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Bio-hydrogen Production in an EGSB Reactor Under Mesophilic, Thermophilic and Hyperthermophilic Conditions

A.A. Abreu, A.S. Danko and M.M. Alves

IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal.
E-mail: (*angela_abreu@deb.uminho.pt*, *madalena.alves@deb.uminho.pt)*

Abstract

Mesophilic, thermophilic and hyperthermophilic bio-hydrogen production with an expanded granular sludge blanket (EGSB) fed with glucose and arabinose, without methane production, was demonstrated. Homoacetogenesis was observed on reactor when operated under mesophilic (37ºC) conditions but not under thermophilic (55ºC) and hyper-thermophilic conditions (70ºC). It was also found that under thermophilic and hyper-thermophilic conditions glucose is preferentially consumed than arabinose.

Keywords Anaerobic granular sludge; Bio-hydrogen; Mesophilic; Thermophilic; Hyperthermophilic

INTRODUCTION

Hydrogen is a CO_2-neutral energy source with a very promising future as an alternative to fossil fuels for energy production. The sustainable generation of hydrogen may be achieved by a range of technologies including biological processes. These include photolysis carried out by algae and cyanobacteria and also dark fermentation by anaerobic bacteria. However, the rate of hydrogen production from fermentation is greater compared to photolysis (Levin et al, 2004). Most anaerobic fermentation studies are carried out in a continuously stirred tank reactor (CSTR) system (Reith et al., 2003). However, a CSTR is very sensitive to fluctuations in environmental parameters (Lee et al, 2004). These limitations may be overcome using an upflow anaerobic sludge blanket (UASB) or an expanded granular sludge blanket (EGSB) reactor. There have been several studies that examined bio-hydrogen production using a UASB reactor (Chang and Lin, 2004; Yu et al., 2002, Kotsopoulos, 2006). However, studies have not been previously reported using an EGSB reactor for bio-hydrogen production.

Fermentative hydrogen production has generally focused on mesophilic (37ºC) and thermophilic (55ºC) conditions . Nevertheless, hydrogen production under hyper-thermophilic conditions (70ºC) is gaining more interest due increased pathogenic destruction of residues coming from anaerobic digesters (Sahlstrom, 2003). In addition, hyper-thermophilic conditions are more favourable for anaerobic fermentation due to the better thermodynamic conditions (Kadar et al., 2004; van Niel et al., 2002). Furthermore, extremely high temperatures (70 – 80ºC) make the system less prone to be contaminated with methanogenic bacteria (van Groenestijn et al., 2002). It is known that for bio-hydrogen production methanogenesis has to be inhibited, although, another very important pathway that consumes hydrogen is homoacetogenesis. Acetate is a potential hydrogen sink in acetogenesis. Homoacetogenic bacteria use hydrogen to reduce carbon dioxide to produce acetate as followed (Lovley and Klug, 1983).

$$HCO_3^- + 2H_2 + 0.5H^+ \longleftrightarrow 0.5\ CH_3COO^- + 2H_2O \quad (1)$$

Until now there has not been much research on homoacetogenesis. There are few reports of the existence of homoactegenesis in continuous systems under mesophilic, thermophilic, and hyperthermophilic conditions. Homoacetogensis was usually observed under psychrophilic

conditions as homoacetogens easily adapt to low temperatures (Kotsyurbenko e tal. 2001; Nozhevnikova et al. 2003). This study is the first to examine the effect of temperature on bio-hydrogen production using an EGSB with a pentose and a hexose as the substrates.

METHODS

Experimental set-up

The experiments were carried out in Plexiglas Expanded Granular Sludge Blanket (EGSB) reactors (Fig. 1) with a height of 1.95 m and internal diameter of 21 mm. Working volume was 1.30 L and the superficial velocity was set at 8.0 m/h by means of an internal recirculation. Reactors were operated continuously with an hydraulic retention time (HRT) of 13 h. Temperature was kept at 37 ± 1 ºC, 55 ± 1 ºC and 70 ± 1 ºC by means of an external jacket for water circulation.

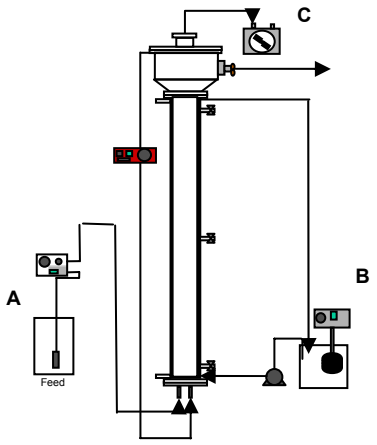

Figure 1 – Experiment set-up, (A) feed container, (B) water recirculation tank and (C) biogas flow-meter.

Inoculum and substrate

400 mL of granular sludge from brewery wastewater treatment plant that was autoclaved for 45 min was used as the inoculum of the EGSB 37ºC reactor (R1). 150 mL of granular sludge from the final operation of 37ºC reactor (R1) was used as the inoculum for EGSB 55ºC (R2) and 70ºC (R3).
All the reactors were fed with glucose and L-arabinose (1/1) at a final concentration of 5 $gCOD.L^{-1}$. During the first 37 days, the R1 was operated with a HRT of 21 hours in order to acclimatize the biomass to the substrate. Sodium bicarbonate was added as the alkalinity source (1 to 2 g/L) and macronutrients (1.5 mL/L) (MgSO4.7H2O: 30 g/L; KH2PO4: 28.3 g/L; NH4Cl: 170 g/L) for cell growth.

Routine analysis

The COD and VSS were determined according to Standard Methods (Standard Methods, 1998). Biogas flow rate was measured by a *Ritter Milligascounter* (Dr. Ing. Ritter Apparatebau GmbH, Bochum, Germany). Methane content of biogas was determined by gas chromatography using a *Porapack Q* (100 - 180 mesh) column, with Helium as the carrier gas at 30 mL/min and thermal conductivity detector. Temperatures of the detector, injector and oven were 110, 110 and 35 ºC, respectively. Hydrogen content of biogas was determined by gas chromatography using a Hayesep Q column (80/100 mesh) and thermal conductivity detector (Varian 3300 Gas Chromoatograph) with nitrogen (30 mL/min) as the carrier gas. The injector, detector, and column temperatures were 120, 170, and 35 ºC, respectively .Volatile fatty acids (VFA), ethanol, L-arabinose, glucose were determined by high performance liquid chromatography using an *HPLC* (Jasco, Japan) with a *Chrompack column* (6.5 x 30 mm^2); sulfuric acid (0.01 N) at a flow rate of 0.7 $mL.min^{-1}$ was used as mobile phase. Column temperature was set at 60 ºC. Detection of VFA and ethanol, L-arabinose, glucose was made sequentially with an UV detector at 210 nm and a RI detector, respectively.

RESULTS AND DISCUSSION

R1 Performance

Figure 2a shows the organic loading rate (OLR) applied to the reactor (influent OLR), the OLR of the exit effluent (effluent OLR) and the pH during reactor operation. During the first 37 days, an influent OLR of 5KgCOD/m^3/d was applied in order to adapt the biomass to the substrate (PI). After day 37, the finluent OLR was increased to a rate of 10kgCOD/m^3/d (PII). COD removal efficiency was approximately 20%. The pH decreased from 7.6 to 4.6 during the first 17 days of operation. When the influent OLR was changed (PII) the pH increased to 5.3 and it was maintained at approximately 5.0 until the end of operation.

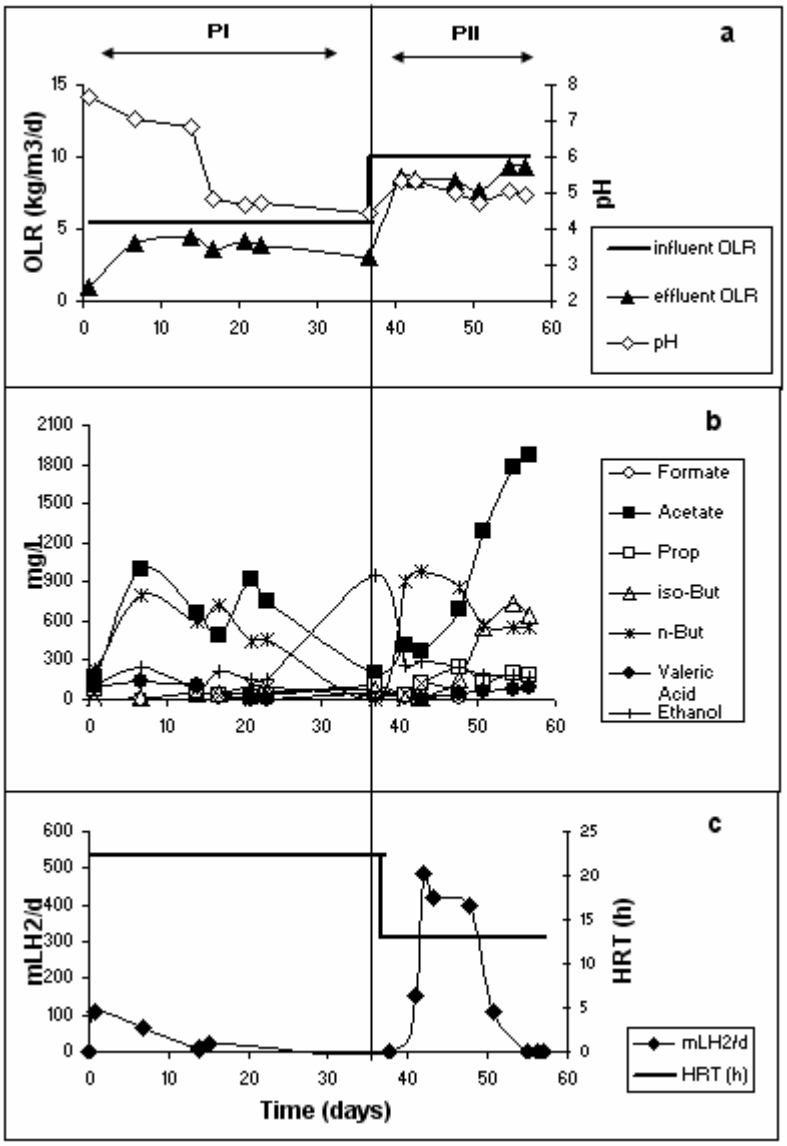

Figure 2 – Time course of reactor (R1) performance, influent OLR, effluent OLR and pH (a); profile of volatile fatty acids and ethanol (b); profile of hydrogen production during the experimental period (c).

From day 0 to day 23 the major fermentation products were acetate and n-butyrate (Fig 2b). Some ethanol was also produced (less than 300 mg/L). In addition, formate, propionate, iso-butirate and valerate were detected at concentrations smaller than 100 mg/L. Hydrogen was produced at a rate of 100 mLH_2/d during the first hours of operation and then decreased (Fig 2c). From day 23 acetate and n-butyrate started to decrease. Ethanol was the most prevalent of VFA (900 mg/L) that was produced during this period. This fact was coincided with the decrease in hydrogen production. From day 37 (PII), ethanol concentration decreased, but acetate and n-butyrate concentrations increased. An increase in n-butyrate concentrations (980 mg/L)

corresponded to the higher hydrogen production rate (500 mL/d). From day 43 until the end of operation (day 57) n-butyrate concentrations decreased slightly from 980 to 571 mg/L, while concentrations iso-butyrate (0 to 731 mg/L) and propionate (37 to 244 mg/L) increased. At day 43, acetate concentrations started to increase reaching a maximum concentration of 1878 mg/L at the end of operation.

Hydrogen production decreased drastically starting at day 48 from 420 mLH_2/d to zero suggesting that hydrogen is being consumed. One possible explanation is hydrogen consumption by methanogens. However, methane was not detected during any operational period. Another possible explanation is homoacetogenesis as acetate concentrations increased significantly as hydrogen production decrased. decreased. Glucose and arabinose were not detected in the effluent. VSS was kept at approximately 260 mg/L (data not shown) during reactor operation. In addition, no washout event was observed.

R2 Performance

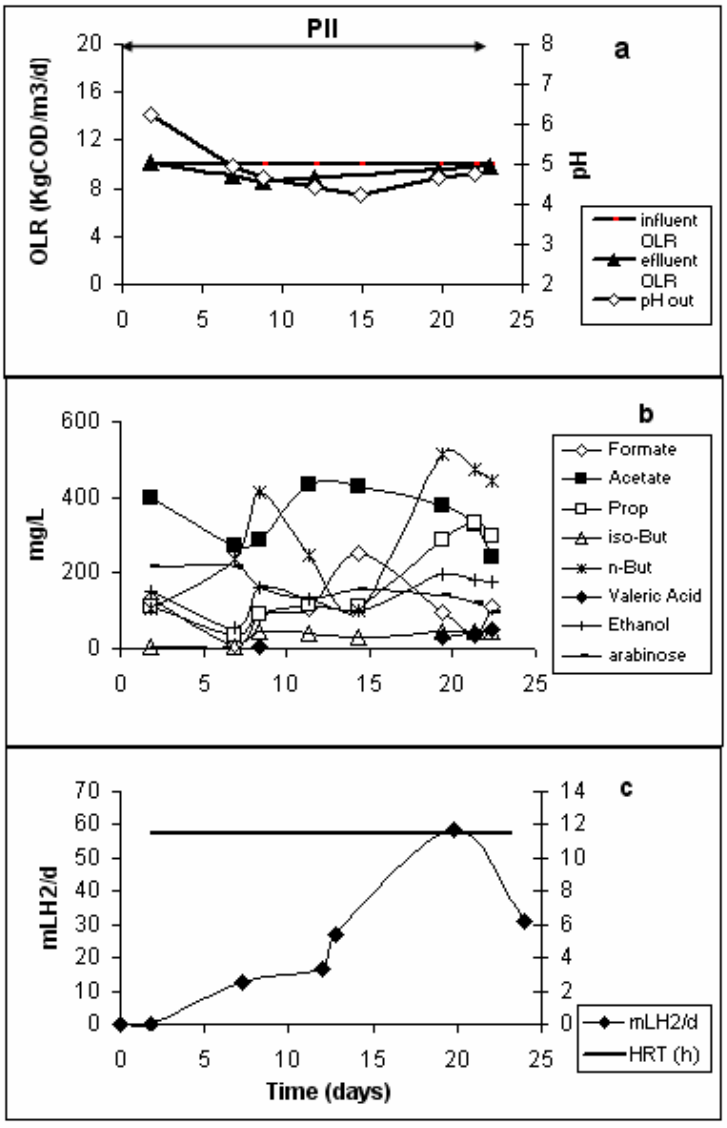

Figure 3 – Time course of reactor performance (R2) influent OLR, effluent OLR and pH (a); profile of volatile fatty acids, ethanol and arabinose remaining (b); profile of hydrogen production (c).

R2 was operated continuously with an OLR of 10 kgCOD/m3/d (PII) at 55ºC. Figure 3a shows the reactor performance. The effluent OLR followed the same behaviour as influent OLR, and the COD removal efficiency was approximately 11%. pH at the beginning of the experimental period was approximately 6 and decreased to 4.2 at day 15. Then, the pH increased slightly, achieving the final value of 5.1 at the end of experimental period.

Acetate was the most prominant VFA throughout all the experimental period, except between day 7 and day 10 and from day 17 until the end (day 23), when n-butyrate was present at higher concentrations (Fig. 3a). All the other VFA's were lower than 200 mg/L except formate and propionate, which were only produced at concentrations of 250 mg/L, at day 15 and 332 mg/L, at day 21, respectively. Arabinose was detected in the effluent during the entire operational period. Arabinose was approximately 215 mg/L from start-up until day 7. Then, concentrations of arabinose decreased until the end of operation (day 23) achieving a value of 97 mg/L. Arabinose consumption at 55ºC was not as efficient when compared against R1. Glucose was not detected in the effluent. The total amount VFA's was lower when

compared against R1 since arabinose was not completely degraded.

Hydrogen production was observed since day 2 reached a maximum rate at day 20 (59mLH_2/d) (Fig.3c). Hydrogen rates obtained in R2 was smaller than R1, however the hydrogen production was sustained for longer period of time. It was observed that the amount of substrate available is lower in R2 when compared to R1 since arabinose was not completely consumed. This can be the reason for smaller rates of hydrogen production. High acetate concentrations were not observed in the effluent, suggesting that homoacetogenesis was not favoured under thermophilic conditions when compared against mesophilic conditions (R1) During the experiment period VSS concentrations increased from 160 to 355 mg/L (data not shown) and no methane was detected.

R3 performance

R3 was operated continuously with an OLR of 10 kgCOD/m3/d (PII) at 70ºC. The reactor performance is shown in Figure 4a. The effluent OLR decreased until day 13, and increased afterwards until day 15 corresponding to a higher hydrogen production rate (314 mLH_2/d) (Fig. 4c). The effluent OLR was decreased at day 20 corresponding to the decrease in hydrogen production. The initial pH was approximately 8 amd decreased until day 9, achieving a final value of approximately 5. This pH level was constant until day 15 and which the pH decreased to 4.25 at day 22. This was followed by an increase to 4.75 at the end of experimental period (day 23).

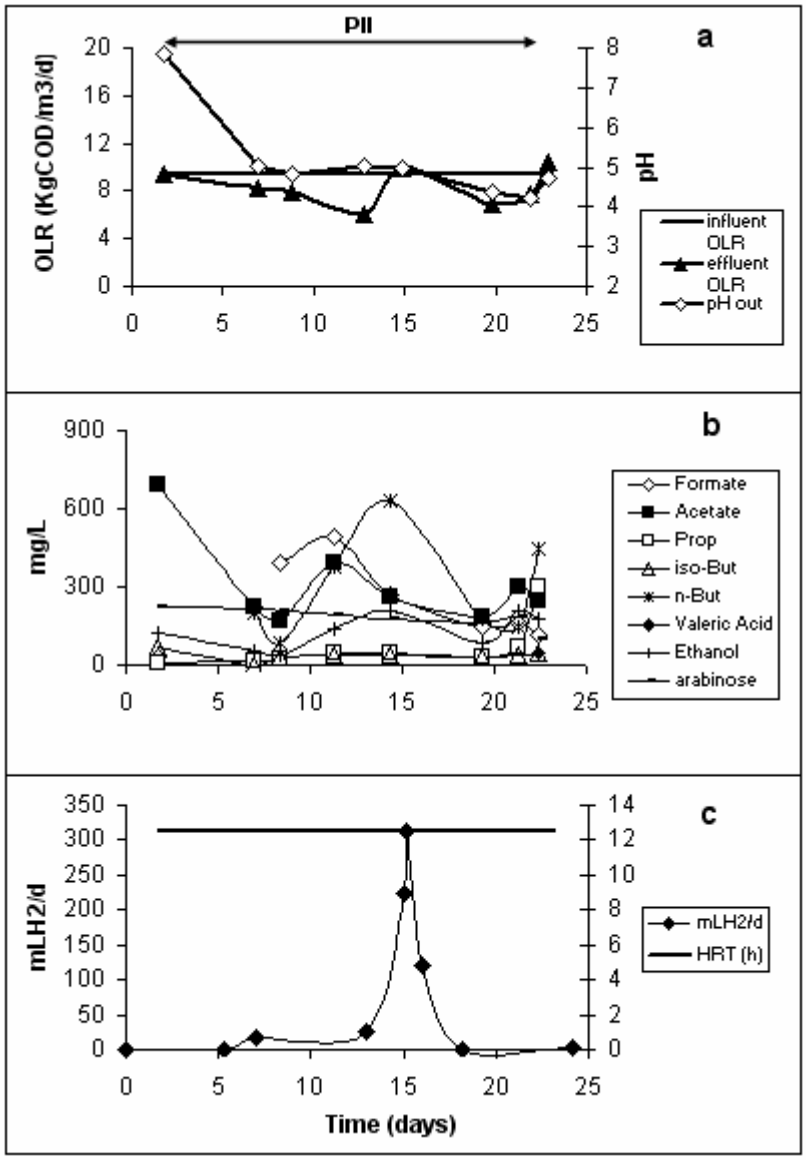

Figure 4 – Time course of reactor performance (R3) influent OLR, effluent OLR and pH (a); profile of volatile fatty acids, ethanol and arabinose remaining (b); profile of hydrogen production (c).

Acetate was the most predominant VFA produced at the beginning of operation, while formate was the most prominat between day 8 and day 12 and n-butyrate between day 12 and day 20 (Fig. 4b). The periods of low hydrogen production corresponded to the smaller amount of VFA's produced. Conversely, higher hydrogen production was observed at approximately day 15 corresponding to the higher n-butyrate concentrations (630 mg/L). In addition, the presence of an unknown compound was observed and was inversely proportional to the production of hydrogen since hydrogen production ceased when this compound was clearly present.

Arabinose was detected during all experimental periods with a concentration around 200 mg/L. Glucose was not detected in the effluent.

As observed in R2, high acetate concentration in the effluent was not detected, suggesting that homoacetogenesis is not favoured under hyper-thermophilic conditions. During the experimental period, VSS concentrations were kept at approximately 210 mg/L (data not show). In addition, no washout event nor methane production was observed.

CONCLUSIONS

1 - Hydrogen was produced using a EGSB reactor fed glucose and arabinose under mesophilic, thermophilic, and hyper-thermophilic conditions, but a stable continuous hydrogen production rate was not observed.

2 - Homoacetogenesis was observed for the reactor operated at mesophilic (37ºC) conditions but not under thermophilic (55ºC) or hyper-thermophilic conditions (70ºC).

3 - Glucose was utilized preferentially over arabinose at thermophilic and hyper-thermophilic conditions.

4 – More research is needed to determine methods that inhibit homoacetogenesis under mesophilic conditions.

5 – Decreased of hydrogen production under hyper-thermophilic conditions was linked to the presence of an unknown compound.that must be identified in future research.

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