Method Article

Biohydrogen production from alkaline wastewater: The stoichiometric reactions, modeling, and electron equivalent

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A B S T R A C T

Hydrogen gas (H_2) is the cleanest energy carrier with 142 kJ/g energy content and without toxic byproducts release during combustion. There is interest to H_2 production by biological process from sustainable resources including municipal and industrial wastewater and also solid waste. Here, we describe the biohydrogen production that involves first survey the effect of alkalinity on biohydrogen production based on stoichiometric reaction, followed by the electron equivalent balances determination and examination of prediction capability of Gamperts model for biohydrogen production.

- The method uses a dark fermentation biological process for H_2 production from wastewater.
- As the influent alkalinity increased, the hydrogen production increased and then promptly descended.
- The predicted gas volume, based on Gamperts model confirmed good agreement with experimental value.

A R T I C L E   I N F O

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Specifications Table

Subject Area:	• Chemical Engineering • Environmental Science
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Method name:	Biohydrogen production from wastewater
Name and reference of original method:	E. Taheri, M.M. Amin, A. Fatehizadeh, H. Pourzamani, B. Bina, H. Spanjers, Biohydrogen production under hyper salinity stress by an anaerobic sequencing batch reactor with mixed culture, J. Environ. Health Sci. Eng., 16 (2018) 159-170.
Resource availability:	NA

Method details

Introduction

In the biological processes, the H_2 produce via photo and dark fermentation. In dark fermentation processes, the energy carrier produced by anaerobic acidogenic bacteria during organic matter consumption [1]. As the carbohydrates were used as an original electron source, the theoretical yield of hydrogen was 4 and 2 mol H_2 per mol of glucose based on the acetate or butyrate pathways, respectively. When the propionate was produced as soluble fermentation end products, the H_2 consumed with conversion of acetate and H_2 to propionate. In addition, ethanol and lactic acid produced in a pathway without any H_2 production [2].

Application of wastewaters as an organic matters source show great potential for biohydrogen production via biological process [3]. Different studies showed that the biohydrogen production rate highly related to wastewater characteristic [4–8].

The key wastewater characteristic that influenced on hydrogen production was alkalinity content [9]. The effect of alkalinity on methanogenic process is well known and established that not only alkalinity concentration but also the ratio of alkalinity to COD must be optimized. The reported ratio was fluctuated from 0.8 to 1.6 g $CaCO_3$/g COD, and the lower limit was 0.3 g $CaCO_3$/g COD. The required alkalinity for biohydrogen processes was lower than methanogenic process however was not exactly established [10,11].

Initial alkalinity in the influent substrates has strong effect on hydrogen producing bacteria by effecting on major metabolites. The high alkaline condition is essential for good performance of anzymatic system of hydrogenase bacteria. The alkaline content must be optimized due to the negative effect of osmotic pressure on fermentation process. The excessive alkalinity concentration can led to hydrogen producing bacteria poisonous and reducing H_2 amount [10,11].

Almost no studies have provided alkalinity effect on fermentative biohydrogen based on stoichiometric reaction for up to now. In this study, we studied the effect of alkalinity on biohydrogen production during anaerobic sequencing batch reactor (ASBR) operation. In addition, the electron equivalent balances have been determined and prediction capability of Gamperts model examined. The method's details have been described, step by step in follow.

Experimental design

ASBR set up. The cylindrical ASBR from Plexiglas that used in this study described elsewhere [12].

The anaerobic sludge was collected from the South wastewater treatment plant (Tehran, Iran) and used as parent inoculums. As our previously paper [8], the anaerobic sludge was sieved in order to

elimination of debris and pretreated for 45 min and 95 °C in order to inactivation of methanogenic bacteria. When the sludge temperature reached environmental temperature, the sludge inoculated to ASBR reactor and first cycle of operation started by synthetic wastewater injection.

Synthetic wastewater. The original electron donor in this study was Glucose. The ASBR was operated at organic loading rate (OLR) 0.5 g COD/L.d and influent chemical oxygen demand (COD) 4.5 g/L. the essential macro and micro elements for microorganism growth were also added to the synthetic substrate [13]. In this study, $NaHCO_3$ was used as alkalinity source at 1125 mg/L in first stage and gradually increased to 2225, 3750, and 4500 mg/L during 120 day operation of ASBR. The mentioned influent alkalinity was corresponding to 670, 1325, 2232, and 2678 mg/L as $CaCO_3$ of influent alkalinity concentration. Each stage continued until reach to the steady state condition.

Volatile fatty acids (VFAs) and alcohols analysis. The ASBR effluent was filtered through filter paper with pore size 0.45 μm (Whatman No. 42) and stored in glass container in the freezer until analysis time. The VFAs (acetic, propionic, butyric and valeric acid) were extracted and analyzed via liquid-liquid extraction method and gas chromatograph equipped with flame ionization detector (GC-FID) based on Manni et al., [14] as below. We added 2 mL of diethyl ether to each 2 mL melted sample and shacked for 30 s. The upper phase transferred to other glass container that contains 0.4 g anhydrous $MgSO_4$ for adsorbtion probable water in the extracted sample. After 10 min, the liquid separated from the magnesium sulfate and transferred to the other vial with gastight cap. The GC system syringe used for derived and injection 10 μL of extracted sample to the GC-FID.

An Agilent 7890A GC with Varian Cp- Sil5cb column was used to determine the content of acids in the extracts. The chromatographic program was as follows: The helium gas at flow rate of 1 mL/min (19.086 cm/s) was used as a carrier gas; oven temperature was 70 °C (3 min), first ramp as 10 °C/min to 130 °C (0 min), second ramp as 5 °C/min to 180 °C (5 min), post run 250 °C (1 min). The nitrogen gas was used as a makeup at flow rate of 30 mL/min.

The extraction and quantification of solvents (methanol, ethanol and acetone) was done by pouring 2 mL sample in a standard vial (10 mL) containing 1 g of NaCL, 70 μL isobutanol solution 1 g/L, 200 μL 2 M H_2SO_4 solution and analyzed with derived method from Adorno et al. [15]. The vials were incubated for 25 min at 100 °C (5 s mixing and 2 s ideal). The chromatographic program was as follows; The helium gas at flow rate of 1.5 mL/min (26.686 cm/s) was used as a carrier gas; oven temperature was 35 °C (0 min), ramp$_1$ as 2 °C/min to 38 °C (0 min), ramp$_2$ as 10 °C/min to 75 °C (0 min), ramp$_3$ as 35 °C/min to 120 °C (1 min), ramp$_4$ as 10 °C/min to 170 °C (1 min), post run 250 °C (1 min). Temperature of split/splitless injector was 250 °C.

Monitoring. Influent and effluent COD, pH, alkalinity, and carbohydrate were routinely measured by closed reflux colorimetric method, precalibrated glass body pH probe (CG 824 SCHOTT), titration method, and phenol-sulfuric acid methods [16,17]. In the headspace of the ASBR, the H_2 percentage was determined by a hydrogen analyzer (COSMOS-XP-3140 model, Japan).

Method evaluation

Biohydrogen production. The variation of biohydrogen production regard with different influent alkaline concentration is shown in Fig. 1. As depicts in Fig. 1, with increasing influent alkalinity, the hydrogen production increased and then promptly descended. At studied influent alkalinity, the average volume of hydrogen production were 57.91, 220.02, 204.65, and 92.51 mL/d respectively. As the initial alkalinity was 1325 mg $CaCO_3$/L, The highest volume of biohydrogen produced. This observation may be due to effect of hydrogen ion on ATP level. The H^+ ion was essential for adjusting ATP level but when its amount excesses from optimum level, the sever environmental condition occurred and, the most sever condition, the most ATP consumed for cell neutration so the H_2 production decreased [18].

Geng et al., reported that as the amount of $KHCO_3$ increased from 0 to 40 mM, the biogas production increased and when the alkalinity reached to 60 mM, biogas production decreased [7]. Choi and Ahn reported that when the pH and alkalinity were 8.95 and 3.18 g $CaCO_3$/L, respectively; anaerobic bacteria can produce the highest volume of hydrogen. At alkalinity higher than 4 g $CaCO_3$/L,
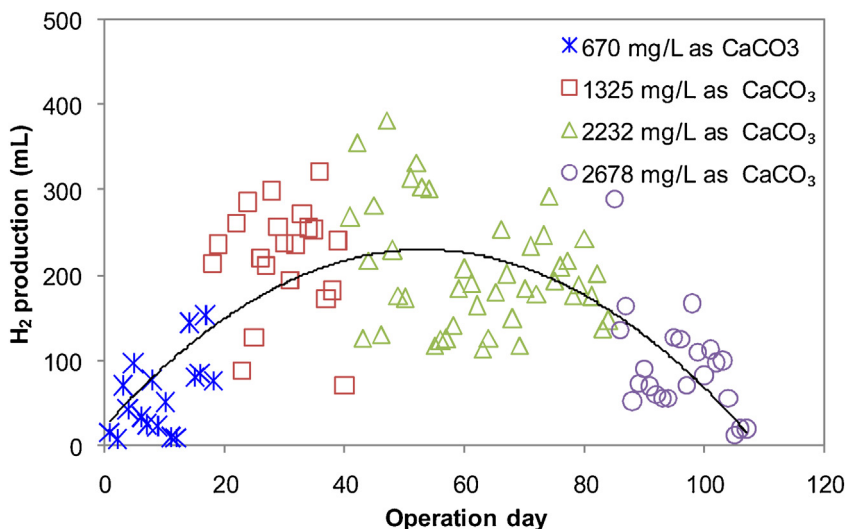
Fig. 1. H_2 production at different initial alkalinity over the operation time.

the lactate type fermentation bacteria started activity and resulted in increasing the amount of propionate, reducing of butyrate, and finally stopping the hydrogen production [10]. luo et al., demonstrated that the influent alkalinity of 6 g $NaHCO_3$/L and HRT 24 h were optimal condition for hydrogen production in rate of 3215 mL H_2/L/d [9].

Mohammadi et al., studied the alkalinity range of 200–2000 mg $CaCO_3$/L and found that the maximum hydrogen yield (124.5 mmol H_2/g COD) was obtained at alkalinity 1100 mg $CaCO_3$/L with 3000 mg/L of initial COD concentration [19]. Therefore, the highest hydrogen yield observed at the ALK/COD ratio 0.37 and increasing influent COD with constant alkalinity, the hydrogen yield decreased. The required ALK/COD ratio for methanogenic processes was varied from 0.11 to 0.30 g $CaCO_3$/g COD but the lower ALK/COD ratio reported for hydrogenogenic processes by Valdez-Vazquez et al., [11]. This difference may be because of different working condition, Valdez-Vazquez's report was in solid substrate fermentation, not in wastewater. In our paper, the studied ALK/COD ratios were 0.15, 0.3, 0.5 and, 0.6 at constant OLR (0.5 g COD/L.d) and the corresponding calculated hydrogen yield was 0.15, 0.6, 0.5 and 0.24 mmol H_2/g COD_{in}.

When the ALK/COD ratio was 0.3, the maximum H_2 produced. The difference between calculated hydrogen yield in this study and Mohammadi et al., may be related to operation condition including influent COD and pH, batch test condition, parent inoculums and feed substrate [19].

COD removal efficiency. The effect of initial alkalinity on COD removal during ASBR operation was presented in Fig. 2. The average of COD removal at studied initial alkalinity was 18.13, 14.72, 10.46, and 17.36%, respectively.

The average of COD conversion to VFA was 51.42, 65.8, 53.9, and 66.6% that was responsible for 62.8, 67.2, 70.2, and 81.3% of effluent COD (Fig. 3). In the hydrogenogenic phase, the significant portion of the carbon remain in the effluent as released VFAs by acidogenic bacteria [18]. Lee et al., reported that the maximum specific production rate of hydrogen and maximum carbohydrate degradation efficiency was observed simultaneously and depicted that the highest specific production yield for VFAs was 0.7 g COD/g sucrose [20].

The observed glucose conversion by Shida et al., was greater than 70% for all studied HRT and reached up to 94% at HRT 8 – 2 h [6]. Van Ginkel et al., reported that the COD removal during biohydrogen production from four food processing wastewaters was 5–11.1%, same as our study [3]. In addition, Sridevi et al., reported the higher COD removal efficiency around 87.35% by hybrid upflow anaerobic sludge blanket reactor [21]. This deference was presumably related to lower studied OLR in
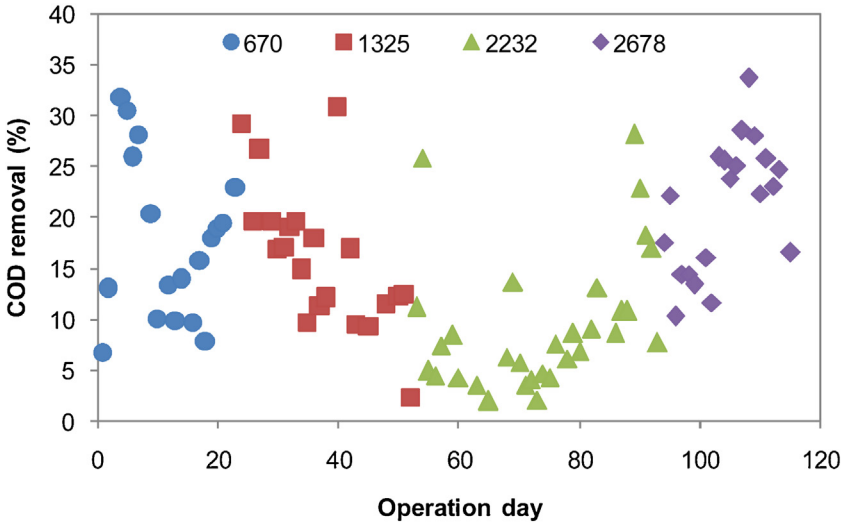
Fig. 2. COD removal at different initial alkalinity over the operation time.
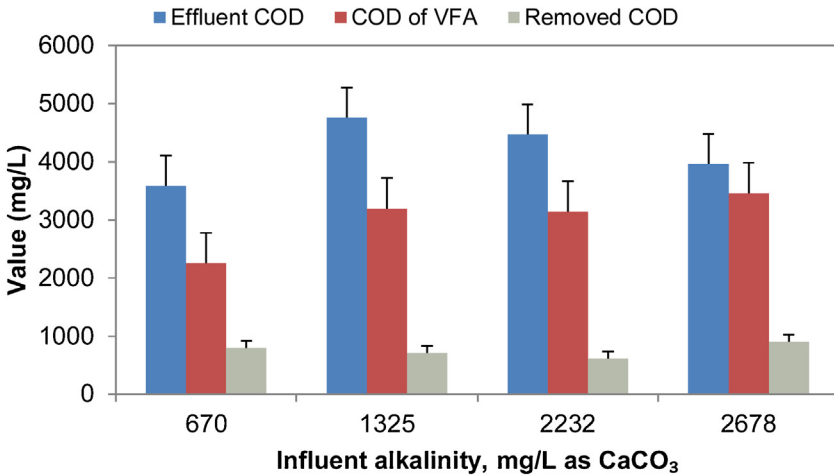

Fig. 3. variation of the Effluent COD, VFAs, COD, and Removed COD at different initial alkalinity.

our study. The maximum and minimum of COD removal efficiency reported by Mohammadi et al., study were 58.3 and 39.6% at 1.1 and 0.2 g CaCO₃/L, resepectively [19].

SEP & solvent production. The variation of soluble end products (SEP) during glucose fermentation by thermal pretreated anaerobic sludge was monitored and depicted in Fig. 4. The dominant SEP in the studied initial alkalinity concentration was acetic acid that was rather than 50% of total VFA. As the initial alkalinity was 2225 mg/L, the acetic acid was 70% of SEPs and the percentage of acetic acid in the other studied initial alkalinity was lower than this value. By application of 1125 mg/L as initial alkalinity concentration, the Valeric acid was 5.8% of SEP but its concentration decreased and not detected in other studied alkalinity.

The highest volume of H₂ was achieved at the highest portion of the acetate acid and butyrate acid and lowest portion of propionic acid and valeric acid. The acetate and butyrate pathways used by acidogenesis
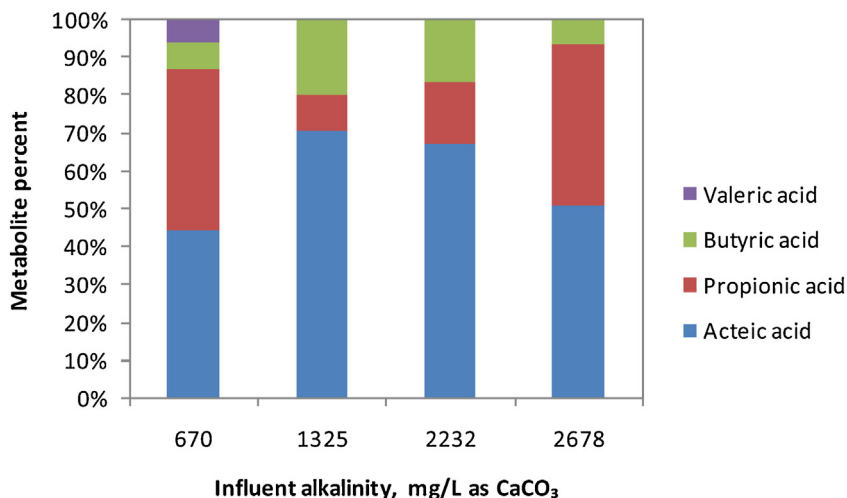
Fig. 4. The contribution of VFAs component at different initial alkalinity.

bacteria for H_2 production but propionate produced when the bacteria used H_2 consuming pathway [18]. With accumulation of propionate in the biological reactor, the hydrogen production was stopped [10]. As illustrated in Fig. 4, the lower hydrogen production was obtained as the higher propionate measured. During 120 d operation of ASBR, the dominant SEP was acetic acid followed by butyric acid and or propionic acid. As shown in Fig. 4, at the studied initial alkalinity, methanol, ethanol and acetone were not detected. The high portion of SEP was related to VFA and demonstrated that the fermentation process in studied ASBR was acidigenes than solventogenis. This finding was in line with shida et al., but showed difference with Lin et al., and Geng et al., [5–7]. As reported by Geng et al., by using monocultures of *C. thermocellum* for hydrogen production, the high concentration of ethanol and acetate were detected and by inducing the *C. thermopalmarium* as co-cultures, the butyrate concentration increased. This finding confirms that by changing the fermentation bacteria species and pathways, the composition and amount of SEP was changed [6,7]·

As the anaerobic bacteria used the solventogenic pathway, the reduced end products such as alcohols formed and synchronize with consumption of additional free electron and low H_2 yields [6,22].

Electron equivalent. We produced the Stoichiometric reactions by converting the amount of electron sink into electron equivalent (e^-eq). The fraction of electron sinks at different influent alkalinity in ASBR was summarized in Table 1. The highest and lowest e^-eq of H_2 was occurred as the initial alkalinity was 1325 and 670 mg/L, respectively. The highest H_2 fraction was coinciding with high e^-eq of acetate. As H_2 fraction of e^-glucose was decreased, the e^-eq fraction of acetate and butyrate decreased and e^-eq fraction of the propionate improved. Previous study reported that the highest conversion efficiency of the initial electron for H_2 was 15%. In fact, the high portion of the initial carbon and energy remained in the effluent [18].

Stoichiometric reactions. The calculation of Stoichiometric reactions for glucose fermentation by ASBR was performed according to our previously published paper [8].The stoichiometric reactions for all studied initial alkalinity were summarized in Table 2. As shows in Table 2, without cell synthesis and production of SEP, conversion of each mol of glucose theoretically produces 12 mol of H_2. The mentioned theoretical value decreased to 4 and 2 mol H_2/mol glucose by using acetate and butyrate fermentation pathway, respectively. As shown in Table 2, In this study when the alkalinity was 670, 1325, 2232, and 2678 mg/L as $CaCO_3$, the H_2 production per mol of influent glucose was 0.19, 0.67, 0.47, and 0.26 mol, respectively. In the other word, the maximum hydrogen production that achieved was

Table 1
Fractions of electron sinks at different influent alkalinity.

Compounds	Influent alkalinity (mg/L as CaCO₃)			
	670	1325	2232	2678
Glucose$_{ini}$	299.74 (100 %)	323.35 (100%)	388.13 (100%)	345.21 (100 %)
Acetate	107.94 (35.61 %)	188.48 (56.53 %)	228.01 (53.68 %)	182.22 (51.77 %)
Propionate	148.32 (48.93 %)	30.55 (9.16 %)	62.73 (14.77 %)	101.56 (28.85 %)
Butyrate	28.73 (9.48 %)	81.30 (24.38 %)	101.31 (23.85 %)	38.71 (11 %)
Formate	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Lactate	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Acetone	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Methanol	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Ethanol	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Biomass	8.00 (2.46 %)	6.75 (2.02 %)	4.38 (1.03 %)	6.25 (1.78 %)
Res-glucose	5.60 (1.85 %)	8.53 (2.56 %)	12.26 (2.89 %)	15.99 (4.54 %)
H₂	4.52 (1.49 %)	17.81 (5.34 %)	16.06 (3.78 %)	7.26 (2.06 %)
Total	302.10	333.42	424.75	351.99
Δ e⁻equiv (%)	−1.12	−3.11	−9.34	−1.97
Effluent pH	5.31 ± 0.43	5.58 ± 0.44	6.18 ± 0.2	6.4 ± 0.07

* Units are in e⁻eq (%).

Table 2
Stoichiometric reactions under different influent alkalinity.

Condition	Overall reaction without cell synthesis	Reference
the complete glucose conversion reaction to hydrogen	$C_6H_{12}O_6 + 12H_2O \rightarrow 6HCO_3^- + 6H^+ + \mathbf{12H_2}$	[23]
the acetic acid fermentation pathway	$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + \mathbf{4H_2}$	[23]
the butyrate fermentation pathway	$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + \mathbf{2H_2}$	[23]
Influent alkalinity: 670 mg/L as CaCO₃	$C_6H_{12}O_6 + 0.40\ H_2O = 1.12\ C_2H_3O_2^- + 0.88\ C_3H_5O_2^- + 0.12\ C_4H_7O_2^- + \mathbf{0.19\ H_2} + 0.66\ CO_2 + 3.19\ H^+$	This study
Influent alkalinity: 1325 mg/L as CaCO₃	$C_6H_{12}O_6 + 0.80\ H_2O = 1.78\ C_2H_3O_2^- + 0.16\ C_3H_5O_2^- + 0.31\ C_4H_7O_2^- + \mathbf{0.67\ H_2} + 0.73\ CO_2 + 3.14\ H^+$	This study
Influent alkalinity: 2232 mg/L as CaCO₃	$C_6H_{12}O_6 + 0.64\ H2O = 1.67\ C_2H_3O_2^- + 0.26\ C_3H_5O_2^- + 0.30\ C_4H_7O_2^- + \mathbf{0.47\ H_2} + 0.67\ CO_2 + 3.14\ H^+$	This study
Influent alkalinity: 2678 mg/L as CaCO₃	$C_6H_{12}O_6 + 0.65\ H_2O = 1.66\ C_2H_3O_2^- + 0.53\ C_3H_5O_2^- + 0.14\ C_4H_7O_2^- + \mathbf{0.26\ H_2} + 0.54\ CO_2 + 3.38\ H^+$	This study

only one eighteenth of the theoretical H₂. This reduction can be related to amount and composition of intermediate fermented products [23].

Modified Gompertz model. In the modified Gompertz equation (Eq. 1) by drawing the cumulative hydrogen production (H_t as mL) during the incubation time (t as h), the maximum hydrogen production potential (H_{max} as mL), the maximum hydrogen production rate (R_{max} as mL/h), and the

Table 3
Estimated kinetic parameters with modified Gompertz equation under different influent alkalinity.

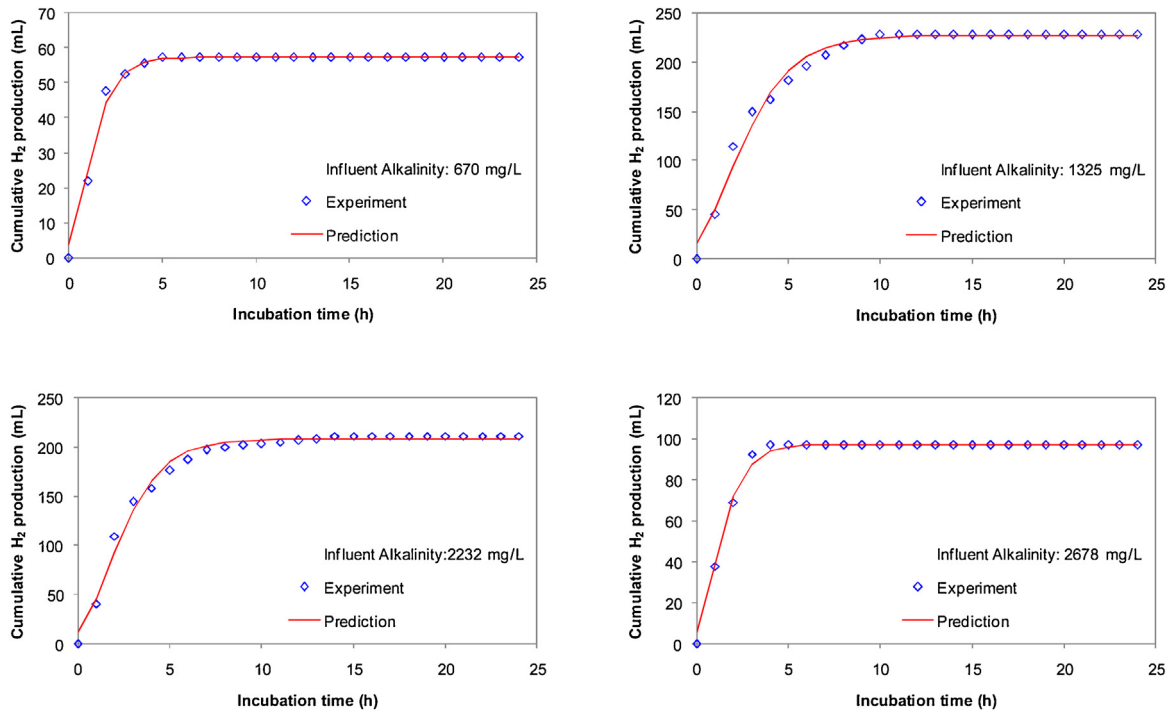| Influent alkalinity (mg/L as CaCO₃)	Model simulation			
	λ, h	R_{max}, mL/h	H_{max}, mL	R^2
670	0.01	25.07	57.27	0.99
1325	0.01	45.56	227.51	0.99
2232	0.07	48.48	208.68	0.99
2678	0.01	39.35	97.30	0.99

Fig. 5. Experimental hydrogen production and predicted H2 production via Gompertz model.

lag-phase time (λ as h), can be achieved [5]. Originally, this equation used for describe the cumulative hydrogen production process in a batch experiment [24].

$$H_t = H_{max} \times \exp\left\{-\exp\left[\frac{R_{max} \times e}{H_{max}}(\lambda - t) + 1\right]\right\} \qquad (1)$$

In the end of each stage, the H_2 production was monitored in the time interval (1 h) and then the solver function of excel software used for optimization of H_{max}, R_{max}, and λ and depicted in Table 3. The experimental and predicted result of H_2 was shown in the Fig. 5. The R^2 was higher than 0.99 for all studied alkalinity and confirmed good agreement between experimental and predicted values. The estimated lag phase in this study was significantly shorter than previously published studies. The reported lag phase by Gadhe et al., Rasdi et al., and Zhang et al., studies was 4.08, > 3, and > 21 h [25–27], as shown in Table 3, we observed the shorter lag phase around 0.7 h. Same short lag phase (0.5 h) observed in continuous stirred anaerobic bioreactor in Xing et al., study [28].This deference presumably related to reactor type, influent COD and solution pH, substrate type and operation condition. As the reactor operated for continuous long day, some portion of gas trigged in the sludge and released by waiting and resulted in the shorter lag phase.

Conclusion

The alkalinity effect on fermentative biohydrogen based on stoichiometric reaction was provided in this study, In addition, the electron equivalent balances were determined and prediction capability of Gamperts model examined. The following results were achieved.

- The average of hydrogen production at studied alkalinity 670, 1325, 2232, and 2678 mg/L as CaCO$_3$, were 57.91, 220.02, 204.65, and 92.51 mL/d.
- As the ALK/COD ratio was 0.3, the highest hydrogen yield (0.6 mmol H$_2$/g COD$_{in)}$ was achieved and the required ALK/COD ratio for methanogenic and hydrogenogenic processes was about alike.
- The highest H$_2$ fraction was coinciding with high e$^-$eq of acetate. The highest and lowest e$^-$eq of H$_2$ was occurred as the initial alkalinity 1325 and 670 mg/L, respectively.
- According to stoichiometric reactions, the maximum hydrogen production was only one eighteenth of the theoretical H$_2$.
- The estimated lag phase in this study was significantly shorter than previously published studies, because of reactor type, influent COD, solution pH, and substrate type and operation condition.

Acknowledgement

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