Energy from Sewage Sludge under Thermophilic Conditions

IlknurSenturkHanifeBuyukgungor*

Environmental Engineering Department, Engineering Faculty, OndokuzMayis University, Atakum/Samsun 55270, Turkey

e-mail: hbuyukg@omu.edu.tr

Abstract The impacts of different pH values (4-9) on hydrogen production under thermophilic conditions were discussed in detail. It was understood from the results that biohydrogen production varies according to the characterization of activated sludge. In the experiments performed at different pH values at 45±1°C, lag time was insignificant (~2h). After 1-2 hours lag time, the hydrogen gas content was increased with time. After a period of approximately 4-5 hours, it was observed that the hydrogen gas production began to increase. But, after 24 hours, hydrogen gas production was begun to decline. The hydrogen productions depending on the initial pH value was changed in form pH 5>pH 8>pH 4>pH 9>pH 6>pH 7 at the end of the first day.Maximum hydrogen production was achieved at pH 5 within the first 24-30 hours of the fermentation (92894 mL m^{-3} H_2). Therefore, it is recognized that higher digestion efficiencies of the sewage sludge were obtained at pH 5. On the contrary of other pH values, the hydrogen concentration was also decreased after the second day. In general, with the increase in methane bacteria in the medium, hydrogen producing ability and hydrogen content of the sludge gradually decreased. Hydrogen production at almost all pH values after the third day was less than 1000 mL m^{-3}.

KeywordsBiohydrogenproduction·Dark fermentation·Renewable energy·Sewage sludge·Thermophilic condition

Introduction

The utilization of current energy sources has been generating environmental pollution of air, water and soil through the years. These negative effects have increased interest in the development of new technologies to obtain clean energy, mainly through the utilization of renewable energy sources. Currently, the world consumes about 15 terawatts of energy per year and only 7.8% of this is derived from renewable energy sources (Senturk&Buyukgungor 2013).

Hydrogen is known as a clean renewable energy source. Combustion of hydrogen produces no greenhouse gases, and has a high energy yield of 142.35 kJg^{-1}, which is 2.75 fold greater than that of hydrocarbon fuels. Thus, utilization of hydrogen as a clean energy source seems to be promising (Cai et al. 2004, Guo et al. 2010). Currently, hydrogen has been widely recognized as an ideal alternative source of energy to substitute fossil fuels, as it is renewable and zero-pollutant-emission energy (Liu et al. 2011). Hydrogen generationcan be classified into two ways: chemical-physical and biological methods. The chemical-physical methods (e.g., through fossil fuel processing, water electrolysis using solar power) are energy intensive and expensive. In contrast, the biological methods are environmentally favorable and consume less energy. The biological wastewater treatment processes are used worldwide. However, large amounts of sewage sludge are produced from these biological processes (Cai et al. 2004).

The strict requirement of wastewater effluent standard and a good design for wastewater treatment process increased the quantity of waste activated sludge, commonly known as sewage sludge. In 2008, about 2.38 billion m^3 $year^{-1}$ of municipal and industrial wastewater was treated in wastewater treatment plants in Turkey, producing about 1,075.000 t $year^{-1}$ of sewage sludge (TUIK 2008, Senturk&Buyukgungor 2014).

Sewage sludge is an important renewable energy source, which unlike others can be more harmful to the environment if not utilized or properly disposed. Sewage sludge from a wastewater treatment plant is rich in carbohydrates and proteins and thus it is a potential substrate for producing hydrogen(Cai et al. 2004, Guo et al. 2008, Senturk&Buyukgungor 2010, 2013a). 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. The supply is plentiful and can be found wherever there are human settlements (Nicolau et al. 2008). The prime advantage is expensive management and disposal of sewage sludge can be surmounted. The amount required for disposal can be converted into a credit against the cost of hydrogen production (Kotay& Das 2010).

The sewage sludge of wastewater treatment plants is composed largely of organic matters (59-88%) that can decompose and produce offensive odors. These organics are mainly the microbial matters and the microorganisms include hydrogen producing ones and hydrogen consuming ones. The treatment and disposal of the excess sludge has become an important problem and a great challenge for many plants. Anaerobic digestion is an appropriate technique for 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. Additionally, anaerobic digestion can recover partly the bioenergy of sludge through producing methane. Hydrogen is an intermediary metabolite of anaerobic digestion, which is rapidly taken up and converted to other products by the hydrogen consuming microorganisms in the third stage of anaerobic digestion. On the other hand, the use of hydrogen is more extensive than that of methane (Xiao & Liu 2009).

In this study, it is expected to address the problems associated with waste activated sludge disposal through simultaneous generation of clean gaseous energy in the form of hydrogen. A synchronous objective was to investigate the usability of sewage sludge, a waste from waste water treatment facility, at the stable thermophilic temperature and different pH conditions in the biohydrogen production by dark fermentation.Biohydrogen production could be enhanced and maintained stable by the combination of suitable fermantation temperature and pH.

Material and Methods

Anaerobic Fermentation of the Sludge

Activated sludge used in the study was obtained from return sludge pumping station of a sewage treatment plant located in Bafra, Samsun and its characteristics are shown in Table 1. The collected sludge samples were gravitationally settled for about 2-3 days and the sediments were stored at 4ºC before being used (Wang et al. 2003).

Table 1 Mean characteristics of was used in experiments

Item	**Value (mg L^{-1})**
pH	6-7
Tprotein	7037
Sprotein	157
Tcarbohydrate	1474
Scarbohydrate	16.5
TCOD	16394
SCOD	316

Before the sludge samples were used to anaerobic fermentation, pH of samples was adjusted from 4 to 9 with 4 M NaOH or 2 M HCl. The pH adjusted sludge samples (750 mL) were added into 2 liter bioreactor, respectively. No extra-seeds and extra-feeds were added into these reactors. The bioreactor was equipped with two ports for gas and sludge sampling. 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±1 °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.

Analytical Methods

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 $mLmin^{-1}$. 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 the samples obtained at different times (before and after fermentation), characteristics of the sludge in the fermenter was identified. All experiments continued until hydrogen production stopped or decreased.

The pH of sludge was measured by a pH meter (Sartorius PB-20). The TCOD and SCOD concentrations of sludge were determined with closed reflux titrimetric method according to the standard method (APHA 2005). 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 proteins in the liquid phase were measured by the Lowry's method using bovine serum albumin as a standard solution (Lowry et al. 1951), and soluble carbohydrates by the phenol-sulfuric acid method using glucose as a standard solution (Dubois et al. 1956).

Results and Discussion

The effects of pH, the content of sewage sludge in the reactor and the changes occurring in the reactor in time on biohydrogen production from sewage sludge via anaerobic fermentation were investigated in studies conducted at 45±1°C. The results obtained are described in detail below.

Due to hydrolysis, Tprotein and Tcarbohydrates concentration increased within the first 24hour and then decreased at pH 4 (Figs. 1, 2). The changes occurring in the concentrations during fermentation are due to the balance between the amounts released from the solid phase and that consumed. Protein and carbohydrates are consumed during hydrogen production. Therefore, 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).

At pH 4, Sprotein, Scarbohydrates and SCOD concentration increased during fermentation (Figs. 4-6). Studies have shown that the increases in Scarbohydrates concentration positively affect hydrogen production from sewage sludge (Nicolai et al. 2008,Kotay& Das 2009, Guo et al. 2010). However, as the amount of Sprotein decreased, reduction in hydrogen production was observed. After 2 hour fermentation, Sprotein concentration reduced from 180 mg L^{-1} to 125 mg L^{-1} (Fig. 4). Decreased Sprotein concentration and increased amount of methane bacteria in the reactor result in reduction in hydrogen gas concentration. The amount of SCOD continued to increase during trial hydrogen production (Fig. 6).

In the study conducted at pH 5, when the reactor content against time was examined, we see reductions in Tprotein, Tcarbohydrates and the TCOD concentrations during fermentation (Figs. 1-3).

The concentration of dissolved matter showed alteration as shown in Figs. 4-6. While Scarbohydrates and SCOD concentrations continued to increase during fermentation, Sprotein began to decrease after a time period of 24 hours. The increase in Sprotein within the 24-30 hour, as seen in Fig. 8, affected hydrogen production positively. Similarly, the increase in SCOD concentration during fermentation influenced hydrogenproduction and food supply that is required by the microorganisms. As shown in Fig. 6, the SCOD concentration in the reactor increased during fermentation.

The general result obtained from the studies conducted at pH 6, 7, 8 and 9 respectively is that Tprotein, Tcarbohydrates and TCOD concentrations decreased with time. However, in the study conducted at pH 6, Tprotein concentration increased from 7952 mg L^{-1} to 9095 mg L^{-1} after ~ 51 hour fermentation (Fig. 1). The solute concentrations generally showed increases in the first days of fermentation and then decreased in time. The alteration in the concentration is a factor that affects biohydrogen production.

When Figs. 4-6 was examined, despite some exceptions (at pH 7, Sprotein concentration continuously decreased during fermentation), it was observed that the amount of soluble nutrients released from the solid phase of sewage sludge was higher during hydrogen fermentation. However, these results do not show parallelism with the hydrogen production graph in Fig. 7. The result shows that not all dissolved organic matter was converted into the hydrogen. 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, hydrogen yield from filtrate was higher than that obtained from sludge and this suggested that not all dissolved organic matter was converted into the hydrogen.

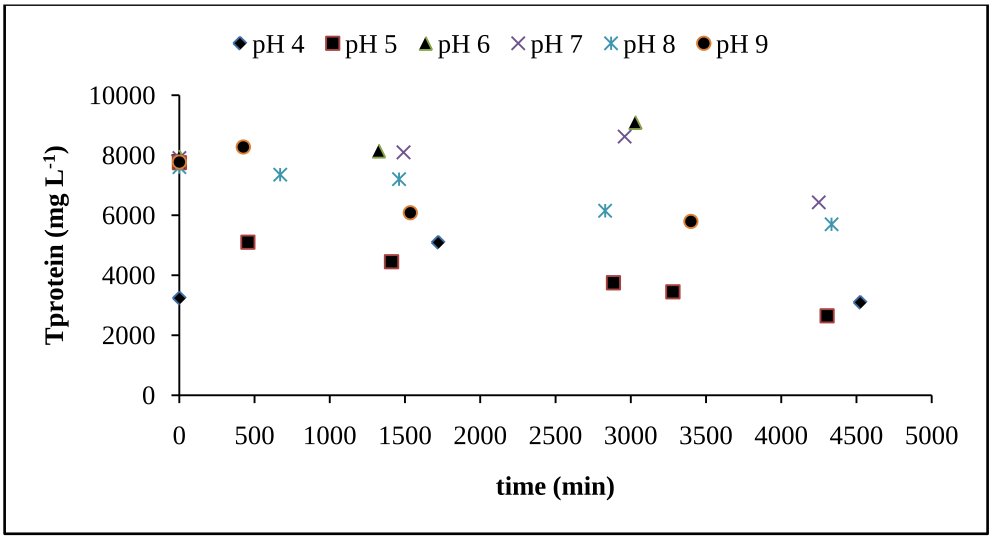

Fig. 1 Alterations in Tprotein concentration in the reactor during fermentation depending on the pH

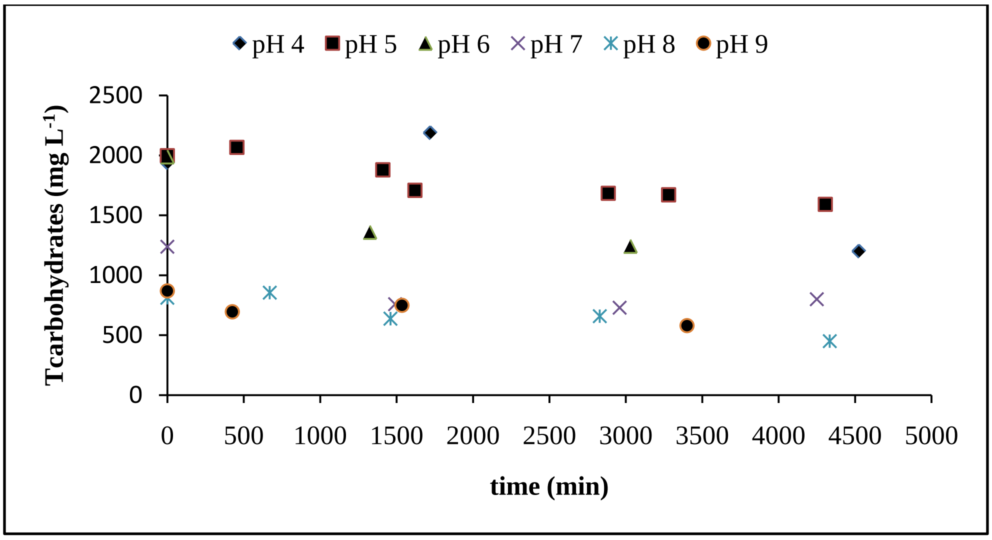

Fig. 2 Alterations in Tcarbohydrates concentration in the reactor during fermentation depending on the pH

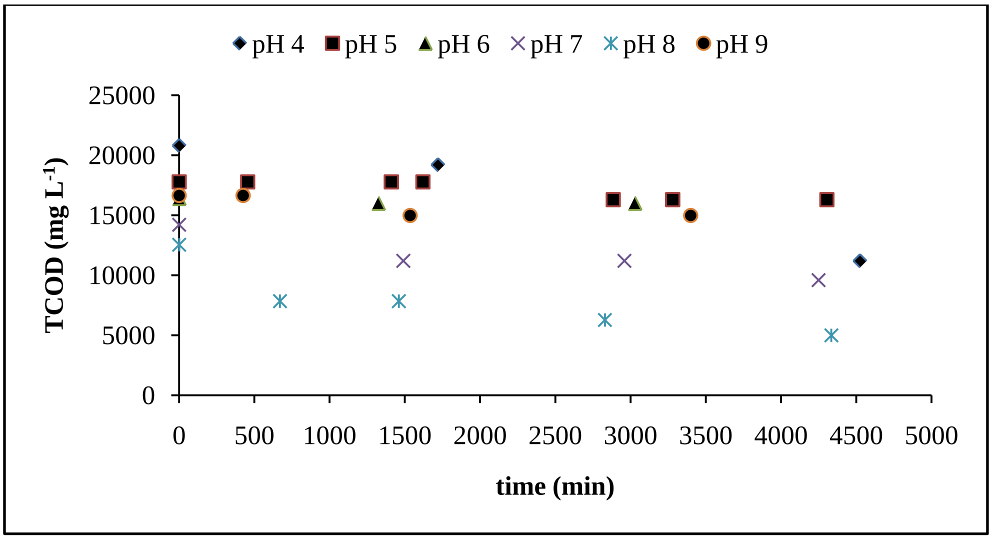

Fig. 3 Alterations in TCOD concentration in the reactor during fermentation depending on the pH

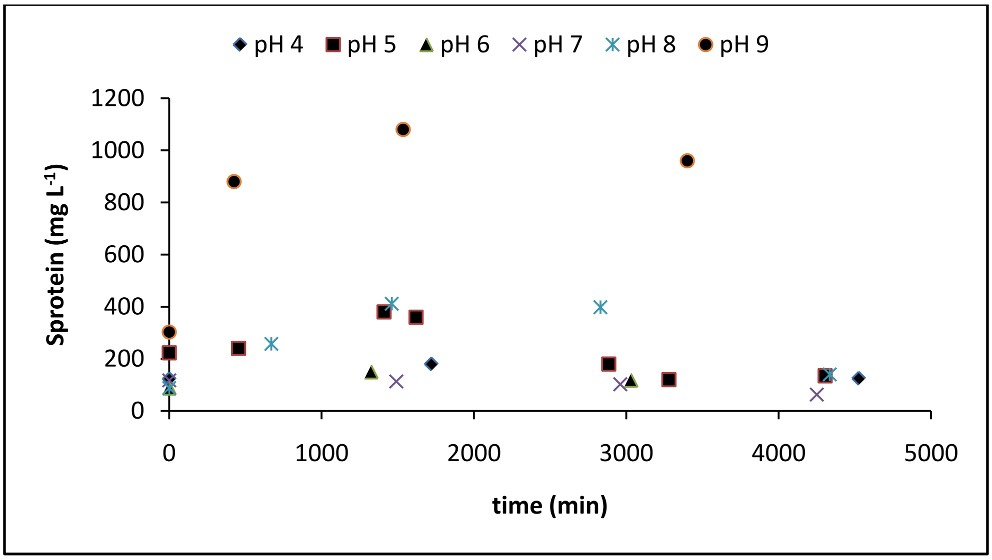

Fig. 4 Alterations in Sprotein concentration in the reactor during fermentation depending on the pH

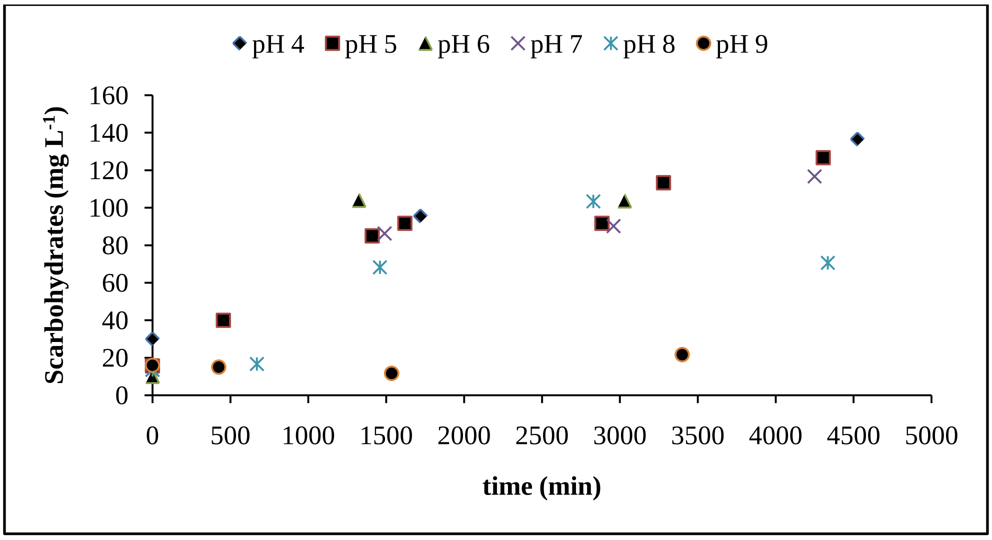

Fig. 5 Alterations in Scarbohydrates concentration in the reactor during fermentation depending on the pH

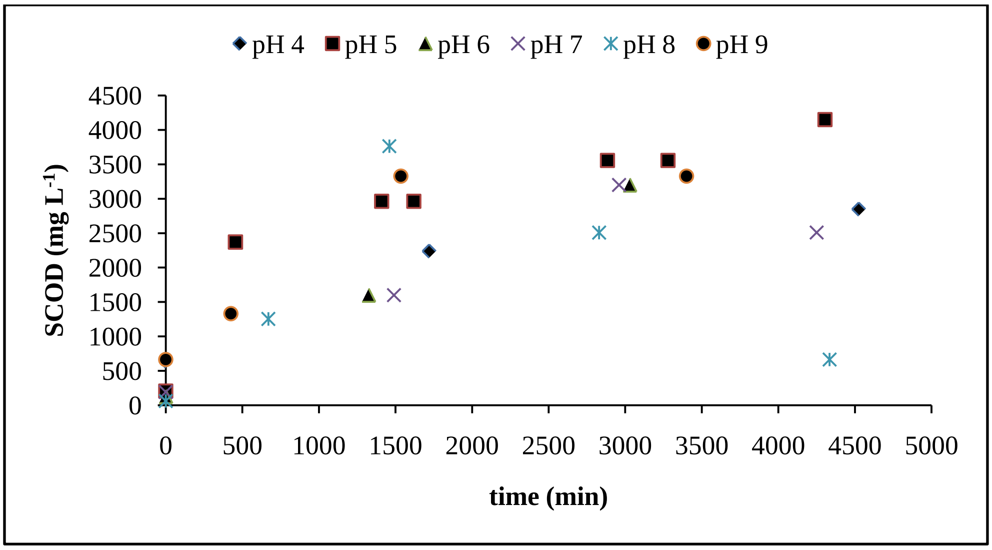

Fig. 6 Alterations in SCOD concentration in the reactor during fermentation depending on the pH

pH studies using untreated sewage sludge show that Sprotein, Scarbohydrate and SCOD concentrations reach their maximum concentrations within first 24 hours due to hydrolysis. Despite gradually decreases in the concentration in time, the concentrations at the end of the test were higher in comparison to baseline in almost all tests (except for Sprotein concentration in the reactor at pH 5 and pH 7) (Fig. 4).

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

Proteins are the principal constituents of the animal organism and they contain carbon, which is common to all organic substances, as well as 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 Sprotein concentration (Nah et al. 2000). As clearly seen from Fig. 4, analysis of Sprotein concentrations within the first 24 hours, depending on the pH, showed alterations as pH 9 (1080 mg L^{-1}) > pH 8 (411 mg L^{-1}) > pH 5 (380 mg L^{-1}) > pH 4 (180 mg L^{-1}) > pH 6 (150 mg L^{-1}) > pH 7 (113 mg L^{-1}). Although, the order is not full compromised with the graph, it supports the hydrogen production graph given in Fig. 7.

Again, as seen from the studies, Sprotein concentration show increases in the beginning of hydrogen production fermentation. However, with the protein fermentation process, the concentration decreased in time, because protein was used for hydrogen production. Alteration in carbohydrates content during fermentation is similar to that in protein. But, while the amount of carbohydrates released increases, the total amount of carbohydrate reduces and hydrogen production becomes slightly lower compared to the use of proteins which shows that the organic matter used for hydrogen production in the sewage 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 & Liu (2009) reported that 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 as 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). They concluded that the protein is produced by hydrogen fermentation. 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 & Liu 2009).

In Figure 7, hydrogen concentrations obtained as a result of anaerobic fermentation studies conducted at 45±1°C with different initial pH values are given. In contrast with the results obtained by Cheng et al. (2000) by using a time lag of 66 h, the lag time in this study is insignificant for anaerobic fermentation (~ 2 hours). Chemical and biological contents of the substrate may be led to the shortening of the time. In all pH tests, hydrogen concentration in the gas phase showed a fluctuating curve with peaks observed between 7 and 24 hours. This shows that 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).

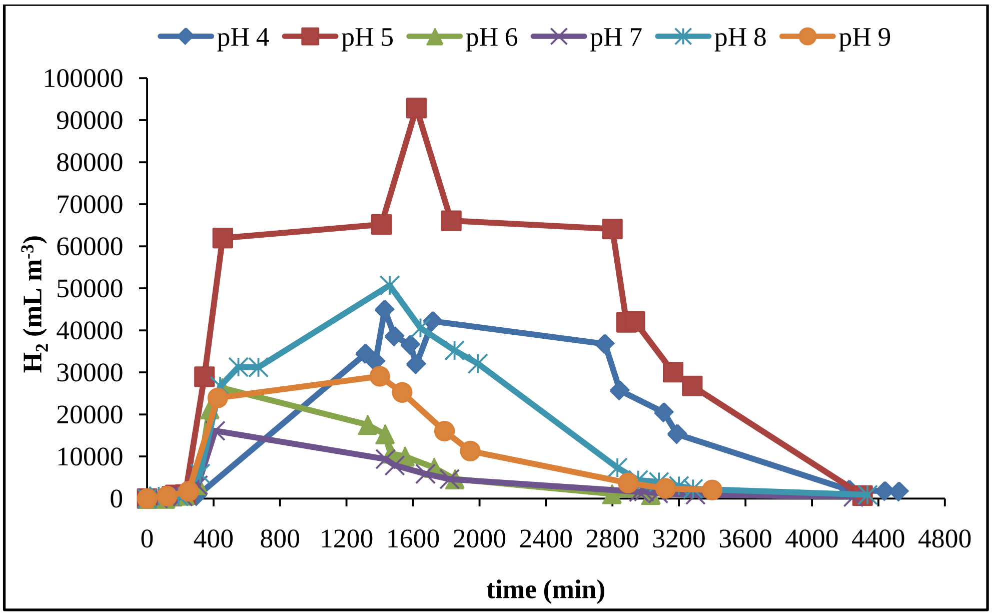

Fig. 7 Hydrogen concentrations obtained at different pH values

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

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

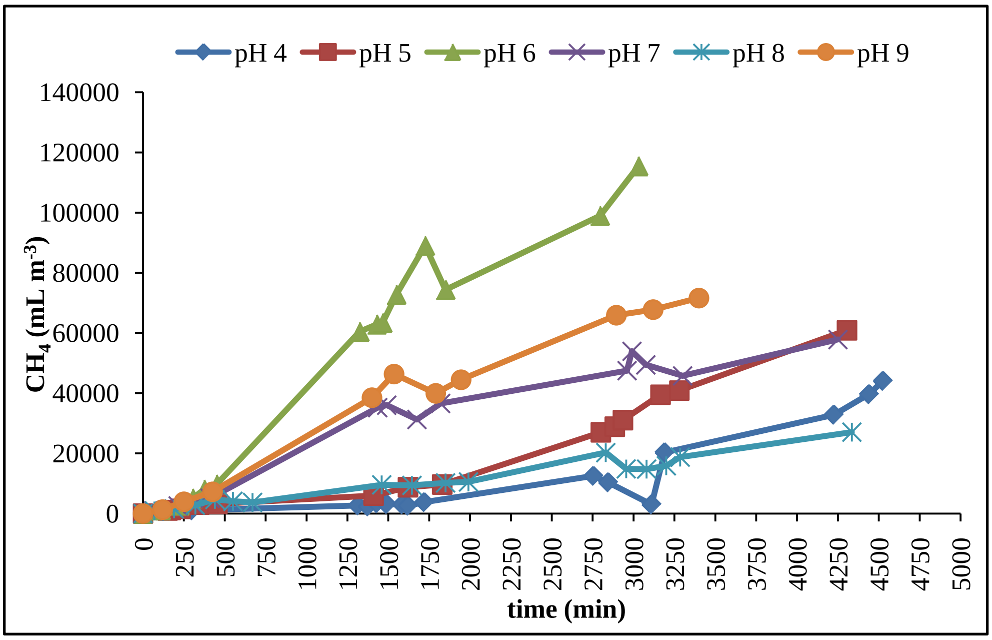

Figure 8. Methane concentrations obtained at different pH values

The Relationship between pH, Rate of Solubility, and HydrogenProduction

In Table 2, SCOD/TCOD (%) rates which were obtained at the end of approximately 66 hour fermentation were calculated and compared with the values that were calculated before fermentation. In order to determine that whether alterations in SCOD/TCOD ratio (%) has an influence on hydrogen gas release, the data in Table 2 were compared with the hydrogen concentration graph in Fig. 7. In all tests, except for pH 8, SCOD/TCOD ratio rose above 20% but remained 13.33% at pH 8. Despite this lower rate at pH 8, hydrogen concentration was higher. At pH 7, hydrogen gas concentration was at the lowest level. From the results, we can conclude that increase in SCOD/TCOD ratio is not proportional to the increase in the hydrogen gas concentration. Therefore, it is clear that not all organic matter released from sewage sludge convert into hydrogen by anaerobic fermentation. In their study of biohydrogen production from sewage sludge using *Clostridium bifermentans*, Wang et al. (2003) reported similar findings.

As is known, 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, pH is reduced. In acidification, namely hydrogen production step, the pH increases and remains constant（Cai et al. 2004).

Chen et al. (2002) indicated that 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 vaccine 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.

Table 2 SCOD/TCOD (%) in the mixture liquid before fermentation (I) and after fermentation (II) at different initial pH values

Initial pH	**I** **SCOD/TCOD (%)**	**II** **SCOD/TCOD (%)**
4	3.08	25.45
5	1.17	25.45
6	0.78	20.00
7	1.35	26.13
8	0.50	13.33
9	4.00	22.22

Similarly, as a result of fermentation experiments conducted at 45±1°C, we observed that when initial pH is between 7 and 9, pH value decreased at the end of fermentation but increased at 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 VFA and NH_4^+-N is the result of organics (SCOD) degradation (BenYi&JunXin 2009).

In all the tests, at the end of 2-3 hours, hydrogen production began. Depending on initial pH, hydrogen production at the end of 24 hours is as follows; pH 5> pH 8> pH 4> pH 9> pH 6> pH 7. Maximum yield was obtained at the 24-30th hours of fermentation at pH 5 (92894 mL m^{-3} H_2). However, 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. Hydrogen gas production and consumption ranking revealed that better results were obtained in studies using initial pH value of 5. Although hydrogen is produced at pH 8, a rapid decrease was observed in hydrogen production in a short period of time. The studies in the literature support our results. pH values of 5 and 6 were preferred in most of the studies on fermentative hydrogen production (Nicolau et al. 2008).

pH value also influence the solubility of organic matters found in sewage sludge structure. In this study, higher solubility was obtained at pH 5 compared to other pH values. At pH 5, protein, carbohydrate and COD in the structure of the sewage sludge in the reactor become more soluble and increased hydrogen production efficiency. Additionally, because methanogenic activity reduces or stops at pH values lower than 6.3, 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 (Fig. 8).

Considering these results and hydrogen production graph given in Fig. 7, it was concluded that setting the pH of reactor contents to 5 would be appropriate.

Conclusion

In the 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. Hydrolysis, a step occurring during anaerobic fermentation, has a very important role in converting the organic matter in the solid phase of the sludge into the free-state. 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. Especially, increase in the amount of Sprotein positively affects hydrogen production.

Another important factor affecting hydrogen production is 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.

Future studies should continue investigating the issues such as removal of hydrogen consuming microorganisms from the environment, detainment of methane production and increment of the solubility of the substance in the fermentor.

AcknowledgmentThe authors thank OndokuzMayis University, Scientific Research Project Funding (OMU BAP) for their financial support [Project number: PYO.MUH.1904.12.006].

References

American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF) (2005). Standard Methods for the Examination of Water and Wastewater, 20 th Ed., Washington DC, USA.

BenYi, X., JunXin, L. (2009) Effects of various pretreatments on biohydrogen production from sewage sludge. Chinese Science Bulletin, 54, 2038-2044

Cai, M.L., Liu, J.X., Wei, Y.S. (2004) Enhanced biohydrogen production from sewage sludge with alkaline pretreatment. Environmental Science &Technology, 38, 3195-3202

Chen, C.C., Lin, C.Y., Lin, M.C. (2002) Acid-base enrichment enhances anaerobic hydrogen production process. Applied Microbiology and Biotechnology, 58(2), 224-228

Cheng, S.S., Bai, M.D., Chang, S.M., Wu, K.L., Chen, W.C. (2000) Studies on the feasibility of hydrogen production hydrolyzed sludge by anaerobic microorganisms. The Twenty-fifth Wastewater Technology Conference, Yunlin, Taiwan (in Chinese).

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. (1956) Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28(3), 350-356

Guo, L., Li, X.M., Bo, X., Yang, Q., Zeng, G.M., Liao, D., Liu, J.J. (2008) Impacts of sterilization, microwave and ultrasonication pretreatment on hydrogen producing using waste sludge.Bioresource Technology, 99, 3651-3658

Guo, L., Li, X.M., Zeng, G.M., Zhou, Y. (2010) Effective hydrogen production using waste sludge and its filtrate. Energy, 35, 3557-3562

http://www.tuik.gov.tr/PreTablo.do?alt_id=1019(2008)

Kotay, S.H., Das, D.(2010) Microbial hydrogen production from sewage sludge bioaugmented with a constructed microbial consortium. International Journal of Hydrogen Energy, 35, 10653-10659

Kotay, S.M., Das, D. (2009) Novel dark fermentation involving bioaugmentation with constructed bacterial consortium for enhanced biohydrogen production from pretreated sewage sludge. International Journal of Hydrogen Energy, 34, 7489-7496

Liu, Q., Zhang, X., Yu, L., Zhao, A., Tai, J., Liu, J., Qian, G., Xu, Z.P. (2011) Fermentative hydrogen production from fresh leachate in batch and continuous bioreactors.Bioresource Technology, 102(9), 5411-5417

Lowry, O.H, Rosebrough, N.J., Farr, A.L., et al. (1951) Protein measurement with the Folin phenol reagent.The Journal of Biological Chemistry, 1993, 265-275

Nah, I.W., Kang, Y.W., Hwang, K.Y., Song, W.K. (2000) Mechanical pretreatment of waste activated sludge for anaerobic digestion process. Water Research, 34, 2362-2368

Nicolau, M., Dinsdale, J.R., Guwy, A. (2008) Hydrogen production from sewage sludge using mixed microflora inoculum: Effect of pH and enzymatic pretreatment. Bioresource Technology, 99, 6325-6331

Senturk, I., Buyukgungor, H. (2010) An examination of used different waste materials and biohydrogen production methods. Sigma Journal of Engineering and Natural Sciences, 28(4), 369-395

Senturk, I., Buyukgungor, H. (2013)Evaluation of biohydrogen production potential from marine macro algae, pp. 117-124. Black Sea Energy Resource Development and Hydrogen Energy Problems (Nato Science for Peace and Security Series-C: Environmental Security), edited by A. Veziroglu, and M. Tsitskishvili. Springer.

Senturk, I., Buyukgungor, H. (2013a)Arıtmaçamurlarındanbiyohidrojeneldesindeişletmeparametrelerininverimeetkisi. IX. Clean Energy Symposium, UTES’13, 25-28 December, Konya

Senturk, I., Buyukgungor, H. (2014)Evaluation of biohydrogen production potential from sewage sludge, pp. 943-950. Progress in Exergy, Energy, and the Environment, edited by I. Dincer, A. Midilli, and H. Kucuk.Springer.

Wang, C.C., Chang, C.W., Chu, C.P., Lee, D.J., Chang, B.V., Liao, C.S. (2003). Producing hydrogen from wastewater sludge by *Clostridumbifermentans*.Journal of Biotechnology, 102, 83-92

Xiao, B., Liu, J. (2009) Biological hydrogen production from sterilized sewage sludge by anaerobic self-fermentation. Journal of Hazardous Materials, 168, 163-167