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Full Length Research Paper

Effect of retention time on biohydrogen production by microbial consortia immobilised in polydimethylsiloxane

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An investigation on biohydrogen production from palm oil mill effluent (POME) was conducted in a continuous stirred tank reactor seeded with polydimethyl-siloxane (PDMS) immobilised mixed cultures at adjusted retention time. The hydrogen-producing bacteria obtained from an anaerobic digester used for treating POME were acclimatised and immobilised in PDMS. The immobilised cultures were assessed for their effectiveness in generating hydrogen in a continuous system. In this study, the PDMS cultures were fed with raw POME at hydraulic retention times of 6, 4 and 2 days and operated at controlled pH and temperature of 5.5 and 55°C, respectively. At hydraulic retention time (HRT) 2 days, the average hydrogen production rate per unit volume of POME was 2.1 NL/L/d. Hydrogen constituted up to 43% of the total gas produced and methane was not detected throughout the 150 days of continuous operation. The soluble carbohydrate degradation efficiency was highest at 81.2% during HRT 4 days and the concentration of soluble metabolites produced, followed the order of acetic > butyric > ethanol > propionic acid. The microbial diversity of the immobilised consortia determined by denaturing gradient gel electrophoresis (DGGE) changed at different HRTs; with increasing dominant species phylogenetically related to *Clostridaceae.*

Key words: Hydrogen fermentation, palm oil mill effluent, polydimethylsiloxane (PDMS), immobilisation.

INTRODUCTION

Dark fermentation of renewable sources have received broad attention as the process is regarded as green technology with potential in reducing greenhouse gases (GHG) impact and relatively low energy intensive compared to the typical hydrogen generation via steam methane reforming (SMR). The GHG emission of hydrogen production via the SMR process is estimated at 9.7 to 13.7 kg CO_2/kg H_2 produced (Spath and Mann, 2000; Muradov and Veziroğlu, 2008) which is one magnitude order than other known industrial polluters such as in fertiliser, steel and cement production. It is envisaged that the biomass-based hydrogen production by anaerobic heterotrophic fermentation is commercially viable by integrating the system with current wastewater treatment system for concomitant waste reduction and bioenergy generation (Hawkes et al., 2002).

Many research works on biohydrogen production from complex wastewater such as palm oil mill effluent

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Abbreviations: GHG, Greenhouse gases; **SMR,** steam methane reforming; **POME,** palm oil mill effluent; **COD,** chemical oxygen demand; **EVA,** ethylene vinyl acetate copolymer; **PMMA,** polymethyl methaacrylate; **PVA,** polyvinyl alcohol; **PDMS,** polydimethylsiloxane; **PCR-DGGE,** polymerase chain reaction coupled with denaturing gradient gel electrophoresis; **VSS,** volatile suspended solid; **HRT,** hydraulic retention time; **TSS,** total suspended solids; **VFA,** volatile fatty acids; **TCD,** thermal conductivity detector; **CPO,** crude palm oil; **PEO,** polyethylene-octene; **ASBR,** anaerobic sequencing batch reactor.

Table 1. Comparative study on the hydrogen yield per unit volume of raw POME using different reactor configuration systems.

Reactor system and operating conditions	Inoculum	Hydrogen yield (LH_2/ L POME)	Reference
Fed batch, CSTR 60°C, pH 5.5	Mixed cultures	2.4	Atif et al. (2005)
Continuous, ASBR 60°C, pH 5.5	Mixed cultures	4.4	O-Thong et al. (2007)
Batch, CSTR, 37°C, pH 5.5	*Clostridium butyricum* EB6	3.3	Chong et al. (2009)
Continuous, CSTR 55°C, pH 5.5	Mixed cultures	2.6	Ismail et al. (2010)
Continuous, CSTR 55°C, pH 5.5	Immobilised PDMS-mixed cultures cubes	2.1	This study

CSTR, Completely stirred tank reactor; ASBR, anaerobic sequencing batch reactor.

(POME) focused on suspended cell culture system either under batch or continuous mode of operation (Table 1). The hydrogen production rate per unit volume of raw POME was in the range of 2.4 to 4.4 at typical POME chemical oxygen demand (COD) concentration of 70 to 100 g/l. It was perceived that recycling of the washout biomass is necessary in order to maintain sufficient biomass concentration in the reactor. Another option will be to immobilise the cells as immobilised cells system not only enhance the biomass retention but also provide localised anaerobic environment for strict anaerobes in the hydrogen fermentation process. The immobilisation system enhances the separation of solid–liquid in the settling tank and therefore allows for further recycling of the biomass.

Entrapment of cells in polymeric matrixes e.g. ethylene vinyl acetate copolymer (EVA), polymethyl methaacrylate (PMMA) and polyvinyl alcohol (PVA) (Wu et al., 2005; Wu and Chang, 2007; Zhang et al., 2007) for biohydrogen production from simple sugars have been successful and the materials are widely used as they are relatively less expensive, high mechanical strength and durability with ease of handling (Park and Chang, 2000). Acclimated sludge entrapped in composite polymeric matrix (PMMA/collagen/ activated carbon) displayed good H_2-producing activity with H_2 production rate of 1.8 L/L/h and a H_2 yield of 2.0 mol H_2/mol sucrose. Polydimethylsiloxane (PDMS) elastomeric derivative such as Sylgard 184 (Dow Corning) is a polymer with additional merits (Colas and Curtis, 2004) due to its low toxicity, ease of processing in standard laboratory conditions, low curing temperature, optical transparency and cost effectiveness. PDMS is also highly hydrophobic and many researchers have reported that bacteria attached more readily to hydrophobic materials (Parkar et al., 2001; Pasmore et al., 2002). Solid PDMS can be used in combination with water, methanol or glycerol without material deformation or swelling which is an added advantage as the polymerised PDMS cells cubes will remain intact in the fermentation broth over long period.

In this study, the performance of PDMS-immobilised microbial consortia on the hydrogen production at different hydraulic retention time was investigated. Anaerobic digested sludge was acid treated at pH 3 overnight, to inhibit the growth of hydrogen–consuming methanogens (Chen et al., 2002; Zhu and Béland, 2006; Dong et al., 2010) and to selectively enriched the growth of hydrogen-producing bacteria such as *Clostridium* sp. (Lee et al., 2010) and *Bacillus* sp. (Liu et al., 2009). A standard seed inoculum was produced by acclimatising the acid-treated sludge with synthetic wastewater as described by many researchers (Lin and Lay, 2005; O-Thong et al., 2009) prior to the immobilisation in PDMS. The PDMS-culture cubes were subjected to complex wastewater, POME, under continuous system. Microbial community dynamics during the hydrogen fermentation by PDMS–cells was studied using polymerase chain reaction coupled with denaturing gradient gel electrophoresis (PCR-DGGE) analysis. This is the first report on the performance of immobilised mixed cultures on the thermophilic, hydrogen fermentation using POME as substrate.

MATERIALS AND METHODS

POME substrate

Fresh POME was collected from the receiving tank of an oil palm mill in Carey Island Selangor, Malaysia and kept at 4°C prior to use. The raw POME used in this study had the following characteristics (in g/L): Total carbohydrate 12.7 to 20.9, soluble carbohydrate 8.1 to 11.7, total protein 15.2, oil and grease (O&G) 3.4, total suspended solids 27.5 and pH of 4.5.

Acclimatisation of seed

Digested sludge originating from a mesophilic digester treating POME was used as inoculum. The collected sludge was sieved using 500 µm mesh and acid-treated with 1N HCl to pH 3 and left overnight. The pH was restored to pH 5.5 by 1N NaOH and the 2 L volume of filtrate was transferred to the fermentor. The initial volatile suspended solid (VSS) and total solid concentration of the sludge were 8.0 and 11.4 g/L, respectively. The specific hydrogen production of the acid treated sludge was pre-determined at 10 ml/gVSS/ h when fed with 20 g/l sucrose under batch condition of pH 5.5 and temperature of 55°C. The sludge was then acclimatised to synthetic wastewater in continuous stirred tank reactor (CSTR) at the above-mentioned operating conditions. The synthetic wastewater consisted of sucrose (20 g/l) and the following nutrients and trace elements as reported by Zhang et al. (2006) (in mg/L): 200 NH_4Cl, 200 peptone, 200 KH_2PO_4, 30 $FeCl_3.6H_2O$, 20 $MgCl_2.6H_20$, 10 $MnCl_2.4H_20$, 5 $COCl_2.6H_2O$, 6 $CaCl_2.2H_2O$, 4.5 $CuCl_2.2H_2O$, 5 $ZnSO_4.7H_2O$ and 4 $NiCl_2.6H_2O$. The reactor was operated in fed batch system for one month at hydraulic retention time (HRT) of 2

days after which the acclimatised sludge was subjected to cell immobilisation procedure.

PDMS-immobilisation

H_2 producing sludge was centrifuged at 5,000 rpm for 10 min and the pellet was rinsed twice with 0.1M phosphate buffer saline (PBS, pH 5.5). The pre-weighed sludge was mixed with powdered (250 µm) coconut shell activated carbon (Casitan Pte. Limited., Malaysia) at ratio of 10 to 1 on weight basis as described by Wu et al. (2005). The slurry was then mixed with the PDMS elastomer (Sylgard 184, Dow Corning) at a ratio of 1 to 8. The PDMS precursor and curing agent were mixed according to the manufacturer specification and the mixture was left for 30 min before being poured into the sludge mixture. The silicon elastomer was cured at room temperature for 15 h and cut into small cubes of 0.15 ± 0.01 g. The immobilised sludge contained about 70 mg VS/g PDMS cubes with average density of 1.12 g/cm^3. In this study, powdered activated carbon was used as filler material to provide attachment sites for microbial consortia while improving the mechanical properties of the synthetic polymer as suggested by Chang et al.(2005).

Continuous H_2 fermentation

A CSTR (Biostat B Plus, Sartorius Stedim Biotech Germany) with a working volume of 4 l was used in this study. The pH of the mixed liquor was controlled at pH 5.5 with 2 M NaOH and 2 M HCl, constantly stirred at 200 rpm and temperature maintained at 55°C through water jacket. The feed was stored at 4°C and pumped into the reactor using a peristaltic pump (Masterflex L/S, Cole Palmer Instrument, USA). A level sensor and effluent peristaltic pump were used to control the reactor working volume at constant value. The gas line was connected to the gas holder to measure the daily biogas production by water displacement method. At the start up, the fermentor was loaded with 10% (w/v) of PDMS cubes (that is, equivalent of 7 g VS/L initial biomass content) and fed with 20 g/l of sucrose-containing wastewater in fed-batch mode over 7 days. This is to reactivate the microbial consortia to generate biohydrogen before changing to continuous operation. POME was then fed at HRT of 6 days and stepwisely reduced to HRT 2 days upon reaching steady state at each run. In this study, steady state conditions were considered attained when hydrogen gas concentration and effluent VFAs concentrations were stable at less than 10% variation. The gas volumes were corrected to standard temperature and pressure (STP) (273.15 K and 101.325 kPa).

Analytical methods

Samplings of biogas and liquid metabolites were conducted five and two times per week, respectively. pH, total suspended solids (TSS) and VSS were analysed according to the Standard methods (APHA, 2005). Carbohydrate and protein were measured according to the phenol-sulphuric and Folin Lowry methods, respectively. Soluble sample were centrifuged and filtered with 0.45 µm cellulose-acetate syringe filter prior to analyses. Volatile fatty acid (VFA) was analyzed by FID gas chromatograph (Hewlett Packard 5890, Column: H-FFAP, Carrier gas: Helium). The biogas composition was analyzed by a thermal conductivity detector (TCD) gas chromatograph (Shimadzu GC-2014, Packing material: Unibeads-C60/80mesh, Column temperature: 145°C, Carrier gas: Argon). The biomass content of the immobilised cubes was determined by mechanical disruption followed by protein analysis.

Total genomic DNA extractions were carried out from samples collected during various stages of the bioreactor operation. Liquid samples were centrifuged at 10,000 rpm to collect settled solids, immobilised cubes were cut into small pieces and all samples were washed twice with phosphate buffer pH 7.0 prior to DNA extraction using UltraClean Soil DNA Kit (MoBio Laboratory Inc.USA). The 16S rRNA genes were amplified from the DNA using PCR with forward primer (F968, 5'-AA CGC GAA GAA CCT TAC-3') with GC clamps at the 5" end and a broadly inclusive bacterial reverse primer (R1401, 5'-CGG TGT GTA CAA GAC CC-3'). Each 25 µl PCR mixture contained 1 x PCR buffer, 1.5 mM $MgCl_2$, a 0.2 mM concentration of each deoxynucleoside triphosphate, 0.5 µM concentration of each primer, 1.25 U of *Taq* DNA polymerase and DNA template. Thermal cycling consisted of the following steps: 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1.5min, and a final step at 72°C for 10 min. The PCR products were used to perform DGGE at constant temperature of 58°C for 5 h at 130 V through a 6% acryl/bis gel (37.5:1) containing denaturant ranging from 40 to 65%. The polyacrylamide gels were visualised with Kodak EDAS 290, selected bands were excised from the gel and re-amplified with primers without GC clamps. PCR products for partial 16s rRNA sequences were identified using Basic Local Alignment Search Tool (BLAST) in GenBank.

RESULTS AND DISCUSSION

Process performance of immobilised-CSTR reactor

Raw POME with COD of 70 to 85 g/l was fed continuously at variable HRT by increasing stepwise the feed flow rate. The whole experimental duration was divided into 3 HRTs (6, 4 and 2 days) during which sampling of immobilised cubes and the effluent were carried out for chemical and molecular analyses. Laboratory-scale experiment was conducted for 150 days, and the results on process performance were depicted in Figure 1 and 2.

H_2 production with immobilised fermentative consortia

The H_2 production rate and gas phase H_2 content did not vary considerably regardless of changes from HRT 4 to 2 days. The average H_2 production rate per unit volume of POME at HRT 2d was 2.1 NL/L/d as shown in Figure 1(a). Figure 1(b) depicts the H_2 production profile of the immobilised cultures with gas phase products were H_2 and CO_2 which accounted for 43 and 57%, respectively. Methane was not detected during the course of the experiment which signifies that acid treatment was effective in inhibiting methanogens in the mixed cultures. The low percentage of H_2 generation coupled with high acetate production (details are as described below) accounts for potential hydrogen sink due to homoacetogenesis in which hydrogen was used to reduce carbon dioxide to acetate. This concurs well with studies conducted by other researchers (Siriwongrungson et al., 2007; Massanet-Nicolau et al., 2010) that hydrogen loss via homoacetogenesis has significant impact in reducing total hydrogen yield even though methanogenesis has been successfully avoided. Based on the highest H_2 production of 0.09 mol/l POME/d and the heating value of H_2 is 242 kJ/mol, the energy yield in this study is

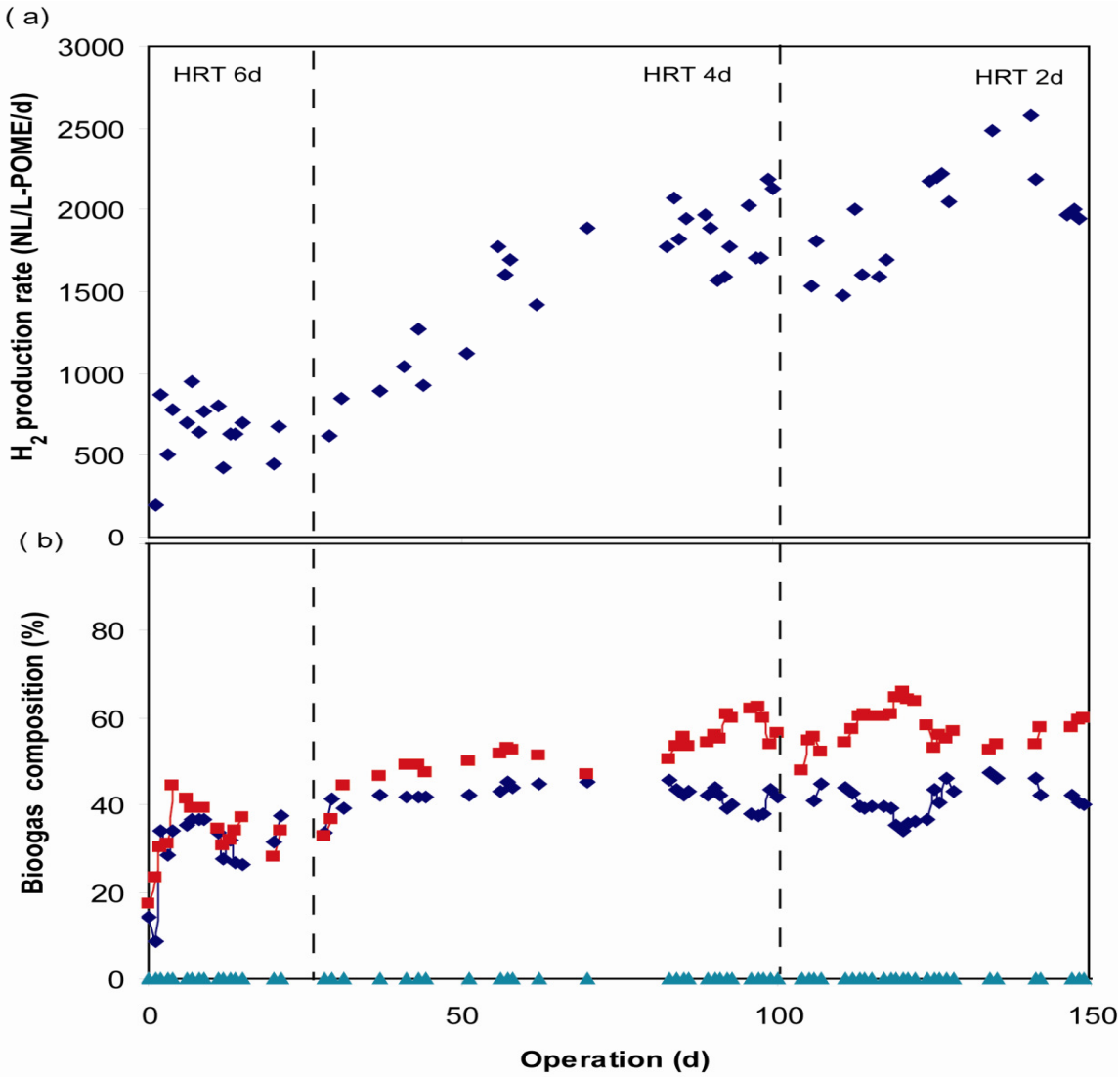

Figure 1. Time course of (a) Biohydrogen production and (b) biogas composition over the 150 days operation. (■) carbon dioxide, (♦) hydrogen and (▲) methane.

calculated to be 22.7 MJ/m^3 POME. Hence, for every ton of crude palm oil (CPO) produced it is estimated that 72 to 103 MJ of hydrogen-based energy can be harnessed via this process. This is far less than the estimated total biogas energy of 1.5 GJ (per ton CPO) from the methanogenic anaerobic digestion of POME (Yusoff, 2006). Therefore, implementing two step processes of biohydrogen production followed by methanogenic digestion is recommended in order to increase the total energy yield from POME.

Liquid-to-gas mass transfer limitation in anaerobic processes has been reported by Pauss et al. (1990) in which despite a good mixing of the liquid phase in the CSTR operating at 400 rpm and 35°C, concentration of dissolved hydrogen in the liquid phase was at 35 times of the equilibrium value. A steady state with no mass transfer limitation is characterised by a dissolved gas concentration approaching the equilibrium value of 1. In anaerobic process where gaseous species are formed in the liquid phase and tend to escape into the gas phase, transfer limitations imply that dissolved-gas concentrations are higher than the corresponding values at equilibrium. Such over concentration can be detrimental to the biological process as H_2 must be maintained at less than 10 Pa to allow degradation of propionate and to reduce its negative thermodynamic effect. The gas-to-liquid mass transfer limitation is further implicated in this study as POME is highly viscous and concentrated substrate.

Liquid metabolites production

The VFA results indicated that the acetic acid fermentation

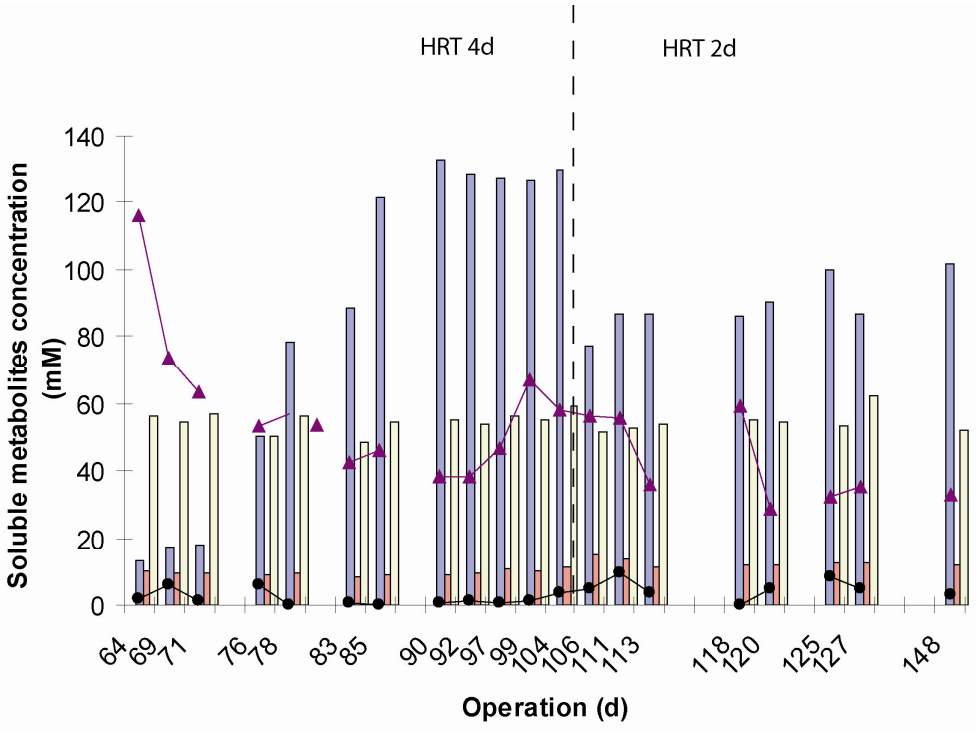

Figure 2. Time course of soluble metabolites production during the fermentation of POME by immobilised cultures. (■) acetic acid, (■) propionic acid, (■) butyric acid, (▲) ethanol and (●) lactic acid.

Table 2. The soluble and gases metabolites produced during the CSTR operation.

HRT (d)	Soluble metabolites[a]						Gases metabolites		
	SMP (mM)	(HAc + HBu) (%)	HPr (%)	HLa (%)	EtOH (%)	Soluble carbohydrate conversion (%)	H_2 (%)	CO_2 (%)	H_2 yield (mol/mol hexose)
4	245.7± 1.5[b]	75.4	4.1	0.4	20.2	81.2 ± 5.3	41.2 ± 5.8	57.2 ± 5.9	1.58
2	216.3 ± 2.2	68.0	5.5	2.7	23.8	69.6 ± 7.9	42.3 ± 5.8	56.9 ± 5.2	2.85

[a]Soluble metabolites in % based on the concentration of metabolites over the SMP; SMP, soluble microbial products; HAc, acetic acid; HPr, propionic acid; HBu, butyric acid; HLa, lactic acid; EtOH, ethanol; [b] Mean ± relative standard deviation RSD (%); whereby *n* in the range of 4 to 23.

pathway (accounting for 52.6% of the total soluble metabolites) was the main pathway in continuous H_2 fermentation of POME. This suggested that the dominant H_2 producers in the mixed cultures were the acetate-producing acidogens. HAc pathway corresponds to the maximum hydrogen yield of 4 moles hydrogen per mole glucose. In Figure 2, prior to reaching steady state (that is, day 64 to 78) at HRT 4 days, the fermentative pathway was dominated towards ethanol production but gradually decrease with time. The production of HAc increased considerably and stabilised at average of 129.2 mM (That is, 90 to 104 day) with butyric acid (HBu) (55.1 to 55.9 mM) and propionic acid (HPr) (10 to 12 mM) productions were fairly constant throughout the whole fermentation period (Table 2). The stoichiometry of hydrogen production from the immobilised cultures utilising POME is constructed based on the HBu/HAc molar ratio of 0.4;

$$4C_6H_{12}O_6 + 2H_2O \rightarrow 5C_2H_4O_2 + 2C_4H_8O_2 + 7H_2 + 6CO_2 \quad \text{(Eq. 1)}$$

This resulted in molar ratio of hydrogen to hexose of 1.75 and the average experimental hydrogen yield of 1.58 mol H_2/mol hexose was determined in the current work. Only 90% of the estimated H_2 yield was recovered as gas phase products; mainly because part of the carbohydrate was also converted to ethanol, propionic acid and cell biomass. The simultaneous ethanol and hydrogen production

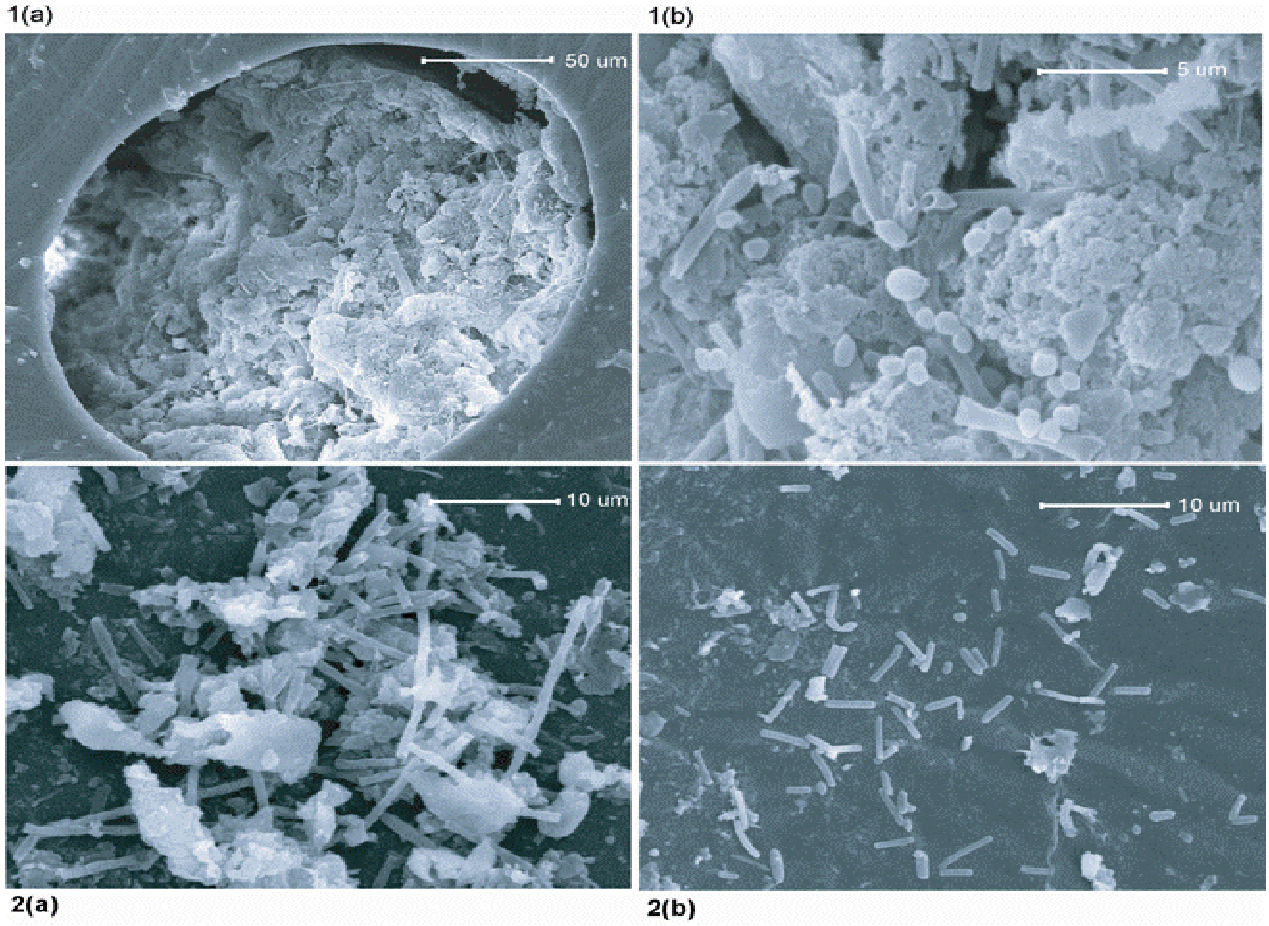

Figure 3. Scanning micrographs of immobilized cultures (1) Directly after immobilisation with (a) magnification 500x, (b) magnification 5000x and (2) at HRT of 4 days with (a) and (b) at magnification 2000x.

could have resulted from different sugars metabolic pathway by the H_2-producing bacteria in the mixed cultures. Wu et al. (2007) has also reported the possible presence of *Enterobacter* and *Klebsiella* species in the polyethylene-octene (PEO) immobilised sludge that might contribute significantly to ethanol production and *Clostridiae* sp. that produced H_2 and ethanol/VFAs mixtures. It is also likely that the lower pH or higher H_2 concentration occur within the entrapped cells due to accumulation of acidic metabolites and gas products, resulting in unfavourable H_2-producing kinetics. This is in agreement with Wu and Chang (2007).

The volatile suspended solids were reduced at 62% under HRT 4 days compared to 33% at HRT 2d (data not shown) which indicates that hydrolysis and subsequently, acetogenesis were implicated possibly due to solid phase mass transfer resistance between immobilised cultures and the particulates. Cubas et al. (2007) has also indicated that the solid phase mass transfer is directly related to the size of the immobilised cubes. Polyurethane foam cubes of 0.5 to 2 cm sides slightly affect the cycles time of the anaerobic sequencing batch reactor (ASBR) as compared to bigger 3 cm biocubes resulting in high concentration of discharge effluent. In order to maintain high carbohydrate conversion (> 80%) with current size of PDMS cubes, the HRT should be maintained at 4 days and ultimately reduce the solid mass transfer limitation. Optimisation of the activated carbon concentration in the future work is deem necessary in order to maintain high availability of hydrogen producing bacteria within the immobilised cubes while sustaining the porosity of PDMS. Increased dosage of PAC will hinder gas- and nutrient transfer.

Bacterial community shift

Scanning electron microscopy (SEM) photos as shown in Figure 3(1a and b) showed that the exterior and interior section of the immobilised cubes was initially dominated by rod-like bacteria and other cocci-like bacteria. However, at HRT 4 days, only rod-like bacteria were observed on the surface of the immobilised cubes. DGGE was then conducted to monitor the changes in microbial population

Table 3. Microbial community of samples as analysed by PCR-DGGE.

Samples	HRT	Sequenced band	Closest relatives/ Accession no	Similarity
Acid treated sludge	NA	A1	*Verrucomicrobia* sp./ CU918373	97%
		A2	Firmicutes / CU918096	99%
		A3	Firmicutes / CU922938	99%
		A4	Uncultured unclassified bacterium/CU921348	93%
		A5	Planctomycetes / GQ356146	99%
Immobilised sludge	4	B1	Uncultured bacterium /AB486727	93%
		B2	Uncultured bacterium /AB486838	98%
		B3	*Enterobacter* sp/GQ418128	95%
		B4	*Clostridium* sp./AB288647	99%
		B5	*Paenibacillus stellifer* strain Bs63/ GU321996	98%
		B6	Gamma Proteobacterium/ GU169052	98%
		C1	Uncultured bacterium clone / EF655642	99%
	2	D1	Uncultured bacterium clone VKW-TB-7/GQ849508	98%
		E1		
		E2	Uncultured bacterium clone/ AB34901	86%
Effluent	4	F1	Uncultured bacterium clone VKW-TB-7/GQ849508	98%
		G1		
	2	H1	Lachnospiraceae/ FJ542922	99%
		H2	Uncultured bacterium clone VKW-TB-7/GQ849508	99%

NA, Not applicable; sequenced bands as indicated in Figure 4.

of the immobilised cubes and in the effluent at different hydraulic retention times. The anaerobic digester sludge pre- treated with acid (Table 3) has microbial diversity which was directly related to bacteria species involved in the mesophilic anaerobic digester and anaerobic ammonium oxidation bioreactor (Li et al., 2009). However, the microbial population altered during the continuous operation at thermophilic condition and selectively induced other dominant bacteria species which play significant roles in hydrogen production. *Enterobacter* sp. and *Clostridium* sp. have been previously reported (Kumar and Das, 2001; Shin et al., 2007; Pattra et al., 2008), as effective hydrogen producers and also found in the biological degreasing system and used in microbial fuel cell, respectively. Presence of these bacteria species explained on the POME-carbohydrate metabolic pathway which was directed to production H_2 coupled with VFAs and ethanol.

At HRT 2 days, the DNA extract of the immobilised cubes and effluent showed further reduction of microbial diversity and was dominated by bacteria species phylogenetically related to the uncultured bacterium clone VKW-TB-7 (Lee et al., 2010) obtained from a 55°C digester producing hydrogen from kitchen waste and also to family of *Lachnopiraceae*. VKW-TB-7 was closely related to the acetogenic hydrogen producing; *Clostridiaceae* bacterium FH052 (accession no AB298768). Angenent et al. (2004) has compiled species from the families *Lachnospiraceae*, *Clostridiaceae*, *Streptococcaceae* and *Thermoanaerobacteriaceae* as examples of spore-forming hydrogen fermenting bacteria that are able to withstand at high temperature.

The DGGE profile of the effluent samples (Figure 4) also showed increasing strong band of dominant bacteria species responsible for hydrogen production at reduced HRTs which also explained the wash out of biomass from the reactor. The VSS/TSS ratio of the effluent observed was 1 compared to the initial influent ratio of 0.88 (data not shown). Constant abrasion between particulates (of about 27.5 g TSS/L) in the POME and immobilised cubes could have caused the sloughing of the entrapped micro organisms in particular from the surface of the cubes.

Conclusion

To our knowledge, this is the first reported attempt of using PDMS as immobilisation matrix for biohydrogen production from POME under continuous operation. The operation of the completely stirred tank reactor at HRT 4 days with PDMS immobilised mixed cultures showed efficient soluble carbohydrate consumption and VSS removal at 81 and 62%, respectively. The immobilised reactor performance was also evaluated based on the by products; volatile fatty acids concentration and the generated gases profiles whereby the main liquid metabolites were mixtures of acetic and butyric acid. Acetic acid attributed to 53% of the total accumulated soluble metabolites. It was also noted that simultaneous ethanol production from the carbohydrate fraction plays a role in

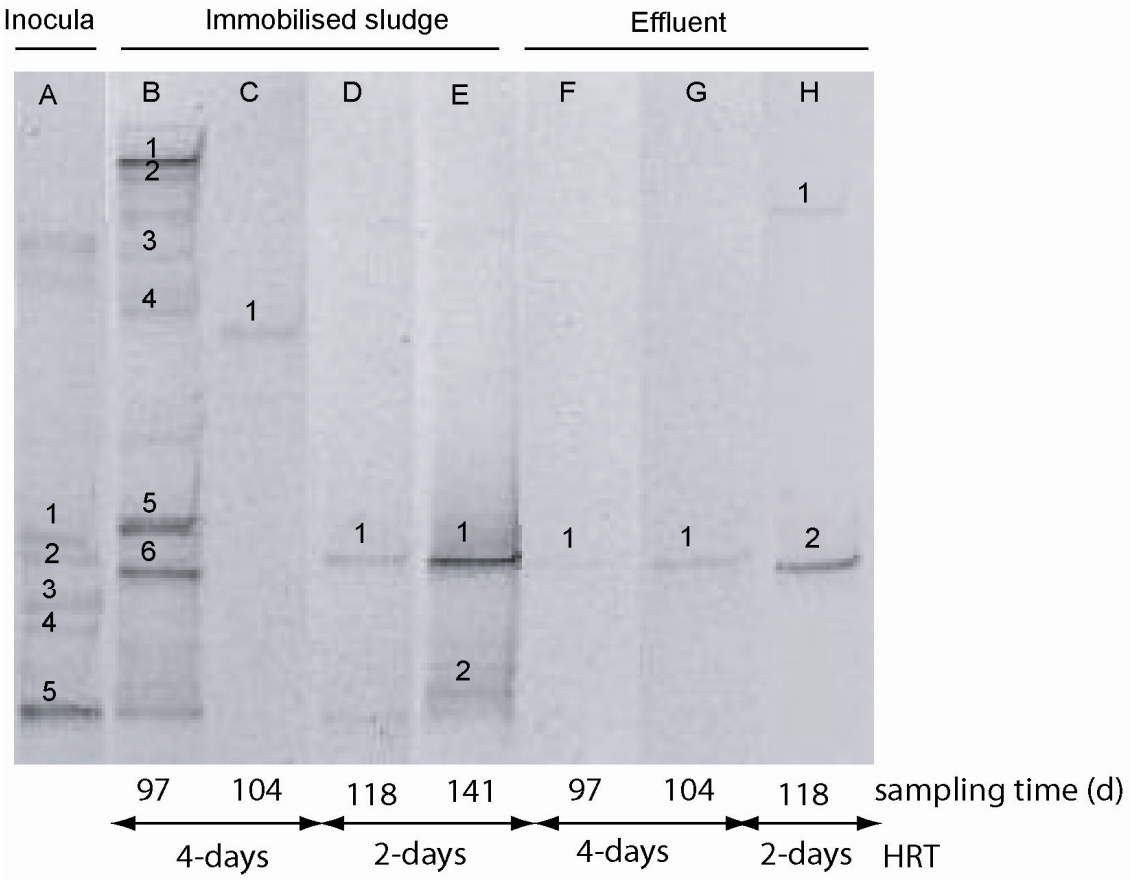

Figure 4. DGGE profiles of various samples taken at different sampling time. Lanes: A, Acid treated sludge; B to C and D to E, immobilised cubes at HRT 4d and 2d, respectively; F to G and H, effluent samples at HRT 4d and 2d, respectively. Numbers denote the excised bands and DNAs analysed for nucleotide sequences.

reducing hydrogen generation from POME. The major cause for the low carbohydrate conversion at reduced HRT could be due to the declining of microbial community structure within the PDMS cubes and as seen increased in the washout. It may also be due to the mass transfer limitation arising from the cells entrapment.

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