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Thermophilic dark fermentation of sewage sludge for biohydrogen production - influence of pH

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Abstract

This study investigates the usability of sewage sludge, waste from a waste water treatment facility, at the stable thermophilic temperature and different pH conditions in the biohydrogen production by dark fermentation. Without the addition of a pure hydrogen producer and nutrient source, the effect of a different constant pH in the range of pH 4-9 on biohydrogen production using sewage sludge was compared with that of a different initial pH. It was understood from the results that biohydrogen production varies according to the characterization of sewage sludge. In the experiments, the lag time was insignificant (~2 h). The maximum hydrogen production was achieved at pH 5 within the first 24-30 hours of fermentation (92894 mL m^{-3} H_2). Therefore, it was determined that the higher digestion efficiencies of the sewage sludge were obtained at pH 5. In general, with the increase in methanogens in the medium, the hydrogen producing ability and hydrogen content of the sewage sludge gradually decreased. Hydrogen production at almost all the pH values after the third day was less than 1000 mL m^{-3}.

Keywords: Biohydrogen production, dark fermentation, initial pH, sewage sludge, thermophilic condition.

1. Introduction

Hydrogen is known as a clean renewable energy source. The combustion of hydrogen produces no greenhouse gases, and has a high energy yield of 142.35 kJ g^{-1}, which is 2.75 fold greater than that of hydrocarbon fuels (Cai *et al.,* 2004; Guo *et al.,* 2010). Currently, hydrogen is widely recognized as an ideal alternative source of energy to substitute fossil fuels as it is renewable and has a zero-pollutant-emission energy (Liu *et al.,* 2011). Hydrogen generation can be classified in two ways: chemical-physical and biological methods. The chemical-physical methods are energy intensive and expensive. In contrast, the biological methods are environmentally favorable and consume less energy (Cai *et al.,* 2004).

Attention is very much focused on the hydrogen production from microbial conversion of industrial wastes and residues. This is not only because hydrogen is a clean energy, but also because it can be a process for waste reduction and reclamation. Alternative uses of wastes are encouraged to reduce environmental pollution. Sewage sludge from a wastewater treatment plant as a municipal solid waste is rich in carbohydrates and proteins and thus, it is a potential substrate for producing hydrogen (Guo *et al.,* 2008; Senturk and Buyukgungor, 2010; Liu *et al.,* 2011). Using sewage sludge as the substrate for fermentative hydrogen production offers several advantages over the use of other biomass sources. It is available at little or no cost (Nicolau *et al.,* 2008). The prime advantage is expensive management and the disposal of sewage sludge can be surmounted. The amount required for disposal can be converted into credit against the cost of hydrogen production (Kotay and Das, 2010).

Anaerobic digestion is an appropriate technique for the reduction in the volume and weight of excess sludge before final disposal, and it is employed worldwide as the oldest and most important process for sludge stabilization. Hydrogen is an intermediary metabolite of anaerobic digestion, which is rapidly consumed and converted into other products by the hydrogen consumption of microorganisms in the third stage of anaerobic digestion. Anaerobic digestion can be operated at different temperatures: mesophilic (25-40^{o}C), thermophilic (40-65^{o}C), extreme-thermophilic (65-80^{o}C) or hyper-thermophilic (>80^{o}C) (Levin *et al.,* 2004).

H_2 production through sewage sludge is influenced by many factors, including the type of inoculum, pretreatment, pH, temperature, and wastewater specificity (Tang *et al.,* 2008). The results so far have indicated that the control of pH is crucial to hydrogen production due to the effects of the pH on the hydrogenase activity and/or on the metabolism pathways (Fang and Liu, 2002). Thus, it is important to control pH at an optimal range to maintain hydrogen production. On the other hand, if the pH is not maintained in the desired range, it could inhibit hydrogen production or cause a microbial population shift resulting in the cessation of hydrogen production (Khanal *et al.,* 2004;

Lin *et al.,* 2011). Along with pH, temperature is also an important environmental parameter which strongly affects biohydrogen production. Most of the studies on biohydrogen production are conducted under mesophilic and thermophilic conditions.

So far, very little work on fermentative hydrogen production from sewage sludge has been conducted. However, it should be noted that so far all these studies used pure cultures of bacteria as inoculum. Although this may result in higher yields it would be expensive and impractical to attempt at full scale due to the time, cost and energy requirements of preparing and storing enough bacterial biomass to seed a digester containing thousands of liters of sewage sludge. Despite this limitation however, work published so far has demonstrated the feasibility of producing hydrogen fermentatively from sewage sludge (Nicolau *et al.,* 2008).

In the work reported here, the inoculum used to begin hydrogen production was provided directly from sewage sludge. The experiments were carried out not using additional anaerobic inoculum and extra nutrient without any pretreatment or enzyme addition to the sludge. Also, the sewage sludge properties are very important in this kind of studies. Content of treated municipal wastewater affect characteristics of sewage sludge. Hence hydrogen production is also affected. Obtained every result for this reason adds a different novelty to the literature. The present study is designed in order to investigate the usability of sewage sludge, waste from a waste water treatment facility, at the stable thermophilic temperature and different pH conditions in the biohydrogen production by dark fermentation.

2. Materials and methods

2.1. Sewage sludge

Sewage sludge used in the study was obtained from return sludge pumping station of the secondary sedimentation tank of a biological municipal wastewater treatment plant located in Samsun, Turkey and its main characteristics are shown in Table 1. The sludge was first concentrated by settling at 4°C for about 2-3 days, and the sediments were stored at 4°C before being used (Wang *et al.,* 2003).

Table 1. Mean characteristics of sewage sludge used in experiments

Item	Value (mg L^{-1})
pH	6.59±0.19
Total chemical oxygen demand (TCOD)	16394.00±2615.04
Soluble chemical oxygen demand (SCOD)	315.96±242.85
Total protein	7037.17±1702.87
Soluble protein	157.00±79.53
Total carbohydrate	1473.69±518.30
Soluble carbohydrate	16.46±6.36
Total solid (TS)	17785±3585.00
Volatile solid (VS)	12322.78±2137.45
Volatile fatty acid (mg CH_3COOH/L)	1144±205.68

2.2. Dark fermentative hydrogen production

The sewage sludge was used as the substrate and the seed without the addition of a pure hydrogen producer for hydrogen production in the experiment. Experiments of biohydrogen production from sewage sludge by fermentation were carried out in a 2-liter bioreactor with a working volume of 750 mL. Before the sludge samples were used to anaerobic fermentation, the pH value of sludge samples was adjusted to 4, 5, 6, 7, 8, 9 respectively by adding 2 M hydrochloric acid (HCl) or 4 M sodium hydroxide (NaOH). The pH adjusted sludge samples (750 mL) were added into 2-liter bioreactor, respectively. No extra nutrients were added into the tested sludge. The bioreactor was equipped with two ports for gas and sludge sampling (Figure 1a-1b). Before fermentation, the internal part of the reactor was purged with nitrogen gas for 3 minutes to provide anaerobic conditions. After quickly sealed, the reactor was placed into an incubator operating at 45°C and 150 rpm. In the whole process; pH, COD, protein and carbohydrate concentrations of the influent and the effluent with the hydrogen and methane concentration were monitored every day. The anaerobic digestion was continued until hydrogen production stopped or decreased (Senturk and Buyukgungor, 2017).

2.3. Analytical methods

During incubation, biogas production was measured periodically by displacement of saturated aqueous 10% NaCl with 2% H_2SO_4 in a graduated cylinder. The biogas in the headspace of digesters was sampled with a 1 mL gastight syringe. The hydrogen and methane contents of biogas were analyzed by a gas chromatograph (Shimadzu, GC-2010) equipped with a thermal conductivity detector (TCD) and Rt® - Msieve 5A (19723) capillary column. The temperatures of injector, detector and column were kept at 200°C, 200°C and 70°C, respectively. Helium gas was used as the carrier gas with a flow rate of 19.3 mL min^{-1}. The concentration of hydrogen and methane was tested frequently during fermentation and the biogas production was also recorded during the whole examination. The measured values were expressed as mL m^{-3} (ppmv = gas gas^{-1}). With samples obtained before and after fermentation, characteristics of the sludge in the fermenter were identified (Senturk and Buyukgungor, 2017a).

The pH of sludge was measured by a pH meter (Sartorius PB-20). The total COD and soluble COD concentrations of sludge were determined with closed reflux titrimetric method according to the standard method (APHA, 2005). Total and soluble proteins in the liquid phase were measured by the Lowry's method using bovine serum albumin as a standard solution (Lowry *et al.,* 1951), total and soluble carbohydrates by the phenol-sulfuric acid method using glucose as a standard solution (Dubois *et al.,* 1956). The samples were filtered through a 0.45 µm membrane and centrifuged at 4000 rpm for 30 min before determining the concentrations of SCOD, soluble protein, and soluble carbohydrate.

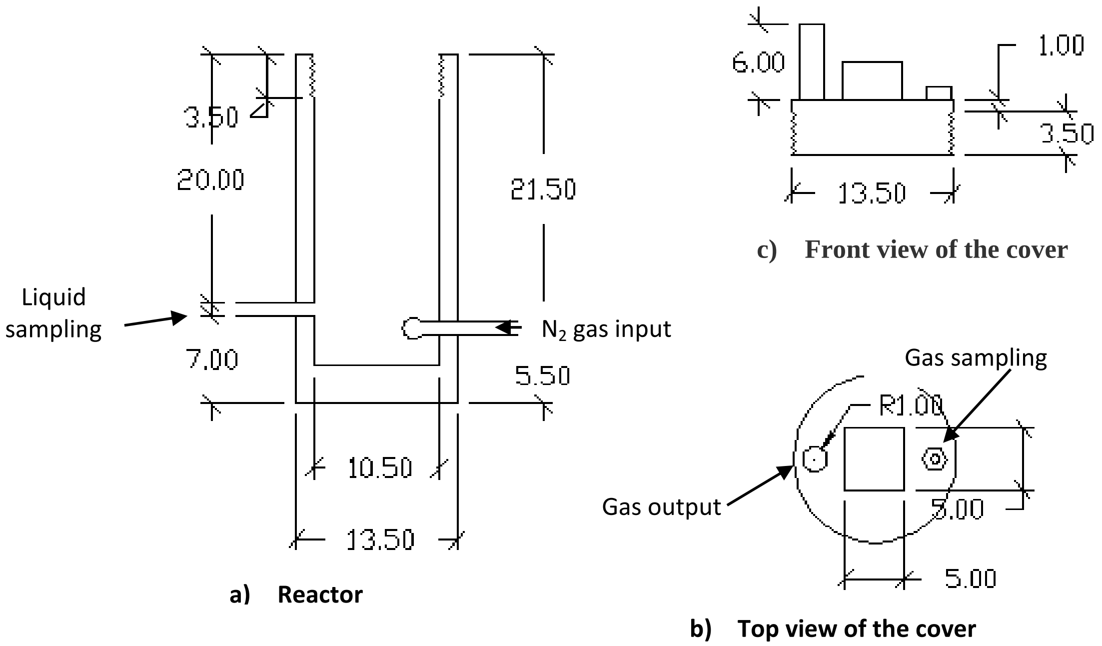

Figure 1a. Reactor used in fermentation experiments

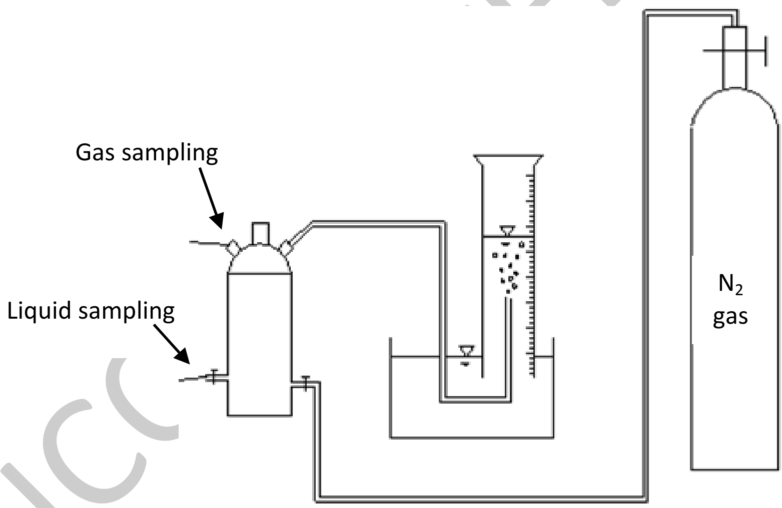

Figure 1b. Experimental set-up

3. Results and discussion

3.1. Effect of initial pH on solubilization of sewage sludge

Many factors such as temperatures and the initial pH can influence the fermentative hydrogen production because they can affect the activity of hydrogen-producing bacteria by influencing the activity of some essential enzymes such as hydrogenases for the fermentative hydrogen production. It has been demonstrated by various one-factor-at-a-time experiments that in an appropriate range, increasing temperatures and the initial pH respectively could increase the ability of hydrogen-producing bacteria to produce hydrogen (Wang and Wan, 2011).

The effects of pH, the content of the sewage sludge in the reactor and the changes occurring in the reactor at the time of biohydrogen production from the sewage sludge via anaerobic fermentation were investigated in studies conducted in a thermophilic temperature. During the sludge fermentation for the biohydrogen production, these organic materials usually undergo solubilization, hydrolysis, and acidification. The changes in the soluble protein and carbohydrate were applied in the current study to express the solubilization of the sludge's main particulate organic matters. The results obtained are described in detail below.

Due to hydrolysis, the total protein and total carbohydrates concentrations increased within the first 24 h at pH 4. However, as the fermentation process progressed, there was a decrease in the total protein and the total carbohydrates concentration and increased C_o/C ratios (Table 2). The changes occurring in the concentrations during the fermentation are due to the balance between the amounts consumed and released from the solid phase. Hydrolysis, a phase occurring between anaerobic fermentation and biohydrogen production, plays an important role in the release of organic matters in the solid phase (Chen *et al.,* 2002).

Table 2. The change in total matter concentrations versus time at different pH values

		C_o/C ratio		
	Total matter	**24 h**	**48 h**	**72 h**
pH 4	Protein	0.64	-	1.05
	Carbohydrates	0.89	-	1.61
	COD	1.08	-	1.86
	Mean	0.87		1.5067
	Std. Deviation	0.22068		0.41477
pH 5	Protein	1.74	2.07	2.92
	Carbohydrates	1.06	1.19	1.25
	COD	1.00	1.09	1.09
	Mean	1.2667	1.45	1.7533
	Std. deviation	0.41102	0.53926	1.01353
pH 6	Protein	0.98	0.87	-
	Carbohydrates	1.46	1.60	-
	COD	1.03	1.03	-
	Mean	1.1567	1.1667	
	Std. Deviation	0.26388	0.38371	
pH 7	Protein	0.98	0.92	1.23
	Carbohydrates	1.63	1.70	1.55
	COD	1.27	1.27	1.48
	Mean	1.2933	1.2967	1.42
	Std. deviation	0.32563	0.39068	0.16823
pH 8	Protein	1.06	1.24	1.33
	Carbohydrates	1.27	1.23	1.81
	COD	1.60	2.00	2.51
	Mean	1.31	1.49	1.8833
	Std. deviation	0.27221	0.4417	0.59341
pH 9	Protein	1.28	1.34	-
	Carbohydrates	1.16	1.50	-
	COD	1.11	1.11	-
	Mean	1.1833	1.3167	
	Std. Deviation	0.08737	0.19604	

Unread C_o = Initial total matter concentration C = total matter concentration at any time

Studies have shown that the increases in the soluble matter concentration positively affect the hydrogen production from sewage sludge (Nicolau *et al.,* 2008; Kotay and Das, 2009; Guo *et al.,* 2010). In the study conducted at pH 5, when the reactor content against time was examined, we see a decrease in total protein, total carbohydrates, and TCOD concentrations during fermentation (Table 2). The concentration of the dissolved matter showed an alteration as shown in Table 3. While soluble carbohydrates and SCOD concentrations continued to increase during the fermentation, the soluble protein began to decrease after a time period of 24 hours. The increase in soluble protein within the first 24 hours, as seen in Figure 2, affected the hydrogen production positively.

Table 3. The change in soluble matter concentrations versus time at different pH values

		C_o/C ratio		
	Soluble matter	**24 h**	**48 h**	**72 h**
pH 4	Protein	0.68	-	0.98
	Carbohydrates	0.31	-	0.22
	COD	0.29	-	0.22
	Mean	0.4267		0.4733
	Std. deviation	0.21962		0.43879
pH 5	Protein	0.59	1.24	1.65
	Carbohydrates	0.18	0.17	0.12
	COD	0.07	0.06	0.05
	Mean	0.28	0.49	0.6067
	Std. deviation	0.27404	0.65184	0.90423
pH 6	Protein	0.59	0.75	-
	Carbohydrates	0.10	0.10	-
	COD	0.08	0.04	-
	Mean	0.2567	0.2967	
	Std. deviation	0.28885	0.39374	
pH 7	Protein	1.04	1.15	1.87
	Carbohydrates	0.16	0.15	0.12
	COD	0.12	0.06	0.08
	Mean	0.44	0.4533	0.69
	Std. deviation	0.52	0.60501	1.02211
pH 8	Protein	0.21	0.22	0.63
	Carbohydrates	0.20	0.13	0.19
	COD	0.02	0.03	0.09
	Mean	0.1433	0.1267	0.3033
	Std. deviation	0.10693	0.09504	0.28729
pH 9	Protein	0.28	0.32	-
	Carbohydrates	1.37	0.74	-
	COD	0.20	0.20	-
	Mean	0.6167	0.42	
	Std. dDeviation	0.65363	0.28355	

Unread C_o = Initial soluble matter concentration C = soluble matter concentration at any time

The general result obtained from the studies conducted at pH 6, 7, 8 and 9 respectively is conclude that the total protein, total carbohydrates, and TCOD concentrations change with time. The soluble matter concentrations generally showed increases in the first 24 hours of fermentation. However, after 24 hours the soluble protein concentration decreased at all the pH values while the carbohydrates and COD concentration either increased or did not change. The increase in the solute COD and carbohydrate concentration did not have an effect on enhancing the hydrogen production, indicating that the soluble protein was effective in the hydrogen production in the first 24 hours.

When Table 3 was examined, despite some exceptions (at pH 7, the soluble protein concentration continuously decreased during the fermentation), it was observed that the amount of soluble nutrients released from the solid phase of the sewage sludge was higher during the hydrogen fermentation. However, these results do not show any parallelism with the hydrogen production graph in Figure 2. Therefore, it is clear that all the organic matter released from the sewage sludge did not convert into hydrogen through anaerobic fermentation. Similar results were reported by Guo *et al.* (2010). In their study, Guo *et al.* (2010) performed fermentation tests by using pretreated sewage sludge via sterilization and filtrate obtained from the sludge. The amount of organic matter released from the filtrate was less than that released from the sludge during fermentation. However, the hydrogen yield from filtrate was higher than that obtained from the sludge and this suggested that not all the dissolved organic matter was converted into hydrogen.

For statistical evaluation of the change in soluble and total matter concentrations versus time at different pH values, the one-way analysis of variance (ANOVA) test was conducted to test the significance of experimental data shown in Tables 2 and 3. The corresponding ANOVA for Tables 2 and 3 showed that the F-values were 1.015, 0.305 and 0.350, respectively. It implies that the data were statistically not significant because P>0.05 (α-level) (Table 4).

3.2. The effect on the production of H_2 and CH_4 in the change in the concentration of the soluble matter in the reactor at different pHs

For the biohydrogen production, the soluble organics are very important since the hydrogen producing microorganisms need to use them in order to grow and produce hydrogen (BenYi and JunXin, 2009). Among all the organic solid matters, carbohydrates are the compounds which undergo the maximum degradation during the anaerobic fermentation. However, proteins and lipids also contribute to hydrogen production to some extent. In this study, we observed that the dissolution of the carbohydrates was higher than that of the proteins during the fermentation.

Proteins are the principal constituents of an animal organism and they contain carbon, which is common in all organic substances, as well as in hydrogen and oxygen. In addition, they contain as their distinguishing characteristic, a fairly high and constant proportion of nitrogen. In many cases sulfur, phosphorus, and iron are also constituents. Therefore, the efficiency of anaerobic fermentation is expected to increase with the increase in soluble protein concentration (Nah *et al.,* 2000). As clearly seen from Figure 2, the increase in soluble protein concentrations within the first 24 hours increased the hydrogen production.

Table 4. ANOVA for the change in soluble and total matter concentrations versus time different pH values

	Parameters	Source of variation	SS	DF	MS	F-value	P-value
Total matter	24 h	$Groups_{between}$	.402	5	.080	1.015	.451
		$Groups_{within}$	.950	12	.079		
		Total	1.352	17			
	48 h	$Groups_{between}$	.201	4	.050	.305	.868
		$Groups_{within}$	1.648	10	.165		
		Total	1.849	14			
	72 h	$Groups_{between}$	.415	3	.138	.350	.790
		$Groups_{within}$	3.159	8	.395		
		Total	3.574	11			
Soluble matter	24 h	$Groups_{between}$	.422	5	.084	.553	.734
		$Groups_{within}$	1.832	12	.153		
		Total	2.254	17			
	48 h	$Groups_{between}$	.263	4	.066	.317	.860
		$Groups_{within}$	2.071	10	.207		
		Total	2.334	14			
	72 h	$Groups_{between}$	.257	3	.086	.160	.920
		$Groups_{within}$	4.275	8	.534		
		Total	4.531	11			

SS, sum of squares; DF, degrees of freedom; MS, mean square

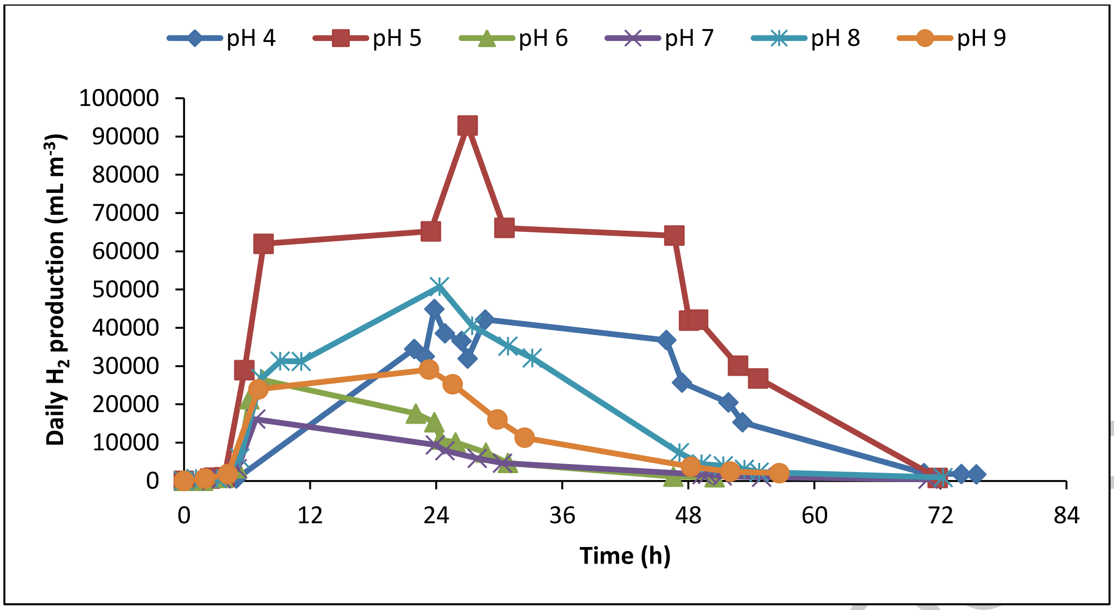

Figure 2. Effect of different initial pH on hydrogen production during sludge fermentation

Again, as seen from the studies, soluble protein concentration shows increases at the beginning of hydrogen production fermentation. However, the protein concentration decreased with time during fermentation due to soluble protein being used for hydrogen production. An alteration in the soluble carbohydrates content during fermentation is similar to that in protein. However, while the amount of released soluble carbohydrates increases, the total amount of carbohydrate reduces, and the hydrogen production becomes slightly lower when compared to the use of proteins, which shows that the organic matter used for hydrogen production in the sewage sludge is protein and then carbohydrate. Guo *et al.,* 2008 used pretreated sludge and *Pseudomonas sp.* GZ1 (EF551040) for hydrogen production via anaerobic fermentation. Similarly, they reported that protein fermentation was more effective in hydrogen production.

In their study, Xiao and Liu (2009) reported that the hydrogen production stage was associated with the decrease in the dissolved carbohydrate concentration and carbohydrates were the main material for hydrogen production via anaerobic fermentation. Nicolau *et al.* (2008) indicated that carbohydrate fermentation to hydrogen is a source of hydrogen production in the anaerobic fermentation of sewage sludge. These results are different from those obtained from Cai *et al.* (2004) and Guo *et al.* (2008). These different results obtained from these studies can be attributed to the different types of substrate and inoculums used in hydrogen production and their sources (Xiao and Liu, 2009).

In Figure 2, hydrogen concentrations obtained as a result of anaerobic fermentation studies conducted at 45°C with different initial pH values are given. In contrast to the results obtained by Cheng *et al.* (2000) by using longer lag periods such as 66 h, the lag periods in this study are insignificant for anaerobic fermentation (~2 h). The chemical and biological content of the substrate may have led to the shortening of the time. In all the pH tests, the hydrogen concentration in the gas phase showed a fluctuating curve with peaks observed between 7 and 24 hours. This shows that a significant amount of hydrogen produced is consumed somewhat. Usually at very low hydrogen concentrations, the hydrogen consuming methanogenic bacteria can convert the formed hydrogen to methane if the methanogenesis step goes smoothly. This also causes a rapid decrease in the amount of hydrogen (Cai *et al.,* 2004).

In these tests, after a 1-2 hour lag time, it was observed that the hydrogen content increased in time and the hydrogen gas release showed an increase after a period of approximately 4-5 hours. However, especially at pH 6 and 7, the hydrogen concentration rapidly decreased after 7-8 hours. At pH 8 and 9, the concentration was higher compared to pH 6 and 7. However, at the end of 24 hours, the hydrogen began to decrease at these pH values. At pH 4 and 5, a decrease in the hydrogen concentration was observed at the end of 48 hours. At pH 5, the highest hydrogen gas concentration was read. The hydrogen gas release remained at higher levels and lasted longer when compared to pH 4. Overall, the hydrogen content decreased monotonically over time and dropped below 1000 mL m^{-3} at almost all pHs at the end of 3 days.

Figure 3 provides the amount of methane concentration in the reactor during anaerobic fermentation. In all the pH studies, significant increases were observed in the methane concentration after 24 hours. At pH 6, approximately 90000 mL m^{-3} of methane was produced at the end of the 28-29 hour of the fermentation, which is the highest concentration obtained in all tests. The amount of methane produced from the sewage sludge after a 48 hour of fermentation, as seen from Figure 3, is as follows; pH 6>pH 9>pH 7>pH 5>pH 8>pH 4. The active methane bacteria in the environment leads to a depletion of hydrogen gas in the reactor, and thus, the hydrogen gas concentration decreased in a short time. Although sewage sludge produces hydrogen, microorganisms in the sewage sludge consume hydrogen rapidly.

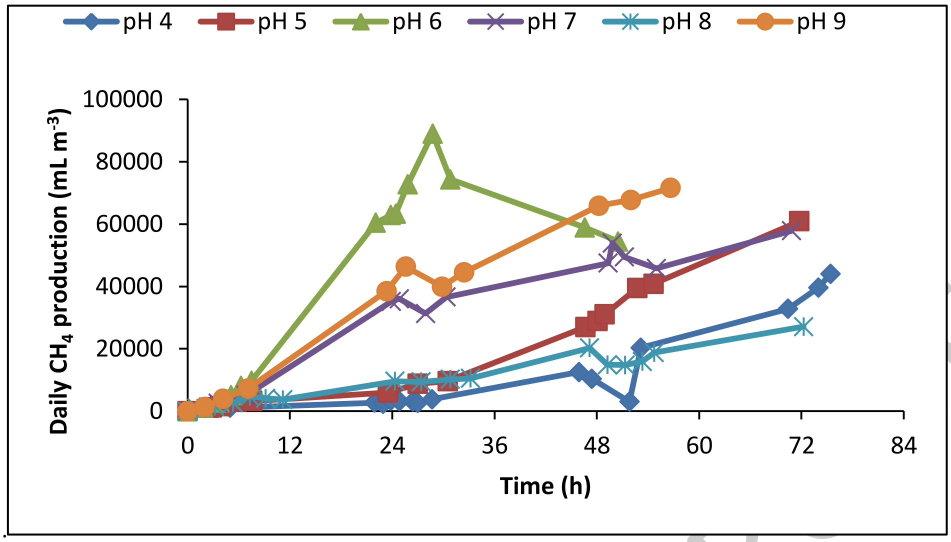

Figure 3. Effect of different initial pH on methane production during sludge fermentation

As is known, the anaerobic fermentation process of waste water or the waste biosolid involves three stages as hydrolysis, acidification, and methane production. Hydrogen is produced in the acidification step and pH affects biohydrogen fermentation. In the hydrolysis step, the pH is reduced. In the acidification, namely hydrogen production step, the pH increases and remains constant (Cai *et al.,* 2004).

Chen *et al.* (2002) indicated that the proper pH level could shorten the lag time and be useful for producing hydrogen in acclimating anaerobic microorganisms; pH 6.5-7.0 was the best levels for enhancing the hydrogen production. Cai *et al.* (2004) performed their studies in a serum bottle (a volume of 125 mL). They used waste water as the substrate without adding extra nutrients or seed and studied at 36±1°C at 150 rpm and pH 3-12.5. The final pH decreased at the initial pH of 7.0-12.5, but increased at the initial pH of 3.0-6.0.

Similarly, as a result of the fermentation experiments conducted at 45°C, we observed that when the initial pH is between 7 and 9, the pH value decreased at the end of the fermentation, but increased at the pH values ranging from 4 to 6. The differences in the change of pH were concerned with the SCOD of the sludge samples since the productions of the VFA and NH_4^+-N is the result of the organics (SCOD) degradation (BenYi and JunXin, 2009).

In all the tests, at the end of 2-3 hours, the hydrogen production began. Depending on the initial pH, the hydrogen production at the end of 24 hours is as follows; pH 5> pH 8> pH 4> pH 9> pH 6> pH 7. The maximum yield was obtained at the 24-30th hours of fermentation at pH 5 (92894 mL m^{-3} H_2). However, the hydrogen accumulated in the upper part of the reactor, is consumed by hydrogen consumers in the upcoming days of fermentation. At pH 6, 7 and 9, a rapid consumption in the amount of hydrogen in the reactor was observed after the first 4 hours. At the same time, the highest methane production was obtained at pH 6, pH 9, and pH 7, respectively. The hydrogen gas production and consumption ranking revealed that better results were obtained in studies using the initial pH value of 5. Although hydrogen is produced at pH 8, a rapid decrease was observed in the hydrogen production in a short period of time. The studies in the literature support our results. The pH values of 5 and 6 were preferred in most of the studies on fermentative hydrogen production (Nicolau *et al.,* 2008).

The pH value also influences the solubility of organic matters found in sewage sludge structure. In this study, high solubility was obtained at pH 5 when compared to other pH values. At pH 5, the protein, carbohydrate, and COD in the structure of the sewage sludge in the reactor become more soluble with increased hydrogen production efficiency. Additionally, because methanogenic activity reduces or stops at the pH values lower than 6.3, the methanogenic activity is expected to be inhibited at pH below 6. Not surprisingly, the methane concentration remained at lower levels at pH 4 and 5 (Figure 3).

Considering these results and the hydrogen production graph given in Figure 2, it was concluded that setting the pH of reactor contents to 5 would be appropriate.

4. Conclusions

In light of all the information given throughout this paper, wastewater sewage sludge was preferred as a raw material for biohydrogen production under thermophilic conditions via anaerobic fermentation. Total and dissolved substances are used to meet the nutritional needs of microorganisms in the fermentor, and consequently this also increases its hydrogen production efficiency.

In particular, an increase in the amount of soluble protein positively affected the hydrogen production within the first 24 hours.

Another important factor affecting hydrogen production is the SCOD/TCOD ratio. However, this increase is not directly proportional. In this study conducted under thermophilic conditions, solubility was higher at pH 5 in comparison to other pH values suggesting that pH 5 is more appropriate for biohydrogen production. At an initial pH 5.0, the maximum cumulative biohydrogen production (92894 mL m^{-3} H_2) was attained. The favorable initial pH for hydrogen producing anaerobic bacteria was found to be 5.0. It was also concluded that the characteristics of wastewater and the initial pH affected the fermentative biohydrogen production.

The results obtained in this study are useful for designing pH operating conditions for an anaerobic reactor in order to produce biohydrogen. Future studies should continue investigating the issues such as the removal of the hydrogen consuming microorganisms from the reactor, the delay in the methane production, and increment of the solubility of the substance in the fermentor.

Acknowledgments

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