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Non-sterile bio-hydrogen fermentation from food waste in a continuous stirred tank reactor (CSTR): Performance and population analysis

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ABSTRACT

Bio-hydrogen production from food waste by anaerobic mixed cultures was conducted in a continuous stirred tank reactor (CSTR). The hydraulic retention time (HRT) was optimized in order to maximize hydrogen yield (HY) and hydrogen production rate (HPR). The maximum hydrogen content (38.6%), HPR (379 mL H_2/L. d) and HY (261 mL H_2/g-VS_{added}) were achieved at the optimum HRT of 60 h. The major soluble metabolite products were butyric and acetic acids which indicated a butyrate-acetate type fermentation. Operation of CSTR at HRT 60 h could select hydrogen producing bacteria and eliminate lactic acid bacteria and acetogenic bacteria. The microbial community analyzed by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) revealed that the predominant hydrogen producer was *Clostridium* sp.

1. Introduction

Diminishing fossil fuel supplies and greenhouse gas emissions are the major reasons for recent research activities on finding the sustainable energy sources that could replace fossil fuels [1]. Methane and hydrogen are renewable fuels but hydrogen has more advantages than methane due to its cleanness, efficiency and non-polluting characteristics [2] i.e., when hydrogen is combusted with oxygen, water is obtained as a by-product [3]. Bio-hydrogen production process can be divided into two main categories i.e., photo production process by photosynthetic bacteria and algae and dark fermentation process by anaerobic bacteria [4]. Dark fermentation has shown a great potential as a practical bio-hydrogen production process due to less energy consumption, cost effective and various kinds of substrate can be used to produce hydrogen including energy crops [5], agricultural waste [6], industrial waste [7] and solid waste [8].

Among these feedstocks, food waste has drawn our attention to use as the substrate for bio-hydrogen due to its high organic content, easily hydrolysable nature and availability. Food waste consists mainly of starch, protein, and fat, with a small amount of cellulose and hemi-cellulose which are possible sources for bioenergy production [9]. In Thailand, the generation of food waste reached about 20,041 tons per day, accounting for 50% of municipal solid waste [10].

The continuous stirred tank reactor (CSTR) is the most frequently used reactor type because it is simple to operate

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[11] and the biomass is well suspended in the mixed liquor; hence the bacteria have a good efficiency to use substrate [11]. However, bio-hydrogen production with CSTR reactor is usually very sensitive to environmental shock such as high substrate concentration which limits a high organic loading rate (OLR) or a short hydraulic retention time (HRT) [12]. An HRT is the important parameter for continuous hydrogen production process. With appropriate HRT, efficient hydrogen production could be achieved which will make the hydrogen production process more applicable [13]. Optimal HRT for continuous fermentative hydrogen production, even for the same type of reactor, are varied. For example, the optimum HRT for a CSTR used to produce hydrogen from glucose by Zhang et al. [14] was 0.5 h, while the optimal HRT for a CSTR used to produce hydrogen from starch by Arooj et al. [15] was 12 h. Wu et al. [16] produced hydrogen from sucrose using the immobilized sludge as the inoculum in CSTR. They found that a reduction of HRT from 4 to 2 h did not significantly change the hydrogen production rate (HPR) but when the HRT varied from 2 to 0.5 h, the HPR increased significantly. In addition, HRT showed an influence on the gas, solid and liquid holdups. Chu et al. [17] reported that when the HRT was shortened the gas holdup and solid holdup increased but liquid holdup decreased. Therefore, these previous findings indicate the needs to optimize the HRT when the continuous hydrogen production is operated.

The aims of this study were to determine the suitable HRT for a continuous bio-hydrogen production from food waste as well as the effects of HRT on CSTR performance and its associated microbial community.

2. Materials and methods

2.1. Food waste

Food waste was collected from the food center of Khon Kaen University campus, Khon Kaen, Thailand. It was mainly made up of rice, vegetables, fruits and meats. Bones were removed from the food waste before being mixed with tap water at the volumetric ratio of 1:3 and then grinded in a food blender. The pH of the resulting food waste slurry was 7.2. The chemical characteristics of the resulting food waste slurry are shown in Table 1. The food waste slurry was stored at −17 °C and thawed in a refrigerator prior the usage.

Table 1 – Chemical characteristics of food waste slurry.

Parameter	Concentration (mg/L)
Total chemical oxygen demand (COD)	116,000
Total carbohydrate	64,093
Total nitrogen	14,081
Total phosphate	1.98
Magnesium	7.94
Manganese	0.25
Iron	0.27
Copper	0.03
Sodium	36.00
Cobalt	0.003
Volatile solid (VS)	10,100

2.2. Inoculums

Anaerobic sludge was obtained from a full-scale anaerobic digester of upflow anaerobic sludge blanket (UASB) reactor of the brewery company and used as the seed inoculums. The seed sludge were prepared following the method of Sreela-or et al. [18]. The volatile suspended solids (VSS) concentration of the seed inoculum was 7.4 g/L.

2.3. Reactor operation and start up

The CSTR was made from acrylic with a 1 L total volume and a 0.7 L working volume (Fig. 1). The reactor was started up using 2.30 g-VSS/L of inoculums, 2.54 g-volatile solid (VS)/L of food waste (equivalent to 29.17 g-COD/L) and 0.11 M of citrate buffer which was the optimum conditions obtained from our previous batch experiments [18]. The head space of the reactor was flushed with nitrogen gas for 15 min to create an anaerobic condition. The reactor was operated at 35 ± 3 °C. In order to control the pH of fermentation medium at 5.0 ± 0.3, the solution of NaOH (2 mol/L) or HCl (2 mol/L) was manually added to the reactor when the pH fermentation medium is lower than 4.7 or higher than 5.3, respectively. pH was monitored by pH meter (pH 190 series, Eutech Instruments, Singapore). The CSTR was continuously stirred at 120 rpm on the magnetic stirrer using the magnetic bar. The oxidation–reduction potential (ORP) was monitored using ORP meter (ORP 190 series, Eutech Instruments, Singapore). The CSTR was firstly operated at the HRT of 84 h and subsequently changed to HRT of 72, 60 and 48 h by changing the volumetric feeding rate when steady state of each HRT was reached. The steady state was justified by a variation of biogas production, hydrogen content, hydrogen yield (HY) and hydrogen production rate (HPR) of less than 10%.

2.4. Analytical methods

Biogas composition was measured by a gas chromatograph (GC) (GC-2014, Shimadzu) equipped with a thermal conductivity detector (TCD) and 2 m stainless column packed with Unibeads C (60/80 mesh) followed the method of Fangkum and Reungsang [19]. For volatile fatty acids (VFAs) and alcohols analysis, the liquid samples were centrifuged at 6000 rpm for 10 min, acidified by 0.2 mol/L oxalic acid and filtered through 0.45 μm cellulose acetate membrane before being analyzed by the high performance liquid chromatography (HPLC) (Shimadzu LC-10AD) with an Aminex HPX-87H column using the protocol of Fangkum and Reungsang [19].

Food waste concentration was represented by VS. The VS and VSS were measured according to the procedures described in standard methods [20].

The volume of biogas was continuously measured by a gas counter connected to the reactor head space. The gas counter was calibrated by injecting a known volume of nitrogen into the head space to determine the volume of gas per count which allows the calculation of the biogas production rate (BPR) (L biogas/$L_{substrate}$. d). In order to calculate HPR (L H_2/L. d), the BPR was multiplied by the content of hydrogen in the biogas. HY (mL H_2/g-VS_{added}) was calculated by divided the HPR by organic loading rate (g-VS_{added}/L. d).