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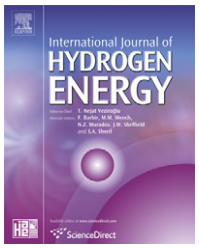

Effect of arabinose concentration on dark fermentation hydrogen production using different mixed cultures

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ABSTRACT

Dark fermentation hydrogen production from arabinose at concentrations ranging between 0 and 100 g/L was examined in batch assays for three different mixed anaerobic cultures, two suspended sludges (S1, S2) obtained from two different sludge digesters and one granular sludge (G) obtained from a brewery wastewater treatment plant. After elimination of the methanogenic activity by heat treatment, all mixed cultures produced hydrogen, and optimal hydrogen rates and yields were generally observed for concentrations between 10 and 40 g/L of substrate. Higher concentrations of arabinose up to 100 g/L inhibited hydrogen production, although the effect was different from inoculum to inoculum. It was evident that the granular biomass was less affected by increased initial arabinose concentrations when calculating the rate of decrease in hydrogen yields versus arabinose concentrations, compared against the two suspended sludges.
The largest amount of soluble microbial product produced for all three inocula was for *n*-butyrate. Also, valeric acid production was observed in some samples.

1. Introduction

Hydrogen appears to be an ideal candidate as an alternative to fossil fuels. It has the highest energy content per unit of weight for any known fuel, it is fifty percent more efficient than gasoline in automobiles, and it can be used to generate electricity by fuel cell technology [1,2]. Hydrogen can be obtained via non-biological and biological processes. Non-biological processes use fossil fuels as a source for hydrogen production [3]. In this case, however, hydrogen cannot be considered an alternative energy source. Conversely, hydrogen can be obtained biologically from photolysis carried out by algae and cyanobacteria and also via fermentation by anaerobic bacteria. However, the rate of hydrogen production from fermentation is greater compared to photolysis [3].

Dark fermentation of hexoses has been extensively studied using a variety of anaerobic inocula under different growth and operational conditions while biohydrogen production from pentoses has been less well characterized [4]. Few reports have demonstrated biohydrogen production directly from arabinose, one of the most common pentoses and a component of various hemicellulosic and plant polysaccharides. Two studies have successfully resulted in the isolation of *Clostridia* species that produced hydrogen using arabinose as the substrate [5,6]. However, the effect of substrate concentration on hydrogen production was not determined and the products of arabinose fermentation were not identified.

Previous studies carried out with other sugars have shown that different substrate concentrations have an effect on the amount of hydrogen produced [7–13]. In addition, different

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sources of inocula may also lead to different yields of hydrogen with varying production rates [4,12,14,15]. The work presented herein examines the effect of different concentrations of arabinose on hydrogen production for three different anaerobic mixed cultures.

2. Materials and methods

2.1. Inocula

Anaerobic sludge was obtained from three different wastewater treatment plants in Portugal. Sludge S1 was dispersed sludge obtained from a sludge digester supplemented with fat, located in a municipal wastewater treatment plant in Coimbra. Sludge S2 was dispersed sludge obtained from a municipal wastewater treatment plant digester located in Oporto. Sludge G was obtained from an upflow anaerobic granular sludge (UASB) reactor treating brewery wastewater. Sludges S1, S2 and G were autoclaved in order to suppress the methanogenic hydrogenotrophic activity.

2.2. Batch culture inoculation and operation

Batch experiments were conducted in 125-mL serum bottles containing 20 mL total of innocula and media. The media composition was as previously described [16,17]. The initial biomass concentration was approximately 10 g/L of volatile suspended solids.

Prior to inoculation, suspended heat treated sludge was centrifuged (5000 rpm for 5 min), washed in media, centrifuged (5000 rpm for 5 min), and added to serum bottles. Heat treated granular sludge was first filtered using a 0.2-mm sieve. Then, the sludge remaining on top of the sieve was added to serum bottles. The final concentration of arabinose in each bottle was 0, 10, 20, 30, 40, 50, 75, and 100 g/L. The initial pH of the batch experiments was adjusted to 6.5 by flushing the headspace of each batch reactor with 100% CO_2 for several minutes. Batch cultures were placed on a rotary shaker (150 rpm) and incubated at 37 °C (±2 °C). Experiments at each substrate concentration were conducted in triplicate.

2.3. Monitoring and analysis

Soluble microbial products (formate, acetate, propionate, *n*- and *i*-butyrate, valerate, and ethanol) and arabinose were determined using a high performance liquid chromatograph (Jasco, Japan) with a Chrompack column (6.5 × 30 mm^2). Sulfuric acid (0.01 N) was used as the mobile phase at a flow rate of 0.7 mL/min. The temperature of the column was set at 60 °C. Detection of VFA, ethanol, and arabinose was accomplished by using a UV detector at 210 nm and a Refraction Index (RI) detector, respectively.

Samples of biogas (0.1 or 0.2 mL) were removed using a gas-tight, gas-locking syringe. Hydrogen concentrations were monitored using a Hayesep Q column (80/100 mesh) and a thermal conductivity detector (Varian 3300 Gas Chromatograph) with nitrogen (30 mL/min) as the carrier gas. The injector, detector, and column temperatures were 120, 170, and 35 °C, respectively. Methane concentrations were monitored using a Porapak Q (180–100 mesh) column and a thermal conductivity detector (Chrompack), with helium as the carrier gas (30 mL/min) and having the injector, detector, and oven temperatures set at 110, 110, and 35 °C, respectively. The quantity of each gas was corrected to 1 atm and 0 °C. Gas pressure was released using the Owen method [18] via a 20-mL or 50-mL glass syringe. The amount of gas present in the headspace of each batch reactor was determined before and after releasing gas pressure. Hydrogen, VFA, and ethanol concentrations for the control inocula (0 g/L of arabinose) were subtracted from the values obtained in the tests with 10–100 g/L arabinose. Volatile solids and volatile suspended solids were measured according to standard methods [19].

Hydrogen production rates and potential were determined using the modified Gompertz equation (Eq. (1)) [14,20]:

$$H(t) = P\exp\left\{-\exp\left[\frac{R_m e}{P}(\lambda - t) + 1\right]\right\} \quad (1)$$

where $H(t)$ is the cumulative hydrogen production (mL); P is the hydrogen production potential (mL); R_m is the maximum hydrogen production rate (mL/h); e is approximately 2.718; λ is the duration of the lag phase (h); and t is time (h).

3. Results and discussion

Hydrogen production occurred for all three sludges but there were differences in the yields, lag times, and rates. Methane production was not detected in any of the batch cultures. An example of the hydrogen production for the three different inocula for an initial arabinose concentration of 75 g/L is shown in Fig. 1.

Granular sludge produced the most hydrogen (50 mL) with the shortest lag phase (15 h) followed by S2 (34 mL and 29 h) with S2 biomass producing the least hydrogen (approximately 15 mL) with the longest lag phase (approximately 45 h). The modified Gompertz equation was used to calculate the values for the maximum hydrogen production rate, hydrogen production potential, and duration of the lag phase for all batch reactors. In addition, the R^2 values listed are the ranges

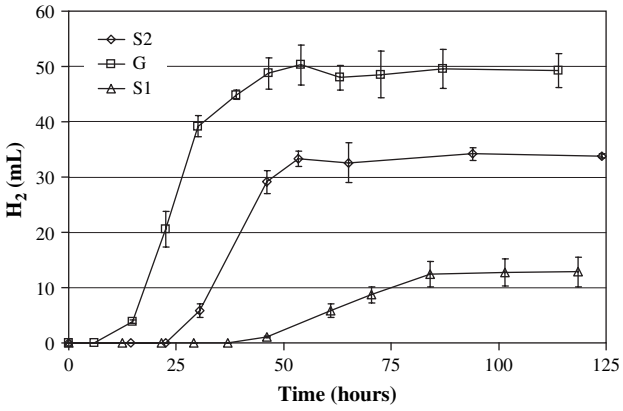

Fig. 1 – Biohydrogen production from three different sludges with an initial arabinose concentration of 75 g/L. Error bars represent one standard deviation of triplicate bottles.

of the values obtained for modelling the individual triplicate bottles. The results are shown in Table 1.

For the S1 biomass, the largest amount of hydrogen production and the maximum rates were obtained for concentrations of arabinose of 30 and 40 g/L, respectively. Similar values were also obtained for arabinose concentrations of 20 and 50 g/L. However, the lag phase was longer for 10 and 20 g/L arabinose compared to concentrations between 30 and 50 g/L. The rate of hydrogen production and hydrogen production potential decreases for 75 g/L and reaches a minimum at 100 g/L. This suggests an inhibitory effect by high concentrations of arabinose. Previous studies have shown that hydrogen production and rates peak at 20 g/L COD xylose (another pentose) and decrease significantly when initial concentrations were increased [11]. Possible reasons for the decrease in hydrogen production include substrate inhibition, product inhibition, a combination of both types of inhibition, and osmolality [21–24]. Similar results were observed for the S2 biomass. Maximum hydrogen production did not differ significantly for arabinose concentrations between 10 and 50 g/L, though peaking at 40 g/L. The amount of hydrogen production from 75 g/L arabinose was much higher for S2 than for the S1 sludge. The highest concentration of arabinose tested (100 g/L) yielded the lowest hydrogen production, lowest rate, and the longest lag time further indicating the inhibitory effects caused by high concentrations of substrate.

Table 1 – Modified Gompertz equation parameters for the three different sludges with varying amounts of arabinose where P = the hydrogen production potential, R_m = maximum hydrogen production rate, and λ = lag phase. The R^2 values listed are the range of the values obtained for modelling the individual triplicate bottles

Arabinose (g/L)	P (mL)	R_m (mL/h)	λ (h)	R^2
Dispersed sludge S1				
10	35.9 ± 1.7	1.5 ± 0.5	37.5 ± 1.1	0.9919–0.9937
20	53.9 ± 1.3	2.3 ± 0.1	38.2 ± 4.8	0.9669–0.9720
30	59.7 ± 4.2	2.8 ± 0.3	16.0 ± 1.5	0.9994–0.9996
40	53.6 ± 3.3	2.5 ± 0.0	17.8 ± 0.4	0.9959–0.9998
50	49.1 ± 1.1	2.4 ± 0.2	17.5 ± 0.4	0.9964–0.9987
75	13.1 ± 2.6	0.4 ± 0.1	47.0 ± 4.3	1.0000–1.0000
100	2.9 ± 0.1	0.3 ± 0.0	58.1 ± 0.6	0.9962–0.9966
Dispersed sludge S2				
10	51.0 ± 6.2	3.5 ± 0.5	20.3 ± 1.9	0.9937–1.0000
20	52.9 ± 1.8	2.3 ± 0.2	13.5 ± 6.3	0.9582–0.9992
30	46.7 ± 1.2	3.1 ± 0.4	15.5 ± 1.0	0.9375–1.0000
40	53.1 ± 2.6	3.8 ± 0.0	19.6 ± 0.5	0.9999–1.0000
50	50.8 ± 0.9	3.3 ± 0.8	18.4 ± 1.1	0.9993–1.0000
75	33.8 ± 1.8	2.3 ± 0.3	28.7 ± 5.3	0.9996–1.0000
100	9.0 ± 1.8	0.4 ± 0.4	87.5 ± 3.8	1.0000–1.0000
Granular sludge G				
10	46.8 ± 0.3	3.8 ± 0.3	14.7 ± 0.7	0.9999–1.0000
20	48.7 ± 0.4	3.5 ± 0.1	10.8 ± 4.1	0.9983–0.9999
30	60.3 ± 0.2	3.6 ± 0.2	13.2 ± 0.4	0.9991–0.9999
40	50.6 ± 0.4	2.9 ± 0.4	11.0 ± 1.8	0.9999–1.0000
50	52.7 ± 1.8	2.7 ± 0.6	11.5 ± 0.3	0.9926–0.9989
75	50.1 ± 4.3	2.9 ± 0.4	14.9 ± 0.4	0.9956–0.9998
100	24.9 ± 0.7	2.7 ± 0.0	23.0 ± 0.4	1.0000–1.0000

The granular sludge produced similar amounts of hydrogen for concentrations between 10 and 75 g/L, reaching a peak production of 60 mL of H_2 for 30 g/L arabinose. It also endured similar lag times with the shortest lag of 10 h for 20 g/L arabinose. The lowest amount of hydrogen produced (25 mL) was for 100 g/L arabinose. Arabinose concentrations of 75 and 100 g/L generated the largest hydrogen production rates and potentials and shortest lag times when compared against the results from the other two sludges. One possible explanation for the smaller inhibitory effect is the granular nature of the sludge. Hydrogen producing populations just beneath the surface of the granule would be exposed to a substrate concentration gradient that, at decreased concentrations of arabinose or metabolic by-products, is possibly no longer inhibitory. The high degree of correlation between the data and the model for all three biomasses suggested that the modified Gompertz equation adequately described the data.

Hydrogen yields were calculated for all batch reactors based on the amount of arabinose consumed and the amount of hydrogen produced. Fig. 2 depicts the changes in the maximum rate of hydrogen production (R_m) and hydrogen yields versus initial arabinose concentrations. In general, as the initial concentration of arabinose increases, hydrogen yields and rates decrease. However, there are differences between the three different biomasses. Significant decreases in yields and rates were observed for the initial arabinose concentrations of 75 and 100 g/L and 100 g/L for the S1 and S2 biomasses, respectively. However, for the granular biomass, rates of hydrogen production potential were similar for concentrations between 10 and 100 g/L. Therefore, it was evident that the G biomass was less affected by increased initial arabinose concentrations when calculating the rate of decrease in hydrogen yields versus arabinose concentrations (slope = –0.10; $R^2 = 0.9946$), compared against S2 biomass (slope = –0.16; $R^2 = 0.9181$) and S1 biomass (slope = –0.19; $R^2 = 0.9279$) (data not shown).

In addition, the consumption of arabinose decreased for higher concentrations of arabinose suggesting inhibition (Table 2). The highest hydrogen yield was observed for the S2 biomass (1.98 ± 0.31 mol H_2/mol substrate consumed) for 10 g/L arabinose. Granular biomass produced the highest hydrogen yield for 10 g/L arabinose when compared to other concentrations for the same inoculum. However, the amount (1.56 ± .01 mol H_2/mol arabinose) was lower than the value for the S2 biomass. The highest hydrogen yield for the S1 sludge was produced at 20 g/L arabinose and was the smallest of all the inocula tested (1.46 ± 0.09 mol H_2/mol arabinose).

The yields and rates of hydrogen production are different when compared against the values obtained for pure culture *Clostridium* sp. No. 2 fed arabinose [6]. *Clostridium* sp. strain No. 2 produced 3600 mL H_2/L culture with a hydrogen yield of 2.2 mol H_2/mol of arabinose consumed with an initial arabinose concentration of 10 g/L. This value is approximately 1.6× greater than the average amounts produced by S2 (51 mL H_2/20 mL culture), S1 (36 mL H_2/20 mL culture) or G (47 mL H_2/20 mL culture) inocula for the lowest concentration of arabinose tested (10 g/L) (average of 44 mL H_2/20 mL culture or 2200 mL H_2/L culture). The amount of hydrogen produced from the three sludges in this study is similar to the amount of hydrogen produced in unacclimated sewage and distillery

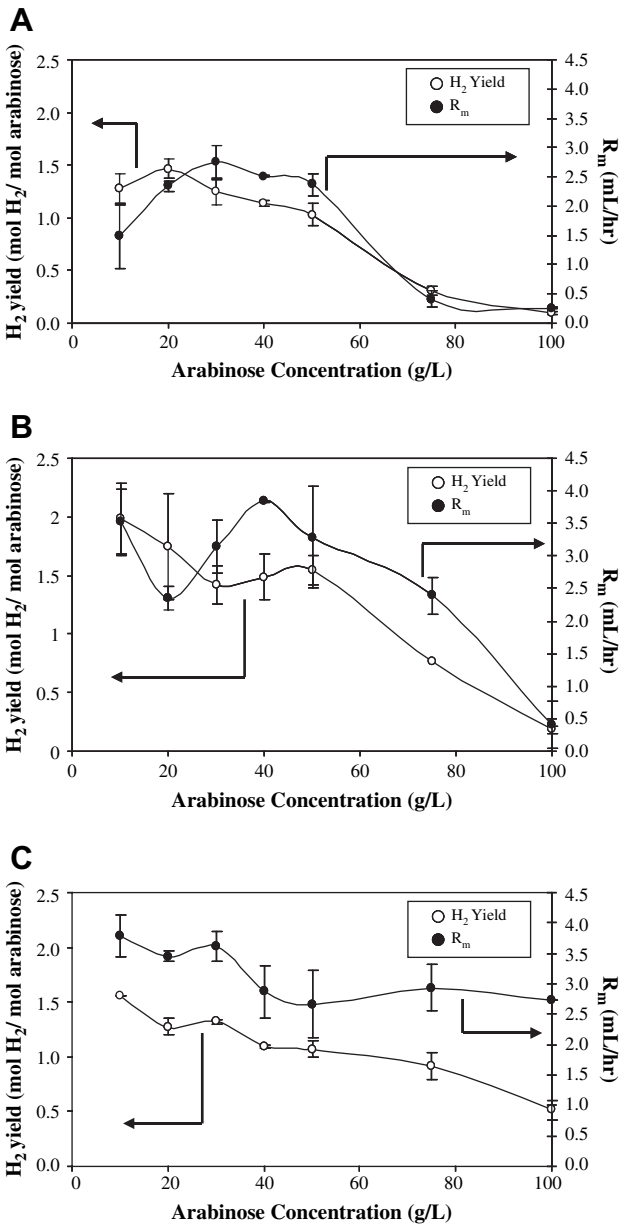

Fig. 2 – Hydrogen yields and maximum hydrogen production rates (R_m) versus different arabinose concentrations for S1 (A), S2 (B), and G (C). Error bars represent one standard deviation of triplicate bottles.

sludge (approximately 150 mL H_2/60 mL culture or 2500 mL H_2/L culture fed 20 g/L xylose) [15]. The rates of hydrogen production for an initial concentration of 10 g/L of arabinose are higher in strain No. 2 (550 mL H_2/L culture-h) compared to the three inocula used in this study (average of 2.6 mL H_2/20 mL culture-h or 130 mL H_2/L culture-h) [6].

These differences in the amounts and rates of hydrogen may occur from non-optimal conditions within the batch reactors of the mixed cultures. For example, temperature and pH were shown to impact the rates and yields of hydrogen in strain No. 2 [6]. In addition, micronutrient, macronutrient and buffer concentrations can also influence the rates and yields of hydrogen production [25–27]. Also, autoclaving to inhibit methanogenic activity may have depressed hydrogen producing activity compared to alternative methods such as bromoethanesulfonate (BES) or iodopropane [28].

3.1. *Volatile fatty acid (VFA) and ethanol production*

Soluble microbial products (SMP) released during fermentation are often used to evaluate the efficacy of hydrogen production. SMP for all batch reactors are shown in Table 2. For the S1 biomass, SMP increased to a maximum concentration of approximately 18 200 mg/L COD for 30 g/L arabinose and then decreased to a minimum concentration of approximately 2500 mg/L COD for 100 g/L arabinose. *n*-Butyrate was the most prevalent of the SMP for arabinose concentrations between 10 and 50 g/L. Valeric acid production was observed for concentrations between 30 and 75 g/L arabinose, ranging from 15 to 30% of the total SMP-COD. Production of valeric acid did not decrease the rate or the quantity of hydrogen as the highest amount of hydrogen production, rates, and shortest lag times were observed at initial arabinose concentrations of 30 and 40 g/L corresponding to valeric acid concentrations of 2200 and 2300 mg/L, respectively.

Previous studies have shown production of high concentrations of valeric acid with UASB reactors [29,30]. In addition, high valeric acid concentrations (approximately 2500 mg/L) were observed for acid pre-treated sludge batch reactors fed sucrose while batch reactors containing either heat treated (0 mg/L) or alkaline treated biomass (250 mg/L) produced little or none [31]. Rates and yields of hydrogen production decreased in a sucrose-fed UASB reactor when concentrations of valerate increased above 275 mg/L [24]. In contrast, impact of valeric acid on biohydrogen production was inconclusive on this sucrose-fed UASB because hydrogen production rates and yields were at their highest and lowest at concentrations of valeric acid observed in the UASB reactor between approximately 450–500 mg/L [25].

The total amount of SMP produced for S2 biomass was less than that produced for the S1. For arabinose concentrations between 10 and 50 g/L, the SMP produced were within 10% of each other, with the highest amount of SMP obtained for 40 g/L arabinose (approximately 10 000 mg/L COD). Similarly to the S1 biomass, the most-prominent of the VFA that were produced was *n*-butyrate, with relative amounts between 60–67% of the total SMP for arabinose concentrations ranging between 10 and 75 g/L. Unlike the S1 sludge, valeric acid was not detected for any arabinose concentrations.

For the granular biomass, the SMP production increased to a maximum concentration of approximately 12 000 mg/L COD. The total amount produced was generally higher when compared against the values for the S2 sludge but less than the amount produced for the S1 biomass. The largest percentage of *n*-butyrate occurred for 10 g/L arabinose and valeric acid was also produced. However, unlike the S1 inocula, production was observed for all concentrations of arabinose tested.

A COD balance for S1, S2, and G indicated that all of the metabolic products were identified (Table 2).

Butyrate to acetate ratios (Bu/Ac) are often used to as an indicator of the extent of biohydrogen production. Previous studies indicate that efficient hydrogen production occurs

Table 2 – Production of soluble microbial products (SMP) during fermentation with three different sludges under different initial substrate concentrations

Arabinose (g/L)	Arabinose consumed (%)	SMP (mg/L COD)	Formate (%)	Acetate (%)	Propionate + i-Butyrate (%)	*n*-Butyrate (%)	Valerate (%)	Ethanol (%)	COD balance (%)	Bu/Ac
Dispersed sludge S1										
10	100.0	10725	0.8	16.7	0.1	66.0	0.0	16.4	113.4	4.0
20	62.5	10567	0.8	16.4	0.8	64.4	0.0	17.6	96.2	3.9
30	56.9	18171	0.9	11.3	1.8	48.0	25.6	13.6	107.0	4.3
40	40.0	16684	1.2	11.5	1.9	46.7	27.0	12.9	103.8	4.1
50	32.8	14616	1.7	10.4	1.9	45.2	29.6	12.6	98.5	4.3
75	19.3	7721	4.5	47.8	14.8	15.4	14.6	5.6	109.0	0.3
100	11.1	2572	8.1	81.4	5.8	1.5	0.0	3.3	106.4	0.0
Dispersed sludge S2										
10	89.4	9269	1.3	15.5	8.0	59.5	0.0	16.1	111.1	3.8
20	53.4	9061	1.4	14.5	3.6	63.8	0.0	17.0	98.3	4.4
30	38.5	9913	1.2	14.3	0.1	66.7	0.0	17.7	98.0	4.7
40	31.7	9982	1.4	15.9	0.0	65.2	0.0	17.8	109.1	4.1
50	23.4	9521	2.2	14.2	5.1	61.9	0.0	16.9	115.6	4.4
75	18.2	6252	1.8	19.3	0.3	61.9	0.0	17.0	113.0	3.2
100	175	3386	3.8	25.6	5.3	5.8	0.0	60.4	100.2	0.2
Granular sludge G										
10	100.0	7361	0.7	15.8	0.6	73.6	9.9	0.0	87.4	4.7
20	65.1	9174	1.3	11.8	0.8	54.3	18.3	14.2	93.9	4.6
30	51.1	10556	1.6	12.9	1.0	59.7	9.5	15.7	105.8	4.6
40	38.8	11197	1.8	10.7	1.4	55.0	17.5	14.5	108.2	5.1
50	33.5	10632	1.6	10.9	1.3	53.9	18.5	14.4	111.7	4.9
75	25.0	8853	1.8	15.5	1.6	62.0	3.2	16.7	110.1	4.0
100	18.0	5897	4.3	13.7	2.6	59.7	5.2	16.5	113.0	4.4

for Bu/Ac ratios between 2.6 and 4.0 [15]. Ranges of Bu/Ac varied between 0.0 for the S1 inoculum at 100 g/L and 5.1 for the granular inoculum at 40 g/L arabinose (Table 2). When the maximum hydrogen production was observed to be larger than 25 mL (or approximately 40% of the maximum) then the Bu/Ac ratio was between 3.2 and 5.1.

4. Conclusions

The potential of dark fermentation hydrogen production from arabinose by two suspended (S1, S2) and one granular (G) anaerobic sludge was assessed in batch assays and optimal hydrogen rates and yield were generally observed for concentrations between 10 and 40 g/L of substrate. Arabinose concentration of 100 g/L inhibited the hydrogen production although the granular sludge exhibited better hydrogen yields and production rates for concentrations between 50 and 100 mg/L than the suspended sludges. The largest amount of SMP produced for all three inocula was for *n*-butyrate. Also, valeric acid production was observed in some samples. In addition, hydrogen production increased when the Bu/Ac ratios were between 3.2 and 5.1.

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REFERENCES

[1] Moore RB, Raman V. Hydrogen infrastructure for fuel cell transportation. Int J Hydrogen Energy 1998;23:617–20.
[2] Ramachandran R, Menon RK. An overview of industrial uses for hydrogen. Int J Hydrogen Energy 1998;23(7):593–8.
[3] Das D, Veziroglu TN. Hydrogen production by biological processes: a survey of literature. Int J Hydrogen Energy 2001; 26:13–28.
[4] Li C, Fang HHP. Fermentative hydrogen production from wastewater and solid wastes by mixed cultures. Crit Rev Environ Sci Technol 2007;37:1–39.
[5] Taguchi F, Chang JD, Mizukami N, Saito-Taki T, Hasegawa K. Isolation of a hydrogen-producing bacterium, *Clostridium beijerinckii* strain AM21B, from termites. Can J Microbiol 1993; 39:726–30.
[6] Taguchi F, Mizukami N, Hasegawa K, Saito-Taki T. Microbial conversion of arabinose and xylose to hydrogen by a newly isolated *Clostridium* sp. No. 2. Can J Microbiol 1994;40:228–33.
[7] Chen W-H, Chen S-Y, Khanal SK, Sung S. Kinetic study of biological hydrogen production by anaerobic fermentation. Int J Hydrogen Energy 2006;31:2170–8.
[8] Fang HHP, Li C, Zhang T. Acidophilic biohydrogen production from rice slurry. Int J Hydrogen Energy 2006;31:683–92.
[9] Islam R, Cicek N, Sparling R, Levin DB. Effect of substrate loading on hydrogen production during anaerobic fermentation by *Clostridium thermocellum* 27405. Appl Microbiol Biotechnol 2006;72(3):576–83.
[10] Kyazze G, Martinez-Perez N, Dinsdale R, Premier GC, Hawkes FR, Guwy AJ, et al. Influence of substrate

concentration on the stability and yield of continuous biohydrogen production. Biotechnol Bioeng 2005;93(5):971–9.
[11] Lin C-Y, Cheng C-H. Fermentative hydrogen production from xylose using anaerobic mixed microflora. Int J Hydrogen Energy 2006;31(7):832–40.
[12] Van Ginkel S, Sung S, Lay J-J. Biohydrogen production as a function of pH and substrate concentration. Environ Sci Technol 2001;35:4726–30.
[13] Wu S-Y, Hung C-H, Lin C-N, Lee A-S, Chang J-S. Fermentative hydrogen production and bacterial community structure in high-rate anaerobic bioreactors containing silicone-immobilized and self-flocculated sludge. Biotechnol Bioeng 2006;93(5):934–46.
[14] Lay J-J, Lee Y-J, Noike T. Feasibility of biological hydrogen production from organic fraction of municipal solid waste. Water Res 1999;33(11):2579–86.
[15] Lin C-Y, Hung C-H, Chen C-H, Chung W-T, Cheng L-H. Effects of initial cultivation pH on fermentative hydrogen production from xylose using natual mixed cultures. Process Biochem 2006;41:1383–90.
[16] Abreu ÂA, Costa JC, Araya-Kroff P, Ferreira EC, Alves MM. Quantitative image analysis as a diagnostic tool for identifying structural changes during a revival process of anaerobic granular sludge. Water Res 2007;41:1473–80.
[17] Zehnder AJB, Huser BA, Brock TD, Wuhrmann K. Characterization of an acetate-decarboxylating, non-hydrogen-oxidizing methane bacterium. Arch Microbiol 1980;124:1–11.
[18] Owen WF, Stuckey DC, Healy JB, Young LY, McCarty PL. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Res 1979;13:485–92.
[19] APHA, AWWA, WPCF. Standard methods for the examination of water and wastewater. 17th ed. Washington, DC: American Public Health Association; 1989.
[20] Zwietering MH, Jogenburger I, Rombouts FM, Vaṅt Riet K. Modeling of the bacterial growth curve. Appl Environ Microbiol 1990;56(6):1875–81.
[21] Kotay SM, Das D. Microbial hydrogen production with *Bacillus coagulans* IIT-BT S1 isolated from anaerobic sewage sludge. Bioresour Technol 2006.
[22] O-Thong S, Prasertsan P, Karakashev D, Angelidaki I. Thermophilic fermentative hydrogen production by the newly isolated *Thermanobacterium thermosaccharolyticum* PSU-2. Int J Hydrogen Energy 2008.
[23] Van Niel EWJ, Claassen PAM, Stamms AJM. Substrate and product inhibition of hydrogen production by the extreme thermophile, *Caldicellulosiruptor saccharolyticus*. Biotechnol Bioeng 2003;81:254–62.
[24] Van Niel EWJ, Martens AA, Claassen PAM. Effect of carbon dioxide and temperature on H_2 producing cultures of the extreme thermophile, *Caldicellulosiruptor saccharolyticus*. In: Proceedings of the 15th World Hydrogen Energy Conference, Yokahama, Japan, 2004.
[25] Lin CY, Lay CH. Carbon/nitrogen ratio effect on fermentative hydrogen production by mixed microflora. Int J Hydrogen Energy 2004;29:41–5.
[26] Lin CY, Lay CH. Effects of carbonate and phosphate concentrations on hydrogen production using anaerobic sewage sludge. Int J Hydrogen Energy 2004;29:275–81.
[27] Yang H, Shen J. Effect of ferrous iron concentration on anaerobic bio-hydrogen production from soluble starch. Int J Hydrogen Energy 2006;31:2137–46.
[28] Zhu H, Béland M. Evaluation of alternative methods of preparing hydrogen producing seeds from digested wastewater sludge. Int J Hydrogen Energy 2006;31:1980–8.
[29] Wang Y, Mu Y, Yu H-Q. Comparative performance of two upflow anaerobic biohydrogen-producing reactors seeded with different sludges. Int J Hydrogen Energy 2007;32:1086–94.
[30] Zhao Q-B, Yu H-Q. Fermentative H_2 production in an upflow anaerobic sludge blanket reactor at various pH values. Bioresour Technol 2008;99:1353–8.
[31] Mu Y, Yu H-Q, Wang G. Evaluation of three methods for enriching H_2-producing cultures from anaerobic sludge. Enzyme Microb Technol 2007;40:947–53.