostridium* species. In this research, the new isolate of *Bacillus coagulans* is isolated from molasses from bioethanol plant in Kanchanaburi Province. Reducing sugar, sucrose and glucose were analyzed in molasses results sugar content 0.61, 3.0, and 0.08 g/100g, respectively. In this research, *B. coagulans* is focused on the ability to convert sugar into hydrogen. It is a gram positive, rod-shape bacterium that known to be an ideal biocatalyst for lignocellulosic biomass fermentation for biofuels production. Gas production were observed after 168 hr fermentation in modified sucrose-based synthetic medium. The isolate produces hydrogen gas 1.634 mol H_2/mol hexose (48 mL in 30 mL synthetic medium supplemented with 15 g/L sucrose). Soluble metabolite such as volatile fatty acid (VFA) are observed.

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Keywords: biohydrogen; dark fermentation; *Bacillus*; wastewater; molasses

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Introduction

Hydrogen is one of the interested cleaner fuels for future. Combusion of hydrogen yields water as the final product (Hallenbeck and Benemann, 2002) The advantages of hydrogen engine using are various such as reduced CO, CO_2, NO_x, SO_x, and other hydrocarbon released to the city (Johnston et al., 2005; Das and Veziroglu, 2001; Adamson, 2004; Scott, 2004). The energy content of hydrogen gas is higher than that in the other fuels (122 kJ/g) (Madawar, Garg and Shah, 2000; Chandrasekhar, Jik Lee and Woo Lee, 2015; Chang and Lin, 2004) Hydrogen can be applied in altered pattern such as convert to be electricity and applied in hydrogenation processes (Quakernaat, 1995).

Biohydrogen production has a key on biocatalysts application on process. Microorganisms that related to the hydrogen production performance can be divided in to 5 groups (Nandi and Sengupta, 1998). First of all, anarerobic heterotrophs group such as *Clostridia*, rumen bacteria and Archea eg. *Caldicellulosiruptor* (Yilmazel, Johnston and Duran, 2015). The second, facultative anaerobes group belongs to species of *Escherichia coli* and Enterobacter. Third, Aerobes belong to species *Alcaligenes* and *Bacillus*. The other one, photosynthetic bacteria which is anoxygenic phototrophic organism. For examples, sulfur and non-sulfur bacteria both green and purple bacteria group. The last one is cyanobacteria group.

Dark fermentation to produce hydrogen gas is one of the efficient techniques for this application. Facultative anaerobe which is *Escherichia coli, Enterobacter*, *Citrobacter* and strictly anaerobe in genus *Clostridia* and methanogenic bacteria have been reported for the ability (Ghimire et al., 2015). The organisms contain the same criteria on hydrogenase enzyme activities and the ability to convert varied carbon sources such as sugar and cellulosic materials to be organic substances (acetic acid, butyric acid and CO_2) in parallel of hydrogen production. However, the yield of production is still not reach to the breakeven point of the bioprocess because of material prices and product collection process. This work pay attention to screen the efficient microorganisms from natural resources which can be adapted to the lower cost material such as industrial agricultural waste.

1.Material and methods

1.1. Sampling area

Collect the samples from agricultural industrial area in Thailand included Sugar cane factory, coconut milk factory, pineapple can fruit factory. Sugar cane samples were derived from Thai Kanchanaburi sugar factory (Kanchanaburi province, Thailand). Coconut waste was collected from Thep Padungpon coconut milk factory (Nakhonpathom province, Thailand). Pineapple waste was picked from Siamfood canned-fruit factory in Chonburi province. Samples were kept and seal at 4 degree celcius before analysis and screening for microorganisms. To collect the samples, 3 points of the area were selected to take the samples by chance. Wastewater were kept by plunge and mixed. Observed physical data of samples by color, temperature, pH from waste tank/pool area before wastewater treatment process.

1.2. Wastewater analysis

Collected wastewater were analyzed for chemical and physiological properties (pH, Total solid (TS), Total suspended solid (TSS), Total Dissolved Solid (TDS), Total Kjeldahl Nitrogen (TKN) by Macro-Kjeldahl Method 4500-Norg B., Total Organic Carbon (TOC), Biological Oxygen Demand (BOD) by 5-Days BOD Test 5210 B., Chemical Oxygen Demand (COD) by Open Reflux Method 5220 B. Total carbohydrates were analyzed by AOAC method (AOAC 2012: 982.14).