ISOLATION OF HYDROGEN PRODUCING BACTERIA FROM SLUDGE OF ANAEROBIC BIOGAS REACTOR

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ABSTRACT

Hydrogen-producing bacteria can be found in the activated sludge generated from anaerobic waste treatment processes. Microbes in it consists of acetogenesis, metanogenesis and hydrogen-producing bacteria such as *Bacillus* and *Clostridium*. The purpose of this study was to isolate, characterize and identify a hydrogen-producing bacteria from sludge of anaerobic biogas reactor. Hydrogen production was analyzed by a gas chromatograph (GC-Shimadzu). Organics acid and ethanol productions were analyzed by HPLC, whereas reducing sugar was analysis by DNS method. Isolates identification was done based on the 16S rDNA gene sequences. Five isolates bacteria have been obtained from enrichment culture (culture C4) were developed using liquid minerals medium (HM medium). Based on the examination on the similarities of the isolates morphology obtained, three isolates have been selected. They were BYM1, BYM2, and BYM3. According to the sequence of their 16S-rDNA, the BYM1, BYM2, and BYM3 were identified as *Bacillus circulans* with 97% similarities. The three isolate bacteria were then designated as *Bacillus circulans* BYM1, *Bacillus circulans* BYM2 and *Bacillus circulans* BYM3. The acid production by the isolate and their combination was higher than the C4 culture. The mixed cultures *Bacillus circulans* BYW2- *Bacillus circulans* BYW3 had higher ability to produce hydrogen than the C4 culture. The fermentation by-products were ethanol, acetic acid and propionic acid, and acetic acid is the major metabolite.

Keywords: biogas sludge, *Bacillus*, bio-hydrogen.

INTRODUCTION

Hydrogen (H_2) is considered to be a promising fuel in the future, because environmentally friendly and high calorie content (122 kJ/g), compared with the calorie content of hydrocarbons only (2-4 kJ/g). Some bacteria have the ability to convert carbohydrates to hydrogen in anaerobic conditions. Especially species of spore-forming bacteria such as Clostridium, Bacillus sp, Enterobakter sp. and some are of thermopiles bacteria. Along with hydrogen is also produced organic acids, methane and carbon