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Biohydrogen Production from Xylose by Aanaerobic Mixed Cultures in Elephant Dung

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Abstract

Xylose was used to produce hydrogen by anaerobic mixed cultures in elephant dung. The elephant dung was subjected to heat shock (90 ºC for 3 h) and acid (pH 3.0 - 4.0 for 24 h followed by neutralization) pretreatments before using it as a seed inoculum. The results showed that the seed inoculum pretreatment by heat shock produced higher hydrogen gas than acid seed inoculum pretreatment, while untreated seed inoculum gave the lowest hydrogen production. Therefore, seed inoculum by heat shock was suitable for hydrogen production from xylose, arabinose and glucose. It was found that xylose was a preferred pentose sugar for hydrogen production, in which the results were comparable to those of glucose. The initial pH of 8.0 was found to be optimal for hydrogen production from xylose, in which a maximum hydrogen production of 371 mL H_2/g VSS and a yield of 1.62 mol H_2/mol xylose were obtained. Microbial community analysis by denaturing gradient gel electrophoresis (DGGE) revealed that, under the optimum initial pH of 8.0, the predominant hydrogen producers were *Clostridium acetobutylicum* and *Ethanoligenens* sp. In addition, lactic acid bacteria i.e. *Bifidobacterium minimum* and *Bifidobacterium* sp. were observed, which coincided with the small amount of lactic acid detected at this optimum initial pH.

Keywords: Hydrogen production, xylose, elephant dung, anaerobic fermentation

Introduction

Hydrogen is a sustainable energy source and has great potential as an alternative energy source to replace fossil fuels [1-4]. It can be produced by electrochemical and biological processes [5,6]. Biological hydrogen production by dark fermentation is a promising method for commercial use in the future, since it can be produced from renewable sources.

Lignocellulosic materials are considered to be potential feedstock for bioenergy production because they are in abundance and available in most countries. Lignocellulosic materials consist of cellulose, hemicellulose and lignin. Pretreatment of lignocellulosic materials yields a hydrolysate containing mainly pentose (xylose and small amounts of arabinose) and hexose (glucose). These sugars can be used as substrates for hydrogen production [7,8]. Therefore, the investigation of hydrogen production from xylose, arabinose and glucose could be employed to develop a method of lignocellulosic materials utilization in order to produce hydrogen effectively.

Most biological hydrogen production studies have been conducted using mixed cultures of bacteria, and just a few have employed pure cultures in the studies. An advantage of using mixed cultures over pure cultures in hydrogen production is that non-sterile fermentable organics can be used as a substrate. The mixed cultures from natural sources, such as activated sludge [9,10] and cow dung [11,12], have been widely used as an inoculum for fermentative hydrogen production. These mixed cultures of bacteria have been reported to contain mostly clostridia. However, the mixed cultures from natural sources often contain unwanted metanogenic bacterium, which consume hydrogen and convert it to methane and acetate. In order to enhance hydrogen yields, it is necessary to avoid the presence of the microorganisms utilising hydrogen. By pretreating anaerobic seed inoculums under extreme conditions, spore forming hydrogen-producing bacteria could have a better chance to survive than non-spore-forming methanogenic bacterium. Several methods for preparing hydrogen-producing seeds, including physical [13,14] and chemical [15,16] pretreatment methods, have been reported. Heat shock has been the most common and effective method for eliminating methanogenic bacterium. It is relatively easy and inexpensive. Methanogens cannot form spores; therefore, they do not survive. Acid and alkali pretreatment has been a common method for eliminating methanogenic bacterium. The bio-activity of methanogens during the conventional anaerobic process treatment of organic matters happens in neutral to slightly alkaline environments (pH 6.8 - 8.0) [17]. Limiting methanogenesis can be achieved by adjusting the acidity of the seed inoculums away from the preferable range to either pH 3.0 - 4.0 or pH 12.0. In this study, the mixed cultures present in elephant dung were used as seed inoculums for hydrogen production. Since cellulase activities have been detected in elephant dung [18], it is possible to use elephant dung in the fermentation of lignocellulosic materials into hydrogen. Heat shock and acid pretreatment was applied for seed inoculum preparation.

Environmental factors have a great influence on dark hydrogen fermentation process. The initial pH is one of the important factors for batch-type hydrogen production, since it affects the hydrogenase enzyme responsible for hydrogen production, microbial growth, etc. [19]. The initial pH for maximum hydrogen production varies due to the difference in the inoculum type and the carbon source. Therefore, an investigation into the influence of initial pH on hydrogen production is necessary for efficient hydrogen fermentation.

The aim of this investigation was to study the efficiency of biological hydrogen production from model substrates, i.e. xylose, arabinose and glucose, by mixed cultures in the elephant dung. Two methods for inoculum pretreatment, i.e. heat shock and acid pretreatment, were investigated in this study. The potential of model substrates for hydrogen production was studied. The initial pH was also investigated in order to achieve high hydrogen production. The investigation of hydrogen production from model substrates by mixed cultures in the elephant dung could be employed to develop the method of lignocellulosic materials utilization in order to produce hydrogen effectively. The information from this study could pave the way for industrialization of biohydrogen production from lignocellulosic materials, especially xylose.

Materials and methods

Seed inoculum preparation

Elephant dung was collected from the elephant village of Surin province, Thailand. It was pretreated before cultivation in order to inhibit methanogens. The enrichment was conducted in a 1 L serum bottle with a working volume of 700 mL. The inoculum was prepared by cultivating 210 g of pretreated elephant dung in 10 g-COD/L xylose as a carbon source with the supplementation of 0.35 mL of nutrient stock solution. Each liter of the nutrient stock solution contained 200 g NH_4HCO_3, 100 g KH_2PO_4, 10 g $MgSO_4 \cdot 7H_2O$, 1 g NaCl, 1 g $Na_2MoO_4 \cdot 2H_2O$, 1 g $CaCl_2 \cdot 2H_2O$, 1.5 g $MnSO_4 \cdot 7H_2O$ and 0.278 g $FeCl_2$ [20]. The culture was incubated at 37 ºC for 24 h before using it as the inoculum in batch experiment. The volatile suspended solid (VSS) of the elephant dung was 8.38 g VSS/L.

Experimental procedure

Three series of batch experiments were carried out to determine (1) the best method for seed inoculum preparation, (2) the potential of sugars for hydrogen production, and (3) the optimum initial pH for maximum hydrogen production from xylose. All batch fermentation was conducted in a 70 mL serum bottle with a working volume of 40 mL. The fermentation broth contained 28 mL of substrate solution and 30 % (v/v) inoculum. The substrate solution was the nutrient stock solution supplemented with 10 g-COD/L of carbon source. The serum bottles were capped with rubber stoppers and aluminum caps after purging with nitrogen gas to create an anaerobic environment. The serum bottles were incubated at 37 ºC. All treatments were conducted in triplicate. First, 2 methods of seed inoculum treatment were conducted. For heat shock pretreatment, the elephant dung was heated in the oven at 90 ºC for 3 h. For acid pretreatment, the elephant dung was adjusted to pH 3.0 - 4.0 with 0.1 N HCl, mixed for 24 h, then adjusted back to pH 7.0 using 0.1 N NaOH. Seed inoculum of non-pretreated elephant dung was used as a control. The initial pH and xylose concentration, at 6.0 and 10 g-COD/L respectively, were prepared. The best pretreatment that provided the highest yield of hydrogen will be used to prepare seed culture in further experiments. In order to study the potential of sugars for hydrogen production, the experiments were performed using xylose and arabinose as carbon sources. In the control, glucose was used as a substrate. The initial pH was 6.0 and the initial concentration of carbon sources was 10 g-COD/L. Then, the effect of the initial pH was studied. The investigated initial pH was varied, ranging from 5.0 to 9.0. The pH of the nutrient solution was adjusted to the designated value using 2N HCl or 2N NaOH.

Analytical methods

The volume of biogas was determined using wetted glass syringes of 20 - 50 mL. The hydrogen and methane contents in the gas phase were determined by using a Shimadzu GC 17-A with a thermal conductivity detector (TCD). Nitrogen was used as the carrier gas with a flow rate of 25 mL/min. The column was a 2 m × 4 mm diameter molecular sieve 5A column. The temperatures of the injector port, detector and column oven were 100, 100 and 60 ºC, respectively. Hydrogen gas production was calculated from the bioreactor headspace measurements of gas composition and the total volume of hydrogen produced at each time interval using the mass balance equation [15]. The specific hydrogen production (ml H_2/L·g VSS) was calculated from the cumulative hydrogen gas volume divided by the fermentation volume (L) and the volatile suspended solid of the elephant dung (g VSS). Hydrogen production yield was calculated as the total molaric amount of hydrogen divided by the molaric amount of sugar consumed (mol H_2/mol sugar consumed). The total molaric amount of hydrogen was calculated using the ideal gas law: molar hydrogen production (mol H_2/L) = volumetric hydrogen production (ml H_2/L)/(RT), where R = 0.08205784 L·atm/K·mol, and T = 310 K [21]. The cumulative hydrogen production in the batch experiment followed the modified Gompertz equation [22].

The liquid samples were centrifuged at 10,000 rpm for 5 min, acidified by 0.2 N oxalic acid and filtered through 0.45 μm cellulose acetate membrane. The concentrations of volatile fatty acids (VFAs) and ethanol were analyzed by a Shimadzu GC-14B with a flame ionization detector (FID) and a 2 m × 2 mm diameter packed column (Porapak Q 80 - 100 mesh). The temperatures of the injector, column and detector were 230, 175 and 250 ºC, respectively [8]. Nitrogen was used as a carrier gas with a pressure of 150 kPa. Lactic acid was determined by a Shimadzu HPLC LC-10AD with a reflection index detector and Aminex HPX-87H column. The oven temperature was 45 ºC, and 5 mM H_2SO_4 was used as a mobile phase at a flow rate of 0.6 mL/min. The concentrations of the total sugar were detected as glucose, xylose and arabinose concentrations, which were measured by phenol-sulfuric method [23].

The microbial community analysis from hydrogen fermentation broth, obtained at a steady state of the optimum pH condition, was conducted by using the PCR-DGGE technique [24].

Results and discussion

The best seed inoculum preparation for hydrogen production

Two methods for inoculum pretreatment, namely heat shock and acid pretreatment, were investigated. **Table 1** summarizes the maximum cumulative hydrogen production, hydrogen production rate and lag-phase time, estimated using the modified Gompertz equation, the maximum specific hydrogen production, and the hydrogen yield of various seed inoculum preparations. The R^2 values of empirical models for all of the experiments were higher than 0.97. The high degree of correlation suggested that the empirical models fit well to the actual data. After a lag-phase time of about 4 - 18 h, hydrogen was detected. However, during 144 h of fermentation, both of pretreatments showed different amounts of hydrogen production. After a lag-phase time of 11.22 h, heat shock pretreated inoculum began to produce hydrogen with a rapid increase in production, actually improving the hydrogen yield, compared to other systems, and gave the highest hydrogen production of 93 ml H_2/g VSS, with a yield of 0.55 mol H_2/mol xylose. The results indicated that the heat shock pretreatment completely repressed methanogenic activity and could enhance hydrogen-producing microorganism activity, which was evidenced by the fact that there was no methane produced from the process. Acid pretreatment gave the lower hydrogen production (62 ml H_2/g VSS), yield (0.40 mol H_2/mol xylose) and the maximum hydrogen production rate (8.50 mL/L·h) and the longest lag-phase time (18.26 h). No methane was detected in the acid pretreatment samples during the whole fermentation process. Although the acid pretreatment would suppress the methanogenic activity, the ability of hydrogen-producing bacteria to produce hydrogen was also limited. It might be due to the protonation of undissociated weak acid, which could pass through the cell membrane into cytoplasms and inhibit the growth of hydrogen-producing bacteria, as well as their hydrogen producing activities [25]. It was found that only the control sample generated methane (data not shown). The shortest lag time to produce hydrogen of 3.72 h was observed; methane was detected after 12 h of cultivation time. The results indicated that metanogens still survived and converted the produced hydrogen to methane.

Table 1 Kinetic parameters of hydrogen production from various seed inoculum preparation methods; total cultivation time was 144 h.

Seed inoculum preparation method	Cumulative hydrogen production (mL/L)	Hydrogen production rate (mL/L·h)	Lag-phase time (h)	Maximum specific H_2 production (mL/g VSS)	Hydrogen yield (mol H_2/mol xylose)	Correlation coefficient (R^2)
Untreated elephant dung	182	4.50	3.72	22	0.14	0.9764
Heat shock	778	17.26	11.22	93	0.55	0.9990
Acid	523	8.50	18.26	62	0.40	0.9926

The produced soluble metabolite products (SMPs) from various seed inoculum preparation methods are shown in **Table 2**. For heat shock pretreatment, the SMPs contained acetic, propionic and butyric acids and ethanol. In biological hydrogen production, acetic and butyric acids are produced, along with hydrogen, while ethanol is produced by consuming electrons, which can reduce the H^+ into H_2 [26]. The butyric acid/acetic acid ratio could be a quantitative indicator of substrate metabolism and hydrogen production by anaerobic microorganism [27]. The results showed that the ratios of butyric acid/acetic acid in fermentation broth were higher than 8.0 for all seed inoculum pretreatments (**Table 2**). Therefore, the hydrogen production by mixed cultures of elephant dung was of the butyrate-type fermentation. Generally, the production of acetate and butyrate favors the production of hydrogen, according to Eqs. (1) and (2). Theoretically, 3.33 mol of hydrogen are produced from 1 mol of xylose if acetate is produced as the fermentation byproduct (Eq. (1)). Alternatively, only 1.67 mol of hydrogen are obtained when butyrate is produced, as shown in Eq. (2) [28].

$$3C_5H_{10}O_5 + 5H_2O \rightarrow 5CH_3COOH + 5CO_2 + 10H_2 \quad (1)$$

$$6C_5H_{10}O_5 \rightarrow 5C_3H_7COOH + 10CO_2 + 10H_2 \quad (2)$$

Ethanol obtained in the fermented broth at the end of the fermentation time (**Table 2**) indicated the occurrence of solvent production, which resulted from a development of a new enzyme system when the pH dropped to about 4.0 due to acetic and butyric acids accumulation in the medium solution. The shift to solvent production may be a mechanism for the cell to detoxify from undissociated acid end products [2,29]. These results indicated that seed inoculum pretreatment affected the hydrogen production potential, the maximum hydrogen production rate, and the lag-phase time. In conclusion, the heat shock pretreatment was a promising method for preparing efficient hydrogen-producing seeds based on the maximum hydrogen yield and specific hydrogen production.

Table 2 Soluble metabolite products from hydrogen production using various seed inoculum preparation methods.

Seed inoculum preparation method	Soluble metabolite products (g/L)				HBu/HAc ratio
	HAc	HPr	HBu	EtOH	
Untreated elephant dung	0.28±0.01	0.04±0.01	2.46±0.04	0.10±0.01	8.80±0.02
Heat shock	0.24±0.02	0.02±0.02	2.25±0.03	0.12±0.01	9.38±0.03
Acid	0.11±0.01	0.03±0.02	1.27±0.01	0.02±0.02	11.55±0.02

HAc = acetic acid; HPr = propionic acid; HBu = butyric acid; EtOH = ethanol

Potential of sugars for hydrogen production

Xylose and arabinose are hydrolyzed pentose sugars from lignocellulosic materials. These sugars were used as carbon sources for hydrogen production. Glucose was used as a control because it is a main representative sugar in lignocellulosic materials, and has been widely used as model substrate [4,7]. **Table 3** summarizes the parameters of the modified Gompertz equation for various sugars. The results showed that the carbon source affected all of the kinetic parameters. Mixed cultures from elephant dung could effectively convert glucose and xylose to hydrogen. Glucose and xylose were converted to pyruvate by glycolysis and pentose phosphate pathways, respectively. Then, pyruvate was converted to hydrogen. However, the lag-phase time for xylose was longer than that of glucose. It could be explained that glucose was easily biodegraded during hydrogen production [30]. Xylose was determined to have the maximum cumulative hydrogen production of 778 mL/L and the hydrogen production rate of 17.26 mL/L·h, while a longer lag-phase time (11.22 h) was detected. Maximum specific hydrogen production and a hydrogen yield of 93 mL/g VSS and 0.55 mol H_2/mol xylose were obtained. These values were comparable to those obtained from glucose. Arabinose exhibited poor hydrogen production. The complexity of the metabolic pathways and the involvement of various enzymes in arabinose fermentation could be one of the reasons for poor hydrogen production, and led to the long lag-phase time (15.91 h). Arabinose fermentation requires various enzymes and, consequently, its biochemical reactions are relatively complex. Arabinose was converted to xylulose 5-phosphate and ribose 5-phosphate via the isomerase, kinase and epimerase, while xylose was directly isomerized to xylulose by xylose isomerase [31]. Xylulose 5-phosphate and ribose 5-phosphate were subsequently converted to glyceraldehydes 3-phosphate and fructose 6-phosphate via transaldolase and transketolase. Then, glyceradehydes 3-phosphate and fructose 6-phosphate entered the glycolysis pathway and continued their degradation [32]. Therefore, arabinose was the unfavored substrate for hydrogen production by mixed cultures of elephant dung. Since mixed

cultures in elephant dung could effectively utilize xylose, the results implied that the production of hydrogen from hydrolysate of lignocellulosic material is possible.

Table 3 Kinetic parameters of hydrogen production from various sugars; total cultivation time was 144 h.

Sugar	Cumulative hydrogen production (mL/L)	Hydrogen production rate (mL/L·h)	Lag-phase time (h)	Maximum specific H_2 production (mL/g VSS)	Hydrogen yield (mol H_2/mol substrate)	Correlation coefficient (R^2)
Glucose	839	19.56	7.27	100	0.70	0.9962
Xylose	778	17.26	11.22	93	0.55	0.9990
Arabinose	37	2.11	15.91	4	0.04	0.9996

The total amount of produced SMPs from xylose was higher than that of arabinose. The most predominant VFA produced was butyric acid, which represented 87 % of the total SMPs for xylose. The ratios of butyric acid/acetic acid in the fermentation broth were higher than 2.0 for all cases (**Table 4**). Therefore, the hydrogen production by mixed cultures of elephant dung in this study was the butyrate-type fermentation.

Table 4 Soluble metabolite products from hydrogen production using various sugars.

Sugar	Soluble metabolite products (g/L)				HBu/HAc ratio
	HAc	HPr	HBu	EtOH	
Glucose	0.32±0.03	0.03±0.01	2.32±0.02	0.10±0.03	7.25±0.03
Xylose	0.20±0.01	0.02±0.01	2.22±0.03	0.12±0.02	11.10±0.02
Arabinose	0.03±0.01	0.01±0.02	0.07±0.01	0.01±0.01	2.33±0.01

HAc = acetic acid; HPr = propionic acid; HBu = butyric acid; EtOH = ethanol

Effect of the initial pH on hydrogen production

The effect of the initial pH of synthetic medium on hydrogen production was investigated at a fixed initial xylose concentration of 10 g-COD/L. The initial pH values were ranged from 5.0 - 9.0 with an interval of 0.5. **Table 5** summarizes the parameters of the modified Gompertz equation at various initial pH values. The results showed that the initial pH affected all of the kinetic parameters. The lag-phase time decreased with the pH ranging from 5.0 to 6.5, and then increased to 41.31 h at pH 8.5. This finding was in agreement with the previous results [33]. The hydrogen yield increased with the initial pH ranges from 5.0 to 8.0, and then decreased as the initial pH further increased to 9.0. The maximum hydrogen production of 371 ml H_2/g VSS and the yield of 1.62 mol H_2/mol xylose were obtained at the initial pH of 8.0. The results indicated that the metabolism of hydrogen-producing bacteria strongly depended on the pH of the medium.

Table 5 Kinetic parameters from hydrogen production at various initial pH values; total cultivation time was 144 h.

Initial pH	Cumulative hydrogen production (mL/L)	Hydrogen production rate (mL/L·h)	Lag-phase time (h)	Maximum specific H_2 production (mL/g VSS)	Hydrogen yield (mol H_2/mol xylose)	Correlation coefficient (R^2)
5.0	484	8.48	20.79	58	0.47	0.9952
5.5	625	14.65	14.94	75	0.35	0.9954
6.0	778	17.26	11.22	93	0.55	0.9990
6.5	999	17.43	11.37	114	0.55	0.9965
7.0	1374	28.98	19.79	164	0.81	0.9952
7.5	1777	54.46	35.21	212	1.03	0.9973
8.0	3106	75.83	41.30	371	1.62	0.9980
8.5	2854	73.12	41.31	341	1.49	0.9985
9.0	2835	55.79	30.97	338	1.46	0.9959

The optimal initial pH for hydrogen production from xylose in this study was found to be at pH 8.0, which was much greater than that at the other pH values. The results from the present study were different from those of the previous ones [20,30,34-36], where a pH range for maximum hydrogen production was between 5.0 and 6.0 due to the difference in the inoculum type and the carbon source. However, it was in close agreement with other previous studies, namely that the optimum culture pH values for hydrogen production were determined to be 7.5 and 8.5 [12,33,37]. If the initial pH did not inhibit bacterial growth, a higher initial pH value would have the superiority of delaying the onset of the pH inhibition to the hydrogen production caused by the metabolic shift from acidogenesis to solventogenesis [37]. The results suggested that control of optimum pH was required in order to obtain high hydrogen production.

The production of soluble metabolites, including ethanol, acetic, propionic and butyric acids are summarized in **Table 6**. The results indicated that the higher the initial pH value, the higher the amount of soluble metabolites produced. The hydrogenic activity of mixed cultures of the elephant dung was the highest for butyric acid. The range of the butyric acid/acetic acid ratio was found to be between 2.83 to 14.85, and was dependent on the initial pH (**Table 6**). This ratio may vary with microbial growth conditions during the fermentation process. In this study, the minimum butyric acid/acetic acid ratio was 2.83 at the initial pH of 8.0, which implied that efficient hydrogen-producing metabolism was observed. It has been reported that the butyric acid/acetic acid ratio 2.60 to 4.0 indicated an efficient hydrogen production by anaerobic microflora [10,38]. The most predominant VFA that was produced was butyric acid, which represented between 70.53 and 88.72 % of the total SMP in this study.

Table 6 Soluble metabolite products from hydrogen production at various initial pH values.

Initial pH	Soluble metabolite products (g/L)				HBu/HAc ratio
	HAc	HPr	HBu	EtOH	
5.0	0.13±0.03	0.01±0.01	1.93±0.010	0.12±0.05	14.85±0.05
5.5	0.20±0.03	0.02±0.01	2.22±0.05	0.14±0.03	11.10±0.04
6.0	0.21±0.02	0.02±0.01	2.26±0.04	0.11±0.02	10.76±0.03
6.5	0.20±0.01	0.03±0.01	2.80±0.03	0.12±0.02	14.00±0.02
7.0	0.73±0.02	0.03±0.01	2.38±0.02	0.12±0.02	3.26±0.02
7.5	0.52±0.03	0.03±0.01	2.31±0.01	0.11±0.01	4.44±0.02
8.0	0.87±0.02	0.03±0.01	2.46±0.02	0.12±0.01	2.83±0.02
8.5	0.70±0.02	0.04±0.01	2.33±0.03	0.09±0.02	2.33±0.01
9.0	0.64±0.02	0.06±0.02	2.28±0.02	0.07±0.02	4.38±0.01

HAc = acetic acid; HPr = propionic acid; HBu = butyric acid; EtOH = ethanol

In conclusion, the optimum pH for hydrogen production from xylose in this study was found to be at pH 8.0, and the pH control at the optimum initial pH is necessary in order to obtain high hydrogen production.

The maximum hydrogen production of 371 ml H_2/g VSS and the yield of 1.62 mol H_2/mol xylose were obtained at the initial pH of 8.0 and the initial xylose concentration of 10 g-COD/L by elephant dung was compared with other literature (**Table 7**). The results indicated that heat shock-pretreatment elephant dung could be used as the seed source for converting xylose, the primary hydrolyzed product from lignocellulosic materials, to hydrogen.

Table 7 Comparison of the hydrogen yield from xylose by different types of the seed source.

Seed source	Initial pH	Initial xylose concentration (g-COD/L)	Temperature (°C)	H_2 yield (mol H_2/mol xylose)	References
Sewage sludge	6.5	20	35	1.30	[10]
Sewage sludge	6-7	20	35±1	1.92-2.25	[39]
Sewage sludge	7.0	20	30-40	1.3	[40]
Clostridium butyricum CGS5	7.5	20	37	0.73	[41]
Clostridium sp. HR-1 isolated from cow dung compost	6.5	12	36±1	1.63	[42]
Elephant dung	8.0	10	37	1.62	This study

Microbial community in elephant dung

The DGGE profile of microorganisms from hydrogen fermentation broth using xylose as a substrate and initial pH 8.0 is depicted in **Figure 1**. The DGGE profile demonstrates that the community of fermented broth cultures had less microbial diversity. Four major bands affiliated with *Clostridium acetobutylicum*, *Bifidobacterium minimum*, *Ethanoligenens* sp., and *Bifidobacterium* sp. were found in the fermented broth. *Clostridium acetobutylicum* and *Ethanoligenens s*p. have been reported as active hydrogen producers, which can increase the hydrogen production efficiency [21,39-41]. The presence of lactic acid-producing bacteria, i.e. *Bifidobacterium minimum* and *Bifidobacterium* sp., could reduce the hydrogen production efficiency [42], which was correlated to the small amount of lactic acid (0.31 g/L) detected at the initial pH of 8.0 (data not shown). *Bifidobacterium* spp. can also produce acetate with lactate, if the bifidum pathway is used [43].

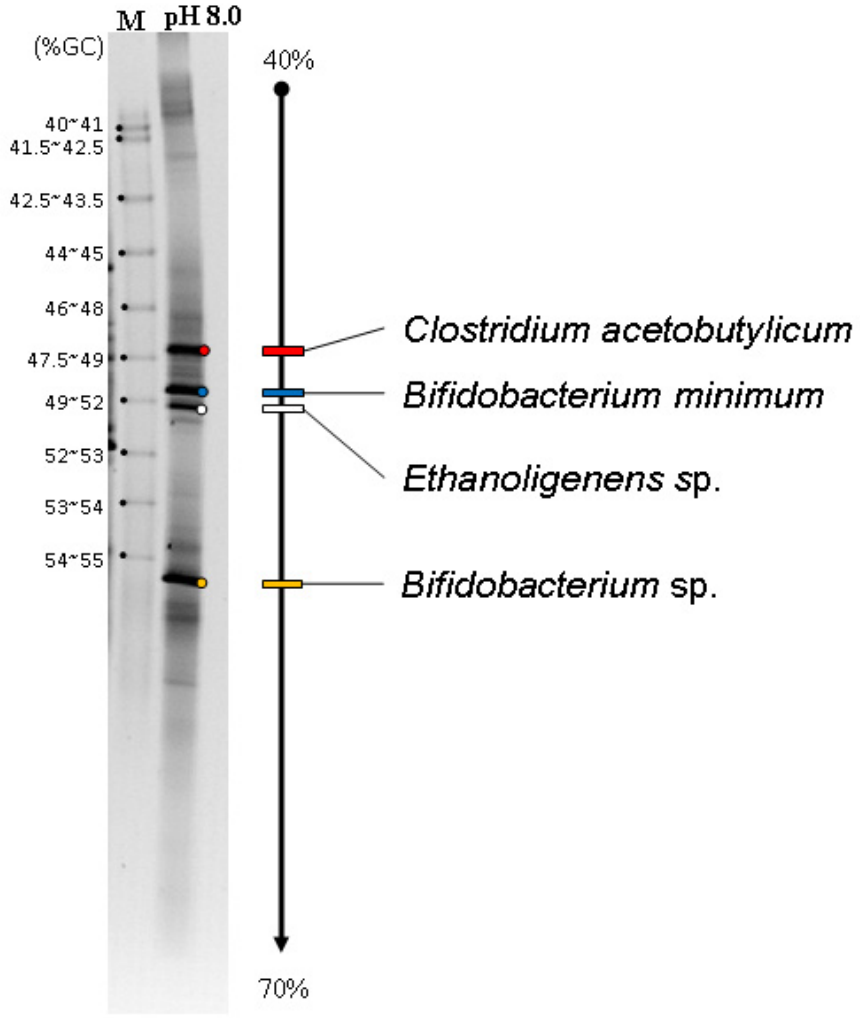

Figure 1 DGGE profile of 16S rDNA gene fragment of microorganism taken from hydrogen production broth at pH 8.0.

Conclusions

The inoculums seed from heat shock pretreatment (90 °C for 3 h) gave the highest hydrogen production and yield. The culture could use xylose for hydrogen production, but arabinose exhibited poor hydrogen production. The maximum hydrogen production and hydrogen yield, of 371 mL H_2/g VSS and 1.62 mol H_2/mol xylose, respectively, were observed at the fixed initial pH of 8.0 and the initial xylose concentration of 10 g-COD/L. The results indicated that the heat shock-pretreated elephant dung was a potentially mixed natural-microbial seed source for hydrogen production from xylose. The predominant hydrogen producers that were found at the optimum initial pH of 8.0 were *Clostridium acetobutylicum* and *Ethanoligenens* sp. The main soluble product was butyric acid, suggesting that the hydrogen fermentation from xylose by mixed cultures in the elephant dung was of the butyric acid-type fermentation.

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