



Production of Biohydrogen and Bioacetic Acid by Using Isolates of *Bacillus circulans*

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Abstract. Biohydrogen and bioacetic acid are very important chemicals. Both of these chemicals can be produced through anaerobic fermentation by using *Bacillus circulans*. *Bacillus sp* can be isolated from biogas solid sludge by growing it in the modified media. The isolation was carried out by boiling the biogas sludge for 2 hours. After it boiled, only the spores-producing microbes would survive, such as *Bacillus sp* and *Clostridium sp*. Therefore, spores were grown in hydrogen media with glucose as a carbon source, for 72 hours, in anaerobic conditions and at 60 °C. During the growth, hydrogen produced was analyzed by using gas chromatography (GC), whereas metabolite liquid products were analyzed by using HPLC to determine the composition of organic acids and other products quantitatively. Finally, the identification of microbes producing hydrogen was conducted through the PCR test. The results of the analysis proved that the microbes matched to *Bacillus circulans* with 97% similarity. The results of GC showed that the hydrogen was produced by *Bacillus circulans*. The highest content of hydrogen was found in samples 8 and 9 (SC IV). They contained acetic acid of 7.800 ppm, propionate acid of 53 ppm. PCR test by comparing the 16S rDNA gene sequences through Blast program showed that the three isolates were most likely identified as *Bacillus circulans* (first isolate) and *Bacillus sp* (second and third isolates), with 97% similarity. *Bacillus circulans* was known as a microbe producing enzyme. Therefore, it could be concluded that this study has successfully found a species of *Bacillus circulans* with a new strain that was able to produce hydrogen and has not been previously discovered.

Keywords: Anaerobic; *Bacillus circulans*; bioacetic acid; biohydrogen; PCR; thermophilic

1 Introduction

Energy consumption is growing because of the increasing world population. The imbalance between supply and demand led researchers and industry to find and produce alternative energy from renewable sources [1].

Hydrogen is one of the renewable energy can be produced by anaerobic bacteria that grow on the substrate that is rich in carbohydrates. The fermentation process can be done in the dark conditions and can be performed at low temperature using mesophile (298-313 K), thermophile (313-338 K), extreme thermophile (338-353 K) and hiperthermophile (> 353 K) bacteria. Gas produced from the fermentation process is a mixture of H₂, CO₂, H₂S and CH₄. Theoretically every mole of glucose can produce 4 moles of H₂. *Bacillus* and *Clostridium* are two species of bacteria that can produce spores and can be found in sludge biogas and can be obtained from anaerobic sludge processing [2].