

Sakchai Pattra. 2009. *Bio-Refinery of Sugarcane for Bio-Fuels Production*.

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ABSTRACT

The research aims to explore the possibility of using sugarcane to produce multibiofuels (hydrogen, methane and ethanol) by *Clostridium butyricum* TISTR 1032 and anaerobic-mixed cultures. Sugarcane juice was investigated for its capacity in hydrogen production by free and the immobilized *C. butyricum* TISTR 1032 on sugarcane bagasse (SCB) using batch and repeated-batch fermentations. A maximum hydrogen yield, hydrogen production rate and hydrogen content of 2.66 mol H₂/mol sucrose consumed, 3.11 L H₂/L.day and 45%, respectively, could be obtained at the initial pH of 6.5 and the initial sucrose concentration of 25 g-COD/L by using the immobilized cells. Repeated batch fermentations using immobilized *C. butyricum* TISTR 1032 were carried out at 3 different end-of-the-batch harvested volumes of 25, 50 and 75%, under the optimal conditions obtained from batch experiments. The highest hydrogen yield, hydrogen production rate and hydrogen content of 3.04 mol H₂/mol sucrose consumed, 2.29 L H₂/L.day and 45%, respectively, were obtained at the suitable end-of-the-batch harvested volume for hydrogen production of 50%. The repeated-batch hydrogen producing system was further connected to a fuel cell unit after the constant hydrogen production between batches was achieved and showed the ability to generate the maximum and a constant electrical power of 49 mW during 8–20 hours of the fermentation. Bio-augmentation of *C. butyricum* in free cell form for continuous hydrogen production from sugarcane juice in the Continuous Stirred Tank Reactor (CSTR) under non sterile condition was examined at different HRT (36-4 h). Results indicated that the maximum hydrogen yield and production rate of 1.00 mol H₂/mol hexose consumed and 1.82 L H₂/L.day, respectively, could be achieved at HRT of 4 h which suggested that hydrogen production under non-sterile condition by *C. butyrcum* could be conducted. However, the hydrogen content

obtained was rather low (less than 25%). The relationship of the augmented microorganism i.e. *C. butyricum* and normal flora in the fermentation system under non-sterile condition were analyzed by the Denaturing Gradient Gel Electrophoresis (DGGE) method at every HRT. The DGGE results revealed that augmented microorganism, *C. butyricum*, was dominant and played an important role on hydrogen production with the support of normal flora, *Klebsiella pneumoniae*, under non-sterile condition. *Lactobacillus harbinensis*, one of a dominant normal flora, was able to grow in the hydrogen fermentation system but played a negative role in reducing hydrogen production efficiency resulting in a low hydrogen content obtained.

In order to implement the total use of sugarcane, SCB was used to produce hydrogen by *C. butyricum*. SCB was hydrolyzed by H_2SO_4 at various concentrations (0.25-7.0% volume) and reaction time (15-240 min) at 121 °C, 15 kg/cm² pressure in autoclave. Optimal conditions obtained were 0.5% H_2SO_4 and 60 min which yielded 24.5 g/L of total sugar. The maximum hydrogen yield of 1.73 mol H_2 /mol sugar consumed and the hydrogen production rate of 1,611 mL H_2 /L.day were obtained at the initial pH 5.5 and the initial sugar concentration of 20 g-COD/L.

Throughout the successful process of hydrogen production from sugarcane juice and SCB, large amount of organic wastewater were generated. Wastewater contained residual organic matter such as butyric and acetic acids and residual sugar, which were valuable as substrates for methane and ethanol productions. Therefore, the possibility of using hydrogenogenic effluent to produce methane by anaerobic sludge and ethanol by carried over microorganism consortium from previous hydrogen fermentation was explored in this study. Response Surface Methodology (RSM) was used as a tool to optimize the factors affecting methane production from hydrogenogenic effluent. Results indicated that substrate concentration, ratio of $NaHCO_3$ to substrate concentration and initial pH significantly ($p < 0.05$) affected methane yield. A maximum methane yield of 1,994.44 mL CH_4 /g- VS_{added} was obtained under the optimum conditions i.e., substrate concentration of 10.09 g-COD/L, $NaHCO_3$ to substrate concentration ratio of 5.53 and initial pH of 7.46. The ethanol production from hydrogenogenic effluent of non-sterile hydrogen fermentation using the CSTR by carried over microorganism consortia from previous

hydrogen fermentation was conducted. The highest ethanol yield of 0.31 mol EtOH/mol sugar consumed and specific ethanol production rate of 0.22 mg EtOH/mg VS.day were achieved at the optimum initial sucrose concentration and pH of 25 g-COD/L and 7, respectively. *C. butyricum*, the augmented microorganism which was carried over from the hydrogen production process was the dominant specie during ethanol fermentation of hydrogenogenic effluent at every pH values (5.0-8.0) and sugar concentration (25-300 g-COD/L). Results from this study demonstrated that sugarcane can be used to produce multibiofuels successfully.

