

Bio-hydrogen Production Potential from Market Waste

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Abstract

This research studied bio-hydrogen production from vegetable waste from a fresh market in order to recover energy. A series of batch experiments were conducted to investigate the effects of initial volatile solids concentration on the bio-hydrogen production process. Lab bench scale anaerobic continuous stirred-tank reactors (CSTR) were used to study the effect of substrate and sludge inoculation on hydrogen production. Three different concentrations of initial total volatile solids (TVS) of organic waste were varied from 2%, 3% and 5% respectively. The pH was controlled at 5.5 for all batches in the experiment. The results showed that bio-hydrogen production depended on feed-substrate concentration. At initial TVS content of 3%, the highest hydrogen production was achieved at a level of 0.59 L-H₂/L at pH 5.5. The maximum hydrogen yield was 15.3 ml H₂/g TVS or 8.5 ml H₂/g COD. The composition of H₂ in the biogas ranged from 28.1-30.9% and no CH₄ was detected in all batch tests.

Keywords: bio-hydrogen; vegetable waste; market waste; organic waste; continuous stirred-tank reactors

1. Introduction

Hydrogen gas shows great promise as a non-polluting fuel. It is clean, recyclable, and an efficient new energy carrier that has a high specific energy on a mass basis (e.g., the energy content of 9.5 kg of H₂ is equivalent to that of 25 kg of gasoline) (Chang *et al.*, 2002). It is expected to play an important role in future energy scenarios. Many believe that hydrogen may replace fossil fuels as the next-generation source of energy supply (Zhang *et al.*, 2003). At present, hydrogen gas is mainly produced from fossil fuels, mostly by steam reforming of natural gas. It is possible to produce hydrogen using other production techniques such as electrolysis of water in which 108.7 kg of hydrogen can be produced from 1 m³ of water (Midilli *et al.*, 2005). Production of hydrogen can be carried out sustainably since hydrogen can be produced from renewable and sustainable energy sources. Many recent studies have shown that hydrogen production via biological means appears to be a feasible alternative (Cheong and Hansen, 2006; Van Ginkel *et al.*, 2005; Han and Shin, 2004; Khanal *et al.*, 2004; Shin *et al.*, 2004). Das and Veziroglu (2001) stated that the hydrogen production from fermentation of organic compounds with anaerobic bacteria yielded higher H₂ production rates than from photolysis of water using algae and cyanobacteria. Wastewater shows great potential for economical production of hydrogen because producing a product from a waste source could reduce

waste treatment and disposal cost (Khanal *et al.*, 2004). Batch tests using various wastes and wastewaters suggest that hydrogen is more efficiently generated from carbohydrates than other materials (Van Ginkel *et al.*, 2005). These carbohydrates can be monosaccharides, starch, and cellulose. Besides carbohydrates, amino acids can also be oxidized to hydrogen by certain strains (Vrije and Claassen, 2003). Simple sugars, such as sucrose and glucose, are converted at elevated temperatures to H₂ at high conversion efficiencies. For example, 61% of the maximum possible biological H₂ recovery from sucrose (assuming a maximum possible yield of 8 mol-H₂/mol-sucrose) was achieved under optimum conditions of temperature (37°C), pH and organic loading (Van Ginkel *et al.*, 2001). Lower yields of 28% were obtained with glucose (maximum yield of 4 mol-H₂/mol-glucose), and 26% with sucrose, at 30°C (Logan *et al.*, 2002). The pH of wastewater has an important impact on hydrogen production from wastewater. At an initial pH of 4.5, the hydrogen production rate peaked at 214 ml H₂/g COD and 125 ml H₂/g COD for sucrose and starch, respectively. At pH lower than 4, the hydrogen production rate was decreased. The optimum pH for H₂ generation was 6-8. At pH lower than 4 or higher than 8, H₂ production was inhibited (Liu and Shen, 2004). Most previous studies on batch and continuous processes were performed using glucose/carbohydrate as feed. There are few reports on biohydrogen production from organic waste.

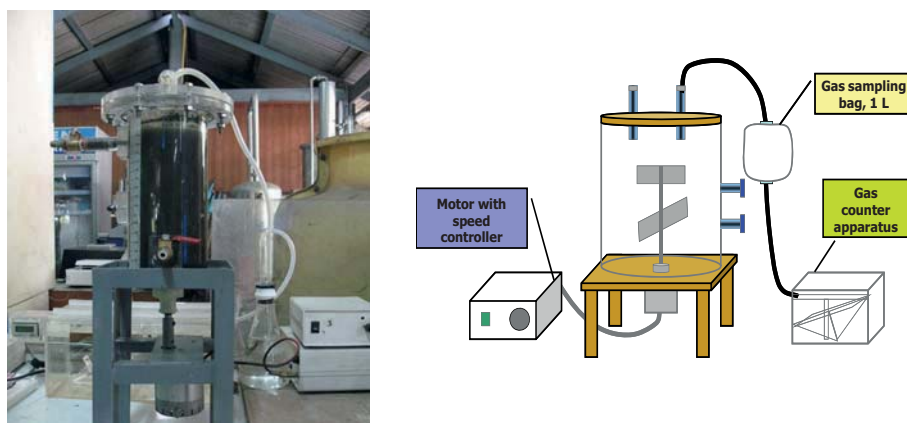


Figure 1. Experimental setup of the bio-hydrogen reactor

The purpose of this research was to investigate the potential for bio-hydrogen production from vegetable waste from a fresh market using CSTRs. The volatile solids content of feed waste were varied in order to determine the impact on hydrogen yield from organic waste.

2. Materials and Methods

2.1. Lab bench-scale reactor

Biohydrogen production experiments were conducted in three 1.45-L lab bench scale anaerobic continuous stirred-tank reactors (CSTRs). The feed vegetable waste used in this experiment was mixed with inoculums at a ratio of 1.16:1 with biomass concentration based on mixed liquor volatile suspended solids (MLVSS) 54 g/L. The total volume of the sample in each reactor was 0.78 liter. The samples were mixed continuously for 40 days. A schematic diagram of the experimental setup is shown in Fig. 1. The initial total volatile solids of waste were varied at 2%, 3% and 5% to find the optimal hydrogen production conditions. The details of the batch experiment in this study are shown in Table 1.

2.2. Heat-treated sludge inoculums

Anaerobic granular sludge from a full-scale up-flow anaerobic sludge blanket (UASB) reactor was used as inoculums for bio-hydrogen production. The sludge inoculums were crushed into slurry and mixed with nutrient solution (ratio of 1:3), which contained (mmol/L) : $\text{AlCl}_3(\text{H}_2\text{O})_6$, 0.002; H_3BO_3 , 0.008; KI, 0.06; $\text{CuCl}_2(\text{H}_2\text{O})_2$, 0.003; $\text{MnCl}_2(\text{H}_2\text{O})_4$, 0.003; $\text{NaMoO}_4(\text{H}_2\text{O})_2$, 0.002; Na_2SeO_3 , 0.003; NH_4VO_3 , 0.004; $\text{NaWO}_4(\text{H}_2\text{O})_2$, 0.002; $\text{FeCl}_2(\text{H}_2\text{O})_4$, 0.2; $\text{CoCl}_2(\text{H}_2\text{O})_6$, 0.04; $\text{NiCl}_2(\text{H}_2\text{O})_6$, 0.002; ZnCl_2 , 0.004; NH_4Cl , 7.48; $(\text{NH}_4)_2\text{HPO}_4$, 0.56; $(\text{NaPO}_3)_6$, 0.02; $\text{Na}_2\text{S}(\text{H}_2\text{O})_9$, 1.25; KCl, 5.37; $\text{CaCl}_2(\text{H}_2\text{O})_2$, 0.3; $\text{MgSO}_4(\text{H}_2\text{O})_7$, 1.62; Cysteine, 0.08; NaHCO_3 , 71.43 (Speece, 1996). The mixture was heated at 100°C for 15 min. to inhibit the hydrogen consuming bacteria and select the spore-forming bacteria. The heat-treated sludge inoculums were cooled down and keep at 4°C for a week. The heat-treated sludge inoculums had a MLVSS of 54.0 g/L.

2.3. Analytical methods

The samples were collected and analyzed every two

Table 1. Details of batch dark fermentation

Details	Values
Temperature	Mesophillic (28-32°C)
Initial pH	5.5
<i>Reactor</i>	
Operation volume	1.45 L
Gas holding capacity	0.57 L
Total volume	2 L
Vegetable waste volume	0.78 L (Initial TVS of 2%,3% and 5%)
Sludge inoculums volume	0.67 L (Initial MLVSS in reactor of 25 g/L)

Table 2. Composition of organic waste sample

Type of organic waste	Weight (kg)	% by weight
Chinese cabbage	15.70	31.4
Cauliflower and leaves	11.10	22.2
Cabbage	7.20	14.4
Chinese kale	6.10	12.2
Lettuce	4.50	9.0
Others (the calyx of round eggplant, Chinese convolvulus, banana flower, etc.)	5.4	10.8
Total	50	100

days for the total solid (TS), TVS, the chemical oxygen demand (COD), filtered COD, the total volatile fatty acid (TVFA) and alkalinity. The temperature and pH of the sample within the reactor were recorded everyday. The analytical methods were determined following standard methods (American Public Health Association, 1995). Biogas production was detected daily by using a gas counter unit. The concentration of hydrogen, methane and carbon dioxide in the biogas was analyzed every two days by using a gas-tight syringe and a gas chromatograph (Varian CP 3800 GC, USA) equipped with a thermal conductivity detector (TCD).

2.4. Organic Waste Sample

The sample of organic waste was collected from a fresh market located in Bangkok. Other contents, such as plastic bags, clothes and wood were discarded from the waste prior to weighing and preparation. The resulting organic composition of waste from the fresh market is shown in Table 2. Most of the organic waste

from the market was various kinds of vegetable such as Chinese cabbage, cauliflower and leaves, cabbage, Chinese kale and lettuce. The vegetable waste was prepared by grinding and then filtering through sieves (No. 10) to separate the particles of sizes less than 2.0 mm for use as organic waste for the H₂ fermentation. The residue particle on the screen was re-crushed to obtain the required particle sizes. The crushed vegetable waste was stored at a temperature of 4°C and maintained for a period of less than a week. Results from the analysis showed that the vegetable waste sample contained a moisture content of 94.1% and total solid content of 5.9% (Table 3). In addition, nitrogen and phosphorus content were 0.28% and 0.039% as shown in Table 3. The vegetable waste sample was diluted to 2%, 3% and 5% TVS, respectively. The samples were mixed with inoculums to 3.6, 4.1 and 4.9% TVS, respectively, and then transferred to three CSTR tanks for batch-dark fermentation.

2.5. Data analysis

Table 3. Characteristics of organic waste samples and the sludge inoculums used in the experiment

Parameters	Values				Sludge inoculums
	Concentrated organic waste sample	2%	3%	5%	
Moisture (% by wet weight basis)	94.10	97.60	96.50	94.10	-
TS (% by wet weight basis)	5.90	2.35	3.53	5.88	-
TVS (% by wet weight basis)	5.02	2	3	5	5.40
TVS/TS	0.85	0.85	0.85	0.85	-
Nitrogen (%)	0.28	0.13	0.16	0.28	-
Phosphorus (%)	0.039	0.018	0.024	0.039	-
Iron (%)	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	-
pH	5.09	4.87	4.88	5.03	8.20
TCOD (g/L)	69.72	46.88	54.38	69.38	61.88

Total gas generated from reactors was collected every day and analyzed for total biogas composition. The cumulative hydrogen gas production was determined by using the following equation:

$$V_{H_2,i} = V_{H_2,i-1} + V_w C_{H_2,i} + V_{G,i} C_{H_2,i} - V_{G,i-1} C_{H_2,i-1} \quad (1)$$

Where $V_{H_2,i}$ and $V_{H_2,i-1}$ are the cumulative hydrogen gas volumes (ml) at the current (i) and the previous measurement (i-1), respectively. $C_{H_2,i}$ and $C_{H_2,i-1}$ are the fraction of hydrogen gas in the biogas at the current and the previous measurements, respectively. V_w is the total gas volume measured by the water displacement method (ml). $V_{G,i}$ and $V_{G,i-1}$ are the volumes of biogas in the head space of the reactor (ml) at the current and the previous measurement, respectively.

The cumulative biogas production curves were obtained over the course of the batch experiment by using a modified Gompertz equation (Logan *et al.*, 2002):

$$H(t) = H_{max} \exp \left\{ - \exp \left[\frac{R_m \epsilon}{H_{max}} (\lambda - t) + 1 \right] \right\} \quad (2)$$

Where $H(t)$ (ml) is the amount of biogas produced at time t , H_{max} (ml) the total amount of biogas produced, R (ml/h) the biogas production rate, λ (h) the lag phase and $\epsilon = 2.718281828$. Constants were obtained by fitting the cumulative biogas production curves by minimizing the ratio of the sum-of-squares error to the correlation coefficient (SSE/r^2) using the 'Solver' function in Microsoft Excel. The hydrogen yield, Y_{H_2} (mole H_2 /mg-COD) was calculated as (Ditzig *et al.*, 2007):

$$Y_{H_2} = \frac{n_{H_2}}{W_g (TVS_i)} \quad (3)$$

Where n_{H_2} is the number of moles of hydrogen recovered in the experiment, calculated as $n_{H_2} = V_{H_2} / (RT)$, R is the gas constant (0.08206 atm-L/mol-K), and T is the absolute temperature (303K), W_g is the weight

of organic waste, and TVS_i is the TVS concentration of the waste at the beginning of the batch test.

3. Results and Discussion

3.1. Bio-hydrogen production from organic waste

It is known that the feedstock type can affect bio-hydrogen production. Most carbohydrates such as monosaccharide, starch, cellulose are a suitable feedstock for dark-hydrogen fermentation. The conversion of carbohydrates to hydrogen and organic acids is preferred because it yields the highest amount of hydrogen per mole of substrate (Vrije and Claassen, 2003). The results of this study show that the amount of H_2 produced per g TVS varied for the different concentrations of vegetable waste under the test conditions, ranging from 4.8–15.3 ml- H_2 /g TVS or 0.19-0.62 mmol H_2 /g-TV S (Table 4). The biogas composition contained an amount of hydrogen ranging from 28.1-30.9% in various test conditions. It is noted that the highest biogas as well as bio-hydrogen production was achieved by using 3% TVS feed (15.3 ml- H_2 /g-TV S). Overall, the maximum biogas produced at different initial TVS concentrations, ranged from 16.2-49.6 ml-biogas/g-TV S at pH 5.5.

The data suggest that vegetable waste from a fresh market showed an effective bio-hydrogen yield production of 0.62 mmol H_2 /g TVS at pH 5.5. Several research studies have analyzed the maximum H_2 yield from carbohydrate wastewater, food waste and other organic wastes at pH 5.0-7.0 with heat-treated and non heat-treated sludge inoculums. Results from these studies are shown in Table 5. Compared to the previous studies, the vegetable waste from a fresh market in this study has potential for bio-hydrogen production when pH is adjusted to 5.5.

3.2. Hydrogen production and hydrogen production rate

Table 4. Bio-hydrogen production for various TVS concentrations of organic waste at pH 5.5.

Initial TVS (Waste) (%)	Mixed TVS ^a (%)	Mixed COD ^b (g)	Biogas production (ml)	H_2 (ml)	Biogas (ml/g TVS)	H_2 (%) ^c	H_2 Produced		
							ml- H_2 /g-TV S	n_{H_2} (mmol/g-TV S)	ml- H_2 /g-COD
5	4.9	69.4	812.2	240.9	16.2	28.1±5.2	4.8	0.19	3.47
3	4.1	54.4	1,489.2	459.7	49.6	30.9±3.5	15.3	0.62	8.50
2	3.6	46.9	921.2	276.5	46.1	30.1±2.1	13.8	0.56	5.90

^a TVS of organic waste and inoculums sludge.

^b Total COD

^c Mean value ± standard deviation.

Table 5. Comparison of H₂ yield in the present study with the findings of previous research.

Substrate	Organism	Experimental conditions			H ₂ (%)	H ₂ yield	Reference
		Reactor operation	pH	Temp. (°C)			
Food waste	Mixed culture ^a	Batch-dark fermentation	5.5±0.1	36±1	- ^b	0.1 L/g-COD	Chen et al. (2006)
Rice slurry	Mixed culture	Batch-dark fermentation	5.5	37	44-55	0.3 L/g-carbohydrate	Fang et al. (2006)
Powdered wheat starch	Mixed culture	Batch-dark fermentation	7.0	37	- ^b	96±2 ml/g-starch	Argun et al. (2008)
Cellulose	Mixed culture	Batch-dark fermentation	6.0	26	- ^b	0.02 mmol/g-cellulose	Logan et al. (2002)
Grass silage	Mixed culture	Batch-dark fermentation	4.9-5.0	35	6-23	0.004 L/g-TVS	Pakarinen et al. (2008)
Organic waste (vegetable waste)	Mixed culture	Batch-dark fermentation	5.5	Room temp. (32±3)	28.1-30.9	0.015 L/g-TVS (0.62 mmol/g-TVS)	This study

^a no heated treatment

^b no data

The Gompertz equation was fitted to hydrogen production curves in order to estimate the biogas production rates and the maximum H₂ production. Representative biogas production curves from batch tests conducted with the TVS concentration in mixed sample (the mixture of organic waste and inoculums) of 3.6–4.9 g/L at pH 5.5 are shown in Fig 2(a). After the lag phase, the H₂ evolved and increased with time to reach a peak level, stabilizing at 192 h (8 days), 312 h (13 days) and 456 h (19 days) for 5%, 2% and 3% TVS, respectively. After that, a small amount of biogas was generated but was undetectable by the gas apparatus. The highest biogas production of 1,489.2 ml and the biogas production rate (R) of 0.2 L-biogas/L-d (0.06 L-H₂/L-d) was obtained at the TVS of 3% with the minimum lag time prior to

experimental H₂ production (λ) of 25.7 h (Table 6). At 2% TVS and 5% TVS, the lag times were 30.5 h and 44.8 h, respectively. The results showed, although the initial TVS of 5% batch test has highest COD, but it has lowest hydrogen production with the long lag time before gas generation and the highest hydrogen production rate (21.5 ml/h, 3.4 ml-H₂/h). This observation indicated that the effect of substrate inhibition, the hydrogen-producing bacteria must to be acclimated at this concentration for substrate degradation and produce hydrogen gas, similar behavior was also observed in Shin *et al.* (2004) and Lin *et al.* (2008) on hydrogen fermentation from food waste and starch, respectively. The H₂ concentration was investigated from the beginning of the biogas production until 3-4 days after the

Table 6. Average kinetic constants for biogas production from various types of wastewater

pH	TVS Waste (%)	Mixed TVS (%)	Mixed COD (g)	Biogas			Hydrogen			R ²
				H _{max} ^a	R (ml/h) ^b	λ (h) ^c	H _{max} ^a	R (ml-H ₂ /h) ^b	λ (h) ^c	
5.5	5	4.9	69.4	812.2	21.5	46.7	240.9	3.4	44.8	0.9927
	3	4.1	54.4	1,489.2	5.8	7.0	459.7	2.0	25.7	0.9902
	2	3.6	46.9	921.2	9.1	29.1	276.5	2.5	30.5	0.9736

^a total amount of biogas produced (ml)

^b biogas production rate (ml/h)

^c lag time prior biogas generation (h)

^d total amount of hydrogen produced (ml-H₂)

^f hydrogen production rate (ml-H₂/h)

^g lag time prior hydrogen generation (h)

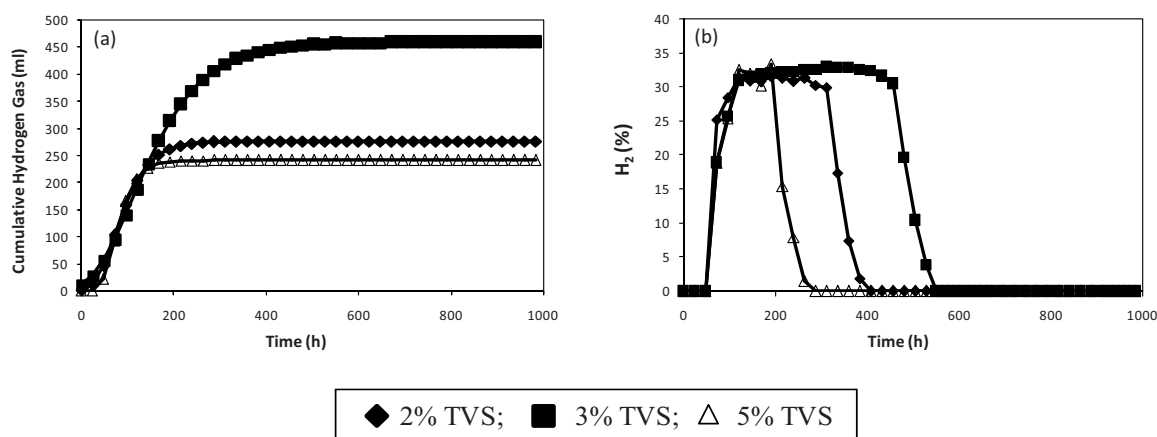


Figure 2. Bio-hydrogen produced from organic waste from fresh market at various TVS.: (a) cumulative H_2 and (b) % H_2 in biogas.

biogas was stopped. The H_2 concentration in various batch tests is shown in Fig. 2 (b). The percentage of H_2 in the biogas at the TVS concentration in mixed sample of 3.6%, 4.1% and 4.9% were 30.1%, 30.9% and 28.1% (mean value), respectively. Whereas, no CH_4 was detected in all batch tests.

3.3. Organic waste removal

TVS-removal efficiency depended on the hydrogen-producing bacteria that produced hydrogen as a final product. The hydrogen fermentation reaction was finished within 8, 13 and 19 days or 312, 456 and 192 h for 5% TVS, 2% TVS and 3% TVS, respectively. Results from each experimental batch showed that organic content of waste declined gradually during hydrogen production. After that, TVS-removal efficiency varied within a narrow range of 28–52%. It is noted that, at 5%, TVS achieved a peak TVS-removal efficiency of

52%, while at 3% TVS yielded the lowest TVS removal efficiency (Fig. 3). This is due to the fact that hydrogen fermentation is an incomplete oxidation process since organic matter is not completely oxidized to CO_2 but to intermediate compounds such as acetic acid.

This observation was confirmed by the finding that the TVFA concentration in each experimental batch increased gradually after hydrogen production was stopped. TVFA accumulated in the reactor at levels of as high as 9,000, 6,000 and 2,500 mg/L at 5% TVS, 3% TVS and 2% TVS, respectively. In addition, filtered COD and alkalinity also increased as shown in Figure 4. However these intermediate products can be further metabolized to methane and CO_2 since acetic acid is among the favorite substrates for methane production. In this case, the hydrogen fermentation was followed by methane fermentation to recover methane from wastewater and, at the same time, reduced COD as well as organic matter in wastewater.

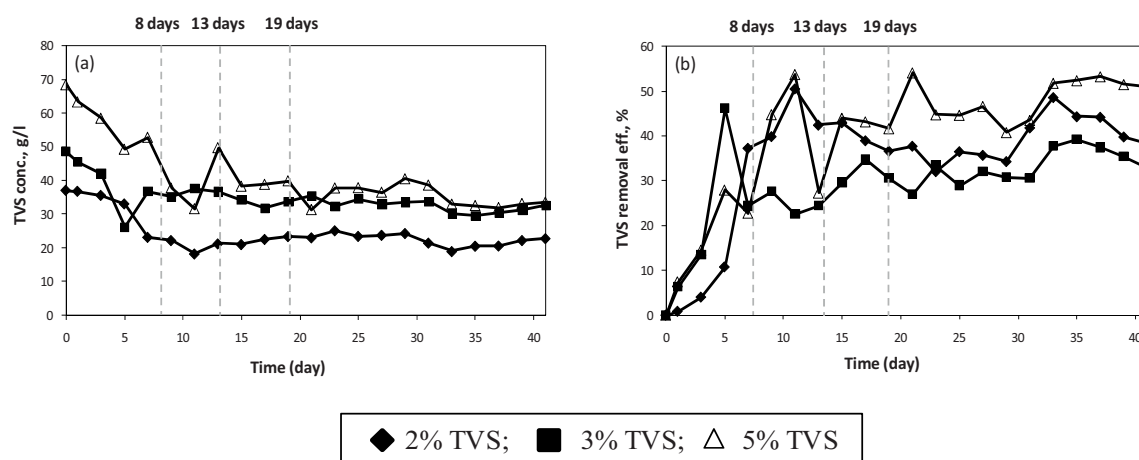


Figure 3. TVS removal efficiency of organic waste at various initial TVS.: (a) TVS concentration and (b) TVS removal efficiency.

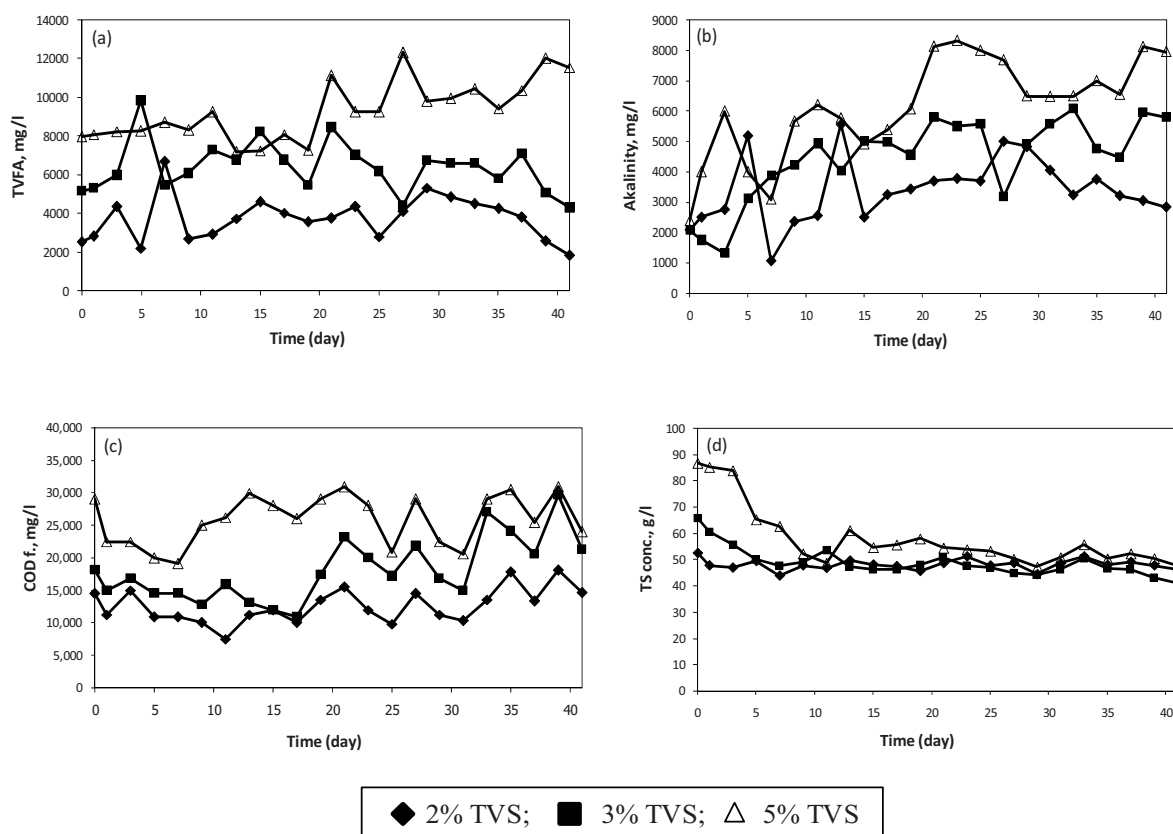


Figure 4. Operational parameter of hydrogen generation from organic waste at various initial TVS.: (a) TVFA, (b) Alkalinity, (c) Filtered COD and (d) TS.

4. Conclusion

Organic waste from a fresh market including various kinds of vegetables has the potential to generate bio-hydrogen. Effective H_2 production from organic waste was achieved using heat-treated sludge inoculums as seed and pH control at 5.5. The maximum H_2 yields were 15.3 ml- H_2 /g-TV S or 0.62 mmol H_2 /g-TV S at 3% TV S. The highest biogas production rate (R) of 0.2 L-biogas/L-d (0.06 L- H_2 /L-d) was obtained with the minimum lag time prior to experimental H_2 production (λ) of 25.7 h.

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