High-rate fermentative hydrogen production from palm oil mill effluent in an up-flow anaerobic sludge blanket-fixed film reactor

Parviz Mohammadi\textsuperscript{a,c}, Shaliza Ibrahim\textsuperscript{a,*}, Mohamad Suffian Mohamad Annuar\textsuperscript{b}

\textsuperscript{a} Department of Civil Engineering, Faculty of Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia
\textsuperscript{b} Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
\textsuperscript{c} Department of Environmental Health Engineering-Kermanshah, Health Research Center (KHRC), Kermanshah University of Medical Science, Kermanshah, Iran

\textbf{A B S T R A C T}

The major problem associated with UASB reactors for biotransformation of organic matter to hydrogen is the long start-up period (2–4 months) required for the growth of the microbial granules. In this study, an integration of granular sludge system and a fixed film reactor in a single reactor was applied to overcome this problem. An up-flow anaerobic sludge blanket-fixed film (UASB-FF) reactor was initially inoculated with heat pretreated seed sludge as inoculum and operated as closed-loop fed-batch for five days (HRT = 24 h; 38 °C; pH 5.5). The reactor was continuously fed with fresh pre-settled POME in order to shorten the start-up period. The organic loading was gradually increased from 4.7 to 51.8 g/L.d. Granular sludge rapidly developed within 22 days. Specific hydrogen production rate was 0.514 L H\textsubscript{2}/g VSS d at the end of the start-up period. Speedy development of bio-granules was attributed to biomass recirculation and the establishment of a fixed film at the upper section of the UASB-FF reactor that resulted in improved interactions among the bacterial consortium.

© 2014 Published by Elsevier B.V. on behalf of The Institution of Chemical Engineers.

Keywords: Fermentative hydrogen production; Granulated sludge formation; UASB-FF; Bioreactor start-up; Palm oil mill effluent; COD removal

1. \textbf{Introduction}

Microbial hydrogen production has received widespread attention from many researchers due to the fact that hydrogen (H\textsubscript{2}) is a renewable energy source along with its non-polluting and environmental friendly nature. Biological H\textsubscript{2} production, which can be operated at ambient temperature and pressure is a less energy intensive alternative to processes like water electrolysis (Najafpour et al., 2004; Lin and Lay, 2004; Atif et al., 2005, Mohan et al., 2008; Younesi et al., 2008; Leite et al., 2008; Mohammadi et al., 2012a). The biological technique requires specific conditions where acidogens (H\textsubscript{2} producing bacteria) and methanogens (H\textsubscript{2} consuming bacteria) are imbalanced in their activities resulting in rapid accumulation of H\textsubscript{2} (Zadariana et al., 2009; Mohammadi et al., 2011). H\textsubscript{2} producing bacteria can utilize various forms of substrates. Glucose (Mizuno et al., 2000; Fang and Liu, 2008; Hallenbeck and Benemann, 2002; Morimoto et al., 2004), sucrose (Chen et al., 2001; Lee et al., 2008) and starch (Shu et al., 2002; Akutsu et al., 2009; Su et al., 2009) were mainly used as substrates for fermentative H\textsubscript{2} production. On the other hand, rice winery wastewater (Yu et al., 2002), palm oil mill effluent (Atif et al., 2005; Chong et al., 2009; Mohammadi et al., 2012b), food waste (Ginkel et al., 2005; Kim et al., 2008), and dairy wastewater (Mohan et al., 2007; Yang et al., 2007) were also investigated as substrates in the biological processes of H\textsubscript{2} production.
due to their ready availability, low cost, high carbohydrate content and biodegradability (Atif et al., 2005; Ginkel et al., 2005; Younesi et al., 2008).

Palm oil mill effluent (POME) is rich in organic carbon with biochemical oxygen demand (BOD) value higher than 20 g/L and nitrogen content around 0.2 and 0.5 g/L as ammoniacal-nitrogen and total nitrogen, respectively (Yang et al., 2007). Three main sources of the POME are sterilization run-offs (36%), clarification (60%) and hydrocyclone (4%) units. Raw POME as a colloidal suspension contained 95–96% water, 0.6–0.7% oil and 4–5% total solids (Ma, 2000; Krishnan and Desa, 2006; Mohammadi et al., 2012c). It is estimated that 5–7.5 tons of water are required for each ton of crude palm oil production, which accounts for more than 50% of the used water converted to POME (Najafpour et al., 2006).

Biological H₂ production from POME have been accomplished using different reactor configurations such as up-flow anaerobic contact filter (Najafpour et al., 2006), anaerobic sequencing batch reactor (ASBR), batch reactors (i.e. fermentors and serum bottles) (Atif et al., 2005; Krishnan and Desa, 2006; Pakarinen et al., 2008; Chong et al., 2009; Ismail et al., 2010; Rasdí et al., 2012; Leaño and Babel, 2012), up-flow anaerobic sludge blanket (UASB) (Singh et al., 2013). The up-flow anaerobic sludge blanket (UASB) system is the most commonly used high rate anaerobic treatment process. The UASB reactor is capable of retaining high microorganism concentration and high rate of waste stabilization and could be an option for a reactor to generate biological H₂. This reactor presents positive features, such as allowing high organic loading rate (OLR), short hydraulic retention time (HRT) and has a low energy demand (Metcalfe and Eddy, 2003). Granulated sludge is the distinct characteristic of UASB reactors as compared to the other anaerobic treatment systems (Thaveesri et al., 1994; Lettinga, 1995). The growth of granulated sludge is affected by the wastewater characteristics, pH, nutrients availability and up-flow velocity (Annachhatre, 1996). However, long start-up period (2–4 months), extreme variation in the up-flow velocities, and granules washout at hydraulic stresses are major issues associated with the conventional UASB reactors (Lin and Lay, 2004). Therefore, modification of the UASB process is needed to eliminate the existing problems in order to encourage high performance H₂ production from POME. In this study, a combination of up-flow anaerobic sludge blanket (UASB) and up-flow fixed film (UFF) in a single reactor was applied as modified up-flow anaerobic sludge blanket-fixed film (UASB-FF) reactor. Several variations of UASB-FF bioreactor have been studied for treatment of different industrial wastewaters, like slaughterhouse, distillery spent wash, starchy, fiberglass manufacturing, whey wastewater, and POME (Fernandez et al., 2001; McHugh et al., 2005; Ismail et al., 2010).

In this study, application of a modified UASB-FF reactor to produce H₂ from POME was investigated. The main objectives of this research were to shorten the start-up period and accelerate the formation of granular sludge in the UASB-FF reactor.

2. Materials and methods

2.1. Experimental set-up

A schematic diagram of the laboratory-scale UASB-FF reactor (total volume 3.5 L, working volume 2.55 L, liquid height 80 cm) rig set-up used in this study is shown in Fig. 1. The glass reactor column was fabricated with an internal diameter of 55 mm at the bottom and middle parts and 75 mm at top part. The reactor comprised three sections. The lowest section of the UASB reactor’s column with the height of 60 cm...
(granular sludge portion) accommodated 67.84% of the total working volume. The middle section with the height of 15 cm (fixed film reactor) accommodated 14.51% of working volume. The top section of the reactor accommodated 17.65% of the working volume consisting a gas–solid separator and an outlet zone for fermented POME. The middle part of the column was packed with 70 plastic Pall rings (diameter and height 16 mm; specific surface area 341 m²/m³). The void space ratio of the packed-bed reactor was 90.91%. The sampling ports (S1–S5) were designed at appropriate intervals along the height of the reactor (Fig. 1).

The gas–liquid–solid separator at the top part is for the separation of the washed out solids and the biogas from the liquid phase. To measure the biogas volume generated, a gas meter was connected to an inverted funnel, cylindrical gas separator. To sample the biogas for the determination of its composition, a gas sampling port with tubing connector was provided. Before connecting the tubing to the gas meter, a water seal was used to separate gas meter unit from the reactor, providing appropriate condition for biogas sampling.

To ensure isothermal operation of the UASB-FF reactor at the selected temperature, water was circulated through the reactor jacket from a thermostated water bath equipped with a centrifugal pump (Lab. Companion, model: CW-05G, Korea). The feeding of substrate (POME) was carried out continuously into the bottom inlet of the reactor using a peristaltic pump (EVELA, model: MP-1000, Japan, 0.24–34.8 L/h) and the effluent went out from the top of the column (S5). An influent liquid distributor was mounted at the base of the column to assist in distributing the feed uniformly into the reactor column.

A cylindrical settling tank was installed in order to settle the washed out suspended solids (SS) from the reactor and also to provide an effluent with low SS for recycling into the reactor. The volume of the settling tank was 880 mL. The effluent was continuously recycled using a peristaltic pump (EVELA, Japan). For recycling the washed out granular sludge (settled at the bottom of the settling tank) into the reactor, a column was fitted to the setup and connected to sampling port #4 (S4) in order to transfer the granular sludge into the anaerobic lower part of the reactor without disintegration. In order to ensure a homogenous substrate supply, a magnetic stirrer was used to agitate the feed reservoir.

### 2.2. Wastewater preparation

The reactor was fed with a pre-settled POME. POME samples were taken from SIME Darby Plantation Palm Oil Industry Sdn. Bhd., Nilai, Malaysia. The samples were transferred to the laboratory and stored in a cold room (4 °C) before use. Various dilutions of POME were prepared using tap water. The pH and alkalinity of the feed was adjusted to 5.5 and 1400–1600 mg CaCO₃/L using NaOH solution (3 N) and NaHCO₃, respectively. Supplementary nutrients such as nitrogen (NH₄Cl) and phosphorous (KH₂PO₄) were added at a final ratio of COD: N: P of 550:7:4.1 (Zinatizadeh et al., 2007). The characteristics of the raw and pre-settled POME are summarized in Table 1.

### 2.3. Seed sludge preparation

The inoculum for the reactor was digested sludge from a palm oil mill (SIME Darby Plantation Sdn. Bhd., Nilai, Malaysia). The sludge was initially passed through a screen to remove debris and solid particles and then heated at 100 °C for 1 h in order to enrich hydrogenic microbes. The initial volatile suspended solids (VSS) concentration of the seed was measured at 4600 mg/L.

### 2.4. Reactor operation

Initially, to make the packing surface sticky for fast development of biofilm in the packed bed section, the pall rings were immersed in a 2% (w/v) agar solution for a few minutes. The reactor was inoculated with 850 mL heat pretreated sludge. For the first five days, the bioreactor was fed in a closed-loop, feed-batch mode for acclimatization of the microorganism with the POME strength (COD 4680 mg/L and HRT 24 h). The effluent was continuously recycled into the reactor until the next feeding cycle. The average VSS concentration within the reactor was increased to 5650 mg/L after a five-day operation.

Subsequently, the reactor was fed in continuous mode with an initial OLR of 9.36 g COD/L·d and a HRT of 12 h. The HRT was maintained constant for the remaining start-up period. The influent COD concentration was increased stepwise to 25,880 mg/L (OLR = 51.8 g COD/L·d) from day 8 to day 22. In order to improve hydrogen production performance and maintain an up-flow velocity of 1.0 m/h (Metcalf and Eddy, 2003), fresh feed with low COD concentration was mixed with recycled effluent at a ratio of 1:10:4. The high recycle ratio helped to eliminate high organic overloading and provide a mixture with suitable alkalinity. The temperature was maintained at 38 °C using thermostated water bath with water circulation system. The pH of the reactor was adjusted to 5.5 using NaOH (1 N) and HCl (1 N). Hydrogen production, COD reduction, total suspended solids (TSS) and VSS concentration were monitored periodically.

### Table 1 – Characteristics of raw and pre-settled POME.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Raw POME</th>
<th>Pre-settled POME</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD₅</td>
<td>±2150</td>
<td>±5250</td>
</tr>
<tr>
<td>TCOD</td>
<td>±4250</td>
<td>±6500</td>
</tr>
<tr>
<td>SCOD</td>
<td>±3220</td>
<td>±4600</td>
</tr>
<tr>
<td>TSS</td>
<td>±1240</td>
<td>±1600</td>
</tr>
<tr>
<td>TKN</td>
<td>±32</td>
<td>±50</td>
</tr>
<tr>
<td>TP</td>
<td>±7.5</td>
<td>±10</td>
</tr>
<tr>
<td>Oil and grease</td>
<td>±62</td>
<td>±120</td>
</tr>
<tr>
<td>pH</td>
<td>±0.2</td>
<td>±0.5</td>
</tr>
</tbody>
</table>

* All in mg/L except pH.
2.5. Analytical techniques

The parameters viz. BOD, COD, TSS, VSS, alkalinity, total Kjeldahl nitrogen (TKN), oil and grease, and pH were analyzed using procedures outlined in the APHA Standard Methods (APHA, 1999). The biogas composition was determined using a gas chromatograph (Perkin Elmer, Auto system GC), equipped with thermal conductivity detector (TCD) and data acquisition system namely Total Chrom® software. H₂ content was also analyzed by GC-TCD fitted with a 1.5 m stainless steel column (SS350A) packed with a molecular sieve (80/100 mesh). The temperature of the injection port, oven and detector were 80, 200, and 200 °C, respectively. Argon was used as a carrier gas at a flow rate of 30 mL/min.

3. Results and discussion

3.1. Start-up and performance of the bioreactor

The UASB-FF reactor was inoculated with heat pretreated seed sludge. After five days of closed-loop, fed-batch operation, the reactor was continuously fed at HRT of 12 h using fresh pre-settled POME. The UASB-FF reactor start-up process was analyzed in terms of hydrogen production, COD removal, and sludge development as follows.

The biogas was found to contain 42–58% hydrogen and 42–58% CO₂ (Fig. 2). No methane production was detected during the experiments, indicating that the heat-shock pre-treatment of sludge had successfully inactivated the hydrogen consuming population. Hydrogen production of 4.61 L/d H₂ for was achieved at the end of start-up period. The hydrogen content of the biogas was increased as the OLR increased. This was attributed to the increase in the reaction rate and the amount of hydrogen gas evolved. The results showed that, even at elevated biogas production rate (8.15 L/d of biogas), the H₂ fraction of the biogas remained substantial at 56.6%. The specific hydrogen production rate was 0.514 L H₂/gVSS d at the end of the start-up period. This represents a marked improvement over the study on fermentative H₂ production using a UASB reactor by Chang and Lin (2004) where they achieved a specific H₂ production rate 53.5 mmol H₂/g VSS day and after a relatively longer period of operation, i.e. 52 days. Their reactor operation was affected by the considerable sludge loss at the beginning of the start-up period (Chang and Lin, 2004). In the present study with the UASB-FF reactor, the flocculated sludge wash out phenomena did not occur. In addition, growth and size enlargement of biological granules were accomplished within a short period of time during the operation of the UASB-FF reactor in this study (see Section 3.2). This process usually takes a long time to achieve ranging from 2 to 4 months (Lettinga, 1995; Schmidt and Ahring, 1996). It is suggested that the closed-loop internal recirculation of dispersed bacterial granules could have assisted in their relatively fast agglomeration.

Fig. 3 showed the performance of the UASB-FF reactor in terms of COD removal efficiency during the start-up period (22 days). The reactor was fed with the POME as substrate at a constant COD concentration of 4680 mg/L (HRT 24 h) in the first five days. The COD removal efficiency was increased from 25.8% to 38.4% on the fifth day. The reactor was then fed with the COD concentration of 4680 mg/L and HRT 12 h. Subsequently, the COD concentration was increased stepwise to 25,880 mg/L for the remainder of the start-up period. The COD removal efficiency decreased when the influent COD concentration was increased from 4680 to 6470 mg/L and 10,350 to 14,650 mg/L. This was attributed to the relative decrease in HRT for an increase in OLR, which may have resulted inorganic shock to the microbial population. Naturally, the microbes need some acclimatization time in order to adapt well to the new condition before they regained their pre-shock treatment efficiency. The COD removal efficiency was 42.5% at an influent COD concentration of 25,880 mg/L corresponding to OLR of 51.8 g COD/L d at the end of the experimental run.

3.2. Granulated sludge formation

In this study, the granulated sludge washout phenomenon was negligible during the UASB-FF operation. Granulation time in the UASB-FF was observed to ensue at shorter time interval (22 days) while it generally requires a longer time in a conventional UASB (>60 days) (Lettinga, 1995; Schmidt and Ahring, 1996). The growth of microbial agglomerates was likely to be promoted by the internal recirculation of the dispersed bacterial population.

Fig. 4 shows considerable growth in the height of granular sludge bed within the UASB-FF during the start-up period. Measurement of the height of sludge bed after discontinuing the feed and settling the sludge in the reactor for 1 h indicated satisfactory sludge growth. At the height of the experiment, the height of the sludge bed was 160 mm. The initial height increase of the granulated bed due to bacterial growth was relatively slow. However, after 10 days, the rate shows significant increase (Fig. 4). This is attributed to the existence of the fixed film section above the sludge blanket acting as a buffer zone against complete washout alongside the provision of the internal sludge recirculation. The height of sludge bed was increased to 174 mm when the OLR was increased from...
Fig. 4 – Growth of sludge blanket height in UASB-FF bioreactor during the start-up period.

4.68 to 51.8 gCOD/Ld. The specific hydrogen production rate was 0.514 L H₂/gVSS d at the end of the start-up period.

Based on COD removal efficiency and hydrogen production in the start-up period of the UASB-FF, it can be concluded that sludge flocculation was satisfactorily achieved and capable of attaining good stability at high organic loading. The primary reason for the accelerated start-up process was the use of hydrogenesis-enriched seed sludge for the reactor inoculation, and the provision made for retaining the dispersed and flocculated sludge in the lower part of the reactor by the fixed bed portion.

The development of hydrogenesis sludge granules was improved by the proportional inoculum seeding to feeding of POME into the reactor. The relatively rapid development of bio-granules also is attributed to biomass recirculation and the existence of a fixed film at the upper section of the UASB that resulted in improved interactions among the bacterial consortium. The visual sequence of sludge granulation in the UASB-FF during the start-up period is shown in Plate 1. The granules average diameter increased to >1 mm at the end of the start-up period. The reactor operating conditions have been shown to affect the development of microbial granules (Liu et al., 2003). In this case, rapid microbial granulation in UASB-FF was hypothesized to be promoted by cell precipitation from the fixed film section, gas bubble generation from sludge blanket and high suspended solids content of POME.

Plate 2a and b shows the fine structure of hydrogen-producing mesophilic granules as observed by scanning electron microscopy (SEM). Plate 2c and d shows a compact granule arising from cellular multiplication of the entrapped bacterial population, and became spherically dense due to the hydrodynamic shear forces caused by the up-flow liquid and biogas generated.

Plate 1 – Sequence of bio-granule formation in the UASB-FF reactor (a) after 10 days, (b) after 20 days, (c) after 30 days.

Plate 2 – SEM images of (a and b) the fine structure of the hydrogen-producing mesophilic granules and (c and d) a full-grown compact granule.
4. Conclusion

This study evaluated the fermentative hydrogen production from POME using the UASB-FF as a novel hybrid reactor. At high organic load and TSS concentration, the reactor showed accelerated anaerobic granulation and high biophydrogen production in a relatively short period of time. Granular sludge was rapidly developed within 22 days. Specific hydrogen production rate was 0.514LH2/gVSS at the end of the start-up period. The rapid development of bio-granules was attributed to biomass recirculation and establishment of a fixed film at the upper section of the UASB that may have resulted in improved interactions among the bacterial consortium.

Acknowledgements

The financial support provided by Universiti Malaya from (1) Biophydrogen production from renewable resource using indigenous microorganisms (53-02-03-1013) and (2) UMRG (RG054 09SUS) is gratefully acknowledged.

References


