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Biohydrogen Production Function of Operating pH and Seed Pre-Treatment

Z. SIDDIQUI. S. A. MEMON, K. M. BROH*

Department of Environmental Engineering, NED University of Engineering and Technology, Karachi, Sindh

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Abstract: Increase in biohydrogen production was carried as function of operating pH and seed pre-treatment using co-blended food waste and sewage sludge. Two batch tests were performed: in first test, three different pH levels (5.5, 6.5 and 7.5) were used and in second test: three different pre-treatments (acid, heat treated and combined heat and acid) were analyzed. The biohydrogen batch study identified the optimised pH level of 5.5 with the highest hydrogen yield of 201.4 mL/g$VS_{removed}$. Proliferation of ammonium nitrogen caused no significant impact on hydrogen yield. The second batch test revealed that heat treatment repressed hydrogen consumers and repeated shock heat treatment was more efficient than shock heat treatment. The highest hydrogen yield of 201.4 mL/g $VS_{removed}$ was observed using acid treatment.

Keywords: Co-blended, pre-treatment, Batch Test, Hydrogen Yield

1. INTRODUCTION

Anaerobic treatment of organic waste is a complex system; first the biodegradable waste is hydrolysed in to fatty acids, which are then transformed to acetate and hydrogen leading to generate methane as a end product. Biological production of hydrogen through a modification of the conventional MAD process appears to offer a lot of promise. The mechanism for hydrogen production during anaerobic digestion is known as dark fermentation (Hawkes *et al*. 2000). The metabolic pathways for hydrogen production favour substrates that are rich in carbohydrates. Due to this reason most of the research has undertaken into the generation of hydrogen from pure feedstocks such as glucose and sucrose or carbohydrate rich substrates such as sugar beet waste (Wang *et al*. 2006), food waste (Siddiqui *et al*. 2013) and the bottom layer from beer manufacturing (Fan *et al*. 2006). Yields from carbohydrate rich wastes are typically in the range 19 to 96 L H_2/kg VS (Okomoto *et al*. 2000) and this compares to yield of 214 L/g COD for sucrose (Khanal *et al*. 2004). Studies have been extended to other biodegradable organic wastes that are not so rich in carbohydrate, such as sludge dewatering liquor (Wang *et al*. 2003), the organic fraction of municipal solid waste (Ueno *et al*. 1995) and bean curd waste (Noike and Mizuno 2000). Although the yields were generally much lower than carbohydrate rich substrates they offered the potential for enhancement by improvements to process conditions.

Key factors responsible for increasing the hydrogen yield from any waste type include the pre-treatment of the seed sludge, the operating pH of the digester, short retention time, seeding with heat shocked or acid treated sludge and the C:N ratio of the feedstock (Siddiqui *et al*. 2011) (Liu and Fang 2003). Pre-treatment of seed inocula is one of the approach helping acceleration in hydrolysis step, reducing the impact of the rate-limiting step and augmenting anaerobic digestion for hydrogen production (Kim *et al*. 2004; Zhu and Beland 2006). Pre-treatment also increases the workability of the seed inocula for fermentative hydrogen production by repressing the hydrogen consumers, principally the hydrogenotrophs (Duangmanee *et al*. 2007). A variety of natural resources are being used to make available inocula for hydrogen generation by mixed microflora including sewage sludge (Fang *et al*. 2002; Chen *et al*. 2002; Zhang and Shen, 2006; Zaib and Kaltwasser 1979), soils (Ginkel *et al*. 2001; Kawagoshi *et al*. 2005; Hawkes *et al*. 2000), cattle dung compost (Fang *et al*. 2004), dairy cow waste slurry (Yokoyama *et al*. 2007; Tang *et al*. 2008) and river-sediments (Zuo *al*. 2005; Zaib and Kaltwasser 1979). At present, practical and efficient hydrogen production is of growing interest amongst the research fraternity (Logan, 2004; Lay, 2001; Lin and Lay 2005).

During the biohydrogen assay test, several methods have been attempted including acid-treatment and shock-heating respectively (Lay, 2001; Mu *et al*. 2007; Chen *et al*. 2002). Both of these techniques have been compared to improve hydrogen production and yield. Oh *et al*. (2003) reported that heat treatment

*Institute of Environmental Engineering Mehran University of Engineering and Technology, Jamshoro, Sindh, Pakistan

favours high hydrogen production and yield compare to acid treatment. In contrast Cai *et al*. (2004) reported that alkaline pre-treatment of SS also helps in increase of hydrogen production. Summarising the same, little information exists to evaluate the effectiveness of these enrichment methods for hydrogen producing using the co-digested food waste and sewage sludge. Therefore, it was the aim of this study to investigate the effects of operating pH and seed pre-treatment on hydrogen production. It was hoped to determine whether control of operating pH and seed pre-treatment can increase the energy yield by optimising the yield of hydrogen production.

2. MATERIAL AND METHODS

2.1 Feedstocks and seed inocula

Feedstocks were selected as described in Siddiqui *et al*. (2011) and seed inoculum for the first experiment was selected as described in Siddiqui *et al.* (2014). For the second experiment, three different seeds were achieved. The first seed, “acid treated (AT)” was obtained from a laboratory scale mesophilic anaerobic digester used in the first experiment. The second seed sludge, termed “heat treated (HT)” was selected from a laboratory scale digester. It was operated modifying Chen *et al*. (2001), fed continuously with the same feed at a concentration of 20g COD/L. To deactivate the spore forming methanogens, repeated boiling was performed for 15 minutes/week (Wang *et al.* 2003). The digester was operated at a temperature of 37ºC±0.2 and HRT of 5 days. The third seed sludge termed “acid and heat treated (AHT)” was acquired by boiling the AT for 15 minutes prior to run of experiment. This helps to deactivate the hydrogen consumers and harvest the hydrogen producers.

2.2 Experimental setup

To investigate the impact of pH and different seeds on biohydrogen potential, an optimised blend for the biohydrogen production was achieved by following Siddiqui *et al*. (2013). To optimise the pH for biohydrogen production three different pH levels (5.5, 6.5 and 7.5) were controlled by adding external alkaline or acid buffers: 6M NaOH and acid 1M HCl (Siddiqui *et al*. 2011a). In second batch test, three different seeds were selected i) acid treated (AT) ii) heat treated (HT) and iii) acid and heat treated (AHT).

Both batch tests were performed following experimental procedure outlined in Siddiqui *et al.* (2014). The quantity of biogas produced was measured following Siddiqui *et al*. (2012).

2.3 Analytical methods

The analytical parameters of total solids (TS), volatile solids (VS), total alkalinity (TA), ammoniacal nitrogen (NH_4-N) and total volatile fatty acids (TVFA) were carried out following the procedures outlined in Siddiqui *et al.* (2014). Headspace gas composition was analysed following procedure outlined by Siddiqui *et al.* (2011a).

3. RESULT AND DISCUSSION

3.1 Impact of pH and pre-treatment of seeds on hydrogen production

The optimum pH and pre-treatment technique were considered after analysing the specific hydrogen potential (SHP), specific hydrogen production rate (SHPR) and characteristics of the biogas. In first experiment, the fermentative hydrogen potential was measured over a range of pH levels from 5.5 to 7.5 **(Table 1).** The SHP decreased with increasing pH (Fig.1A) and was found in the range 17.5 – 86 mL (Table 1). The optimised SHP (86 mL) was noted at pH of 5.5 (Fig.1A). This agrees with the optimised pH of 5.5 for hydrogen production found by Mohan *et al*. 2007; Kim *et al*. 2004; Shin *et al*. 2004 and Cheong and Hansen (2007). A significant drop in pH was found at high pH (6.5 and 7.5). A considerable increase in SHPR was observed during first two days and the highest SHPR [88 mL/(L.d)] was recorded at pH 5.5 **(Fig, 1A)** and was comparable to that of Ginkel *et al*. (2001). Appreciable methane production was observed for all analysed pH values, excluding the initial days for pH 5.5. The methane level was recorded over a range of 5.7 to 37.2%, due to reason the methane yield was smaller and recorded in range of 3.2 to 18.8 mL CH_4/g VS. The methane yield at optimised pH was smaller than 8.1 mL/g VS (Kim *et al*. 2004) and 17.5 mL/g VS (Chu *et al*. 2002).

Table 1 Summary of all three pH and different seed sludges during 5-days BHP test

pH	H_2 Yield $mLH_2/gVS_{removed}$	$VS_{removed}$ %	Max.H_2 (Mean H_2) %	Cum.Biogas (Cum.H_2) mL
5.	201.4	17.8	38.5 (32.05)	264.4 (86)
6.	109.1	15.7	23.4 (11.8)	350.2 (41.2)
7.	24.7	29.6	17.6 (8.9)	198.1 (17.5)
AT	201.4	17.8	38.5 (32.05)	264.4 (86)
HT	91.9	50.2	36.0 (18.9)	580 (110.7)
AHT	0.02	41.5	0.03(0.02)	86.4(0.02)

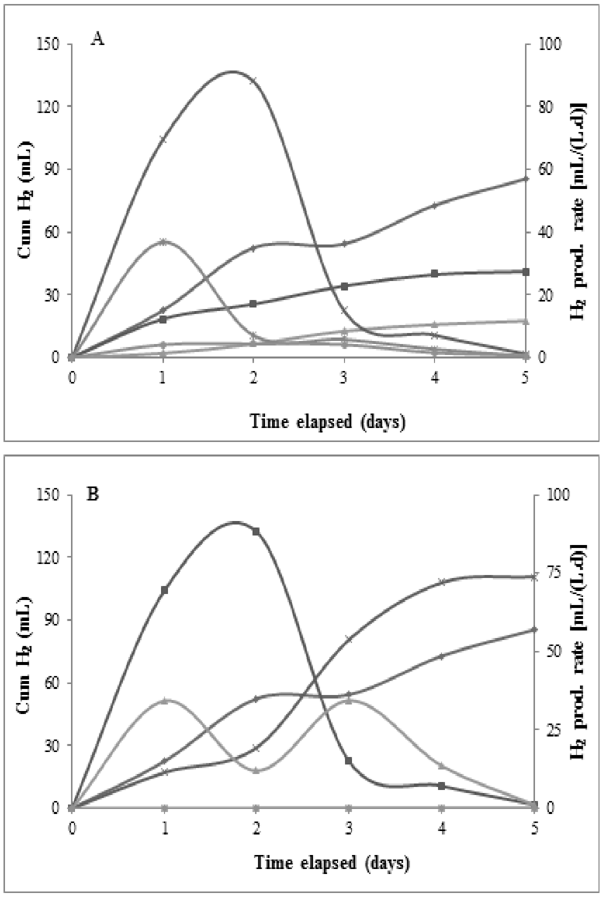

Fig. 1A Cumulative H_2 production and rate for analysed pH
Fig. 1B Cumulative H_2 production and rate for analysed seeds

In second experiment, negligible hydrogen production was observed using the AHT. Compared to this, repeated heat treatment increased the hydrogen production potential. The optimum SHP was noted using HT and subsequently at **(Table 1 and Fig.1B).** The optimised SHP was found higher than earlier reported values of 83 – 93 mL (Chen *et al*. 2002; Oh e*t al*. 2003; Mohan *et al*. 2008). It was observed that the potential for biohydrogen production increased by heat treatment, which corroborates earlier investigations (Duangmanee *et al*. 2007; Kawagoshi *et al*. 2005; Mu *et al*. 2007; Oh *et al*. 2003; Lay *et al*. 2003). Apart from AHT, a significant increase in SHPR was observed during first two days and that decreased during the end of experiment. The highest SHRP [88.1 mL/(L.d)] was noted at (Fig.1B) is comparable to the data of (Ginkel *et al.* 2001). Due to deactivation of spore forming methanogens, no methane levels were detected with HT and AHT. This shows the heat treatment is efficient in suppressing the non-spore forming/methanogenic bacteria (Zhu and Beland 2006). Compared to heat treatment, acid treatment was less effective in deactivating the non-spore forming bacteria and methane with 5.7% concentration was recorded during the third day of the experiment. A negligible methane yield (3.2 mL CH_4/g VS) was recorded with AT and considerably lower than the 8.1 mL/g VS found by Kim *et al*. (2004) and 17.5 mL/g VS by Chu *et al*. (2002).

3.2 Impact of pH and seed sludge on biodegrade-ability (VS destruction) and hydrogen yield

Fermentation efficiency was assessed as a function of volatile solids destroyed or degraded and an increase in fermentation efficiency was observed when increasing the pH level. The highest biodegradability (29.6%) was achieved at pH 7.5 (Table 1). This shows an alkaline pH level encouraged biodegradability more than the acidic pH level and this was also reported by Lee *et al*. (2002). A negligible hydrogen yield was observed at pH 7.5 compared to pH 5.5 and 6.5. This shows that hydrogen production is feasible at acid pH. The hydrogen yield decreased by increasing the pH value and varied in range of 24.7–201.4 mL/g $VS_{removed}$. The highest hydrogen yield (201.4 mL/g $VS_{removed}$) was observed at pH 5.5. This was eight-fold higher than the other pH values (Table 1). It was also higher than earlier reported values of 21–91 mL/g VS (Okomoto *et al*. 2000; Noike and Mizuno 2000; Ginkel *et al*. 2001; Kim *et al*. 2004; Zhu and Beland 2006; Mizuno *et al*. 2000) and 125 – 127 mL/g sucrose COD (Khanal *et al*. 2004; Lee *et al*. 2002).

In second experiment, the optimum biodegradability (50.2%) was noted with HT (Table 1) which is noticeably higher than the earlier reported values of 4 to 24.2 % (Oh *et al*. 2003; Mohan *et al*. 2008). Although the highest biodegradability was observed with HT, by contrast the highest hydrogen yield of 201.4 mL/g $VS_{removed}$ was recorded with AT (Table 1) and this was double the HT seed sludge. It was also higher than earlier reported values of 21–91 mL/g VS (Okomoto *et al*. 2000; Noike and Mizuno 2000; Ginkel *et al*. 2001; Kim *et al*. 2004; Zhu and Beland 2006; Mizuno *et al*. 2000); 125–127 mL/g sucrose COD (Khanal *et al*. 2004; Lee *et al*. 2002) 0.0317 mmol H_2/g COD (Mohan *et al*. 2008).

3.3 Impact of pH on operational characteristics

In batch tests, biohydrogen production rate was significantly relying on operatable pH and ammonium level (Salerno *et al*. 2006). Proliferation of ammonium caused no significant impact on hydrogen yield at acidic pH of 5.5 and 6.5. However SHPR was adversely affected by ammonium levels at pH 7.5 (Fig. 2A). The maximum ammonium level of 294 mg/L (pH 7.5) was recorded and was found lower than earlier reported inhibited limits of 2.0 g/L (Cheong and Hansen 2007; Salerno *et al*. 2006), 5.0 g N/L (Borja *et al*. 1996). A direct relationship was observed between ammonium and alkalinity **(Fig. 2A and B)** whereby an increase in pH increases the alkalinity level. Due to this, the highest alkalinity (10,000 mg/L) was recorded at pH 7.5. By contrast, the lowest alkalinity (4000 mg/L) was recorded at pH 5.5. The increase in alkalinity and ammonium improved the buffering capacity and neutralised acid production at pH (6.5 and 7.5). Compared to this a high acid (48,000 mg/L) production was recorded at pH 5.5 **(Fig. 2C).**

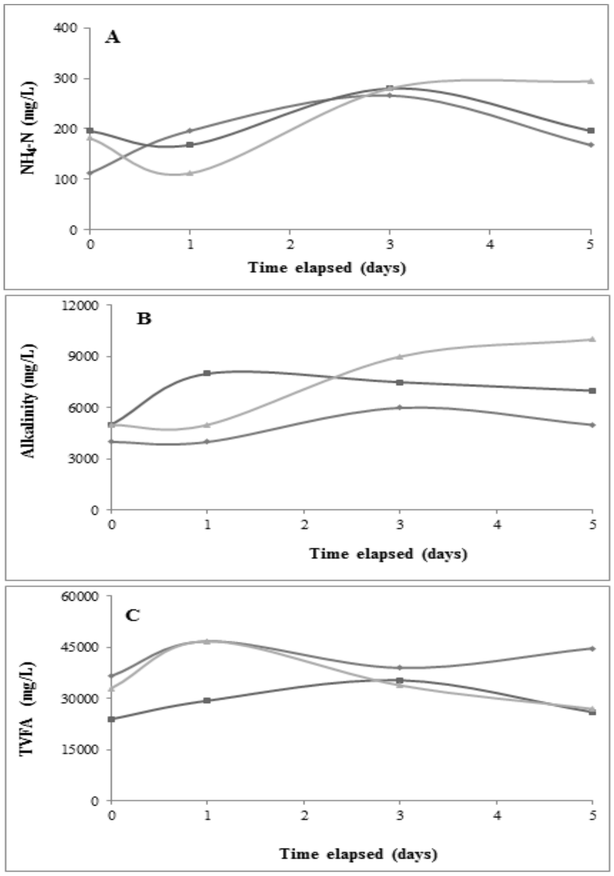

Fig.. 2A Ammonium-N levels at all analysed pH
Fig.. 2B Alkalinity levels at all analysed pH
Fig. 2C TVFA levels at all analysed pH

3.4 Impact of pH on intermediate metabolites

The potential for hydrogen production is reflected by the intermediary products of acids coupled with solvent production. The fermentative intermediates reflect the changes in metabolic pathways of that microorganism involved. The major VFAs produced were acetic and butyric respectively. A gradual increase in acetic and butyric acid was observed at pH 5.5 and 6.5 maximising the potential of hydrogen production **(Fig. 3A and B).** The HAc/HBu ratio was observed in the range of 3 – 4, a similar finding to that of Khanal *et al.* (2004). A proliferation of propionic acid was recorded at pH 5.5 and 6.5 and negligible levels observed at pH 7.5 **(Fig. 3C).** Propionic acid production helps in hydrogen production but it reduces methane production. Due to this a high methane level and low hydrogen production was achieved at pH 7.5.

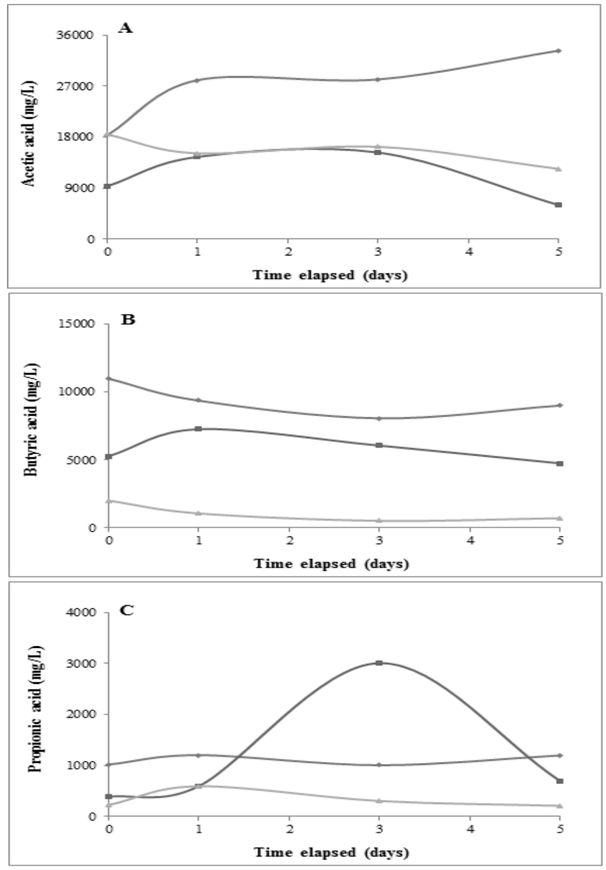

Fig. 3A Acetic acid levels at all analysed pH
Fig 3B Butyric acids levels at all analysed pH
Fig. 2C Propionic acid levels at all analysed pH

4. CONCLUSIONS

The biohydrogen production is a function of pH and type of treatment applied to seed sludge. The optimise pH for a mix of IFW and SS was recorded at 5.5. The optimised SHP (86.0mL) and the highest hydrogen yield (201.4 mL/g $VS_{removed}$) was observed at pH 5.5 followed by pH 6.5 and 7.5.

Fermentation efficiencies increase by increasing the pH level, the highest biodegradability (29.6%) was achieved at alkaline level of pH 7.5. Proliferation of ammonium nitrogen caused no significant impact on hydrogen yield. Compare to this SHPR was adversely affected by high ammonium levels at all pH levels. Acetate, butyrate and ethanol were chief intermediary products and the optimum HAc/HBu ratio was found in the range of 3 – 4.

Heat treatment successfully deactivated the non-spore forming methanogens and repeated shock heat treatment was more efficient than shock heat treatment.

The optimised cumulative hydrogen production of 86.0mL and the highest fermentation efficiency (50.2%) were achieved using the HT type of seed sludge. The highest hydrogen yield (201.4 mL/g VSremoved) was observed using AT followed by HT.

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