African Journal of Biotechnology Vol. 10(27), pp. 5320-5326, 15 June, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1742
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Biohydrogen production from diary processing wastewater by anaerobic biofilm reactors

Ileana Mayela María Moreno-Dávila, Leopoldo Javier Ríos-González*, Yolanda Garza-García, José Antonio Rodríguez-de la Garza and Jesús Rodríguez-Martínez

Departamento de Biotecnología, Facultad de Ciencias Químicas, Universidad Autónoma de Coahuila, Boulevard V. Carranza e Ing. J. Cárdenas Valdés, Col. República Oriente, Saltillo, Coahuila C.P. 25280., México.

Accepted 25 January, 2011

Fermentative hydrogen production was studied in packed bed batch reactors to assess the influence of environmental factors over yield hydrogen production from dairy wastewater. Dried stems of *Opuntia imbricata* were used as substratum adding a pretreated mixed culture for biofilm formation. Experimental results showed that, yield hydrogen production was significantly affected by initial COD concentration, temperature and dairy wastewater pH. Maximum yield obtained was 12.73 mM H_2/g COD_c when initial COD concentration was 21.1 g COD, dairy wastewater pH with no adjustment (11.32) and room temperature of 16 ± 3°C. Methane production was completely inhibit at an initial pH of 4 at all temperature studied (final pH 4.06), meanwhile, with an initial pH of 11.32, with exception for 16°C, methanogenic activity was not completely inhibit when final pH was over 5, showing an increase in methane production of 0.35 to 0.75 g CH_4/l for 35 to 55°C.

Key words: Biofilm, dairy wastewater, hydrogen, *Opuntia imbricata.*

INTRODUCTION

The worldwide energy need has been increasing exponentially and the reserves of fossil fuels have been decreasing (Fan et al., 2008). Environmental pollution due to the use of fossil fuels as well as their shortfall makes it necessary to find alternative energy sources that are environmentally friendly and renewable (Wan and Wan, 2008).

Hydrogen gas is a clean energy source with a high energy content of 122 kJ g^{-1}. Unlike fossil, fuels hydrogen does not cause any CO_2, CO, SO_x and NO_x emissions producing water as its only by-product when it burns reducing green house effects considerably (Chong et al., 2009). Hydrogen is considered to be a major energy carrier of the future and can directly be used in fuel cells for electricity generation (Kargi et al., 2008).

Biological hydrogen production from renewable resources using microorganisms appears to be the most attractive method compared to other hydrogen production processes because it has fewer environmental concerns (Kapdan et al., 2009). Biological method mainly includes photosynthetic hydrogen production (photo fermentation) and fermentative hydrogen production (dark fermentation) (Jin et al., 2009).

Dark fermentation, traditionally known as anaerobic digestion, is considered as a feasible process because it generates bio-H_2 from carbohydrate substrates including biomass and organic waste materials. However, the yield of bio-H_2 is relatively low, since H_2 is produced as an intermediate and can be further reduced to methane, acetate and propionate by hydrogen-consuming bacteria (HCB) during dark fermentation. To increase the production rate of bio-H_2, more attention needs to be given to developing methods that inhibit the activity of HCB and exclusively enrich hydrogen-producing bacteria (HPB) (Wan and Wan, 2008). Some of HPB produce spores under unfavorable conditions and spore forming conditions such as heat shock, chemical stress, pH stress and aeration could suppress the growth of HCB and

*Corresponding author. E-mail: leopoldo.rios@uadec.edu.mx. Tel: +52 844 4155752, +52 844 4162129. Fax: +52 844 4159534.

Table 1. Substratum characteristics.

Parameter	Specification
Identification	*O. imbricata*
Configuration	Packed-bed
Origin	Natural
Dimensions	1.5 x 0.5 cm
Dried weight	10 ± 0.023 g
Density	0.838 g/cm^3
Specific surface area	0.599 m^2/g (from BET)

Table 2. Dairy wastewater composition.

Parameters	Dairy wastewater
pH	11.32 ± 0.240
COD (g/l)	21.1 ± 0.381
Conductivity (m/S)	2640 ± 52.8
TSS (g/l)	21.9 ± 0.557

accordingly, increase the survival ratio of HPB. Heat and chemical pre-treatments have been widely used due to its high efficiency in bio-H_2 production (Hwang et al., 2009).

Critical factors in biological H_2 production are pH, temperature, feed concentration, bacterial population, retention period, etc. (Kalia and Purohit, 2008) Recently, experiments have been carried out to study the possibility of hydrogen production using organic wastes from various industries in combination with the wastewater treatment strategy (Chong et al., 2009). Dairy wastewater contains complex organics such as polysaccharides, proteins and lipids, which on hydrolysis form sugars, amino acids and fatty acids. In subsequent acidogenic reaction, these intermediate products are converted to volatile fatty acids (VFA), which are further degraded by acetogens, forming acetate, CO_2, and H_2 (Mohan et al., 2007).

Dark fermentation process for hydrogen production has been widely reported (Jin et al., 2009; Mohan et al., 2007). However, there are not many reports involving the use of biofilm systems (Mohan et al., 2007). Recent researches carried out by our research group have focused on the use of a natural substratum (*Opuntia imbricata*). *S*ubstratum used on this work has been tested to develop biofilm reactor systems useful for the treatment of different wastewaters (May-Esquivel et al., 2008). Contrary to some synthetic organic or inorganic polymer materials such as resins, gels and fibers conventionally used as substratum, *O. imbricata* is renewable with high grade of reusability and without disposal problems (Ilyná et al., 2008).

The objective of this work was optimize the hydrogen fermentative process from dairy wastewater using packed bed batch reactors, inoculated with a pretreated anaerobic mixed culture and *O. imbricata* as substratum.

MATERIALS AND METHODS

Anaerobic microbial mixed culture pretreatment

Anaerobic microbial mixed culture (500 ml) was obtained from a UASB reactor that treated wastewater from brewery Modelo (Zacatecas, Mexico). Pretreatment was carried out as describe by Chen (2007). In the heat pretreatment, the granules or sludge was heated in boiling water bath for a short period of time (30 min) first and then, cooled down. Heat pretreatment was followed by acidic pretreatment that involved decreasing the pH of the sludge or granule solution to 3.0 using 0.1 N HCl solution for 24 h and a readjustment of pH back to 7.0 by 0.1N NaOH solution.

Substratum pretreatment and preparation

Small pieces of *O. imbricata* dried stems were cut (previously rinse and washed with a pressurized water stream) in rectangular segments of 1.5 cm x 0.5 x 0.5 cm (Table 1).

Biofilm formation on *O. imbricata*

Biofilm formation was carried out in a 1.5 L up-flow plastic (PET) reactor, 520 g (dried weight) of *O. imbricata* (substratum preparation describe previously) was added. Finally, 500 ml of pretreated anaerobic microbial mixed culture was added.

Reactor was fed by a peristaltic pump (MANOSTAT - division of Barnant Company, Simon varistaltic pump, USA) for a period of 30 days with following conditions; initial pH 7.0, temperature 20 ± 3°C (ambient temperature), HRT 2 h and initial wastewater concentration 21.1 g COD/L. During this period, all parameters mentioned earlier were monitored on daily basis. Subsequently, HRT was decreased to 1 h for a period of 30 days, maintaining the rest of the condition (pH, temperature and concentration) as mentioned earlier.

Substrate

The substrate was diary wastewater and was collected from a moderate size commercial milk and cheese factory located in Saltillo, Coahuila, Mexico. After collection, the wastewater was transferred immediately to the laboratory and stored at 4°C. Characteristics of dairy wastewater used are given in Table 2.

Experimental setup

Influence of initial COD concentration on H_2 production

The experiment was performed with 120 ml batch reactor (serum bottles) filled with 40 ml of dairy wastewater, varying the amounts of initial dairy wastewater concentration from the range of 2.9 to 21.1 g COD/L, adding 20 ± 0.020 g of support (containing 0.550 ± 0.10 g biomass). Reactors were purged with helium for 15 min and capped tightly with silicon rubber and aluminum caps to avoid gas leakage. Reactors were then placed in an incubator at 35 ± 1°C.

Influence of initial pH of dairy wastewater on H_2 production

Experiments were performed in a pH range of 4.0 to 7.0 with increments of 0.5 and also, at 11.32 (original pH of wastewater). 120 ml batch reactor were used at an initial COD concentration of 21.1 g COD/l (optimum COD concentration obtained in previous

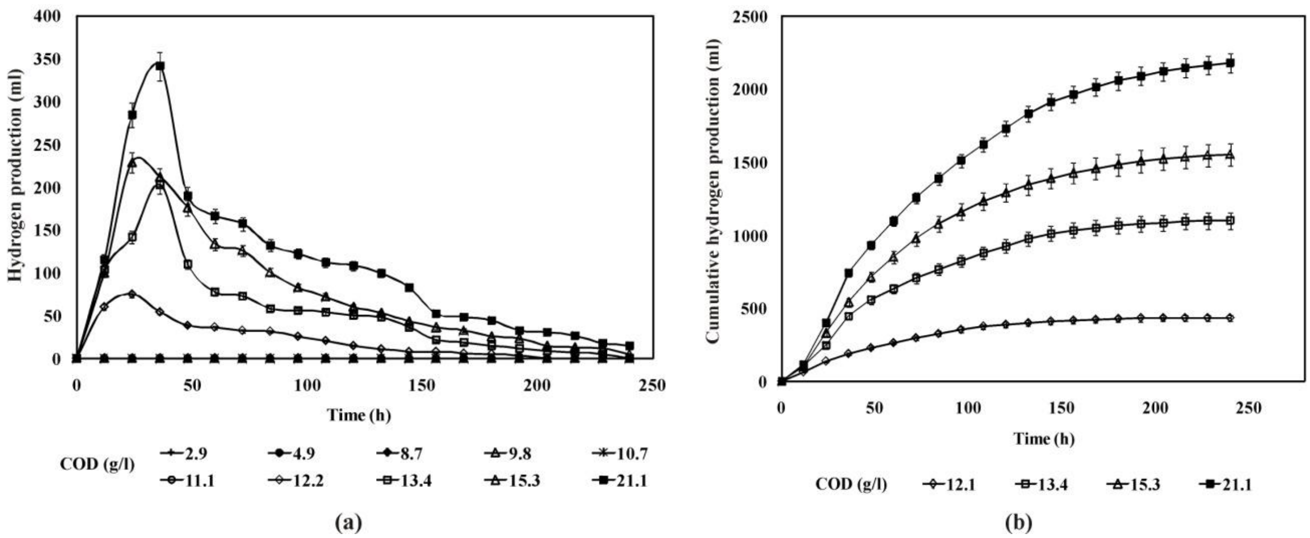

Figure 1. (a) Hydrogen production (ml) and b) cumulative hydrogen production (ml) at different initial COD concentrations (2.9 - 21.1 g/l) at pH 7.0 and temperature 35 ± 1°C.

experimental series) adding 20 ± 0.020 g of support (containing 0.550 ± 0.10 g biomass. Reactors were purged and sealed as previously described and were then placed in an incubator at 35 ± 1°C.

Influence of temperature on H_2 production.

120 ml batch reactor were used at an initial COD concentration of 21.1 g COD/L (optimum COD concentration obtained in previous experimental series) adding 20 ± 0.020 g of support (containing 0.550 ± 0.10 g biomass, pH levels were 4 and 11.32 Experiments were conducted at the following temperatures: 16 ± 3°C, 35 ± 1°C, 45 ± 1°C, 55 ± 1°C. Reactors were purged and sealed as previously described and then, placed at the selected temperatures.

Analytical methods

The amount of hydrogen and methane were determined by gas chromatography (GC TCD) VARIAN 3400, equipped with a Molecular Sieve 5ª packed column injecting 25 µl using a 1 ml syringe. GC conditions were as followed: injector and detector temperature 200°C, column temperature 50°C, using helium as carrier gas with flow rate 6 ml/min. Removal of COD was determined according to standard methods (APHA, 1998). The pH was determined by extracting liquid sample (2 ml) from effluent and measured by potentiometer (WTW, INOLAB-pH/ION Level 2). All reactors were monitored every 24 h. All data presented represents the means from three replications that were kept for each experiment.

RESULTS AND DISCUSSION

Effects of initial concentration (COD) on H_2 production

Figure 1a shows the hydrogen production from dairy wastewater in packed bed batch reactors at different initial COD concentrations (2.9, 4.9, 8.7, 9.8, 10.7, 11.1, 12.2, 13.4, 15.3 and 21.1 g/l) at pH 7.0 and temperature 35 ± 1°C. Hydrogen production was detected starting from initial COD concentration of 12.2 g/l, producing a maximum of 74.8 ml of H_2 after 24 h, with an accumulated H_2 production of 433.43 ml (Figure 1b), whereas, in concentrations under the mentioned earlier, hydrogen was not detected. Hydrogen production increased as initial COD concentration increased (12.2 to 21.1 g/l), producing a maximum hydrogen production of 341 ml of H_2 after 36 h, with an accumulated H production of 2181.34 ml for the case of higher initial COD concentration studied (21.1 g/l COD).

Figure 1a also shows that, for cases where hydrogen production was detected, a decrease in hydrogen production occur between 24 and 36 h as a result of an important diminish of initial COD. During the first 24 h for the case of reactors with initial COD concentration of 21.1 g/l, COD diminished to 8.5 g/l corresponding to removal efficiency of 59.2%, achieving 73.6% removal efficiency after 240 h (Table 3).

This concurs with early reports by Ginkel et al. (2001) and Lin et al. (2006) in which using high concentration of substrate (glucose 15 to 25 g/l) hydrogen production was detected and Kyazze et al. (2006) reported hydrogen production even at higher concentrations of substrate using sacarose up to 40 g/l.

Our test results showed that, the change of the COD concentration remarkably affected the hydrogen production yield, as shown in Table 3, where the maximum yield hydrogen production was 5.73 mM H_2/g COD_c (consumed chemical oxygen demand), for the case of

Table 3. Data obtained in study of influence of initial concentrations over hydrogen production from dairy wastewater after 240 h.

Initial COD (g/l)	pH		COD removal effficiency (%)	H_2 yield (mM/g COD_c)	CH_4 (g/l)
	Initial	Final			
2.9	7.0	6.12	46.98	0	0.52
4.9	7.0	6.18	51.61	0	0.75
8.7	7.0	6.05	65.31	0	1.14
9.8	7.0	6.08	62.18	0	1.36
10.7	7.0	6.1	56.62	0	1.34
11.1	7.0	6.01	58.08	0	0.83
12.2	7.0	5.16	63.24	2.30	0
13.4	7.0	5.11	67.45	4.96	0
15.3	7.0	5.05	69.61	5.97	0
21.1	7.0	5.06	73.63	5.73	0

*COD_c: Chemical oxygen demand consumed.

21.1 g/l initial COD (raw water concentration). Although, an increase in COD concentration could enhance the H_2 yield, but a higher substrate concentration results in accumulation of the volatile fatty acids, which inhibit the growth of hydrogen-producing bacteria. In addition, the partial pressure of hydrogen in the reactor rose with the increases of substrate concentration. An exorbitant partial pressure level of hydrogen in the headspace of reactor would inhibit hydrogen production because the microorganisms could switch to alcohol production (Fan et al., 2006).

Table 3 also shows that, final pH after 240 h was 5.09 in average for cases where hydrogen production was detected (12.2-21.1 g/l COD), whereas at low concentrations (2.9-11.1 g/l COD) hydrogen was not detected and final pH was 6.09 in average.

Fermentative hydrogen production has been reported that, can take place at a wide range of pH from 3.3 (Gadhamshetty et al., 2009) to 5.0 (Kyazze et al., 2006), contributing to inhibition of methanogenic bacteria that cannot tolerate pH below 5 according to different reports (Kim et al., 2009). These reports concur with results obtained on this work, where reactors with a high concentration of COD (where hydrogen was detected) methane was not detected. However, Table 3 shows that in reactors with COD concentrations in the range of 2.9 to 11.1 g/l, methane was observed for all cases, with a maximum production of 1.36 g/l CH_4 for the case of 9.8 g/l COD.

Effects of initial pH on hydrogen production

Optimum COD concentration (21.1 g/l) was used to carried out experimental series of influence of initial pH (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and raw wastewater's pH 11.32) and were kept at 35 ± 1°C. Figure 2a shows hydrogen production, it can be observed that a maximum hydrogen production 302.86 ml after 48 h for cases where initial pH was 5.0. However, accumulated hydrogen production after 468 h was higher for cases were initial pH was 4.0 (2466 ml), compared with 1765 ml produced for the cases were initial pH was 5.0.

Figure 2a and b also shows that, maximum hydrogen production was for the cases were the pH was not adjusted was 251.33 ml with an accumulated hydrogen production of 1473 ml. Table 4 shows that, yield hydrogen production fell in the same proportion that initial pH are increased. It can be observed that, maximum yield was 5.83 mM/g COD_c was for cases of initial pH of 4.0, whereas for the case of pH 7.0 it only achieved maximum yield hydrogen production of 0.41 mM/ g COD_c. Removal efficiency was not significantly affected for all initial pH cases studied (pH's 4.0 to 7.0 with an average of 59.95 %) with exception of initial pH of 11.32 (raw wastewater pH) were the removal efficiency was slightly lower (52.01%).

Results shown on this work are comparable with reported by Mohan et al. (2007) where a high yield hydrogen production was achieved with 1.25 mM H_2/ g COD_c (26 ml H_2/ g DQO_c) at fermentation pH 6 from composite chemical wastewater in batch experiments using sequentially pretreated anaerobic mixed consortia as inoculum. Methanogenic activity was completely inhibit for the cases were initial pH was 4.0 and 4.5. Methane production increased slightly (0.21- 0.75 g CH_4/L) when pH was in range of 5.0 to 6.5 and no difference in methane production was noticed for pH 7.0 and 11.32.

In spite that at the end, final pH for cases were initial pH was in the range of 5.0 to 11.32 was 4.5 to 5.2, respectively; it can be observed that methanogenic activity was not completely inhibit. This behavior can be

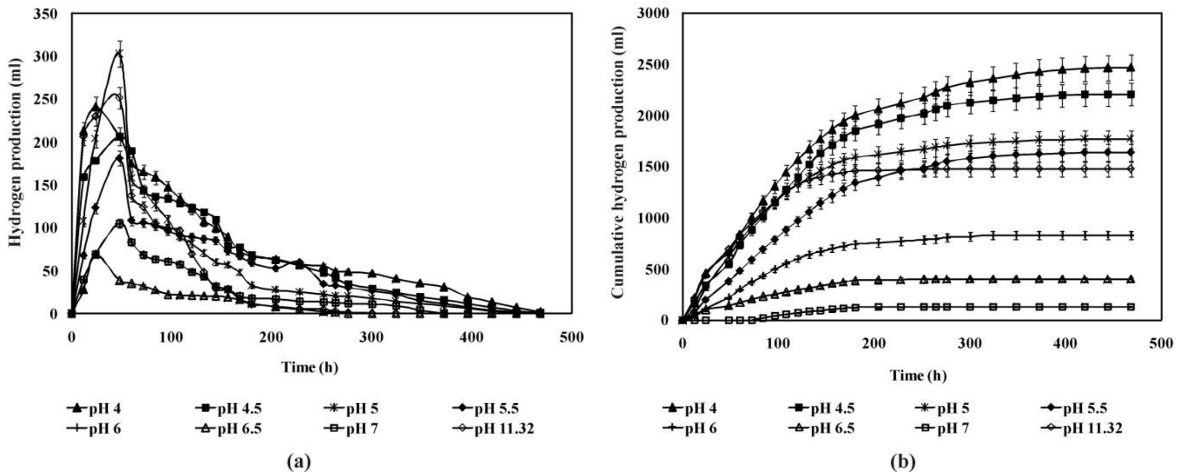

Figure 2. (a) Hydrogen production (ml) and (b) cumulative hydrogen production (ml) at different initial pH (4.0 to 7.0 and raw wastewater's pH 11.32) at 35 ± 1°C.

Table 4. Data obtained in study of influence of initial pH over hydrogen production from dairy wastewater after 468 h.

pH		COD removal efficiency (%)	H_2 yield (mM/g COD_c)	CH_4 (g/l)
Initial	Final			
4.0	4.07	62.58	5.83	0
4.5	4.18	61.28	5.53	0
5.0	4.51	59.92	4.40	0.21
5.5	4.52	60.84	4.31	0.33
6.0	4.54	58.44	2.24	0.55
6.5	4.53	58.51	0.93	0.75
7.0	4.53	58.09	0.41	0.75
11.32	5.19	52.01	3.62	0.75

explained due the use of biofilms system in which regularly the specific activity is increased and also this type of systems offer a higher operational stability (Qureshi et al., 2005). This is mentioned by Wu and Chang (2007), in which the microbial community is protected by the polymeric matrix and allows tolerating extreme environmental conditions.

Influence of temperature on H_2 production

For this experimental stage, four temperature were assessed: 16 ± 3, 35 ± 1°C, 45 ± 1°C y 55 ± 1°C, with an initial concentration of 21.1 g/l COD and initial pH 4.0 and 11.32.

Figure 3a shows hydrogen production with an initial pH of 4.0. It can be observed that maximum hydrogen production was similar for 35, 45 and 55°C (238.67 ml in average). However, the maximums (hydrogen production) for each case were achieved at different times as a result of the presence of different lag phases for each case. This figure shows a lag phase with duration of 72 h for 35 and 55°C and 144 h for 45°C. This behavior can be explained due to formation of semisolids aggregates when pH was adjusted to 4.0, creating conditions with low substrate availability. This conditions decrease as temperature was increased, accelerating its segregation at high temperatures (35 to 45°C). However, at room temperature (16°C) hydrogen production was not detected.

It is well-known that, the polysaccharides must be cleaved into smaller molecules, typically with molecular weight less than 1000 Daltons, before they can be introduced into a cell and used for energy production (Tang et al., 2008). However, at low temperatures hydrolysis rates is slower and therefore, presence of complex or particulate matter can affect biodegradation process, causing biomass problems due to accumulation of solids (Mahmoud et al., 2003).

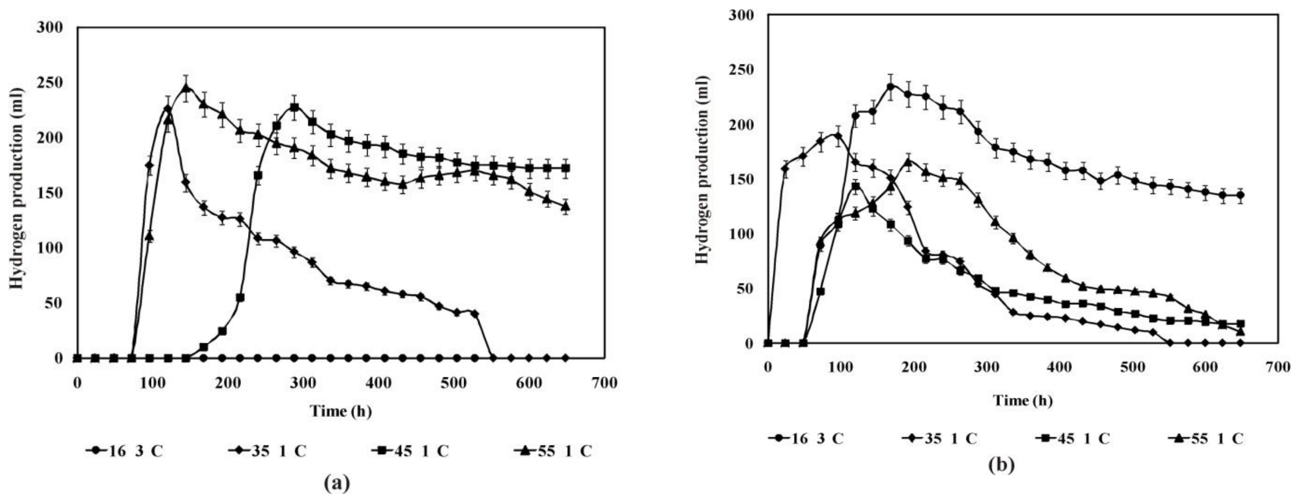

Figure 3. Hydrogen production (ml) a) initial pH 4.0 and b) initial pH 11.3;, initial concentration 21.1 g/l COD at different temperatures (16 ± 3, 35 ± 1°C, 45 ± 1°C and 55 ± 1°C).

Table 5. Data obtained in study of temperature influence on hydrogen production from dairy wastewater after 648 h.

Temperature (°C)	pH		COD removal efficiency (%)	Cumulative H_2 (ml)	H_2 yield (mM/g COD consumed)	CH_4 (g/l)
	Initial	Final				
16 ± 3	4.0	4.03	43.06	0	0	0
16 ± 3	11.3	4.98	49.48	4215.88	12.73	0
35 ± 1	4.0	4.25	64.28	1848.11	5.14	0
35 ± 1	11.3	5.19	47.42	1811.18	5.58	0.35
	4.0	4.05	59.65	2592.69	7.77	0
45 ± 1	11.3	5.03	46.68	1356.21	4.82	0.59
55 ± 1	4.0	3.94	56.42	3814.76	11.05	0
55 ± 1	11.3	5.35	45.37	2130.75	7.89	0.75

Figure 3b shows hydrogen production with an initial ph of 11.32. It can be observed that, maximum hydrogen production detected was 234 ml after 168 h at 16°C. However, Table 5 shows that accumulated hydrogen production was higher (4215.88 ml) compared with hydrogen production obtained for initial pH 4 at 55°C (3814.76 ml). Accumulated hydrogen production for pH 4 followed an increasing pattern as temperature was increased, 35 to 55°C; meanwhile for ph 11.32 this behavior was not observed (Table 5). These differences in accumulated hydrogen production affected directly hydrogen production yields, showing that maximum yield for pH 11.32 was 12.73 mM H_2/g COD_c (16°C) and for pH 4.0 was 11.05 mM H_2/g COD_c (55°C).

Yields obtained on this work are comparable to those by Sun et al. (2008). Tang et al. (2008) reported maximum yield hydrogen production of 319 ml H_2/ g COD_c (15.33 mM H_2/ g COD_c) at 45°C and pH 5.5, from cattle wastewater.

In comparable studies reports, optimal temperature for H_2 production via dark fermentation widely varies, mainly depending on the type of products and the carbon source used (Tang et al., 2003).

Methane production was inhibit completely for initial pH 4.0 at all temperatures studied, due to maintain a low pH at all time during hydrogen production process (pH 4.06 in average), whereas for initial ph 11.32 (with exception at 16°C), methanogenic activity was not completely inhibit (final pH 5.0 in average) with low methane production detected of 0.35 to 0.75 g CH_4/L at 35 to 55°C.

Conclusions

Results obtained on the present work demonstrated that, pretreatment (acidic and thermal) of microbial mixed culture and the use of *O. imbricata* as substratum for biofilm formation under an acidic regime (4.0 to 5.0) contributed to achieved a complete inhibit of methanogenic activity and lead to an increase of yield hydrogen production.

High-yield hydrogen production was obtained with a low initial pH (4.0) at 55°C (11.05 mM H_2/g de COD_c). However, it turned out to be more feasible hydrogen production process using raw wastewater with no modification (21.1 g/l COD, pH 11.32, room temperature 16 ± 3°C) achieving a yield hydrogen production of 12.73 mM H_2/g COD_c.

Overall, results obtained on the present work demonstrate an economical, non-complex and efficient way to produce hydrogen.

REFERENCES

APHA, AWWA, WEF (1998). Standard Methods for the Examination of Water and Wastewater, 20th edn, American Public Health Association, Washington, D.C., USA.

Chen S, Hu B (2007). Pretreatment of methanogenic granules for immobilized hydrogen fermentation. Int. J. Hydr. Energ. 32(15): 3266-3273.

Chong ML, Sabaratnam V, Shirai Y, Hassan MA (2009). Biohydrogen production from biomass and industrial wastes by dark fermentation. Int. J. Hydr. Energ. 34: 3277-3287.

Fan YT, Zhang GS, Guo XY, Xing Y, Fan MH (2006). Biohydrogen-production from beer lees biomass by cow dung compost. Biomass and Bioenergy, 30(5): 493-496.

Fan YT, Pan CM, Xing Y, Hou HW, Zhang ML (2008). Statistical optimization of process parameters on biohydrogen production from glucose by *Clostridium* sp. Fanp2. Bioresour. Technol. 99: 3146-3154.

Gadhamshetty V, Johnson D, Nirmalakhandan N, Smith G, Deng S (2009). Dark and acidic conditions for fermentative hydrogen production. Int. J. Hydr. Energ. 34(2): 821-826.

Ginkel S, Sung S, Lay L (2001). Biohydrogen Production as a Function of ph and Substrate Concentration. Environ. Sci. Technol. 35(24): 4726-4730.

Hwang SJ, Lee MJ, Song JH (2009). Effects of acid pre-treatment on bio-hydrogen production and microbial communities during dark fermentation. Bioresour. Technol. 100(3): 1491-1493.

Ilyná A, Huerta-Guel P, Martínez-Hernández JL, Rodríguez Martínez J, Gorokhovsky (2008). Stability and activity of bovine prostaglandin H synthase immobilized on *Opuntia imbricata* (coyonoxtle). A. J. Mol. Catal B-Enzym., 51: 1.

Kalia VC, Purohit HJ (2008). Microbial diversity and genomics in aid of bioenergy J. Ind. Microbiol Biotechnol. 35(5): 403-419.

Kim IS, Hwang MH, Jang NJ, Hyun SH, Lee SD (2003). Effect of low ph on the activity of hydrogen utilizing methanogen in bio-hydrogen process. Int. J. Hydr. Energ. 29(11): 1133-1140.

Kyazze G, Hawkes F, Hussy I, Dinsdale R, Hawkes D (2006). Continuous dark fermentative hydrogen production by mesophilic microflora: Principles and progress Int. J. Hydr. Energ. 32(2):172-184.

Kyazze G, Hawkes F, Hussy I, Dinsdale R, Hawkes D (2006). Continuous dark fermentative hydrogen production by mesophilic microflora: Principles and progress. Int. J. Hydr. Energ. 3 (2):172-184.

Lin CY, Hung CH, Chen CH, Chung WT, Cheng LH (2006). Biohydrogen Production as a Function of ph and Substrate Concentration. Process Biochem. 41(6):1383-1390.

Mahmoud N, Zeeman G, Gijzen H, Lettinga G (2003). Solids removal in upflow anaerobic reactors, a review. Bioresour Technol. 90(1): 1-9.28

May-Esquivel F, Rios-Gonzalez LJ, Garza-García Y, Rodríguez-Martinez J (2008). Performance of a packed reactor with *Opuntia imbricata* for Municipal Wastewater treatment. Environ. Res. J. 2(5): 238-245.

Mohan SV, Bhaskar YV, Krishna PM, Rao N Ch, Babu VL (2007) Biohydrogen production from chemical wastewater as substrate by selectively enriched anaerobic mixed consortia: Influence of fermentation ph and substrate composition. Int. J. Hydr. Energ. 32: 2286.

Mohan SV, Bhaskar YV, Sarma PN (2007). Biohydrogen production from chemical wastewater treatment in biofilm configured reactor operated in periodic discontinuous batch mode by selectively enriched anaerobic mixed consortia Water Res. 41(12): 2652-2664.

Mohan SV, Rao NCH, Sarma PN (2007). Low-biodegradable composite chemical wastewater treatment by biofilm configured sequencing batch reactor (SBBR). J. Hazardous Mat. 144(1-2): 108-117.

Qureshi N, Annous BA, Ezeji TC, Karcher P, Maddox IS (2005). Biofilm reactors for industrial bioconversion processes: employing potential of enhanced reaction rates. Microbial Cell Factories. 4: 24.

Tang GL, Huang J, Sun ZJ, Tang QQ, Yan CH, Lui GQ (2008). Biohydrogen production from cattle wastewater by enriched anaerobic mixed consortia: influence of fermentation temperature and ph. J. Biosci. Bioeng. 106(1): 80-87.

Wan J, Wan W (2008). Influence of Ni^{2+} concentration on biohydrogen production. Int. J. Hydr. Energ. 99(18): 8864-8868.

Wu KJ, Chang JS (2007). Batch and continuous fermentative production of hydrogen with anaerobic sludge entrapped in a composite polymeric matrix. Process Biochem. 42(2): 279-284.