



Influence of initial pH on hydrogen production from cheese whey

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Abstract

Batch experiments were conducted to investigate the effect of initial pH, between 5 and 10, on fermentative hydrogen production from crude cheese whey (87.5% (v/v) by *Clostridium saccharoperbutylacetonicum*). Hydrogen was produced over the range of pH studied. The hydrogen production rate and yield peaked at an initial pH 6 and then steadily decreased as the pH increased. The highest rate and yield were 28.3 ml h⁻¹ and 7.89 mmol g⁻¹ lactose, respectively. Sugar consumption was unaffected between pH 5 and 9 and remained at 97%. All final pHs were acidic and increased alongside the initial pH. There was no correlation between the initial pH and the fermentation time; the times were shorter (50–52 h) between pH 6 and 8, and longer (62–82 h) outside this range. A modified Gompertz equation adequately described fermentative hydrogen production from cheese whey. The respective maximum hydrogen production rate and hydrogen potential at an optimal pH of 6 were 47.07 ml h⁻¹ and 1432 ml. Lag phase times were much longer at acidic pHs than at alkaline pHs.

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1. Introduction

Hydrogen is now considered as one of the alternatives to fossil fuels. It is preferred to biogas or methane because hydrogen is not chemically bound to carbon, and therefore, burning does not contribute to greenhouse gases or acid rain (Nath and Das, 2004). How-

ever, hydrogen is produced mainly from natural gas, a finite resource, through steam reforming, a process that generates large quantities of carbon dioxide (CO₂) which is a principal cause of global warming. Therefore, it is imperative that to consolidate the benefits of using hydrogen as a fuel or energy carrier, alternative but cleaner processes that rely on renewable feedstock must be developed.

Fermentative hydrogen production (FHP) is a microbial-based anaerobic process, which emits less carbon dioxide (CO₂) than conventional thermochem-

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ical hydrogen production processes do. FHP has been reported from numerous waste and wastewater sources including bean curd manufacturing waste (Zhu et al., 1999; Mizuno et al., 2000), rice and wheat bran (Noike and Mizuni, 2000), rice winery wastewater (Yu et al., 2002), molasses and sugary wastewater (Tanisho and Ishiwata, 1995; Ueno et al., 1996; Wu and Lin, 2004), waste activated sludge (Wang et al., 2003), municipal solid waste (Okamoto et al., 2000), starch wastewater (Yokoi et al., 2001; Zhang et al., 2003); food waste from cafeteria (Han and Shin, 2004), peptone degradation (Cheng et al., 2002); lignocellulose materials such as rice straw, coir and sugar bagasse (Kumar and Das, 2001), and paper sludge (Kadar et al., 2004). These studies have shown that FHP can rely on carbohydrate-rich wastewater and waste as feedstock thereby providing a prospect of integrating pollution reduction with energy generation. However, not only does the sustainability of FHP depend on the availability of locally abundant renewable feedstock but also the establishment of fermentation conditions that increase both the rate and the yield of hydrogen production from these materials.

Cheese whey is the lactose-rich watery by-product of cheese manufacturing. It makes up about 80% of the original fermentation medium, and it retains most of the milk fat, trace minerals, salts and vitamins. Cheese whey contains about 5% lactose, which is sufficient substrate to be used for fermentation purposes. Ghaly et al. (2000) estimated that in 1998 about 137.9 million tonnes of whey was produced worldwide. In Canada, annual cheese production increased by 22% between 1994 and 2004 (CDC, 2005). Total cheese production in Canada in 2004 was estimated at 0.34 million tonnes, which implies that over 0.27 million tonnes of whey was produced that year (CDC, 2005). Even though there are a number of technological developments in the transformation of whey to other useful products, utilisation or disposal of whey is one of the significant problems in the dairy industry (Mawson, 1994; Calli and Yukselen, 2004).

Recently, we demonstrated the feasibility of FHP from crude, defatted or deproteinised cheese whey (Ferchichi et al., in press). We reported that hydrogen production rate and yield were enhanced by whey dilution, supplementation with yeast extract and trace elements (magnesium and iron), and prior heating to kill lactic acid bacteria in whey.

While these results are important, a number of studies over the last 5 years have also shown that the initial pH is one of the most important parameters that influences FHP (Lay, 2000; Van Ginkel et al., 2001; Chen et al., 2002; Fang and Liu, 2002; Zhang et al., 2003; Wu and Lin, 2004). Differences in pH optima reported for hydrogen production could also be attributed to the composition of the medium or waste, the fermentation conditions and the microorganism(s) used. Therefore, this study was conducted to investigate the influence of initial pH on H₂ production from cheese whey by *Clostridium saccharoperbutylacetonicum*. This microorganism can utilise a wide range of sugars including lactose, the principal sugar in cheese whey, for H₂ production (Ferchichi et al., in press).

2. Materials and methods

2.1. Microorganism, maintenance and growth conditions

C. saccharoperbutylacetonicum ATCC 27021 was used in this study. The microorganism was selected in preference to *C. beijerinckii* based on the simplicity of the former's fermentation medium, and to seven new hydrogen-producing isolates because *C. saccharoperbutylacetonicum* produced more hydrogen without pH control. *C. saccharoperbutylacetonicum* was grown in peptone-yeast (PY) medium and maintained at -78°C . The PY medium contained per litre of deionised water: lactose, 10 g; tryptone (FisherBiotech, Fair Lawn, NJ), 5.0 g; Bacto peptone (BD, Sparks, MD), 5.0 g; yeast extract (FisherBiotech, Fair Lawn, NJ), 10.0 g; glucose, 5.0 g; Bacto beef extract (BD, Sparks, MD), 5.0 g; Tween 80, 1.0 ml; K₂HPO₄, 2.0 g; salt solution, 40.0 ml (per litre: CaCl₂·2H₂O, 0.25 g; MgSO₄·7H₂O, 0.50 g; K₂HPO₄, 1.0 g; KH₂PO₄, 1.0 g; NaHCO₃, 10.0 g; NaCl, 2.0 g); hemin solution, 10.0 ml (per litre: 0.5 g hemin, 10 ml 1N NaOH); 0.5% vitamin K₁, 200 µl (per litre: 5 ml vitamin K₁, 970 ml 95% ethanol); and L-cysteine·HCl·H₂O, 0.5 g. The microorganism was activated for fermentation by transferring 1 ml of the stock culture into 10 ml of fresh PY medium followed by anaerobic incubation at 30 °C with the aid of a H₂ and CO₂ generator in a BBL Gas-pak jar (BD, Sparks, MD). Overnight cultures were subcultured in fresh PY medium for 5–7 h and used

as inoculum. The total preculture time was 25–27 h. The inoculum formed 5% of the total volume of the culture.

2.2. Whey collection and characterisation

Fresh crude (unskimmed) cheese whey was obtained from Saputo Cheese in Vancouver, British Columbia. It had a pH of 4.2; COD, 102.1 g l^{-1} ; total sugars (as lactose), 49.2 g l^{-1} ; total solids, 70.9 g l^{-1} ; orthophosphate, 2.4 g l^{-1} and a total nitrogen, 1.76 g l^{-1} . Whey was kept at 4°C until used.

2.3. Experimental procedure, set-up and hydrogen quantification

The effect of the initial pH was investigated in the range of 5–10. Fermentation was carried out at a working volume of 200 in 250 ml media bottles at 30°C and agitated at 50 rpm. Cheese whey used in this work was diluted to 87.5% (v/v) (approximately $41.4 \text{ g lactose l}^{-1}$) with deionised water and supplemented per litre of the medium with $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 g l^{-1} and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 50 mg l^{-1} . Prior to inoculation, the pH of each medium was adjusted with either 2.5 M H_2SO_4 or 5 M KOH and autoclaved at 115°C for 15 min.

Each medium was seeded with a 5% inoculum and sparged with nitrogen gas (99.9%). All the culture bottles were tightly capped with open-top screw-caps, each fitted with a butyl septum and placed in the AER 200× respirometer (Challenge Environmental Systems, AK, USA). The culture pH was not controlled during fermentation. Gas produced in a bottle was channelled through a needle inserted through the butyl septum at the top of the bottle through a tube into a 100 ml solution of 30% KOH contained in a 150 ml bottle inserted inline which stripped CO_2 from the gas stream. The residual gas was then channelled into a bubble counter for the measurement of H_2 gas. To ensure that the 30% KOH solution did indeed remove CO_2 from the gas stream, the H_2 content of the effluent gas was also measured with a H_2 sensor ($\text{H}_2\text{Scan DCH}$, CA, USA). Each bubble counter was pre-calibrated and linked through an interface to a computer. Therefore, the volume of gas measured at any given time by a bubble counter was computed as real-time volumetric data and continuously logged in the computer. All experiments were conducted in triplicate.

2.4. Analytical methods

Total sugar concentration was estimated as lactose by the dinitrosalicylic acid method described by Miller (1959). Samples were centrifuged at 4000 rpm for 10 min and then 1.5 ml of the supernatant was hydrolysed by boiling with $100 \mu\text{l}$ of concentrated hydrochloric acid for 5 min. The resulting solution was then neutralized with $300 \mu\text{l}$ of 5N KOH and used for sugar analysis. Total solids (TS), total nitrogen (TN), orthophosphate, chemical oxygen demand (COD) were estimated according to standard methods (APHA, 1995).

3. Results and discussion

The effect of initial pH on hydrogen production was investigated between pH 5 and 10 in 87.5% (v/v) whey supplemented with FeSO_4 and MgSO_4 . The pH of inoculum used was 5.25. On inoculation, it was observed that for whey media with pre-sterilization pH within the range of 5 and 7, the culture pH remained unchanged; whereas for those between (pre-sterilization) pH 8 and 10, there was a 0.12 pH unit decrease (data not shown). This indicated that the pH of each culture at the onset of cultivation was the same as its pre-sterilization pH.

3.1. Hydrogen production

Fig. 1 shows sugar consumption (a); the volume of hydrogen produced (b); the hydrogen production rate (c); the hydrogen yield (d), as functions of the initial pH. Fig. 1a shows that sugar consumption remained high at 97% between pH 5 and 9, suggesting that the microorganism's ability to consume sugar was not altered within this initial pH range; consumption decreased to 92% at pH 10. However, as shown in Fig. 1b, hydrogen production occurred within the pH range investigated but the volume produced was pH-dependent. Hydrogen production peaked at 1412 ml at pH 6 and steadily decreased to 753 ml at pH 10. A similar trend was observed between the initial pH and the H_2 production rate (Fig. 1c) or the H_2 yield (Fig. 1d). The observations in this study are in agreement with the findings that increasing pH results in a decrease in hydrogen production (Lee et al., 2002; Fang and

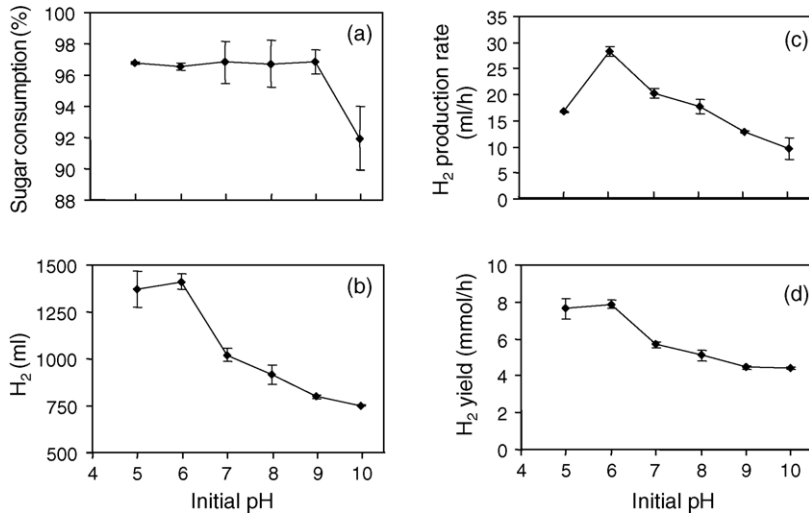


Fig. 1. Relationship between the initial pH and (a) sugar consumption; (b) volume of H₂ produced; (c) H₂ production rate; (d) H₂ yield.

Liu, 2002; Zhang et al., 2003; Wu and Lin, 2004). The relatively stable consumption of sugar of 97% against decreasing hydrogen production observed between pH 5 and 9 suggested a switch in the metabolism of sugar. *C. saccharoperbutylacetonicum* produces alcohols and acids from sugar (Crabbe et al., 2001). Lee et al. (1995) reported that the initial pH affected the levels of the acids and alcohols produced by this microorganism. Hence, it was assumed that by varying the initial pHs the levels of all the metabolites were altered and that could have accounted for the differences observed in hydrogen production.

The respective highest hydrogen production rate and yield of 28.3 ml h⁻¹ and 7.89 mmol g⁻¹ lactose were both obtained at pH 6, which suggested that maximum hydrogen production was located in the region of this pH. The highest yield of 7.89 mmol g⁻¹ lactose obtained in this study was higher than 2.99 mmol g⁻¹-hexose for molasses wastewater (Wu and Lin, 2004), 4.11 mmol g⁻¹-starch for starch wastewater (Zhang et al., 2003) but less than 8.61 mmol g⁻¹-cellulose (Ueno et al., 1996) and 11.4 mmol g⁻¹ monosaccharide from paper sludge hydrolysate (Kadar et al., 2004).

Fig. 2 shows that the final culture pH was also affected by the initial pH. The final pH was in the range of 5.46–6.13 and it increased alongside the initial pH. At an initial pH of 10, the final pH coincided with the optimum initial pH 6 for hydrogen production observed

in this study (Fig. 1b) but there was no resumption in the production of hydrogen. This reason for this is unclear. However, the general observation indicated that cultures which had lower final pHs (Fig. 2) produced higher hydrogen yields (Fig. 1d). The results suggested that besides the initial pH, hydrogen production could further be enhanced by controlling the culture pH at lower values during fermentation in cheese whey by this microorganism. Similar relationships between the initial and final pH have been reported by Zhang et al. (2003) and Wu and Lin (2004).

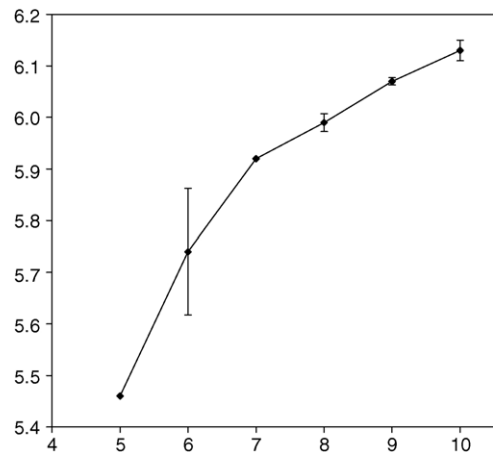


Fig. 2. Relationship between the initial pH and the final culture pH.

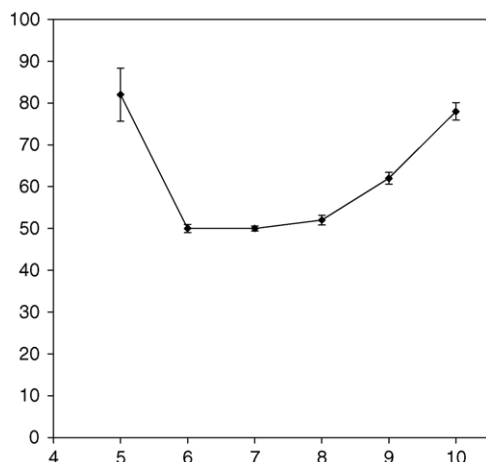


Fig. 3. Relationship between the initial pH and the fermentation time.

Fig. 3 shows the effect of initial pH on the fermentation time. Shorter times (50–52 h) were observed between pH 6 and 8 but outside this range, fermentation times were longer (62–80 h), indicating that there was no direct correlation between the fermentation time and the initial pH.

3.2. Kinetic analysis

To determine the effect of the initial pH on the hydrogen potential, P (ml), the maximum hydrogen production rate, R_m (ml/h), and the duration of the lag phase, λ (h), a modified Gompertz bacterial growth model (Eq. (1)) (Zwietering et al., 1990) was used to fit the cumulative H_2 production data obtained from each batch experiment:

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

$H(t)$ is the cumulative H_2 production during a fermentation time, t (h) and e is 2.71828. This model was originally developed to describe the progress of biogas production but it has since been employed to describe cumulative H_2 production (Lay et al., 1999; Chen et al., 2002; Fang and Liu, 2002; Lee et al., 2002; Zhang et al., 2003; Wu and Lin, 2004). The three parameters were nonlinearly estimated using Sigma Plot 4.0, and the values have been given in Table 1. The hydrogen potential, the maximum hydrogen production rate and the duration of the lag phase were all pH-dependent. The

Table 1

Kinetic parameters of hydrogen production estimated by the modified Gompertz equation

Initial pH	P (ml)	R_m (ml h ⁻¹)	λ (h)	R^2
5	1422.3	38.87	43.26	0.9887
6	1432.0	47.07	13.68	0.9893
7	1030.0	29.32	3.81	0.9936
8	902.6	28.93	3.06	0.9929
9	801.7	23.84	7.00	0.9921
10	759.1	17.78	8.70	0.9960

P : hydrogen potential; R_m : maximum hydrogen production rate; λ : lag phase time; R^2 : regression coefficient.

hydrogen potential (1432 ml) and the maximum hydrogen production rate (47.07 ml h⁻¹) peaked at pH 6, and then decreased with increasing initial pH between pH 6 and 10 (Table 1). This was consistent with trends observed between the initial pH and the hydrogen production rate (Fig. 1c) or the yield (Fig. 1d). Fig. 4 shows that there was a strong correlation ($R^2 = 0.896$) between the hydrogen potential and the maximum hydrogen production rate.

On the other hand, the lag phase time decreased from a highest of 43.26 h at pH 5 to 3.08 h at pH 8 and then increased to 8.70 h at pH 10, suggesting that the duration of the lag phase was longer in acidic than in alkaline pHs. A decrease in the lag phase time, however, did not correspond to an increase in the hydrogen production rate (or the hydrogen potential) (Table 1).

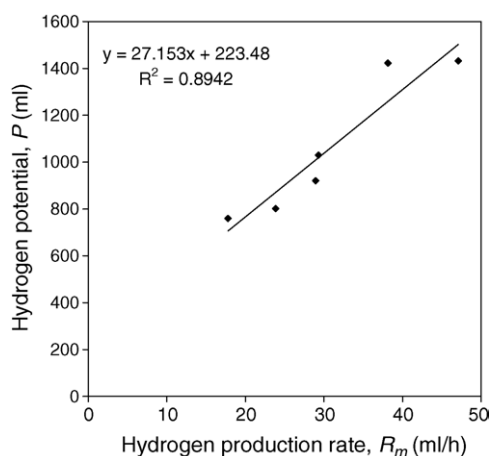


Fig. 4. Correlation between the hydrogen potential and the maximum hydrogen production rate estimated using Eq. (1).

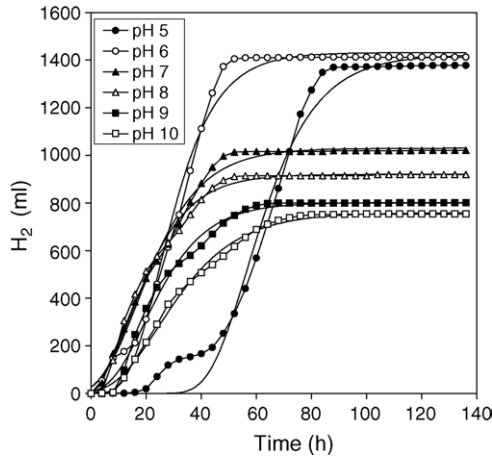


Fig. 5. Cumulative hydrogen production from crude cheese whey at different initial pHs. Cheese whey was diluted to 87.5% (v/v) and supplemented with FeSO_4 and MgSO_4 . The smooth lines were generated using parameters for P , R_m and λ estimated by Eq. (1).

The longer lag phase times at pH 5 and 6 were attributed to an intermediate lag phase, which occurred between 28–45 h at pH 5 and 7–15 h at pH 6, after an initially high production rate (Fig. 5). This second lag phase, which was not observed at pH 7–10, was in response to a rapidly changing environment immediately after the onset of hydrogen production. Chen et al. (2002) also found that the lag phase times were longer at pH 5 and 6 than at pH 7. Zhang et al. (2003) reported a minimum and a maximum lag phase time of 21 h and 72 h at pH 8 and 5, respectively. Studies have shown that addition of acid to fermentation medium results in the protonation of undissociated weak acids in the medium, which may pass freely through the cell's membrane into its cytoplasm (O'Sullivan and Condon, 1999). Since the cytoplasm has a higher pH than the external medium, the weak acid dissociates releasing its proton, which results in acidification of the cytoplasm (Cotter and Hill, 2003). This internal condition could result in loss of activity by the glycolytic enzymes and structural damage to the cell membrane, DNA and proteins and which could down growth. In response, bacteria induce acid tolerance response mechanisms; the clostridia, both constitutive and inducible mechanisms are activated to extrude the excess protons from the cytoplasm (Villarreal et al., 2002). The excretion of excess protons facilitates the resumption of cell growth.

Overall, the magnitudes of the regression coefficients, $R^2 = 0.988\text{--}0.996$, indicated a strong correlation between the experimental data and the fit (Table 1). As shown in Fig. 5, the time courses of hydrogen production generated using the three kinetic parameters were the same as those observed experimentally. Based on the results of this study, pH 6 was identified as the optimum for hydrogen production from cheese whey by *C. saccharoperbutylacetonicum*. This value compared with optimum pH reported for similar investigations in starch wastewater (Zhang et al., 2003) and molasses wastewater (Wu and Lin, 2004). However, it differs from an optimum pH of 7–8.5 obtained for this same microorganism in glucose medium (Ferchichi et al., in press). The results suggested that in cheese whey, hydrogen production at slightly acidic initial pHs preceded by a longer lag phase facilitated higher hydrogen yields. The model has been used to predict hydrogen production by the microorganism from other types of cheese whey, glucose and apple processing waste.

4. Conclusion

Wastewater treatment is an area in which the world can reduce its reliance on fossil fuels and lower the amount of pollution that it generates (Angenent et al., 2004). FHP provides a means of recovering the energy in the waste in the form of hydrogen. This potential exists in places where local agro-food industries generate large volumes of carbohydrate-rich waste or wastewater, such as cheese whey, which can serve as renewable feedstock for FHP. Moreover, these processes can be optimised to facilitate high hydrogen production rates and yields.

In this study, we report on the influence of the initial pH on FHP from crude cheese whey by *C. saccharoperbutylacetonicum*. The results show the importance of optimising the initial pH to facilitate maximum hydrogen production. They show that the initial pH affected the hydrogen potential, lag phase and fermentation times, the maximum average hydrogen production rates and the yield. Slightly acidic initial pH favoured higher yields than alkaline pHs. The highest hydrogen potential, 1432 ml, yield, 7.89 mmol g^{-1} lactose, and the maximum production rate, 47.07 ml h^{-1} , were all obtained at pH 6.

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