Effect of Temperature and Addition of Buffer on Biohydrogen Production from Cheese Whey

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ABSTRACT

One of the principal residues of dairy industry is the cheese whey (CW). CW is a source of contamination if it is discharged without treatment. One treatment of CW could be its fermentation for H_2 production due to its high content of organic matter. The pH and the incubation temperature are variables that could affect the H_2 production, rate of production and metabolites generation. Thus, the aim of this work was to evaluate the H_2 production (with cheese whey as substrate) in batch fermentation at two levels of operational temperature: ambient temperature 18oC average (A) and 35 °C (M) and the presence of absence of phosphate buffer. The fermentation was carried out with intermittent venting and headspace flushing. The reactors consisted in vials with 40 mL of working volume (10 g CW/L) and 1g of inoculum (bioparticles of a fluidized bed reactor). The presence of buffer had positive effects in H_2 production and 48% more H_2 was obtained compared to unbuffered reactors. Two cycles of H_2 production were achieved whereas unbuffered reactors only showed one cycle of H_2 production. Regarding the process temperatures there was no significant difference between the H_2 production of reactors incubated at ambient temperature and a 35ºC. This means that ambient temperature was as effective for H_2 production as mesophilic operation. This is novel result with several practical advantages. Maximum yields of 3.1 mmol H_2/g_{TS} CW were observed at ambient temperature and buffered feedstock. This amount is 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 attractive biofuels, because it is a clean energy carrier [1-2]; it has an energy content per unit mass of 142 kJ/g o 61,000 Btu/lb[3] that is almost 3 times greater than that in some hydrocarbon [4] and it is more secure to utilize than domestic natural gas [3]. An important point to consider

for H_2 generation is the sustainability, that depends on several aspects: (*i*) the availability of low cost, (*ii*) the use of renewable substrate, and (*iii*) the establishment of fermentation conditions that augment both the rate and the yield of hydrogen production, among others [5].

The type of substrate, temperature and alkalinity are fermentation conditions that could allow the increment the H_2 production. [1, 6-8]. The incubation temperature can affect the growth rate and metabolic activity of microorganisms, the distribution of aqueous products and substrate degradation [7]. The optimal temperature for H_2 production by mixed-culture systems varied widely due to the presence of more complex bacterial populations [9]. The selection of the temperature of operation should contemplate that the heat energy required to preserve higher operation temperatures can abate the net energy gain of biofuel production [10].

The pH may influence the microorganisms in several ways: (i) it impacts on the electrical charge of the cell membranes, (ii) could influence the uptake of nutrients and (iii) it has an important effect on enzymatic activities such as (hydrogenasa) ; (iv) determine product distribution in fermentation [11-15]. In H_2 production can also inhibit hydrogen- consuming methanogenic microorganisms [16]. Decay of pH associated to low contents of alkalinity and organic acids concentration imbalances might result in arrest of H_2 production [17]. An alternative is to add the sufficient buffer to compensate for the pH decrease resulting from the generation of organic acids [18]. Ferchichi *et al*., [5] suggested that could be increased the H_2 production by regulating the pH.

Regarding the substrate, several authors have demonstrated that the use of organic waste is viable. One residue that could be employed for H_2 production is the cheese whey (CW). CW is the liquid that apart from the milk during cheese generation, it has the main of the water soluble components that were not integrated in the coagulation of casein. CW is considered a residue of dairy industry and corresponds to around 85–90% of the total volume of processed milk and its cost-effective utilization or disposal has become more and more important due to the legislative demands [19].

There are different schemes of CW recovery: (i) use in fermentative processes, as culture medium for biomass, poly-(3-hydroxybutyrate), enzymes or biofuels production; (ii) beverage manufacture, and (iii) biofilm production [20-22]. Despite the technological developments for CW reuse and reclaiming, its disposal remains to be an important issue in the dairy industry. There are works that produced H_2 from CW at

mesophilic and thermophilic range of temperature [23-26] but little is known on dark fermentation of CW at ambient temperature. Therefore the study the H_2 production at ambient temperature is interesting due to it could save processes energy expenses such as heating.

The aim of this work was to evaluate the effect of buffer addition and process temperature (two levels, ambient (A) and 35 °C (M)), on H_2 production in batch fermentation of CW.

2. Methodology

2.1.Experimental design and bioreactors

The experimental design examined the effect of two factors on H_2 production in batch fermentation (using cheese whey as substrate), i.e., phosphate buffer addition (PB) and process temperature at two levels, ambient (A) and 35 °C (M). The main response variables were: cumulative H_2 production (mmol H_2/g_{TS}), initial rate of hydrogen accumulation (mmol $H_2/(g_{TS}*h)$), lag time of H_2 production (h), A/B (defines as the ratio between the acetic acid production and butyric production) and ρ (defined as the ratio between sum of organic acid production and sum of solvents production).

The bioreactors were glass vials of 60 ml of capacity with 40 mL of working volume and 1 g of inoculum. The fermentation was carried on batch mode with intermittent venting and headspace flushing according to procedures reported by Valdez-Vazquez *et al*. [27]. During the incubation the biogas in headspace was frequently released (intermittently vented) to maintain atmospheric pressure of 0.77 atm., and when a maximum H_2 cumulative production was observed (no more H_2 production), the reactors' headspace was flushed with N_2 to wash-out the accumulated H_2. Afterwards, the bottles were re- incubated; neither fresh inoculum nor substrate was added.

2.2. Inocula and substrate

The inocula were bioparticles from two Anerobic Fluidized Bioreactors (AFBR's) that produced H_2 from cheese whey; one AFBR was operated at ambient temperature and one at 35ºC. The structure of the reactors is similar to those of [28-29]. The substrate was powder cheese whey (10 g CW/L). The reactors without buffer (A-NPB and M-NBP) were fed with a synthetic wastewater with the following composition (mg/L; [30-31]): CH_4N_2O (125); $NiSO_4*6H_2O$ (1); $FeSO_4*7H_2O$ (5); $FeCl_3*6H_2O$ (0.5); $CoCl_2*2H_2O$

(0.08); $CaCl_2*6H_2O$ (47); SeO_2 (0.07); KH_2PO_4 (85); K_2HPO_4 (21.7); $Na_2HPO_4*2H_2O$ (33.4); $NaHCO_3$ (1000). The buffer solution used in buffered units (A-PB and M-PB) was composed by 24.5 g/L of KH_2PO_4 and 3.5 g/L K_2HPO_4.

2.3. Analyses

The main monitoring parameters were hydrogen and methane concentration in biogas; pH and soluble concentration in the effluent. Hydrogen and methane contents in biogas were determined by gas chromatography [32,33] in a Gow-Mac chromatograph (model 350) fitted with a thermal conductivity detector and Molecular Sieve 5A packed column (injector, detector and column temperatures were 25, 100 and 25 ºC, respectively) and argon as carrier gas.

Soluble metabolites (volatile organic acids and solvents) were determined in the effluent, after filtration through a glass-membrane filter in a gas chromatograph (Varian Star 3400) equipped with a flame ionization detector. The injector and detector temperatures were set at 250ºC. 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. A 50 m 0.32 mm internal diameter fused silica capillary column coated with 0.2 mm CP-Wax 57 CB was used. Nitrogen was used as a carrier gas with a 20 mL/min flow rate. Lactic acid was measurement as [34] reported.

3. Results and discussion

3.1. Effect of buffer addition

The addition of buffer showed a positive effect on H_2 production. A 2nd cycle of H_2 production (after the headspace flushing) was observed at both temperatures (Fig. 1). The H_2 production in the 2nd cycle was approximately 60% of that obtained in the 1st cycle alone (Table 1). No CH_4 was observed in biogas of our batch bioreactors. The lag time was less than 24 h in all cases in our work.

In the 1st cycle the units with buffer (PB) had 1.3 times more cumulative H_2 production than non-buffered ones (NPB; 1.85 ± 0.14 and 1.37± 0.07 mmol H_2 /g_{TS}, respectively, Table1) and lower initial rate of H_2 accumulation (0.6 times) than NPB. Higher cumulative H_2 production in PB compared to NPB could be due to a lower ΔpH (1.9; defined as pH initial – pH final) than ΔpH in NPB (2.8). The high ΔpH (lower final pH)

could affect the H_2 producers, the microorganisms could not adjust to the fast change [35] and the fermentation could be driven to solvent production with detriment of H_2 accumulation. Acetic acid (HAc), butyric acid (HBu), propionic acid (HPr), lactic acid (HLac), ethanol (EtOH) and butanol (BuOH) were the soluble microbial products (SMP) detected in this fermentation (Table 2).

The A/B in all reactors were lower than the threshold value (0.79; [36]) and it can be said that the H_2 production via HBu was likely predominant. There was a significant difference in HBu production between buffered and non-buffered reactors.It is important to mention that the HBu production in non- buffered was 1.3 fold higher than in PB reactors despite that there was only one cycle of hydrogen production in non-buffered ones.

There are several studies that relate high concentration of HBu with low H_2 production [37]. The undissociated HBu acid was related with the initiation of solventogenesis [38] and in our work the ratio ρ in non-buffered reactors were less than in PB indicating a high solvent production. It is known that butyrate production regenerates NAD^+ through both H_2 production and butrylphosphate reduction, but, if the butyrate production pathway is inhibited by excess butyrate, and if H_2 production is disadvantageous because of high concentrations of dissolved hydrogen, the only electron sink left is by solvent production which does not result in any H_2 production [37]. Van den Heuvel *et al.* [39] determined that the critical inhibitory concentration of undissociated HBu was 50 mM for a metabolic switch to solventogenesis; in our work the unbuffered units exhibited a HBu concentration nearly that value (49.59) in the first cycle, whereas the PB units had 37.5 after the 2^{nd} cycle.

3.2. Effect of temperature

Regarding the process temperature there was no significant difference between the cumulative H_2 production, H_2 production rate, concentration of organic acid and ρ of units incubated at ambient temperature and a 35ºC. Several authors reported highest H_2 production at higher temperatures [7, 40-42]. Yet, in our work we obtained a good performance with the ambient temperature. It is important to mention that the inocula of batch fermentation comes from Fluidized Bed Reactors that had been operated with sucrose, during the

Table 1.Hydrogen production, initial rate of hydrogen accumulation, pH and ΔpH of the batch fermentation of cheese whey.

Reactor	P_H (mmol H_2/g_{TS})			$R_{i,H}$ (mmol $H_2/(g_{TS}*h)$)		Initial pH	Final pH	ΔpH
	1 Cycle	2 Cycle	Total	1 Cycle	2 Cycle			
A-NPB	1.37 ± 0.14	-------	1.37 ± 0.14	0.031	-----	7.39	4.70 ± 0.08	2.70 ± 0.08
A-PB	1.71 ± 0.02	1.35 ± 0.15	3.07 ± 0.13	0.025	0.028	5.9	4.02 ± 0.03	1.88 ± 0.03
M- NPB	1.36 ± 0.01	0.15 ± 0.13	1.51 ± 0.12	0.040	-----	7.39	4.47 ± 0.01	2.93 ± 0.01
M-PB	1.99 ± 0.03	0.96 ± 0.46	2.95 ± 0.13	0.016	0.02	5.9	3.99 ± 0.02	1.92 ± 0.02
PB*	1.85 ± 0.20	1.16 ±0.28	3.01 ± 0.08	0.035	0.024	5.9	4.00 ± 0.03	1.90 ± 0.03
NPB**	1.37 ± 0.001	0.07 ± 0.10	1.44 ± 0.10	0.021	----	7.39	4.58 ± 0.14	2.81 ± 0.14
A***	1.54 ± 0.25	0.68 ± ---	2.22 ± 1.20	0.023	0.014	6.65	4.36 ± 0.39	2.29 ± 0.47
M****	1.68 ± 0.45	0.55 ± ---	2.23 ± 1.02	0.032	0.01	6.65	4.23 ± 0.28	2.42 ± 0.58

Notes: A-NPB: ambient temperature without buffer ; M-NPB: 35ºC without buffer; A-PB: ambient temperature with buffer; M-PB: 35ºC with buffer. P_H: Cumulative H_2 production, $R_{i,H}$ initial rate of hydrogen accumulation. *Average result calculated by pooling A-PB and M-PB results. ** Average result calculated by pooling A-NPB and M-NPB results. *** Average result calculated by pooling A-NPB and A-PB results. **** Average result calculated by pooling M-NPB and M-PB results. The lag time T_{lag} for hydrogen production onset in all reactors was < 24 h.

Table 2. Acid and solvent concentration of hydrogen production with cheese whey

Concentration (mg COD/ L)	A-NPB	A-PB	M-NPB	M-PB
Acetic acid	1 308 ± 207	1 675 ± 591	1 966 ± 232	1 462 ± 883
Propionic acid	523 ± 105	1 783 ± 845	400 ± 60	671 ± 43
Butyric acid	4362 ± 186	3 136 ± 125	4337 ± 255	3 464 ± 304
Lactic acid	LDL	389 ± 70	LDL	367 ± 7
Acetone	LDL	LDL	LDL	LDL
Methanol	LDL	LDL	LDL	LDL
Ethanol	304 ± 161	55.1 ± 14.7	128 ± 26	173 ± 26
Butanol	1422 ± 109	56.5 ± 41.8	217 ± 47	86.7 ± 38.
EtOH/SMP (%)	3.9 ± 2.2	0.8 ± 0.2	1.8 ± 0.3	2.9 ± 0.7
BuOH/SMP (%)	18.3 ± 1.5	1.2 ± 1.0	3.1 ± 0.5	1.5 ± 0.8
HAc/SMP (%)	15.8 ± 2.2	28.8 ± 1.6	26.9 ± 2.9	30.2 ± 1.2
HPr/SMP (%)	6.2 ± 1.3	27.5 ± 15.0	5.1 ± 0.7	10.5 ± 1.7
HBu/SMP (%)	54.6 ± 1.0	48.8 ± 5.0	61.2 ± 3.7	56.5 ± 8.3
A/B	0.3 ± 0.0	0.5 ± 0.2	0.4 ± 0.1	0.6 ± 0.3
TVOA	6 193 ± 349	5 702 ± 1281	6 743 ± 259	5 842 ± 652
SMP	7 919 ± 214	5 776 ± 1270	7 088 ± 326	6 101 ± 600
ρ	3.6 ± 0.5	79.2 ± 27.1	19.9 ± 2.9	23.6 ± 7.3

Notes: A/B: acetic to butyric acid ratio. EtOH: ethanol; BuOH: butanol; HAc: acetate; HPr: propionate; HBu: butyrate; TVOA: total volatile organic acids= HAc+HPr+HBu; SMP: soluble microbial products= TVOA+EtOH+BuOH. Based in COD/L. LDL: low that detection limit.

operation with sucrose the continuous bioreactors ambient temperature showed better performance than at 35ºC [29]. The favorable H_2 production obtained at low temperature is in agreement with the obtained by Infantes *et al.* [43] who investigated the influence of pH and temperature on acidogenic fermentation and bio-hydrogen production in batch fermentation, using glucose as substrate. They suggested that the net dissociated organic acids. The increase of temperature induces two opposite effects: (*i*) to elevate the microbial metabolism and (*ii*) increased membrane permeability [44-45].

Table 3 shows the H_2 production of several systems that used CW as substrate and it can be observed that the pseudoyield of hydrogen in our work (3.07 at ambient temperature and 2.95 at 35ºC) is in the middle of the reported range.

Table 3. 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	[23]
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	[25]
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	[26]
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	[24]
Bioparticles of AFBR[d]	Batch T: 15-22ºC (ave 18ºC); 35ºC pH: 5.9	Powder 10.04* With phosphate buffer	3.07 At 18ºC 2.95 At 35ºC	Acetate, propionate, butyrate, ethanol, butanol, lactate	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

- The reactors with phosphate buffer showed a second cycle of H_2 production after the headspace flushing.
- H_2 production in the 2nd cycle was approximately 60% of that in the 1st cycle.
- After flushing of the headspace at the end of the 2nd cycle the H_2 production did not resume.
- The initial rate of H_2 accumulation in the 2nd cycle was lower than in the 1st one at both temperatures.
- A high solvent production was observed in non-buffered units as given by low ρ ratios.
- There was no significant difference between the H_2 production of units incubated at ambient temperature and a 35ºC.

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Notation

A/B	ratio production of acetic acid/production of butyric acid
BuOH	butanol
COD	chemical oxygen demand
EtOH	ethanol
HRT	hydraulic retention time
HAc	acetic acid concentration
HBu	butyric acid concentration
HLac	lactic acid
HPr	propionic acid concentration, (mgCOD /L)
SMP	soluble microbial products, (mgCOD/L)
TVOA	total volatile organic acids, (mgCOD/L)
VOA	volatile organic acids
Y’	hydrogen pseudo yield

Greek characters

ρ	Factor or ratio sum of volatile organic acids/sum solvents on COD basis, dimensionless