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

Alissara Reungsang [a,b,*], *Chakkrit Sreela-or* [c], *Pensri Plangklang* [a]

[a] *Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand*
[b] *Research Group for Development of Microbial Hydrogen Production Process from Biomass, Khon Kaen University, Khon Kaen 40002, Thailand*
[c] *Department of Agriculture, Faculty of Food and Agricultural Technology, Phibulsongkram Rajabhat University, A. Muang, Phitsanulok 65000, Thailand*

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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

* *Corresponding author*. Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand.
E-mail address: alissara@kku.ac.th (A. Reungsang).
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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).

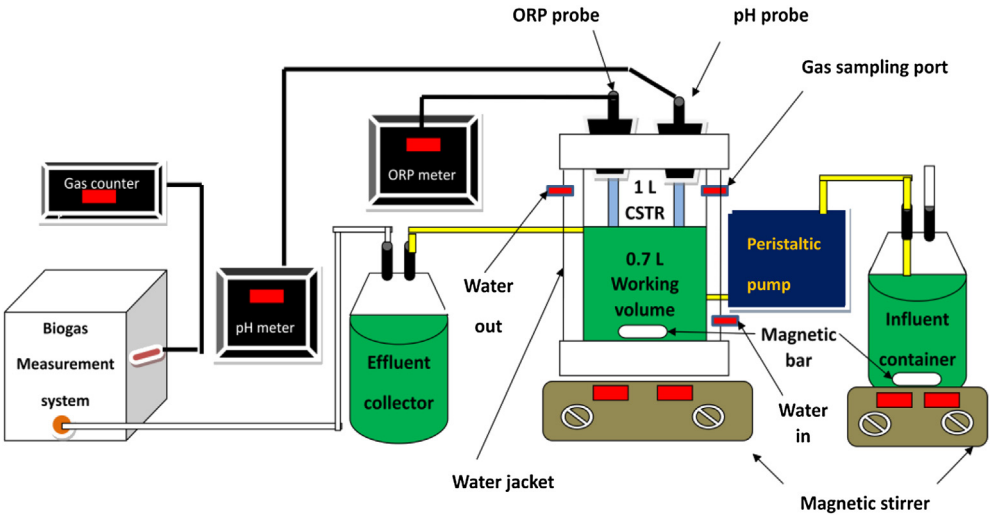

Fig. 1 – Schematic diagram of anaerobic continuous stirred tank reactor for hydrogen production.

At the steady state of each HRT, the microbial community in the sample was analyzed using PCR-DGGE and 16S rDNA sequencing techniques. Briefly, total genomic DNA was extracting using phenol/chloroform method [21]. PCR-DGGE analysis of the extracted DNA and sequencing analysis were performed according to the method described by Sreela-or and Reungsang [18]. Closest matches for partial 16s rRNA gene sequences were identified by database searches in GenBank using BLAST [22].

3. Results and discussion

3.1. *Hydrogen production*

Biogas contained hydrogen and carbon dioxide without the detection of methane throughout the reactor operation. During the reactor operation, the ORP values ranged between −280 and −225 mV (Fig. 2) which indicated that the reactor was operated and maintained under anaerobic condition. A variation of HRT markedly affected the efficiency of biogas and hydrogen production. A decrease of HRT from 84 to 60 h led to an increase in hydrogen content, HY and HPR from 22.9 to 38.6%, 106 to 261 mL H_2/g-VS_{added} and from 110 to 379 mL H_2/L. d, respectively (Fig. 2, Table 2). Biogas production had a similar trend to hydrogen production at this range of HRT. A further decrease in HRT from 60 h to 48 h resulted in a decrease in hydrogen content, HY and HPR to 17.9%, 88 mL H_2/g-VS_{added} and 160 mL H_2/L. d, respectively, while biogas production remain unchanged.

The optimum HRT for hydrogen production from food waste in CSTR was 60 h giving the HY and HPR of 261 mL H_2/g-VS_{added} and 379 mL H_2/L. d, respectively. The HY obtained in this study was higher than those reported in the literature. However, the HPR is considered much lower than the other reports (Table 3). Such a discrepancy might be due to different in hydrodynamic of the type of reactor, operational parameters, compositions of food waste and sources of inocula.

The discussions regarding the results on the effects of HRT on hydrogen and biogas production were provided in the sections of soluble metabolites products (SMPs) production (Section 3.2) and microbial community analysis by PCR-DGGE (Section 3.3).

3.2. *SMPs*

Production and compositions of SMPs at different HRT were used to describe the performance of reactor. Within the HRT ranges of 60–84 h, SMPs and VFAs concentrations showed a positive correlation with hydrogen production (Fig. 2, Table 2) in which the highest SMPs and VFAs of 6404 and 5743 mg/L, respectively, was obtained at the HRT 60 h. A decrease in SMPs and VFAs concentrations at a short HRT of 48 h might be the results of dilution effect and/or a decrease of microbial activity due to some microorganisms were washed out (Table 2) by an increase in substrate feeding rate. This was evidenced by the biomass concentration was reduced from 2804 mg-VSS/L to 2460 mg-VSS/L when the HRT was shortened from 60 to 48 h (data not shown). A product inhibition (or a low hydrogen production) caused by a high OLR (or a high substrate concentration) was reported since a large amount of SMPs was produced and accumulated in the fermentation system, leading to a decline of pH in the reactor and adversely affected the activity of the microorganisms [23–25]. However, in this study, the SMPs concentration did not affect hydrogen production since the concentration of SMPs in the fermentation broth were not increased at the HRT 48 h.

The volume of biogas at HRT 60 and 48 h (Fig. 2) was not different which implied that the growth of hydrogen producers at HRT 48 h could not compete with the dilution rate but some anaerobic microorganisms were still remained and active in the fermentation system. These remained active microorganisms might produce other metabolites (e.g. acetic acid (HAc), butyric acid (HBu), propionic acid (HPr), lactic acid (HLa) and ethanol (EtOH)) yielding carbon dioxide as by products and/or converted the produced hydrogen to other products (e.g. HAc and HPr) leading to a reduction in

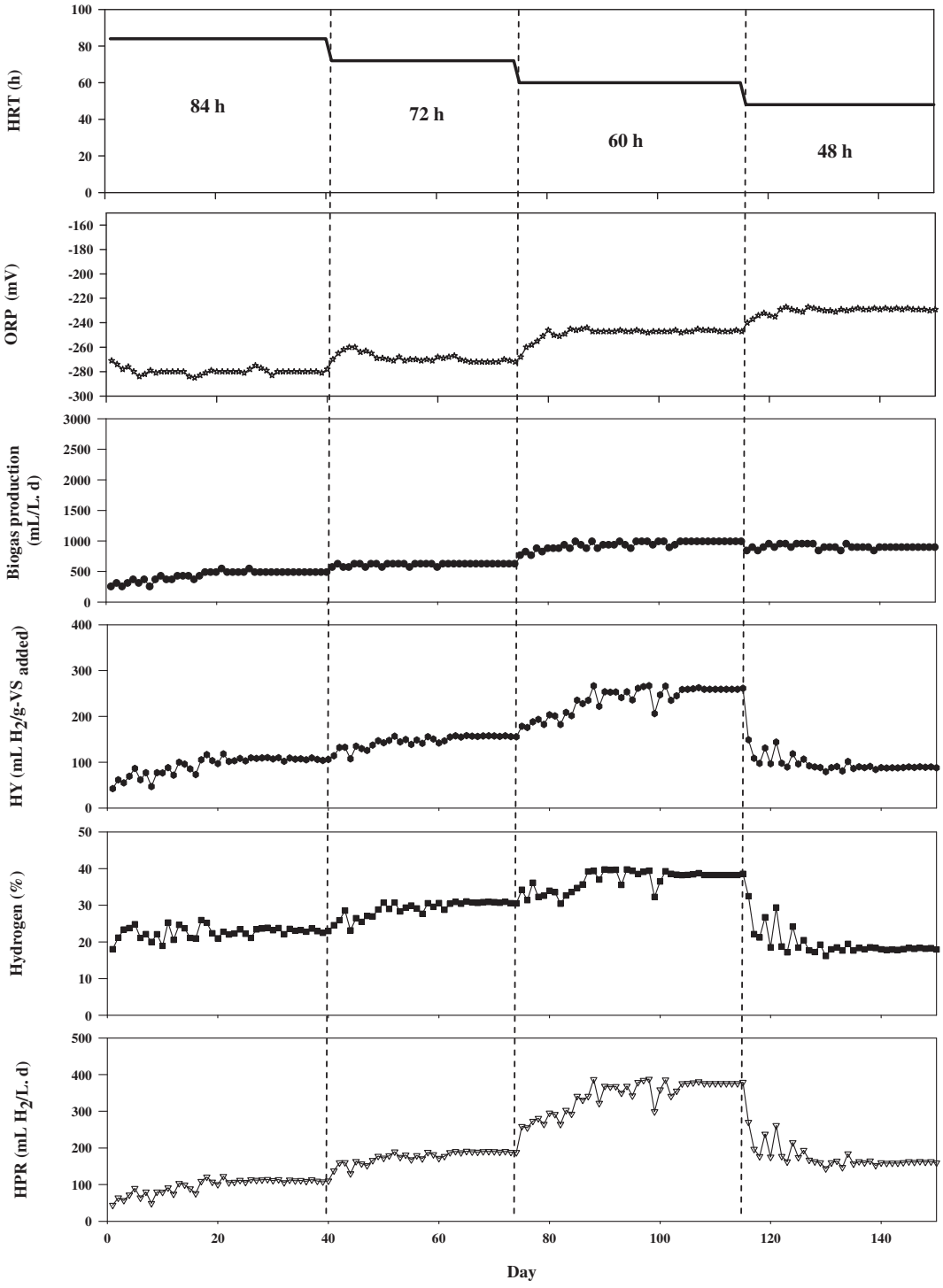

Fig. 2 – Effect of HRTs on biogas production, HY, hydrogen content, HPR and ORP value.

hydrogen content in biogas while biogas production was not decreased [23].

In all ranges of HRT, HBu followed by HAc were the main VFAs accounted for 41.68–51.61% and 30.56–39.18%, respectively (Table 2). HBu concentration showed a positive correlation to both HY and HPR in all ranges of HRT. The highest concentrations of HBu (3305 mg/L) and HAc (2093 mg/L) were obtained at HRT 60 h which coincided with the greatest value of HY and HPR. The high concentrations of HBu and HAc in the liquid phase indicated an efficient hydrogen production from carbohydrate-rich substrates, since HBu and HAc productions were positively correlated to hydrogen production as shown in Eqs. (1) and (2), respectively. However, it should be noted that HAc can be converted from hydrogen (Eq. (3)) by acetogenic bacteria or can be converted from hexose directly to acetate alone by the process of homoacetogenesis (Eq. (4)) leading to a low hydrogen production efficiency [26].

Table 2 – Summary of hydrogen production parameters and SMPs production at steady state of each HRT.

HRT (h)	H_2 content (%)	HY (mL H_2/g-VS_{added})	HPR (mL H_2/L. d)	HAc (mg/L)	HBu (mg/L)	HLa (mg/L)	HPr (mg/L)	EtOH (mg/L)	SMPs (mg/L)
84	22.9	106	110	1993	2271	113	217	493	5087
72	34.2	155	188	2019	2780	385	130	548	5862
60	38.6	261	379	2093	3305	106	239	661	6404
48	17.9	88	160	1812	2471	137	425	1084	5929

HY = hydrogen yield; HPR = hydrogen production rate.
HAc = acetic acid, HBu = butyric acid, HLa = lactic acid, HPr = propionic acid, EtOH = ethanol.
SMPs = soluble metabolites production = HAc + HBu + HLa + HPr + EtOH.

Table 3 – Comparison between HY and HPR from food waste obtained in this study and in the literature.

HRT (h)	Reactor type	Substrate							Operation condition		HY (mL H_2/g-VS_{added})	HPR (L H_2/L. d)	Reference
		Type	Total COD (g-COD/L)	Soluble COD (g-COD/L)	Carbohydrate (g-COD/L)	Nitrogen (g-N/L)	Oil and grease (g/L)	Volatile solid (VS) (g/L)	Temperature (°C)	pH			
30	ASBR	Food waste	44.2	21.9	12.6	1.1	NA	4.4	35	5.3	80.9	2.73	[33]
26	ABR	Food waste	64	32	NA	7.3	9.4	56	26	5–6	370.0	NA	[34]
32	CSTR	Food waste	77	NA	NA	NA	NA	35.8	55	5.5	205.0	NA	[35]
48	CSTR	Kitchen waste	82–106	30–35	5.3–15	1.6–2.5	7.6–11	NA	35	5.3–5.6	NA	2.60	[36]
60	CSTR	Food waste	116	NA	64.09	14.08	NA	10.10	35	5.0	261	0.38	This study

NA: not available.
ABR: anaerobic baffled reactors.

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (1)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (2)$$

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \quad (3)$$

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \quad (4)$$

EtOH, HLa and propionic acid (HPr) were found as minor metabolites. The distribution of EtOH, HLa and HPr concentrations were diverse at different HRT. The highest fraction of EtOH (18.28%, 1084 mg/L) was observed at the HRT of 48 h which was corresponded to a large volume of biogas detected at this HRT (Table 2). EtOH fermentation yielded carbon dioxide without hydrogen to the gas phase (Eq. (5)) [27]; therefore, a large volume of biogas detected at HRT 48 h might be from CO_2. The highest fraction of HPr (7.71%, 425 mg/L) was also observed at HRT 48 h which might contribute to the low hydrogen production efficiency due to HPr production is the hydrogen consuming pathway (Eq. (6)) [23].

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (5)$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (6)$$

The highest fraction of HLa (6.57%, 385 g/L) was observed at the HRT 72 h which implied that lactic acid bacteria could grow and were more active at this HRT than at the other HRT. Production of HLa in the fermentation system lowered the hydrogen production efficiency due to the existing substrates were converted to HLa with no hydrogen produced (Eqs. (7) and (8)) [28].

$$C_6H_{12}O_6 \rightarrow 2CH_3CH(OH)COOH \quad (7)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH(OH)COOH + CH_3COOH + CO_2 \quad (8)$$

Distribution of SMPs in this study indicated the butyrate-acetate-type fermentation. Overall results indicated that HRT had little impact on HBu and HAc productions but had a significant impact on EtOH, HPr and HLa production which directly affected the hydrogen production efficiency.

3.3. *Microbial community analysis by PCR-DGGE*

The presence of distinguishable bands in the different separation patterns were observed in which the bands sequences were affiliated with different species of microorganisms (Fig. 3). Results indicated the shift of microbial population with the changes of HRT which affected the hydrogen production efficiency and SMPs compositions.

Nine bands were obtained from the HRT 84 h, three of which were affiliated with well-known hydrogen producers *Clostridium* sp., i.e., *Clostridium butyricum* (band 7), *Clostridium roseum* (band 9), and *Clostridium diolis* (band 14). Two of which were affiliated with *Enterococcus faecium* (band 1) and

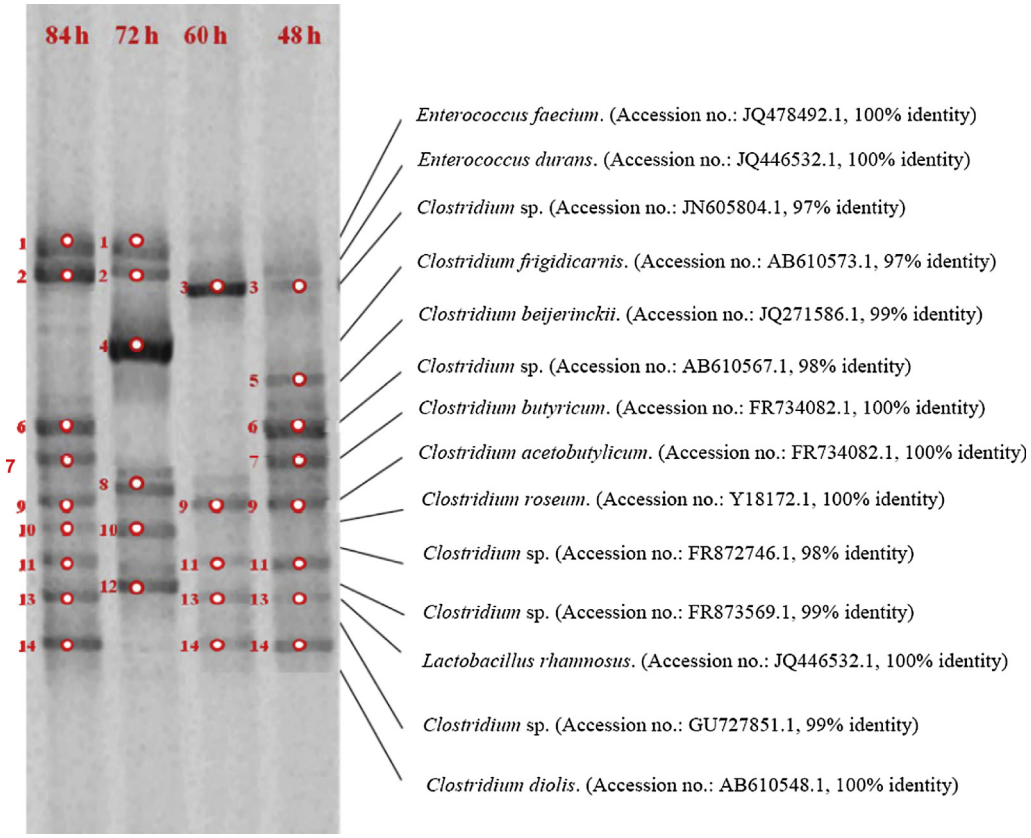

Fig. 3 – PCR-DGGE analysis of microbial community in hydrogen production at different HRT and the affiliated microbial species determined by 16S rDNA sequencing.

Enterococcus durans (band 2) and four of which were affiliated with *Clostridium* sp. K19 (bands 6), *Clostridium* sp. mbf_VZ 132 (bands 10), *Clostridium* sp. DMHC 10 (band 13). These *Clostridium* sp. have not been reported to produce hydrogen. *E. faecium* and *E. durans* were capable of producing HLa and HAc via acetogenesis pathway [29] which contributed to a low hydrogen production at this HRT of 84 h. These bacteria were still retained at the HRT 72 h with the presence of one more acetogenic bacteria i.e., *Clostridium frigidicarnis* (band 4) [30] and one more lactic acid bacterium i.e., *Lactobacillus rhamnosus* (band 12) [31] and one band affiliated with hydrogen-solvent fermentation bacterium i.e., *Clostridium acetobutylicum* (band 8) [32]. Large abundance of lactic acid bacteria was coincided with the high concentration of HLa (Table 2) observed at this HRT of 72 h. Moreover, the abundance of both lactic acid bacteria and acetogenic bacteria could decrease the hydrogen production efficiency as described in Section 3.2. A reduction in HRT from 72 to 60 and 48 h eliminated lactic acid bacteria and acetogenic bacteria. Five bands were obtained at the optimal HRT of 60 h, two of which were affiliated with *C. roseum* (band 9) and *C. diolis* (band 14) and three of which were affiliated with *Clostridium* sp. (bands 3, 11 and 13). Band 3 was the most intensive band at this HRT which implied that *Clostridium* sp. associated with this band might contribute to a production of hydrogen. Most of the bands obtained at HRT 60 h were still retained at the HRT 48 h. However, the intensity of band 3 was apparently decreased. *C. butyricum* (band 7) and *Clostridium* sp. (band 6) reappeared with the presence of new band (band 5) affiliated with *Clostridium beijerinckii*. *C. beijerinckii* was reported as hydrogen and/or solvent producing bacterium in which the types of major product depended on the fermentative conditions [32]. The high concentration of EtOH with the low hydrogen content observed at this HRT might be contributed by the present of *C. beijerinckii*.

4. Conclusions

Variation of HRT provided an opportunity to select the hydrogen producing population in the reactor and consequently achieved the high hydrogen fermentation efficiency. The composition of SMPs indicated a butyrate-acetate-type fermentation. Though the hydrogen producer, *Clostridium*sp., was presence at every HRT, the presence of lactic acid bacteria, acetogenic bacteria and solventogenic bacteria have contributed to a decrease in hydrogen production efficiency. Operation of CSTR at HRT 60 h could select hydrogen producing bacteria and eliminate lactic acid bacteria and acetogenic bacteria. As a result, the highest hydrogen content (38.6%), HY (261 mL H_2/g-VS_{added}) and HPR (379 mL H_2/L. d) were achieved.

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