Karaelmas Fen ve Mühendislik Dergisi
Journal home page: http://fbd.beun.edu.tr

Research Article
Received / Geliş tarihi : 22.09.2016
Accepted / Kabul tarihi : 13.02.2017

The Effects of Incubation and Operational Conditions on Biogas Production

İnkübasyon ve İşletme Koşullarının Biyogaz Üretimine Etkisi

Vedat Yılmaz

Artvin Çoruh University, Department of Environmental Engineering, Artvin, Turkey

Abstract

Anaerobic digestion is leading to environmental benefits such as producing energy and reducing greenhouse gas emissions. Biochemical methane potential (BMP) assays provide a realistic estimate of methane production from the anaerobic digestibility of a given substrate. The information provided by BMP is valuable when planning the optimal conditions for anaerobic digestion and designing the anaerobic digesters. Therefore, recognized and accredited BMP assays will help us for comparing the different literature results. This study focused on the effects of incubation and operational conditions in BMP assay. The effects of headspace pressure, measurement frequency, nutrient addition, pH adjustment, N_2/CO_2 purging and mixing were investigated by using 27 different configured reactors. The results indicated that biogas production had positively affected under low headspace pressure and lesser measurement frequency, but the other modifications didn't create significant variations.

Keywords: Anaerobic digestion, Biochemical methane potential, Operational parameters

Öz

Anaerobik parçalanma ile enerji üretimi ve sera gazı emisyonlarının azaltılması gibi çevresel yararlar elde edilmektedir. Biyokimyasal metan potansiyeli testi (BMP) ile istenilen maddenin metan üretim potansiyeli gerçekçi olarak tahmin edilebilmektedir. Anaerobik reaktörlerin dizaynı ve anaerobik parçalanmanın optimal koşullarının planlanmasında BMP testi önemli bilgi sunmaktadır. Bu sebeple deneysel olarak standart BMP testleri literatür sonuçlarının karşılaştırmasında ve değerlendirilmesinde önemlidir. Bu çalışmada BMP testinin hazırlanması ve BMP testine işletme koşullarının etkisi incelenmiştir. Hava boşluğu basıncı, ölçüm sıklığı, besin ilavesi, pH ayarlaması, N_2/CO_2 gaz karışımına ile ortamın anaerobik yapılması ve karıştırma işlemlerinin etkisi 27 farklı şekilde işletilen BMP reaktörleri kullanılarak incelenmiştir. Düşük boşluk hacmi basıncı ve uzun aralıklı ölçümler yapılması biyogaz üretimine olumlu yönde katkı sağlarken diğer parametrelerin önemli bir etkisinin olmadığı tespit edilmiştir.

Anahtar Kelimeler: Anaerobik parçalanma, Biyokimyasal metan potansiyeli, İşletme parametreleri

1. Introduction

The application of anaerobic digestion technology is growing worldwide because of its economic and environmental benefits. Organic fraction of wastes can be anaerobically digested and its product is biogas, composed mainly of methane (50–80% v/v) and carbon dioxide, of which the former can be used to produce energy and heat. The production of biogas based on biomass generates the reduction of fossil fuel use and supporting the reduction of the greenhouse gases emission especially, not mentioning the use of a local energy resource (Ravuri 2013).

Anaerobic digestion (AD) is a multi-stage biological process in which organic material of waste is degraded by microorganisms in the absence of oxygen (Esposito et al. 2012). This process also minimizes the volume of waste generation and resulted digestate could be used in agriculture as nutrient fertilizers or soil conditioner. AD has become an established and proven technology for the treatment of wastes, coming from including those of domestic/municipal, agricultural and industrial origins (De Baere 2000).

Biochemical methane potential (BMP) is a procedure, first established by Owen et al. (Owen et al. 1979), developed to determine the realistic estimate of methane production of a given organic substrate during its anaerobic decomposition. The BMP assay has demonstrated to be a relatively simple, reliable and powerful tool to obtain the extent and rate of

*Corresponding Author: vedatyilmaz@artvin.edu.tr

Vedat Yılmaz orcid.org/0000-0002-2969-1082

organic matter conversion to methane (Raposo et al. 2011). The BMP test is measuring the maximum amount of biogas produced from per gram of volatile solids (VS) or per gr of COD contained in the organics or in the wastewater used as substrates in batch and anaerobic conditions. As an initial evaluation for an organic substrate, the BMP test requires minimal labor and cost for set-up and monitoring as compared to larger feasibility studies, and it provides more accurate and repeatable information for a specific waste/wastewater than values obtained from literature (Moody et al. 20111). In the last three decades, a huge amount of papers related to BMP assays for different substrates and test conditions have been published (Owen et al. 1979, Angelidaki et al. 2009, Cynoweth et al. 1993, Owens and Chynoweth 1993, Hansen et al. 2004). However, there is no standard protocol to carry out these tests and due to different environmental conditions and experimental setups used during these assays, the comparability of biogas production data in the literature is very difficult.

The BMP tests can be started and operated reliable, if there should be; i) appropriate microbial community and nutrients, ii) optimal environmental conditions, and iii) non-toxic substrate concentration in the reactors (Manjanthrarat 2013). A general description of the process is as follows. The known amounts of organic waste or wastewater, active anaerobic inoculum and the micro and macro nutrients should be added to 60-1000 mL serum bottles. The pH of the bottle content should be adjusted at around 6.5-7.2 and the headspace of bottles gassed with mixture of nitrogen (N_2) and/or carbon dioxide (CO_2). The bottles are placed in an incubator at a constant mesophilic temperature (35 ±2 °C) for 30 days (for simple substrates, e.g. sugars) to 120 days (for recalcitrant substrates, e.g. agricultural wastes), and finally, the volume of gas production and its composition should be measured during the incubation. The methane potential is expressed in terms of standard temperature and pressure (STP) ml CH_4 per 1 g of VS or 1 g of COD added (mL CH_4/g VS or g COD) (Hansen et al. 2004).

Within the anaerobic environment, various important parameters, such as temperature, pH, stirring intensity, physical and chemical characteristics of substrates, substrate/inoculum ratio, liquid volumes, and headspace pressure, affect the rates of the different steps of the digestion process (Esposito et al. 2012, Appels et al. 2008).

Agitation strategy can affect anaerobic digestion of sewage sludge and optimum agitation strategy should be found (Perot et al. 1988). There is no obvious discussion for the influence of mixing on the gas production. Several researchers reported that mixing improve the anaerobic digestion (Stroot et al. 2001, Vedrenne et al. 2008), while some literature results couldn't show the enhancement of biogas production under mixed conditions (Chen et al. 1990, Karim et al. 2005). Most methanogenic bacteria functions in a pH range between 6.7 and 7.4, but optimally at pH 7.0-7.2, and the process may fail if the pH is close to 6.0 (Ravuri 2013). BMP tests are carried out at neutral pH by using some chemicals, like sodium bicarbonate, sodium hydroxide, sodium carbonate and sodium sulphide. Anaerobic microbial activity could be limited by some macro, micro nutrients and trace metals (Takashima et al. 1990). Biogas production of some anaerobically digested wastewater sludges supplemented with trace elements showed stable digestion, while non-supplemented reactors showed methanogenic failure (Climenhaga and Banks 2008, Rittmann and McCarty 2001, Speece et al. 1983). The pressure and headspace relationships were not investigated broadly in the literature. Clark et al. (2012) revealed that control of headspace pressure below atmospheric levels did not have a significant effect on gas production. Oxygen is considered as a potential toxic compound for the acetogens and principally the methanogens, thus, it was believed that reactor instability and low performance might occur due to oxygen entering anaerobic digesters (Kato et al. 1993). Batch reactors could be purged with N_2 or mixture of N_2/CO_2 to get oxygen-free headspace.

The aim of this work was to evaluate the effects of some incubation and operational conditions (headspace volume, measurement frequency, mixing, pH adjustment, addition of macro, micro nutrients and buffer solutions) on the biogas production.

2. Materials and Methods

Batch tests were performed in 500 mL serum bottles with the addition of anaerobic seed sludge, nutrients, buffer solutions and distilled water under mesophilic conditions. In the first part of experiments, BMP tests were operated with 125, 200, 300 and 400 mL liquid volume. Total volume of biogas production in serum bottles was monitored 7-15 times throughout the 30 days of incubation. Biogas productions were measured at every one, two and three day increments. After the first 10 days of incubation, measurements were taken less frequently because of insufficient gas production. In the second part of experiments, 15 reactors were operated with and without nutrients addition, buffer addition, pH adjustment, mixing and N_2/CO_2 purging as provided in Table 1.

Glucose was chosen as the substrate because of its common use and often studied metabolism. Anaerobic biomass samples were supplemented with 2,000 mg/L glucose. Anaerobically digested sludge originally was taken from an anaerobic digester of a municipal wastewater treatment plant located in Antalya, Turkey. The seed sludge had a concentration of 3.6 g/L as VS. Batch BMP reactor is illustrated in Figure 1. Concentrated stock solutions were used for preparing the defined media and are stored at 4°C. The substrate to inoculum ratio was maintained around 0.44.

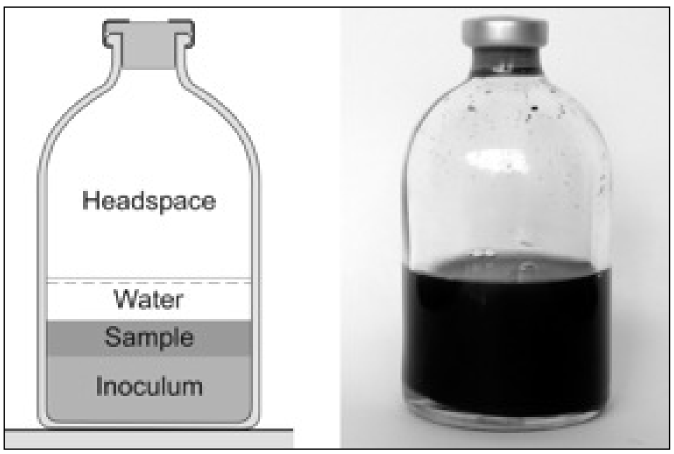

Figure 1. A general view of BMP bottle.

The nutrients and buffer solution supplied in BMP bottles were as follows (the concentrations are presented in mg/L in parenthesis); macro elements: NH_4Cl (172), KH_2PO_4 (65), $MgCl_2 \cdot 6H_2O$ (39), $CaCl_2 \cdot 2H_2O$ (19); micro elements: $FeCl_2 \cdot 4H_2O$ (20), $CoCl_2 \cdot 6H_2O$ (5), $MnCl_2 \cdot 4H_2O$ (1), $NiCl_2 \cdot 6H_2O$ (1), $ZnCl_2$ (0.5), H_3BO_3 (0.5), Na_2SeO_3 (0.5), $CuCl_2 \cdot 2H_2O$ (0.4), $Na_2(Mo)O_4 \cdot 2H_2O$ (0.1); tampon bicarbonate solution: $NaHCO_3$ (2600). The pH of batch experiments was set to neutral pH at the beginning of the BMP test by using HCl. The headspace of BMP bottles was gassed with N_2/CO_2 (70/30 v/v) mixture gas for approximately 30 sec. to obtain anaerobic condition. Each BMP bottle was sealed with butyl rubber septa and sealed with aluminum screw caps to avoid a loss of pressure, headspace pressure in each bottle was set to atmospheric pressure by releasing the headspace gas with a needle, and finally the bottles were placed in an incubator or in a water bath for mixing, both operated at 35±2°C. Each assay was prepared in duplicate.

Total volume of biogas in reactors was measured via a liquid displacement device. The instrument consisted of a needle connected to a 100 mL burette, which is connected to a 100 mL graduated cylinder filled with diluted acid solution. The composition of biogas was determined using gas chromatography (GC, Varian 4900) equipped with a thermal conductivity detector (TCD) and 10 m PPQ column. The temperatures of injector port, detector and column oven were 150, 145 and 150 °C, respectively. Nitrogen was used

Table 1. The operational parameters used in BMP tests.

Reactors	Macro nutrients addition	Micro nutrients addition	Buffer addition	pH adjustment	N_2/CO_2 purging	Mixing
A	-	-	-	+	+	+
B	-	-	-	-	+	+
C	-	-	-	+	-	+
D	+	+	+	+	+	+
E	+	-	+	+	+	+
F	-	+	+	+	+	+
G	+	+	+	-	-	+
H	+	+	+	+	-	+
I	-	-	-	+	+	-
K	-	-	-	-	+	-
L	-	-	-	-	-	-
M	+	+	+	-	+	-
N	+	+	+	-	+	-
P	+	+	+	-	+	-
R	-	-	-	+	-	-

as the carrier gas at a flow rate of 25 mL/min. A gas standard consisting of 60% (v/v) CH_4 and 40% of CO_2 was used as calibration. Total solids and volatile solids concentrations were determined using Standard Method 2540 B and 2540 E (APHA 1998). The pH of samples was determined by a pH meter (Fisher Scientific Instrument, Accumet™ Model 50) equipped with a poly combination electrode with silver/silver chloride references.

3. Results and Discussion

Two different BMP setups were organized. In the first group of experiments, the effect of headspace pressure and biogas measurement frequency was investigated. For this purpose, 12 BMP bottles were operated with 20, 40, 60 and 80 % working volume (corresponding 125, 200, 300, and 400 mL). Biogas measurement were performed with daily, once in two days, and once in three days increments. In the second setup, 15 BMP bottles prepared to evaluate the effects of macro and micro nutrients addition, bicarbonate buffer usage, pH adjustment, headspace purging, and mixing (Table 1).

Effects of Headspace Pressure and Biogas Measurement Frequency on BMP

The BMP assays were performed with the working volume of 125, 200, 300 and 400 mL in 500 mL glass bottles. The lowest working volume reactors produced the maximum methane (Figure 2). In each sets, the amounts of biogas production was increasing with the increasing headspace volumes and continually measurement increments. The average of biogas production of %14-23 obtained in 125 mL working volume bottles is higher than average of the other three sets of assays. These results revealed that the performance of anaerobic digestion was enhanced under low pressure conditions. Logan et al. (2002) observed that greater volumes of hydrogen gas production measured when the gas pressure was continuously released in respirometer tests than when gas pressure was only periodically released. The percent of headspace volume in the reactor should be planned as much as possible to maximize the biogas production.

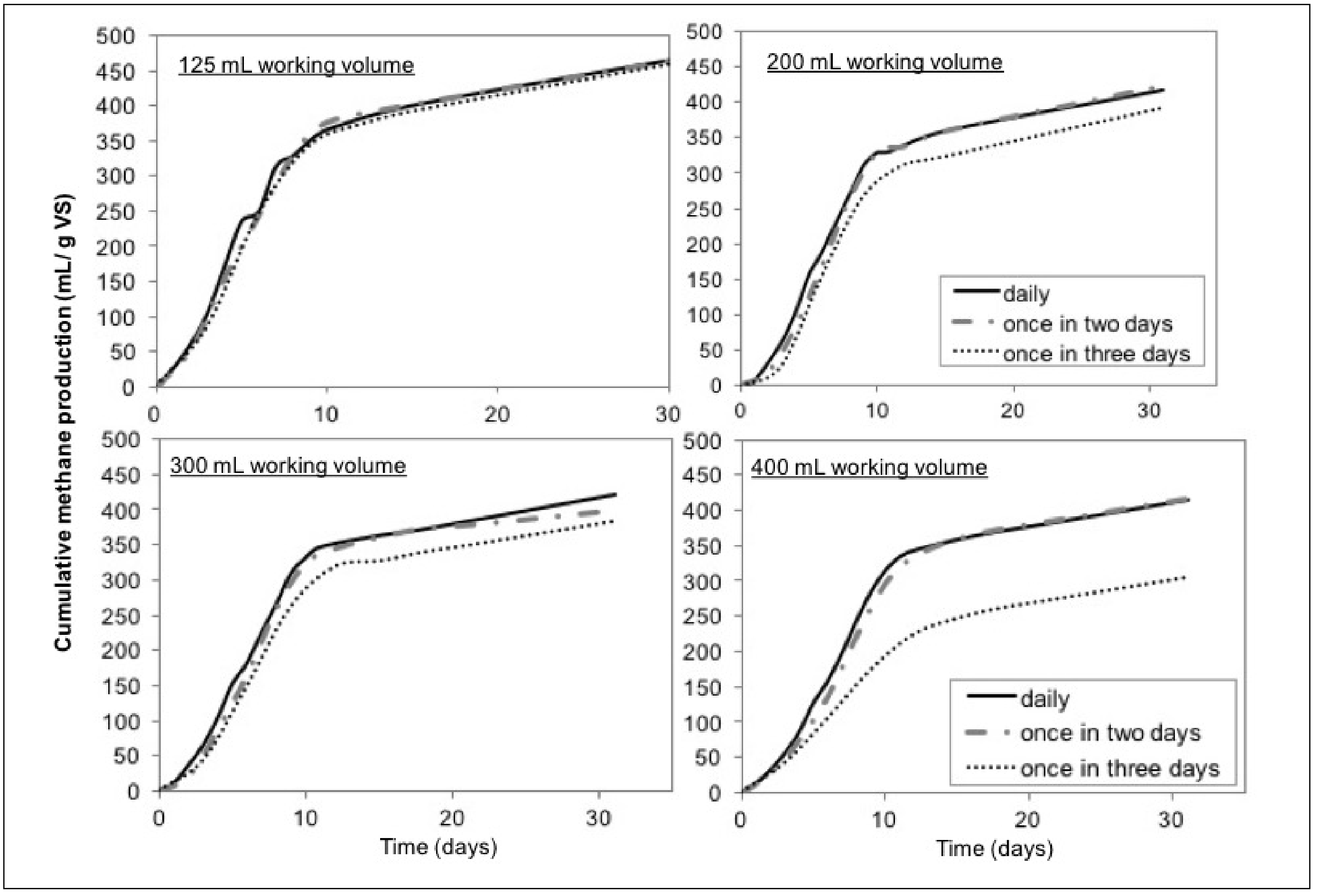

Figure 2. Methane production curves for different working volumes and measurement increments.

Effects of Macro and Micro Nutrients Addition, Bicarbonate Buffer Usage, pH Adjustment, Headspace Purging, and Mixing on BMP

Owen et al. (1979) used the first protocol for BMP tests and the common steps were followed by later researches. In the second part of this study, some of these basic steps were investigated on the biogas production performance. As tabulated in Table 1, fifteen BMP bottles were organized in different characteristics. Some of them fed without addition of nutrients and tampon solution. At the finalize phase of assay preparation, pH adjustment and N_2/CO_2 purging were not applied in some reactors. Eight of the BMP bottles were operated under mixing conditions. The cumulative methane produced versus time was plotted (Figure 3). The fifteen BMP bottles gave a biogas yield range of 460 and 534 mL CH_4/g VS with the average of 507 mL CH_4/g VS. The average of methane production was calculated as 520 mL CH_4/g VS except unpurged reactors and they varied between 507 and 534 mL CH_4/g VS.

One of the most stringer aspects of BMP assays is the preparation of macro and micro nutrients. The addition of macro and micro nutrients didn't result in enhancement of gas production. Although it is well documented that all microbial mediated processes require nutrients and trace elements during organic biodegradation, it is not clear if under the normal conditions of a BMP test sufficient nutrients are available from the sludge and/or organic substrate, or if additional supplements are necessary (Raposo et al. 2011). Park et al. (2010) stated that supplementation of micro and macronutrient cocktails, Vanderbilt media did not seem to stimulate the acetoclastic methanogens. Total working volumes were fixed to 300 mL in this set of experiments. Distilled water substituted instead of equal volumes of nutrients and buffer solution was not added to reactors. Anaerobic microorganisms may favor in more water media instead of chemicals, so that biogas production was determined only 2% reduction in nutrients fed reactors.

Anaerobic digestion is conventionally operated under oxygen-free conditions. A general belief is that anaerobic treatment of wastewaters can face a serious problem due to the possible presence of dissolved oxygen (Kato et al. 1997). In this study, some of the reactors were prepared without purging under N_2/CO_2 mixture. The oxygen-free reactors produced 6.9-8.4% more methane than unpurged reactors. Some previous studies have demonstrated that some methanogens have an oxygen tolerance and dissolved oxygen does not constitute any detrimental effect on reactor treatment performance (Conklin et al. 2007, Kato et al. 1993). Methanogens can adopt and survive under presence of oxygen, and thus, reactors could be started without oxygen-free conditions.

The importance of mixing in achieving efficient substrate conversion has been noted by many researchers, although the optimum mixing pattern is a subject of much debate (Karim et al. 2005-a). Since the energy consumption is one of the important aspects of wastewater treatment, minimal mixing rate will be preferable. In our experiments, mixing had almost negligible effect on biogas performance, cumulative methane productions brought up similar values ranging from ±2% of variations. Continuous mixing was not helpful especially if the digesters fed with less than 5% of solid concentration (Karim et al. 2005-b).

3. Conclusions

The results of BMP assays could represent powerful tools to

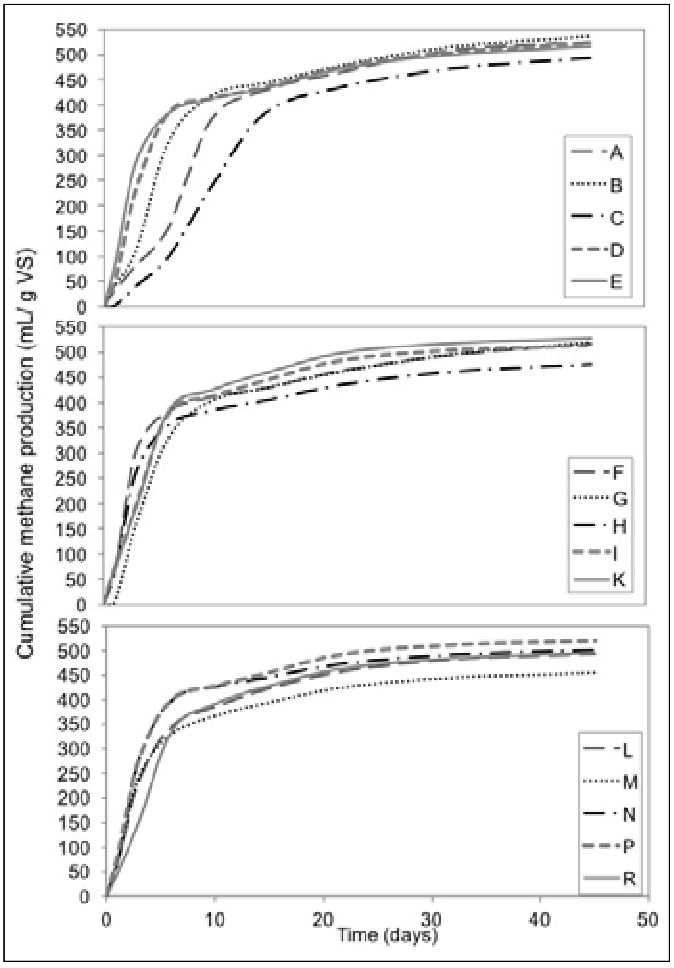

Figure 3. Cumulative methane productions of 15 different BMP bottles configured according to Table 1.

establish effective anaerobic digesters. An inexpensive, easily operated and quick test protocol could be standardized in order to gain comparable results. The enhancement of biogas production was consisted with lowering headspace pressure in the reactor. However, the other investigated parameters didn`t improve the reactor performance. The BMP assays of easily biodegradable wastes could be conducted straight forward and its preparation and operation didn't need to much pay attention.

4. References

American Public Health Association (APHA). 1998. American Water Works Association., & Water Environment Federation. *Standard methods for the examination of water and wastewater*. Washington, D.C: APHA-AWWA-WEF.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, JL., Guwy, AJ., Kalyuzhnyi, S., Jenicek, P., Van Lier, JB. 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Sci. Technol.*, 59: 927-934.

Appels, L., Baeyens,1 J., Degrève, J., Dewil, R. 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. *Prog. Energ. Combust. Sci.*, 34: 755-781.

Chen, T.H., Chynoweth, P., Biljetina, R. 1990. Anaerobic digestion of municipal solid waste in a nonmixed solids concentrating digestor. *Appl. Biochem. Biotechnol.*, 24-25: 533-544.

Chynoweth, DP., Turick, CE., Owens, JM., Jerger, DE., Peck, MW. 1993. Biochemical methane potential of biomass and waste feedstocks, *Biomass Bioenerg.*, 5: 95-111.

Clark, I.C., Zhang, R.H., Upadhyaya, S.K. 2012. The effect of low pressure and mixing on biological hydrogen production via anaerobic fermentation. *Int. J. Hydrogen Energ.*, 37: 11504-11513.

Climenhaga, M.A., Banks, C.J. 2008. Anaerobic digestion of catering wastes: effect of micronutrients and retention time. *Water Sci. Technol.*, 57: 687-692.

Conklin, A., Bucher, R., Stensel, H.D., Ferguson, J. 2007. Effects of oxygen exposure on anaerobic digester sludge. *Water Environ. Res.,* 79: 396-405.

De Baere, L. 2000. Anaerobic digestion of solid waste: state-of-the-art. *Water Sci. Technol.*, 41: 283-290.

Esposito, G., Frunzo, L., Liotta, F., Panico, A., Pirozzi, F. 2012. Bio-methane potential tests to measure the biogas production from the digestion and co-digestion of complex organic substrates. *The Open Environmental Eng. J.*, 5: 1-8.

Hansen, T.L., Schmidt, J.E., Angelidaki, I., Marca, E., Jansen, J.l.C., Mosbæk, H., Christensen, T.H. 2004. Method for determination of methane potentials of solid organic waste, *Waste Manag.*, 24: 393-400.

Karim, K., Hoffmann, R., Thomas Klasson, K., Al-Dahhan, M.H. 2005-a. Anaerobic digestion of animal waste: Effect of mode of mixing. *Water Res.*, 39: 3597-3606.

Karim, K., Klasson, K., Hoffmann, R., Drescher, S.R., DePaoli, D.W., Al-Dahhan, M.H. 2005-b. Anaerobic digestion of animal waste: Effect of mixing. *Bioresource Technol.*, 96: 1607-1612.

Kato, M.T., Field, JA., Lettinga, G. 1993. High tolerance of methanogens in granular sludge to oxygen. *Biotechnol Bioeng.*, 42: 1360-1366.

Kato, MT., Field, JA., Lettinga, G. 1997. Anaerobe tolerance to oxygen and the potentials of anaerobic and aerobic cocultures for wastewater treatment. *Brazilian J. Chemical Eng.*, 14 (4).

Logan, BE., Oh, S.-E., Kim, I.S. Van Ginkel, S. 2002. Biological hydrogen production measured in batch anaerobic respirometers. *Environ. Sci. Technol.*, 36: 2530-2535.

Manjanthrarat, S. 2013. Biochemical methane potential estimation of feed mixtures used for dry anaerobic digestion, M.Sc. Thesis, Asian Institute of Technology School of Environment, Resources and Development., 137 pp.

Moody, L.B., Burns, R.T., Bishop, G., Sell, S.T., Spajic, R. 2011. Using biochemical methane potential assays to aid in co-substrate selection for co-digestion. *App. Eng. Agric.*, 27: 433-439.

Owen, W.F., Stuckey, D.C., Healy, J.B., Young, L.Y., Mccarty, P.L. 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Res.*, 13: 485-492.

Owens, J.M., Chynoweth, D.P. 1993. Biochemical methane potential of municipal solid-waste (Msw) components. *Water Sci. Technol.*, 27: 1-14.

Park, C., Bega, A., Unlu, C., Chadderton, RA., McKean, WR., Kohl, PM., Hunt, JA., Keaney, J., Willis, JL., Duran, M. 2010. Acetoclastic methanogens in an anaerobic digester could be susceptible to trace metal supplementation. *Water Sci. Technol.*, 62: 2905-2911.

Perot, C., Sergent, M., Richard, P., Luu, R.P.T., Millot, N. 1988. The effects of pH, temperature and agitation speed on sludge anaerobic hydrolysis-acidification; Environ. Technol. Lett., 9: 741-752.

Raposo, F., Fernández-Cegrí, V., De la Rubia, M.A., Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B., Fernández-Polanco, M., Frigon, J.C., Ganesh, R., Kaparaju, P., Koubova, J., Méndez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M., Uellendahl, H., Wierinck, I., de Wilde, V. 2011. Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *J. Chem. Technol. Biot.*, 86: 1088-1098.

Ravuri, H.K. 2013. Role of factors influencing on anaerobic process for production of bio hydrogen: Future fuel. *Int. J. Advanced Chemistry*, 1: 31-38.

Rittmann, B.E., McCarty, P.L. 2001. Environmental biotechnology: principles and applications. McGraw-Hill, 768 pp.

Speece, RE., Parkin, GF., Gallagher, D. 1983. Nickel stimulation of anaerobic digestion. *Water Res.*, 17: 677-683.

Stroot, P.G., McMahon, KD., Mackie, RI., Raskin, L. 2001. Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions—I. digester performance. *Water Res.*, 35: 1804-1816.

Takashima, M., Speece, RE., Parkin, GF. 1990. Mineral requirements for methane fermentation. *Crit. Rev. Env. Cont.*, 19: 465-479.

Vedrenne, F., Béline, F., Dabert, P., Bernet, N. 2008. The effect of incubation conditions on the laboratory measurement of the methane producing capacity of livestock wastes. *Bioresource Technol.*, 99: 146-155.