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Inoculum type response to different pHs on biohydrogen production from L-arabinose, a component of hemicellulosic biopolymers

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ABSTRACT

Biohydrogen production from arabinose was examined using four different anaerobic sludges with different pHs ranging from 4.5 to 8.0. Arabinose (30 g l^{-1}) was used as the substrate for all experiments. Individual cumulative hydrogen production data was used to estimate the three parameters of the modified Gompertz equation. Higher hydrogen production potentials were observed for higher pH values for all the sludges. G2 (acclimated granular sludge) showed the highest hydrogen production potential and percentage of arabinose consumption compared to the other sludges tested. Granular sludges (G1 and G2) showed different behaviour than the suspended sludges (S1 and S2). The differences were observed to be smaller lag phases, the percentage of acetate produced, the higher percentage of ethanol produced, and the amount of arabinose consumed. A high correlation ($R^2 = 0.973$) was observed between the percentage of *n*-butyrate and the percentage of ethanol in G1 sludge, suggesting that ethanol/butyrate fermentation was the dominant fermentative pathway followed by this sludge. In S1, however, the percentage of *n*-butyrate was highly correlated with the percentage of acetate ($R^2 = 0.980$). This study indicates that granular sludge can be used for larger pH ranges without reducing its capacity to consume arabinose and achieve higher hydrogen production potentials.

1. Introduction

Hydrogen is now considered one of the alternatives to fossil fuels. It is preferred to biogas or methane because hydrogen is not chemically bound to carbon and therefore, combustion does not contribute to green house gases or acid rain [1]. While there are numerous ways to produce H_2 from renewable energy sources, currently the majority of H_2 is produced from fossil fuels [2]. One alternative to sustainable H_2 energy production from renewable energy sources is through microbiological fermentation or photosynthesis. Dark fermentation produces H_2 at higher rates than photosynthesis and has the potential to combine organic waste management with simultaneous H_2 production [3].

Biological hydrogen production is affected by several environmental factors such as pH [4,5]. Fermentative hydrogen production occurs during the acidification stage and pH is one of the important factors that affect this process. A change in system pH may result in decreased process efficiency. In general, the optimum initial pH for biohydrogen production is generally reported to be between 5.0 and 6.0 [6–8]. However, there have been conflicted reports about the

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optimum pH value because the optimum pH in batch biohydrogen production was determined to be 9.0 with sucrose [9]. There have been many studies examining the effect of pH in fermentative hydrogen production from glucose and sucrose using mixed microflora [6,9–12]. Although the influence of pH on the fermentative biohydrogen production using arabinose, one of the most common pentoses and a component of various biopolymers such as hemicellulose, is not well known. Previous studies reported biohydrogen production from arabinose using mixed cultures but the effect of pH is not described [13,14]. The effect of pH on the biohydrogen production from arabinose was examined using a pure culture but the range of pH values tested was limited and the soluble microbial products were not identified [15]. Understanding the effect of pH is necessary to develop arabinose-based hydrogen fermentation applications, such as the use of agricultural wastes. The purpose of this study was to investigate the effect of initial pH on biohydrogen production from arabinose using mixed cultures in order to evaluate the feasibility of applying arabinose-based hydrogen fermentation in a continuous system.

2. Materials and methods

2.1. Batch experiments

2.1.1. Seed sludges

Four different biomasses were tested for hydrogen production as follows: S1 (disperse anaerobic digester sludge from municipal WWTP), S2 (disperse anaerobic digester sludge from municipal WWTP supplemented with fat), G1 (anaerobic granular sludge from industrial WWTP from brewery waste) and G2 from a hydrogen producing reactor fed glucose and L-arabinose (1/1) 5 g COD l^{-1} final concentration, during 120 d [16]. S1, S2 and G1 sludges were heat treated at 121 °C for 30 min to 2 h to inhibit methanogenic activity.

2.1.2. Experimental procedures

The experiments were conducted using 125 ml serum bottles. L-Arabinose was used as the substrate at an initial concentration of 30 g COD l^{-1}. Four series of batch experiments were conducted, one for each biomass.

Anaerobic buffer [17] (20 ml) was added to each vial containing 10 g VSS l^{-1} of biomass and nutrients for bacterial growth (18 ml l^{-1} of macronutrients – $MgSO_4 \cdot 7H_2O$: 30 g l^{-1}; KH_2PO_4: 28.3 g l^{-1}; NH_4Cl: 170 g l^{-1} and 1 ml l^{-1} of micronutrients – $FeCl_2 \cdot 6H_2O$: 2 g l^{-1}; H_3BO_3: 0.05 g l^{-1}; $ZnCl_2$: 0.05 g l^{-1}; $CuCl_2 \cdot 2H_2O$: 0.038 g l^{-1}; $MnCl_2 \cdot 4H_2O$: 0.5 g l^{-1}; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$: 0.05 g l^{-1}; $AlCl_3 \cdot 6H_2O$: 0.09 g l^{-1}; $CoCl_2 \cdot 6H_2O$: 2 g l^{-1}; $NiCl_2 \cdot 6H_2O$: 0.092 g l^{-1}; $Na_2SeO_3 \cdot 5H_2O$: 0.164 g l^{-1}; EDTA: 1 g l^{-1}; Resazurin: 0.2 g l^{-1}; HCl 37% [18]).

Eight different pHs (4.5; 5.0; 5.5; 6.0; 6.5; 7.0; 7.5; and 8.0) were tested in triplicate. The initial pH of individual bottles was adjusted adding HCl or NaOH and flushing with 100% N_2, 20% CO_2/80% N_2 or 100% CO_2. The bottles were sealed, placed on a rotary shaker (150 rpm), and incubated at 37 °C. Hydrogen, VFA, and ethanol concentrations for the control inoculum (0 g l^{-1} of substrate) were subtracted from values obtained for each pH.

Gas pressure was released using a glass syringe (20 and 50 ml capacity) by the Owen method [19]. The amount of gas present in the headspace of each bottle was determined before and after releasing gas pressure.

2.1.3. Monitoring and analysis

Soluble COD was determined according to Standard Methods [20]. Volatile fatty acids (VFAs) (formate, acetate, propionate, *iso*-butyrate, *n*-butyrate, valerate), ethanol, and L-arabinose were determined by high performance liquid chromatography (Jasco, Japan) with a Chrompack column (6.5 × 30 mm^2). Sulfuric acid (0.01 N) was used as mobile phase at a flow rate of 0.7 ml min^{-1}. The column temperature was set at 60 °C. Detection of soluble products was made sequentially with a UV detector at 210 nm (VFAs) and a Refraction Index (RI) detector (ethanol and L-arabinose), respectively.

Hydrogen in the headspace of bottles was determined by gas chromatography (GC) using a pressure-lock syringe (0.2 ml injection volume) and a Hayesep Q column (80/100 mesh) and thermal conductivity detector (Varian 3300 Gas Chromatograph) with nitrogen (30 ml min^{-1}) as the carrier gas. The injector, detector, and column temperatures were 120, 170, and 35 °C, respectively. Methane was analysed by GC using a Porapack Q (100–180 mesh) column with N_2 as the carrier gas (30 ml min^{-1}) and a thermal conductivity detector. The temperatures of the detector, injector, and oven were 110, 110 and 35 °C, respectively.

The modified Gompertz equation was used to describe the progress of cumulative hydrogen production obtained from the batch experiments [21,22]. Using the cumulative hydrogen production data, corrected to STP conditions (0 °C and 1 atm), the maximum hydrogen production rates were estimated from the fit of the modified Gompertz equation (equation (1)).

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

where $H(t)$ is cumulative hydrogen production (ml), P hydrogen production potential (ml), R_m maximum hydrogen production rate (ml h^{-1}), $e = 2.71828\ldots$, λ lag-phase time (h), and t time (h). R^2 values and the standard errors of each variable were calculated.

2.1.4. Principal Components Analysis (PCA)

Principal components (PC) analysis was used in order to find and interpret hidden complex relationships between features in a data set. PCA is a technique for summarizing the information contained in variables by a few weighted components as a mean of reducing the number of variables needed in an analysis. Correlating features were converted to the so-called factors which are themselves noncorrelated [23]. PCA modelling shows the correlation structure of data matrix X, approximating it by a first term $1 * \overline{X'}$ representing the variables' average plus a matrix product of lower dimension (TP'), called the principal components, plus a matrix of residuals (E).

$$X = 1 * \overline{X'} + TP' + E \quad (2)$$

SIMCA-P (Umetrics AB) software package was used to perform the PCA; it iteratively computes one principal component at a time, comprising a score vector t and a loading vector p. The score vectors contain information on how the samples relate

to each other (matrix *T*). Otherwise, the loading vectors define the reduced dimension space and contain information on how the variables relate to each other (matrix *P*). Usually, a few PC (2 or 3) can express most of the variability in the database when a high degree of correlation among data exists.

The criterion used to determine the model dimensionality (number of significant components) is cross-validation (CV). Part of data is kept out of the model development, and then is predicted by the model and compared with the actual values. The prediction error sum of squares (PRESS) is the squared differences between observed and predicted values for the data kept out of the model fitting. This procedure is repeated several times until the data elements have been kept out once and only once. Therefore, the final PRESS has contributions from all data. For every dimension, SIMCA computes the overall PRESS/SS, where SS is the residual sum of squares of the previous dimension. A component is considered significant if PRESS/SS is statistically smaller than 1.0.

2.1.5. *Partial Least Squares regression (PLS)*

PLS is an iterative algorithm that extracts linear combinations of the essential features of the original data *X* while modelling the *Y* data dependence on the data set, being well suited for multivariate calibration. The most important advantage of this method reports to the non-problematic handling of multicollinearities relying on an iterative algorithm, which makes possible the treatment of data with more features than objects [23].

In this method, the latent variables *u* (matrix *U*) are used for modelling the objects separately in the matrix of *Y* dependent data, whereas, the *t* variables (matrix *T*) are used for modelling the objects separately in the *X* matrix of independent data. The latent variables *U* and *T* are the basis of the regression model and are determined by:

$$U = AT + E \tag{3}$$

(PLS components matrix *A* and error matrix *E*) in an iterative process with the centred matrices of *X* and *Y* as starting points [23].

SIMCA-P (Umetrics AB) software package was used to perform PLS analysis from the data set. This software iteratively computes one PLS at a time, that is, one vector for each of *X*-scores (*t*), *Y*-scores (*u*), weights (*w*) expressing the correlation between *X* and *U*, weights (*c*) expressing the correlation between *Y* and *T* and loadings (*p*). The PLS components are calculated in descending order of importance. For the response variables (*m*) in *Y*, the multiple correlation coefficient (R^2Y_{cum}) or goodness of fit is given by:

$$R^2Y_{cum} = \sum R^2Y_a \tag{4}$$

where R^2Y_a is the sum of squares of the entire Y's explained by each extracted component (*a*).

3. Results and discussion

3.1. *Effect of pH on hydrogen production potentials, rates and lag times*

Biohydrogen production from arabinose was examined using initial pH values ranging from 4.5 to 8.0 for four different anaerobic sludges. The initial substrate concentration was 30 g l^{-1} COD for all experiments with 0 g l^{-1} serving as control. Individual cumulative hydrogen production data was used to estimate the three parameters of the modified Gompertz equation (maximum hydrogen production rate, hydrogen production potential, and duration of the lag phase). Hydrogen production occurred for all four sludges but there were differences in the yields, lag times, and rates (Table 1). Methane production was not detected in any of the batch cultures indicating that methanogenic activity was inhibited. pH was measured at the end of each batch experiment and the values were determined to be approximately 5.0 for all the biomasses tested (data not shown).

G1 was determined to have the highest hydrogen production potential (61.6 ± 0.1 ml) at pH of 6.5 while the highest hydrogen production rate (2.3 ± 0.2 ml h^{-1}) was obtained at a pH of 7.0. Also, the shortest lag time (10.6 ± 2.4 h) was detected at a pH of 8.0 (Table 1). G2 was determined to have the highest hydrogen production potential (137.2 ± 9.6 ml at pH 7.5) when compared with the other sludges tested. G2 showed the highest hydrogen production rate (2.9 ± 0.2 ml h^{-1}) at pH 7.5 and lower lag phase (11 ± 1.8 h at pH 7.0). Concerning the S1 sludge, the highest hydrogen production potential (51.1 ± 1.3 ml) and rate (2.8 ± 0.4 ml h^{-1}) occurred at a pH of 7.0. The shortest lag time was obtained with pH 6.0 (Table 1). For S2 sludge, the highest hydrogen production potential was observed with pH 8.0 (58.1 ± 1.8 ml) and the maximum rate (4.8 ± 1.4 ml h^{-1}) was obtained with pH 7.5 (Table 1).

Higher hydrogen production potentials corresponding to higher pH values have been observed in other studies [9]. When comparing all four sludges, G2 obtained the highest hydrogen production potential (137.2 ml) and S2 obtained the largest hydrogen production rate (4.8 ml h^{-1}) at a pH of 7.5, while G1 obtained the shortest lag time (10.6 h) at a pH of 8.0. Comparing these results with previous studies using mixed cultures [13] a higher hydrogen production potential and hydrogen production rates as well as a significant reduction in lag phases were obtained. Jianzheng et al. [13] reported a cumulative hydrogen yield of 34 ml, hydrogen production rate of 0.8 ml h^{-1} and a lag phase of 68 h using a pH of 6.

3.2. *Effect of pH on arabinose consumption and hydrogen yields*

Hydrogen yields were calculated for all batch reactors based on the amount of arabinose consumed and the amount of hydrogen produced. The results are shown in Table 1. The highest hydrogen yield was obtained with S2 (2.5 mol H_2 $mol^{-1}_{arabinose\ consumed}$) at pH 6.5. The highest hydrogen yield obtained for S1 was 2.0 mol H_2 $mol^{-1}_{arabinose\ consumed}$ with a pH of 7.0 and 8.0 and the highest hydrogen yield obtained for G2 was 1.5 mol H_2 $mol^{-1}_{arabinose\ consumed}$ with pH values of 6.0, 6.5, 7.5 and 8.0. G1 had the smallest hydrogen yield (1.3 mol H_2 $mol^{-1}_{arabinose\ consumed}$; pH = 6.5) when compared with the other biomasses. However, the minimum amount of arabinose consumed for G1 was at least 41% for all pH values. For S2, the highest percentage of arabinose consumed was 39.7% at pH 8.0. The highest percentage for S1 was only 33.3% at a pH

Table 1 – Modified Gompertz equation parameter values, percentage of arabinose consumed, COD balance, hydrogen yields for the different pH's tested.

pH	P (ml)	R_m (ml h^{-1})	Lambda (λ)	R^2	Arabinose consumed (%)	COD balance (%)	Yield (mol H_2 mol^{-1} arabinose consumed)
G1 sludge							
4.5	27.7 ± 0.6	0.7 ± 0.1	21.9 ± 2.3	0.99	42.1	105.3	0.8 ± 0.1
5.0	26.2 ± 0.5	0.9 ± 0.1	17.1 ± 1.2	0.99	41.1	107.4	0.8 ± 0.2
5.5	32.2 ± 0.7	0.9 ± 0.1	15.9 ± 1.9	0.99	44.8	115.3	0.8 ± 0.1
6.0	54.3 ± 1.5	1.2 ± 0.1	17.2 ± 1.9	0.99	50.5	99.6	1.2
6.5	61.6 ± 0.1	2.1 ± 0.1	15.2 ± 0.9	1.00	53.8	99.5	1.3
7.0	56.4 ± 1.2	2.3 ± 0.2	12.3 ± 1.2	0.99	52.0	100.6	1.2 ± 0.1
7.5	51.2 ± 1.1	2.0 ± 0.2	11.6 ± 1.4	0.99	52.6	100.3	1.1 ± 0.1
8.0	41.3 ± 1.7	1.8 ± 0.4	10.6 ± 2.4	0.96	54.6	93.3	0.9 ± 0.1
G2 sludge							
4.5	0.0	0.0	0.0	na	19.9	90.2	0.0
5.0	11.8 ± 0.1	0.9 ± 0.1	50.2 ± 0.7	0.99	24.8	87.0	0.5 ± 0.18
5.5	47.2 ± 0.4	2.1 ± 0.1	19.3 ± 0.6	0.99	50.0	93.7	1.1 ± 0.1
6.0	93.0 ± 0.4	2.6 ± 0.04	18.8 ± 0.3	0.99	72.3	109.8	1.5 ± 0.2
6.5	111.8 ± 1.4	2.9 ± 0.16	14.5 ± 1.1	0.99	80.7	116.4	1.5 ± 0.03
7.0	97.4 ± 1.9	2.3 ± 0.2	11.0 ± 1.8	0.99	75.7	117.0	1.4 ± 0.21
7.5	137.2 ± 9.6	1.7 ± 0.2	13.7 ± 4.7	0.97	92.4	115.8	1.5 ± 0.05
8.0	136.7 ± 6.4	1.9 ± 0.2	15.4 ± 3.3	0.98	97.6	114.5	1.5 ± 0.05
S1 sludge							
4.5	0.4 ± 0.04	1.0 ± 0.2	80.0 ± 0.2	0.89	8.8	104.8	0.1
5.0	11.3 ± 0.1	0.8 ± 0.04	56.1 ± 0.4	1.00	13.2	113.0	0.7 ± 0.1
5.5	19.3 ± 0.8	1.0 ± 0.2	25.9 ± 1.7	0.98	11.3	114.0	2.0 ± 0.6
6.0	24.4 ± 0.5	1.8 ± 0.2	18.7 ± 0.9	0.99	22.2	119.8	1.3 ± 0.3
6.5	38.0 ± 1.1	1.9 ± 0.3	29.5 ± 1.7	0.99	28.8	107.7	1.6 ± 0.1
7.0	51.1 ± 1.3	2.8 ± 0.4	24.1 ± 1.3	0.99	29.6	108.9	2.0 ± 0.1
7.5	47.4 ± 1.8	2.5 ± 0.5	22.6 ± 2.0	0.98	33.3	106.1	1.7
8.0	46.9 ± 1.2	2.2 ± 0.3	24.0 ± 1.4	0.99	28.5	106.5	2.0 ± 0.3
S2 sludge							
4.5	23.2 ± 1.3	0.5 ± 0.1	26.0 ± 2.9	0.98	19.9	95.2	1.3 ± 0.1
5.0	35.7 ± 2.1	1.2 ± 0.2	37.5 ± 2.8	0.98	24.9	95.1	1.5 ± 0.1
5.5	34.5 ± 1.5	1.4 ± 0.2	31.9 ± 2.1	0.99	23.0	101.9	1.7 ± 0.2
6.0	49.4 ± 0.5	1.9 ± 0.1	28.3 ± 0.6	1.00	22.1	101.3	2.5
6.5	54.4 ± 0.4	3.3 ± 0.1	27.9 ± 0.4	1.00	34.5	102.9	1.8 ± 0.2
7.0	47.1 ± 0.5	3.0 ± 0.4	19.4 ± 1.5	1.00	32.4	99.1	1.7 ± 0.1
7.5	56.3 ± 1.5	4.8 ± 1.4	32.8 ± 2.4	1.00	39.6	108.9	1.2 ± 0.7
8.0	58.1 ± 1.8	2.4 ± 0.3	28.2 ± 1.6	1.00	39.7	103.0	1.7

of 7.5. G2 was observed to have the highest percentage of arabinose consumption (97%) at a pH of 8.0.

The yields obtained in this study are less than the theoretical value (3.3 mol H_2 $mol^{-1}_{arabinose}$). Although, compared to the values obtained in a previous study that used xylose (pentose) (20 g COD l^{-1}) as a substrate [24] the maximum yields obtained in this study are slightly higher. The highest yield obtained in the previous study using xylose (2.25 mol H_2 mol^{-1}_{xylose}) was observed at a pH of 6.5, while in the present study we were able to obtain 2.5 mol H_2 $mol^{-1}_{arabinose\ consumed}$ using S2 at pH 6.0. The yields obtained in the present study were significantly higher than those obtained in a previous study that also used arabinose as the substrate (10 g l^{-1}) and mixed culture for hydrogen production (9.7 ml H_2 $g^{-1}_{arabinose\ consumed}$ corresponds to 0.05 mol H_2 $mol^{-1}_{arabinose\ consumed}$) [13]. The yields and amounts for hydrogen production were also different for this study when compared against the pure culture *Clostridium* (strain No. 2) fed with arabinose (10 g l^{-1}) [15]. The maximum yield for the strain No. 2 (2.2 mol H_2 $mol^{-1}_{arabinose\ consumed}$) was similar to S1 and S2 but was higher than the yields obtained with G1 and G2. The maximum amount of hydrogen production from *Clostridium* (strain No. 2) with controlled pH was 3600 ml H_2 l^{-1} culture and with uncontrolled pH was 2000 ml H_2 l^{-1} culture [15]. These values are similar to the maximum amounts of hydrogen production from S1 (2550 ml H_2 l^{-1} culture), S2 (2900 ml H_2 l^{-1} culture), and G1 (3100 ml H_2 l^{-1} culture). However, G2 produced almost twice as much hydrogen (6850 ml H_2 l^{-1} culture) as strain No. 2.

3.3. *Effect of pH on VFAs and ethanol production*

Soluble microbial products (SMPs) released during fermentation are often used to evaluate the efficiency of hydrogen production. The percentage of each VFA and ethanol at the end of each batch test for each pH tested is shown in Table 2.

For G1 sludge, the SMP production achieved a maximum concentration of 19 144 mg COD l^{-1} at pH 5.5 (Table 2). All other pH values produced approximately 15 000 mg COD l^{-1}. The total amount of SMP produced was higher when compared against the values obtained with S1 and S2. G2

Table 2 – Total COD from VFAs and ethanol and percentage of each soluble microbial product (SMP) at the end of each batch test, for the different pHs.

pH	VFAs + ethanol (mg COD l^{-1})	Percentage (%)						
		Formate	Acetate	Propionate	*i*-Butyrate	*n*-Butyrate	Valerate	Ethanol
G1 sludge								
4.5	15 257	0.0	3.4	0.0	0.0	13.8	0.0	82.8
5.0	15 710	0.0	3.8	0.0	0.0	14.4	0.0	81.8
5.5	19 144	0.1	8.4	0.0	0.0	28.6	0.0	62.9
6.0	15 050	0.1	4.7	0.0	0.0	23.4	0.0	71.8
6.5	15 765	0.3	6.1	0.0	0.0	29.6	0.0	63.9
7.0	15 734	0.8	4.9	0.0	0.0	27.7	0.0	66.5
7.5	15 945	0.6	6.2	0.0	0.0	28.4	0.0	64.8
8.0	14 643	0.6	3.7	0.0	0.0	34.4	0.0	61.2
G2 sludge								
4.5	574	0.0	0.0	0.0	0.0	17.8	0.0	82.2
5.0	2862	0.0	10.5	3.1	0.0	22.0	0.0	64.5
5.5	12 278	0.0	9.9	3.8	0.0	30.4	0.0	56.0
6.0	22 931	0.0	10.1	1.4	0.0	31.2	0.0	57.3
6.5	27 274	0.0	9.9	1.2	0.0	31.2	0.0	57.7
7.0	26 276	0.0	8.2	1.5	0.0	32.9	0.0	57.4
7.5	29 400	0.0	8.2	0.9	0.0	31.1	0.0	59.8
8.0	29 717	0.0	8.3	0.7	0.0	29.8	0.0	61.2
S1 sludge								
4.5	993	7.9	64.1	8.3	4.3	6.4	0.0	0.0
5.0	3976	1.6	24.1	3.7	0.9	48.8	0.0	19.7
5.5	5547	1.1	20.9	1.8	0.0	60.8	0.0	15.4
6.0	8119	1.0	17.6	1.6	0.6	64.8	0.0	15.7
6.5	10 224	1.7	23.3	0.0	0.4	57.0	0.2	16.2
7.0	10 410	0.9	19.7	0.0	0.0	62.3	0.2	16.2
7.5	10 779	1.1	19.7	0.0	0.0	61.5	0.2	17.3
8.0	10 340	1.2	17.9	0.0	0.0	62.2	0.4	17.1
S2 sludge								
4.5	4001	0.2	20.5	0.0	0.0	71.0	0.0	8.3
5.0	5168	0.2	23.4	0.0	0.0	70.5	0.0	5.8
5.5	5942	0.4	24.4	0.0	0.0	74.6	0.0	0.6
6.0	5433	0.9	33.5	0.0	0.0	61.0	0.0	4.3
6.5	9974	1.4	25.4	0.0	0.0	72.3	0.0	0.7
7.0	8271	0.9	23.1	0.0	0.0	74.8	0.0	0.4
7.5	11 097	1.8	24.1	1.5	0.3	54.9	0.0	16.9
8.0	11 465	0.7	22.2	0.0	0.3	60.0	0.0	16.5

obtained the highest SMP production compared to the other sludges (29 717 mg COD l^{-1} at pH 8.0). In addition, SMP was higher than 22 931 mg COD l^{-1} when pH values were higher than 5.5. The highest percentage of ethanol for all pH values was observed for G1 and G2 (Table 2). *n*-Butyrate was the second most abundant SMP for all pH values. Acetate was produced but corresponded to less than 6%. The presence of large amounts of ethanol and small amounts of acetate may be one of the reasons for the smaller hydrogen yields obtained with G1 and G2 even though higher percentages of arabinose consumption were observed. This suggests that the system was following an ethanol type fermentation [25,26].

Regarding the S1 sludge, the SMP production achieved a maximum concentration of 10 779 mg COD l^{-1} with a pH of 7.5 (Table 2). The most prominent SMP present for pH values greater than 4.5 was *n*-butyrate, corresponding to values between 50 and 65% of the SMP produced, followed by acetate (approximately 20%) and ethanol (approximately 16%) (Table 2). This suggests that the hydrogen is being produced via butyrate–acetate fermentation [27,28]. Acetate had the highest percentage of SMP production (approximately 70%) at a pH of 4.5. However, the amount of arabinose consumed was very low (8.8%).

S2 produced similar amounts of SMP to S1 although the distribution was slightly different. The highest amount of SMP (11 465 mg COD l^{-1}) was observed at a pH of 8.0 (Table 2). The most prominent SMP present was *n*-butyrate, corresponding to approximately 70% of the total SMP produced at pH values of 4.5, 5.0, 5.5, 6.5 and 7.0, approximately 60% with pH values of 6.0 and 8.0, and 55% with a pH of 7.5 (Table 2). Acetate was the second most abundant VFA for all pH values (approximately 20%), except at pH 6.0 (approximately 34%). This pH value (6.0) corresponded to the highest hydrogen yield (2.5 mol H_2 mol^{-1} arabinose consumed) and the highest percentage of acetate in all experiments, after pH 4.5 from S1 sludge. Ethanol was present in all samples corresponding to less than 10% of the SMP for all pH values except for 7.5 and 8.0. This suggests that the hydrogen is being produced via butyrate–acetate fermentation

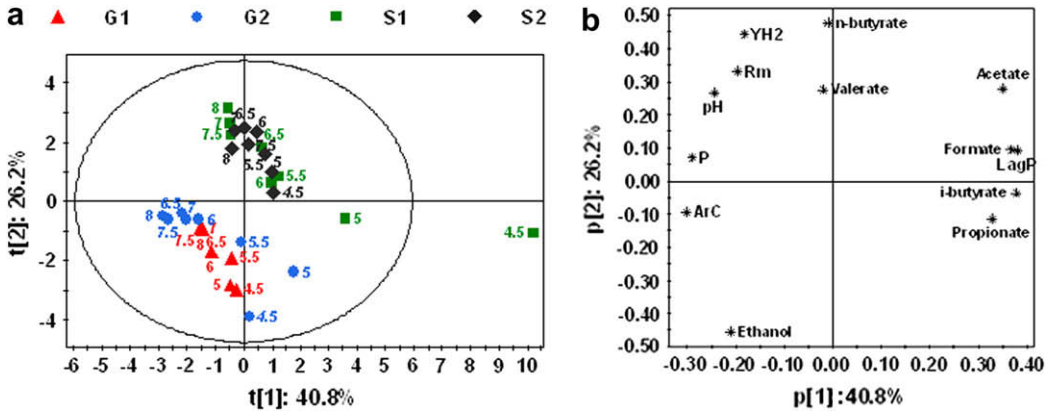

Fig. 1 – Score map (a) and loading map (b) obtained with Principal Components Analysis for all assays.

[27,28]. For all batch tests the COD balance indicated that the major metabolic products were identified (Table 1).

3.4. *Principal Components Analysis (PCA)*

Principal Components Analysis (PCA) was performed to visualize the main differences between the 4 biomasses tested. The data set consisted of 13 variables and 32 samples. All variables were autoscaled to unit variance, avoiding that some variables would be more important than others because of scale effects. The 3 first Principal Components (PC) contained 82.4% of the total variability present in the data set. The use of more components did not significantly improve the robustness of the model. The plane $t[1]$ *vs.* $t[2]$ (Fig. 1a) shows that the granular sludges (G1 and G2) presented different behaviour than the suspended biomasses (S1 and S2). The score (t_i) of an observation (i) on a principal component PC_j ($t_i PC_j$) is the weighted sum of the original variables (x_i). The weights (p_i) are called the loadings of the variables on that PC_j. The loading of a variable is related to its variation [29].

$$t_i(PC_j) = \sum [p_i(PC_j) * x_i] \quad (5)$$

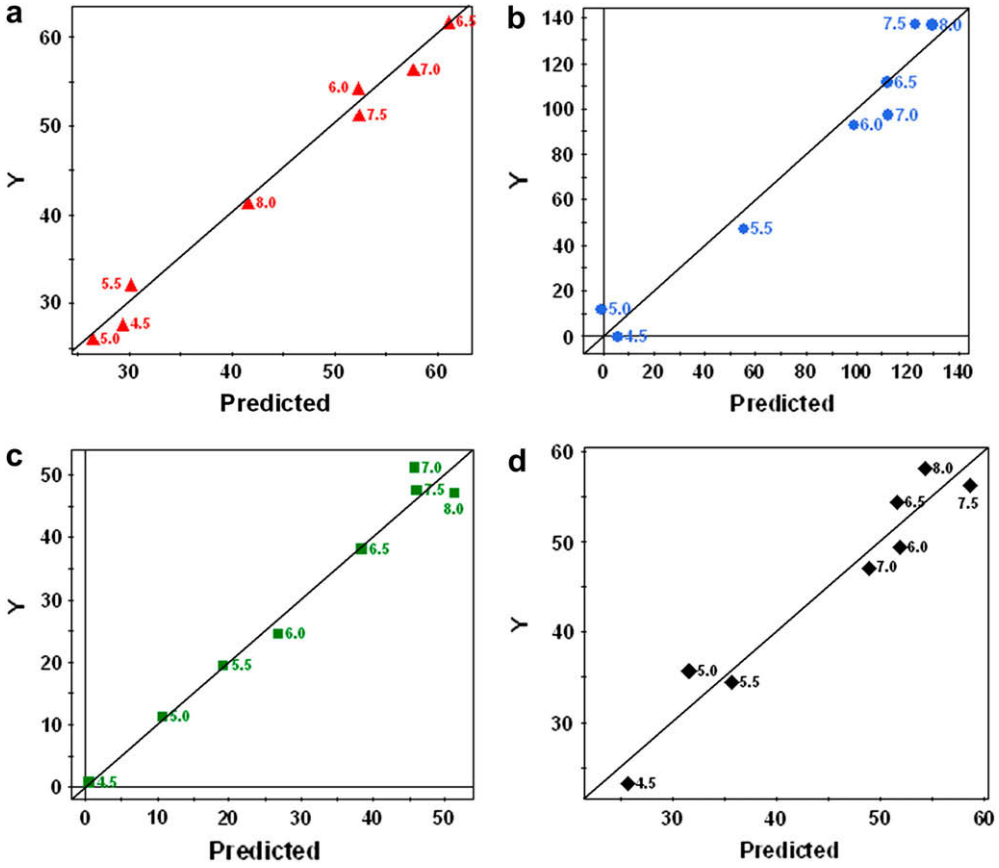

Fig. 2 – Hydrogen production potential (P), observed and predicted, with two latent variables for: (a) G1; (b) G2; (c) S1; and (d) S2.

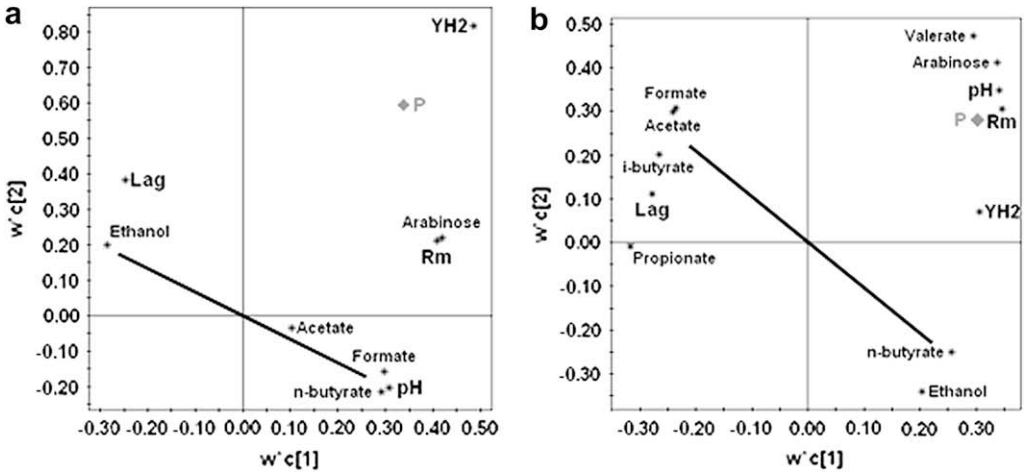

Fig. 3 – Loading maps for G (a), S1 (b), with *P* as *Y* variable.

Therefore, analyzing Fig. 1b (*p*[1] *vs.* *p*[2]) we verify that the differences of granular sludges compared to suspended sludges are explained by smaller concentrations of VFAs, hydrogen yield (Y_{H_2}) and percentages of acetate and *n*-butyrate, and also by higher % of ethanol and arabinose consumed.

The sample corresponding to a pH of 4.5 from S1 (see Fig. 1a) is an outlier of the model because it shows higher percentages of formate, propionate, *i*-butyrate, and acetate, and smaller percentages of ethanol and arabinose consumed, with large lag phases, and small H_2 production potentials (*P*).

3.5. *Partial Least Squares (PLS)*

In order to determine the relationship between parameters, a Partial Least Squares (PLS) regression was performed, individually, to each of the biomasses data sets with *P* (hydrogen production potential) as the *Y* variable, and lag-phase time, R_m (maximum hydrogen production rate), arabinose consumed, volatile fatty acids, and ethanol as the *X* variables.

When the PLS regression was performed no significant improvement in the prediction ability occurred for more than two latent variables in the *P* study attaining a value for the multiple correlation coefficient (goodness of fit) of 97.8, 94.2, 98.9, and 96.2%, respectively for the S1, S2, G1, and G2 sludge data sets (Fig. 2).

The loading plots w^*c display both the correlation between the *X*-weights (w^*) and *Y*-weights (c), and thereby the correlation structure between *X* and *Y*. One sees how the *X* and *Y* variables combine in the projections, and how the *X* variables relate to *Y* and to each other. These weights are selected so as to maximize the covariance between *T* and *U*, thereby indirectly *T* and *Y*. It is important to note that variables with equivalent (positive or negative) weights are highly correlated. The variables with similar weights (w^*c) are directly correlated, and variables are inversely proportional if their weights are symmetric, *i.e.* situated in opposite quadrants of the graph.

A high correlation ($R^2 = 0.973$) was observed between the percentage of *n*-butyrate and the percentage of ethanol for G1 sludge (Fig. 3a). This suggested that the fermentation is following the butyrate/ethanol pathway corresponding to the lower yields of hydrogen obtained.

It is shown in Fig. 3b, that the percentage of *n*-butyrate is highly correlated with the percentage of acetate ($R^2 = 0.980$) for the S1 sludge. This suggests that the system is following butyrate–acetate type fermentation with butyrate in excess.

3.6. *Acclimated granular sludge*

G2 sludge was obtained from hydrogen producing continuous system and the batch experiments revealed that this biomass achieved higher hydrogen production potentials and a higher percentage of arabinose consumption with a very large range of pHs (Table 1). This suggests that biomass acclimatization is very important to achieve higher hydrogen production values and higher percentages of substrate consumption. For a continuous system, high hydrogen production rates and small lag phases as well as tolerance to pH variations are essential. Suspended sludges showed higher yields of hydrogen production when compared to the granular sludges but were observed to have lower hydrogen production potentials and percentages of arabinose consumption and also longer lag phases. In general, granular sludges showed the highest hydrogen production potentials within a larger range of pH values that demonstrated a higher tolerance to pH changes. On the other hand, the maintenance of high biomass concentrations inside the reactors, such as those observed in granule-based systems, is necessary for a stable hydrogen production.

4. Conclusions

In the present study, all the sludges tested showed higher hydrogen production potential values with the utilization of higher initial pH values. Granular sludges obtained smaller lag phases and higher percentages of arabinose consumption. G2 (acclimatized granular sludge) showed highest hydrogen production potential values and percentage of arabinose consumption. Granular sludges (G1 and G2) showed different behaviour than the suspended sludges (S1 and S2). The differences were observed to be smaller lag phases, the percentage of acetate produced, the higher percentage of ethanol produced, and the amount of arabinose consumed.

The percentage of *n*-butyrate is highly correlated with the percentage of acetate ($R^2 = 0.980$) for S1 suggesting an acetate–butyrate main pathway. High correlation ($R^2 = 0.973$) was also observed between the percentage of *n*-butyrate and the percentage of ethanol for G1. This suggested that the fermentation is following the butyrate/ethanol pathways which corresponded to the lower yields of hydrogen obtained. This study suggests that acclimatization of biomass is very important to achieve higher hydrogen production potentials and substrate consumption. Granular sludge can be used for larger pH ranges without losing its hydrogen production potential and arabinose uptake capacity when compared with suspended sludges.

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REFERENCES

[1] Nath K, Das D. Improvement of fermentative hydrogen production: various approaches. Appl Microbiol Biotechnol 2004;65:520–9.
[2] Das D, Veziroglu TN. Hydrogen production by biological processes: a survey of literature. Int J Hydrogen Energy 2001; 26:13–28.
[3] Levin DB, Pitt L, Love M. Biohydrogen production: prospects and limitations to practical application. Int J Hydrogen Energy 2004;29:173–85.
[4] Kondratieva EN, Gogotov I. Production of molecular hydrogen in microorganisms. Adv Biochem Eng 1983;28:139–91.
[5] Dabrock B, Bahl H, Gottschalk G. Parameters affecting solvent production by *Clostridium pasteurianum*. Appl Environ Microbiol 1992;58:1233–9.
[6] Van Ginkel S, Sung S, Lay JJ. Biohydrogen production as a function of pH and substrate concentration. Environ Sci Technol 2001;35:4726–30.
[7] Fang HP, Liu H. Effect of pH on hydrogen production from glucose by a mixed culture. Bioresour Technol 2002;83:87–93.
[8] Sung S, Raskin L, Duangmance T, Padmasiri S, Simmons JJ. Hydrogen production by anaerobic microbial communities exposed to repeated heat treatments. In: Proceedings of the 2002 U.S. DOE hydrogen program review; 2002 [NREL/CO:610-32405].
[9] Kim IS, Hwang MH, Jang NJ, Hyun SH, Lee ST. Effect of low pH on the activity of hydrogen utilizing methanogen in bio-hydrogen process. Int J Hydrogen Energy 2004;29:1133–40.
[10] Mulin C, Junxin L, Yuansong W. Enhanced biohydrogen production from sewage sludge with alkaline pretreatment. Environ Sci Technol 2004;38:3195–202.
[11] Khanal SK, Chen W-H, Li L, Sung S. Biological hydrogen production: effects of pH and intermediate products. Int J Hydrogen Energy 2004;29:1123–31.
[12] Kawagoshi Y, Hino N, Fujimoto A, Nakao M, Fjita Y, Sugimura S, et al. Effect of inoculum conditioning on hydrogen fermentation and pH effect on bacterial community relevant to hydrogen production. J Biosci Bioeng 2005;100(5):524–30.
[13] Jianzheng L, Nanqui R, Baikun L, Zhi Q, Junguo H. Anaerobic biohydrogen production from monosaccharides by a mixed microbial community culture. Bioresour Technol 2008;99: 6528–37.
[14] Danko AS, Abreu AA, Alves MM. Effect of arabinose concentration on dark fermentation hydrogen production using different mixed cultures. Int J Hydrogen Energy 2008; 33:4527–33.
[15] Taguchi F, Mizukami N, Hasegawa K, Saito-Taki T. Microbial conversation of arabinose and xylose to hydrogen by newly isolated *Clostridium* sp. No. 2. Can J Microbiol 1994; 40:228–33.
[16] Abreu AA, Danko AS, Alves MM. Biohydrogen production with an EGSB reactor using chloroform and 2-bromoethanesulfonate as hydrogen-consuming bacteria inhibitors. In: Proceedings of the 10th international chemical and biological engineering conference (CHEMPOR), Braga, Portugal; 2008.
[17] Colleran E, Concannon F, Goldem T, Geoghegan F, Crumlish B, Killilea E, et al. Use of methanogenic activity tests to characterize anaerobic sludges, screen for anaerobic biodegradability and determine toxicity thresholds against individual anaerobic trophic groups and species. Water Sci Technol 1992;25:31–40.
[18] Zehnder AJB, Huser BA, Brock TD, Wuhrmann K. Characterization of an acetate-decarboxylating, non-hydrogen-oxidizing methane bacterium. Arch Microbiol 1980;124:1–11.
[19] Owen WF, Stuckey DC, Healy Jr JB, Young LY, MacCarty PL. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Res 1979;13:485–92.
[20] APHA, AWWA, WPC. Standard methods for the examination of water and wastewater. 17th ed. Washington, DC: American Public Health Association; 1989.
[21] Lay JJ, Lee YJ, Noike T. Feasibility of biological hydrogen production from organic fraction of municipal solid waste. Water Res 1999;33:2579–86.
[22] Zwietering MH, Jongenburger L, Rombouts FM, van't Riet K. Modelling the bacterial growth curve. Appl Environ Microbiol 1990;56:1875–81.
[23] Einax JW, Zwanziger HW, Geiss S. Chemometrics in environmental analysis. Weinheim: VCH; 1997.
[24] Lin CY, Cheng CH. Fermentative hydrogen production from xylose using anaerobic mixed microflora. Int J Hydrogen Energy 2006;31(7):832–40.
[25] Moat AG. Microbial physiology. New York: John Wiley & Sons; 1979. p. 123–89.
[26] Thauer RK, Jungermann K, Decker K. Energy conversion in chemotrophic anaerobic bacteria. Bacteriol Rev 1977;41(1): 100–80.
[27] Fang HHP, Lui H. Granulation of a hydrogen-producing acidogenic sludge. In: Proceedings part 2 of the 9th world congress on anaerobic digestion, vol. 2; 2001. p. 527–32.
[28] Noike T, Mizuno O. Hydrogen fermentation of organic municipal wastes. Water Sci Technol 2000;24(8):17–23.
[29] Massart DL, Heyden YV. From tables to visuals: principal component analysis, part 2. Practical data handling. LC/GC Europe 2005;18(2):84–9.