The effect of substrate concentration and pH in batch hydrogen production from cheese whey at ambient temperature

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ABSTRACT

The cheese whey (CW) is one of the main residues in dairy industry; it has a high content of organic matter that could be used as substrate for H_2 production. The H_2 production is affected by several factors such as substrate concentration and the initial pH. The H_2 production at ambient temperature could be interesting because of the savings of energy associated to process heating compared to operation at 35 or 55ºC. To the best of our knowledge, there are no works on H_2 fermentation of CW at low or ambient temperature.

Thus, the aim of this work was to determine the effect of substrate concentration and pH on batch hydrogen production of CW at ambient temperature and 35ºC. The experiment was a response surface based on 2^2 factorial with central and axial points with substrate concentration (10 to 35 g DQO/L), initial pH (4.8 to 6.2) and incubation temperature (ambient and 35ºC) as factors. The reactors consisted in vials with 20 mL of working volume (with phosphate buffer) and 0.5 g of inoculum (bioparticles of a fluidized bed reactor) with intermittent venting and periodic flushing with inert N_2 gas. At 35ºC only one cycle of hydrogen production was observed whereas two cycles was observed at ambient temperature. There was a significant effect of substrate concentration and pH on hydrogen production at both temperatures. 8.30 ± 1.28 and 4.05 ± 1.89 mmol H_2/g TS CW were observed and ambient temperature and 35ºC, respectively. The good performance at ambient temperature is a promising result for hydrogen production. The yields achieved at both temperatures are in the high side of the range of H_2 productions reported in the open literature for Hydrogen fermentation of CW.

1. Introduction

Hydrogen is one of the most promising biofuel because has the highest energy content per unit mass of any known fuel [1] and it generates only water in a controlled combustion [2-3]. The H_2 production could be performed by physico-chemical and biological processes [4-5]. The biological processes have the advantage that is performance at lower temperatures and pressure than the chemical or thermochemical technologies [6-7]. The biological process could be divided in: (*i*) photolysis; (*ii*) photo-fermentation and (*iii*) dark fermentation [8-9].

In dark fermentation, the pH, temperature and substrate (type and concentration) are fermentation conditions that could allow the yield increase of H_2 generation [10]. The pH has many effects on biohydrogen generation, some of the most important are: (*i*) pH could inhibit methanogenic archea (microorganisms that consume H_2; [11-12]; (*ii*) low pH could drive the fermentation to solvent production (with H_2 consume or not production; [13]; (*iii*) may influence the distribution of metabolites [14]. There are no consensus about which is the optimum pH range for H_2 production, some authors mentioned that the optimum range of pH is 5.2 - 6.0 using pure or mixed cultures of bacteria [15-17]. Khanal *et al.* [18] using sucrose and starch as substrate observed that the initial pH is affected strongly the H_2 production, and the best pH may depend of the substrate, type of inhibition treatment, inocula [19] and other fermentations conditions.

The incubation temperature could affect the growth rate, substrate degradation, metabolic activity of microorganisms and distribution of sub- products [20-21]. Many of the research of biohydrogen production are carried out at mesophilic and thermophilic range of temperature [14, 22] and few works are at ambient temperature. The H_2 production at ambient temperature has the advantage that could save energy that is use to conserve higher fermentation temperatures and allow a higher net energy gain of H_2 production [23].

The type of the substrate is very important due to the use of a cheap and renewable one could allow an economical and sustainable H_2 production [24]. The organic wastes are good candidates for be substrate for H_2 because are cheap and its re-use could help to the waste management [23, 25]. Regarding the substrate concentration, high concentrations could be inhibitory because: (*i*) pH depletion, (*ii*) acid production, or (*iii*) high H_2 partial pressures [26].

One of the organic wastes that could be used as substrate is cheese whey (CW). Cheese whey is the liquid that separates of the coagulation of milk, the CW composition depends of the quality of the milk, the type of technology used for cheese manufacture, type of cheese produced [27-28]. A cheese factory producing 40,000 liters of CW could generate a daily pollution similar to a population of 1,250,000 habitants [29]. It is known that CW has high content of organic matter and low bicarbonate alkalinity [30]; the last characteristics may difficult the CW treatment [29, 31]. For that is interesting to study the optimization of the concentration of the CW and the pH used for H_2 production at low temperature and mesophilic temperature. Thus, the aim of this work was to determine the effect of substrate concentration and pH on batch hydrogen production of CW at ambient temperature and 35ºC. To this goal we applied a statistical model known as response surface analysis [32].

2. Methodology

Experimental design and bioreactors

The experiment was a response surface based on 2^2 factorial with central and axial points with substrate concentration (10 to 35 g DQO/L), initial pH (4.8 to 6.2) and incubation temperature (ambient and 35ºC) as factors, the levels and the terms coded are shown in Table 1. The cumulative H_2 production (mmol H_2/ g_{TS}), the ratio between sum of organic acid production and sum of solvents production (ρ), and the ratio between the acetic acid production and butyric production (A/B) were the main response variables.

Table 1. Levels of independent variables in terms of coded and natural units.

Treatment	Range of levels			
	Coded		Actual	
	X_1	X_2	CW concentration (g COD/L)	Initial pH
1	+	+	35	6.2
2	+	-	35	4.8
3	-	+	10	6.2
4	-	-	10	4.8
5	0	0	22.5	5.5
6	0	0	22.5	5.5
7	0	0	22.5	5.5
8	0	0	22.5	5.5
9	0	0	22.5	5.5
10	+ α	0	40.2	5.5
11	- α	0	4.8	5.5
12	0	+ α	22.5	6.5
13	0	- α	22.5	4.5

Glass vials of 60 ml of capacity were used as bioreactors for batch biohydrogen production with a working volume of 20 mL and 0.5 g of inoculum. During the incubation, the H_2 concentration in the biogas was analyzed, the headspace was frequently released (intermittently vented) to maintain atmospheric pressure of 0.77 atm; when no more H_2 production was observed, the bioreactors' headspace was flushed with N_2 to wash-out the accumulated H_2 and re-incubated neither fresh inoculum nor substrate addition [33].

Inocula and substrate

Bioparticles from two Anaerobic Fluidized Bed Bioreactors that produced H_2 from CW (at ambient and 35ºC) were employed as inocula. The medium support of the bioparticles was activated carbon (average diameter 1-2 mm) colonized by a microbial consortium [34]. The substrate was powder CW, with the following characteristics: 0.85 % fat, 12.6% protein, 7.58% ash and pH of 6.3. The buffer solution used in order to have and maintain certainly pH was a buffer the phosphates.

Analyses

Hydrogen and methane concentration in biogas was determined using a gas chromatograph (Gow-Mac model 350). The chromatograph was fitted with a thermal conductivity detector and Molecular Sieve 5A packed column and argon as carries gas. The temperatures of the injector, detector and column temperatures were 25, 100 and 25 ºC, respectively [33-34].

The pH and soluble metabolites concentration were analyzed in the effluent. Metabolites (volatile organic acids and solvents) were determined in the effluent after filtration through a glass-membrane filter in a gas chromatography (Varian Star 3400) equipped with a flame ionization detector. A 50 m 0.32 mm internal diameter fused silica capillary column coated with 0.2 mm CP-Wax 57 CB was used. The injector and detector temperatures were set at 250ºC., N_2 was used as a carrier gas with a 20 mLmin-1 flow rate. The oven temperature was programmed as follows: 60 ºC for 2 min, increasing to 140 ºC at 5ºC/min, and then kept constant at 140ºC for another 6 min.

3. Results and discussion

Surface response 35ºC vs ambient temperature

There was observed a second cycle of H_2 production (after flushing) in the experiments at ambient temperature but at 35ºC not (Table 2). It is important to observe that most of the values of ratio ρ (organic acid sum/solvents sum) are higher at ambient temperature than at 35ºC, this could indicate that there was more solvents production at 35ºC than at ambient and it is known that the solvent production is related with low H_2 production [35-36].

The ratio A/B acetic-to-butyric acid could be used as parameter that may indicate the metabolic pathway favored in the fermentative process, values higher than the threshold (0.79; [37]) indicate that the H_2 production via HAc is likely predominant and values lower than 0.79 indicate that the H_2 production via HBu is likely predominant. The A/B values at ambient temperature are lower than the values at 35ºC, this may indicate that the main contribution to H_2 production is the HBu path.

Table 2. Cumulative H_2 production, ratio ρ and A/B at 35ºC and ambient temperature

	Conditions (g COD/L)/pH	35ºC			Ambient temperature			
		P_{H2} (mmol H_2/g_{TS})	ρ	A/B	P_{H2}(mmol H_2/g_{TS})		ρ	A/B
					1 cycle	2 cycle		
1	35 / 6.2	1.14 ± 0.58	7.53 ± 3.32	0.56 ± 0.09	2.87 ± 0.20	0.07 ± 0.03	15.33 ± 14. 98	0.027 ± 0.004
2	35 / 4.8	0.10 ± 0.01	12.21 ± 2.49	1.26 ± 0.60	0.59 ± 0.13	0.11 ± 0.08	6.03 ± 1.83	0.28 ± 0.21
3	10 / 6.2	3.53 ± 0.48	57.82 ± 6.07	0.44 ± 0.07	8.35 ± 1.24	0.02 ± 0.02	12.41 ± 8.24	0.02 ± 0.01
4	10 / 4.8	0.59 ± 0.16	2.00 ± 0.91	5.40 ± 1.66	1.30 ± 0.24	0.63 ± 0.37	3.12 ± 1.95	0.39 ± 0.33
5-9	22.5 / 5.5	1.42 ± 0.10	4.04 ± 0.99	0.29 ± 0.17	2.61 ± 0.26	0.29 ± 0.08	11.79 ± 6.38	0.10 ± 0.10
10	40.2 /5.5	0.42 ± 0.06	13.83 ± 1.92	0.11 ± 0.02	1.79 ± 0.15	0.09 ± 0.09	10.53 ± 4.27	0.19 ± 0.19
11	4.8 /5.5	4.05 ± 1.89	1.04 ± 0.09	2.23 ± 0.18	6.51 ± 0.48	0.53 ± 0.16	23.26 ± 23.26	0.24 ± 0.12
12	22.5 / 6.5	2.70 ± 0.51	7.91 ± 1.37	0.48 ± 0.02	4.90 ± 0.45	0.67 ± 0.67	10.84 ± 10.84	0.35 ± 0.04
13	22.5 /4.5	0.00 ± 0.00	4.01 ± 0.10	2.01 ± 0.95	0.14 ± 0.05	0.01 ± 0.01	8.90 ± 1.32	5.88 ± 5.88

Notes: [a] H_2 cumulative production (mmolH_2/gTS); [b] Ratio between sum of organic acid production and sum of solvents production (dimensionless); [c] ratio between the acetic acid production and butyric production (dimensionless).

The cumulative H_2 production at ambient temperature (two cycles) was almost two fold of that obtained at 35ºC. The ANOVA analysis with Design Expert 6.0 (Table 3) shows that the substrate concentration and pH had significant effects on cumulative H_2 production at both temperatures, whereas the interaction of those two factors was only significant at ambient temperature.

Table 3. Significance probabilities of ANOVAs of the response variables

Temperature	Source	Response variables		
		P_{H2} [a]	ρ [b]	A/B [c]
35ºC	Substrate concentration	*< 0.0001* *significant*	*< 0.0001* *Significant*	0.0020 *Significant*
	pH	*< 0.0001* *significant*	0.3733 *not significant*	0.0008 *Significant*
	Interaction	*0.0682* *not significant*	0.0307 *Significant*	0.0060 *Significant*
Ambient	Substrate concentration	*< 0.0001* *Significant*	0.36 *not significant*	0.9607 *not significant*
	pH	*< 0.0001* *significant*	1.10 *not significant*	0.0385 *Significant*
	Interaction	*< 0.0001* *significant*	*5.351E-008* *Significant*	0.9662 *not significant*

Notes: [a] H_2 cumulative production (mmolH_2/gTS); [b] Ratio between sum of organic acid production and sum of solvents production (dimensionless); [c] ratio between the acetic acid production and butyric production.

The final equations in terms of actual factors of are shown in Table 4. The higher cumulative H_2 observed in the experiments at ambient temperature than at 35ºC is congruent with the higher value predicted by the model equations.

Table 4. Final equations in Terms of Actual Factors

Temperature	Source or coefficient	Response variables		
		P_{H2} [a]	ρ [b]	A/B [c]
35ºC	b_o, Independent	*- 11.07854*	*- 253.47070*	+ 25.57137
	b_1, Substrate concentration	*+ 0.21971*	+ 9.28572	- 0.73877
	b_2, pH	+ 2.62221	+ 49.00168	- 4.13335
	b_{12}, Interaction	*- 0.054571*	- 1.72829	+ 0.12157
Ambient	b_o, Independent	- 27.37059	- 6.82911	+ 9.51479
	b_1, Substrate concentration	+ 0.62654	- 0.12214	-0.020254
	b_2, pH	+ 6.13611	+ 3.80946	-1.57997
	b_{12}, Interaction	- 0.13800	+ 9.52381E-005	+ 3.33333E-003

Notes: [a] H_2 cumulative production (mmolH_2/gTS); [b] Ratio between sum of organic acid production and sum of solvents production (dimensionless); [c] ratio between the acetic acid production and butyric production (dimensionless);.

In Figure 1(a) and 2 (a) we can observe the response surface of H_2 accumulation , with low substrate concentration and high pH value it can be produced higher H_2 than with high substrate concentration of CW and low pH. A similar

pattern was observed in the response surface of the ratio ρ at both temperatures (Figures 1b and 2b). This is congruent with the fact that low solvent concentration is related to high H_2 production. It is known that some cultures fed with high substrate concentrations are susceptible to product inhibition [38].

Surface response at 35ºC

The maximum H_2 accumulation (4.05 ± 1.89 $mmolH_2/g$ TS) was obtained with 4.8 gDQO/L and pH of 5.5 (treatment 11) whereas with 22.5 gDQO/L and pH of 4.5 (treatment 13) no H_2 production was detected (Table 2). The treatment 13 has the lower value of pH of all treatments, this value is considered for some authors as the threshold to solvent generation [13, 39] and this is congruent with the higher ratio ρ obtained with treatment 11 than with treatment 13 (1.5 fold).

Surface response at ambient temperature

The maximum H_2 accumulation (8.38 ± 1.28 $mmolH_2/g$ TS) was obtained with 10 gDQO/L and pH of 6.2 (treatment 3) whereas whit 22.5 gDQO/L and pH of 4.5 (treatment 13) almost no H_2 production was detected (Table 2).

Comparison with other H_2 production using cheese whey as substrate.

The maximum cumulative H_2 production at ambient temperature (8.38 $mmolH_2/g_{TS}$) and 35ºC (4.5 $mmolH_2/g_{TS}$) is in the high range of H_2 production using cheese whey as substrate (Table 5). The good performance at ambient temperature is a promising result for hydrogen production.

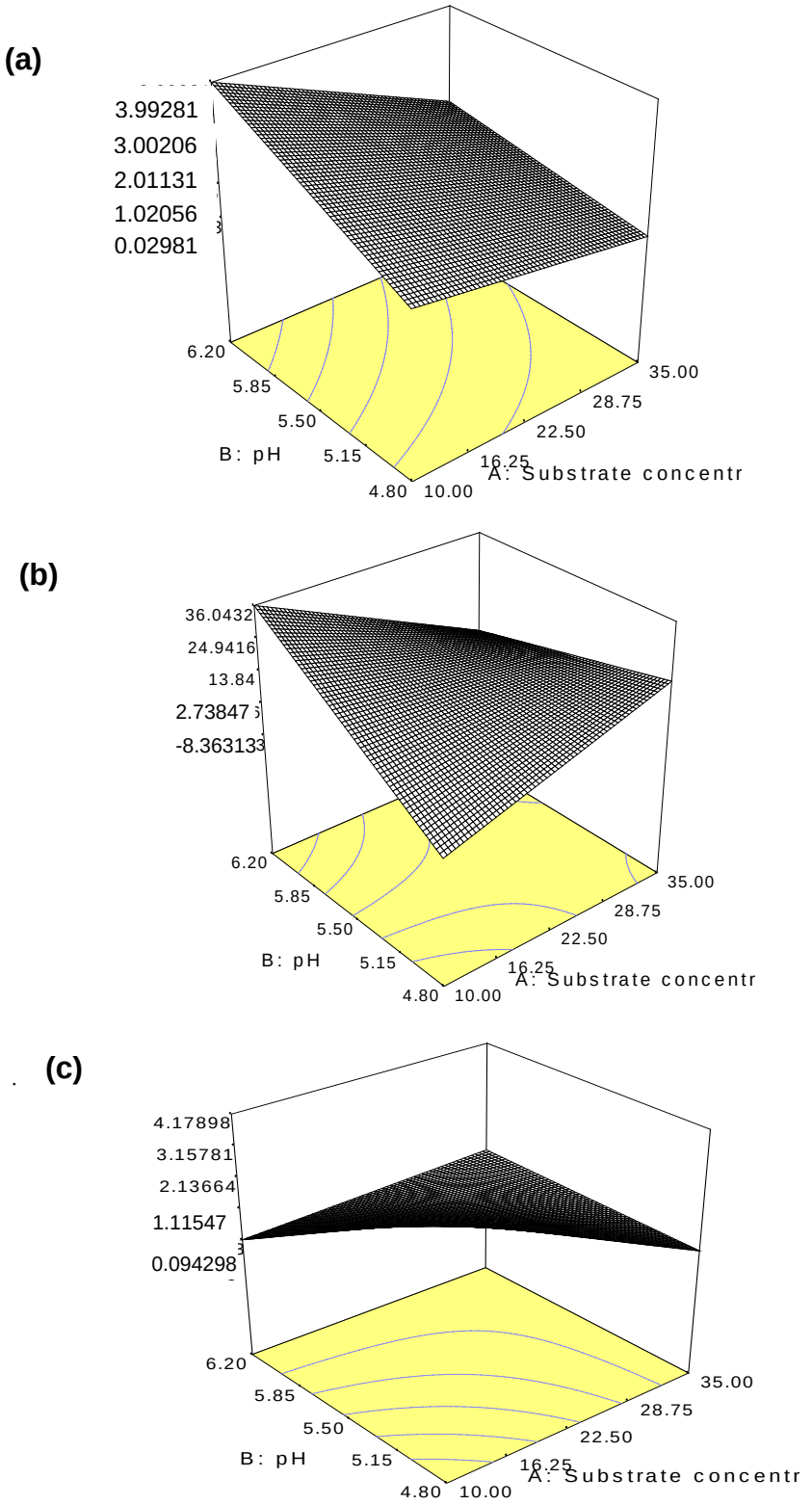

Figure 1. Response surface of : a) Cumulative H_2 production ; b) Ratio ρ and c) Ratio A/B at 35ºC.

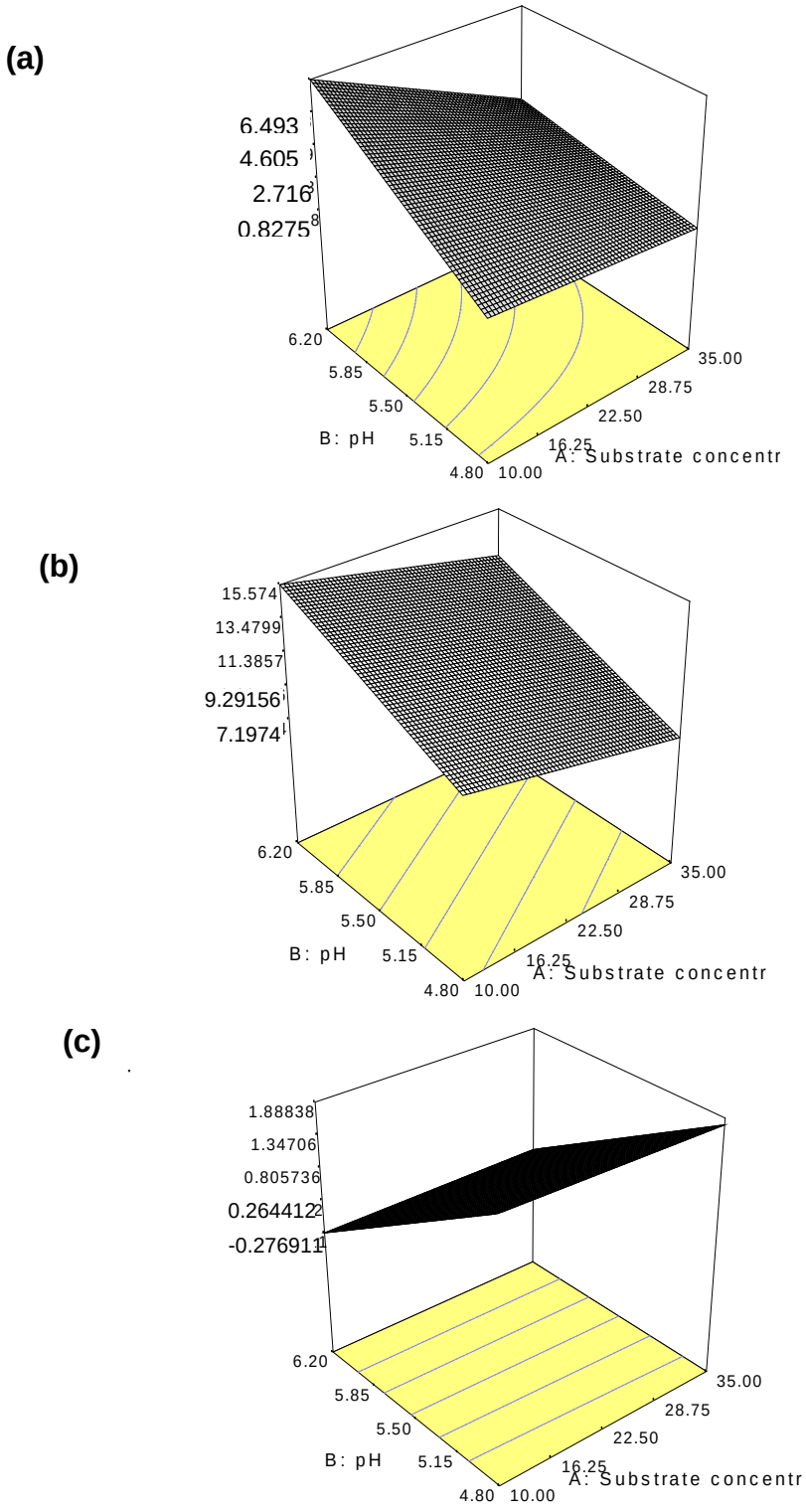

Figure 2. Response surface of : a) Cumulative H_2 production ; b) Ratio ρ and c) Ratio A/B at ambient temperature.

Table 5. Hydrogen production using cheese whey as substrate

Inocula	Conditions	Substrate Volumetric loading rate (gCOD/(L*day))	Y' Hydrogen Pseudoyield ($mmolH_2/gTS^{-1}$)	Soluble microbial products	Ref
Sludge Heat shock treatment (85°C/45 min)	HRT[a]: 1, 2 and 3.5 d T: 55ºC pH 5.5 Continuous	Fresh raw cheese whey with heat shock treatment (105°C/ 5min) 47	0.91	Acetate, butirate, propionate, isobutyrate, isocaprionate, formate, lactate	[40]
Acidogenic lab-scale reactor	HRT=12 h pH=5 T=30°C Continuous	Cheese whey stored at 4ºC 20	0.18	Acetate, butyrate, lactate and valerate	[41]
Digestates of a CSTR[b]	HRT = 24 h T:35°C pH: 5.2 Continuous	Cheese whey stored at -20ºC 30	1.5	Acetate, butyrate, lactate	[42]
Anaerobic mixed microflora from an UASB[c] Heat shock treatment	HRT = 24 h T:55ºC pH=5.5 Continuous	Cheese whey with heat shock pretreatment 85°C / 30 min 30	3.5	Lactate, butyrate	[43]
Bioparticles of AFBR[d]	Batch T: ambient; 35ºC pH: 5.9	Powder 10.04* With phosphate buffer	3.07 At 18ºC 2.95 At 35ºC	Acetate, propionate, butyrate, ethanol, butanol, lactate	[44]
Bioparticles of AFBR[d]	Batch T: ambient; pH: 6.2 35ºC pH: 5.5	Powder 10.04* With phosphate buffer 10 22.5	 8.38 At ambient 4.5 At 35ºC	Acetate, propionate, butyrate, ethanol, butanol	This study

Notes: [a] Hydraulic retention time; [b] continuous stirred tank reactor; [c] upflow anaerobic sludge blanket reactor; [d] anaerobic fluidized bed reactor. *Substrate concentration (g COD/L).

4. Conclusion

- At 35ºC only one cycle of hydrogen production was observed whereas two cycles was observed at ambient temperature in batch bioreactors fed with cheese whey.
- There were significant effects of substrate concentration and initial pH on hydrogen production at both temperatures.
- The maximum yields of 8.30 ± 1.28 and 4.05 ± 1.89 mmol H_2/g dry CW were observed and ambient temperature and 35ºC, respectively.
- The good performance at ambient temperature is a promising result for hydrogen production.

On the one hand, the yields achieved at both temperatures are in the high side of the range of H_2 productions reported in the open literature for Hydrogen fermentation of CW. On the other hand, the highest results obtained at ambient temperature strongly suggests the implementation of a dark fermentation bioH2-producing process with important energy savings.

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