

The Kinetic of Biogas Production Rate from Cattle Manure in Batch Mode

Budiyono, I N. Widiassa, S. Johari, and Sunarso

Abstract—In this study, the kinetic of biogas production was studied by performing a series laboratory experiment using rumen fluid of animal ruminant as inoculums. Cattle manure as substrate was inoculated by rumen fluid to the anaerobic biodigester. Laboratory experiments using 400 ml biodigester were performed in batch operation mode. Given 100 grams of fresh cattle manure was fed to each biodigester and mixed with rumen fluid by manure : rumen weight ratio of 1:1 (MR11). The operating temperatures were varied at room temperature and 38.5 °C. The cumulative volume of biogas produced was used to measure the biodigester performance. The research showed that the rumen fluid inoculated to biodigester gave significant effect to biogas production ($P < 0.05$). Rumen fluid inoculums caused biogas production rate and efficiency increase two to three times in compare to manure substrate without rumen fluid. With the rumen fluid inoculums, gave the kinetic parameters of biogas production i.e biogas production rate constants (U), maximum biogas production (A), and minimum time to produce biogas (λ) are 3.89 ml/(gVS.day); 172.51 (ml/gVS); dan 7.25 days, respectively. While the substrate without rumen fluid gave the kinetic parameters U , A , and λ are 1.74 ml/(gVS.day); 73.81 (ml/gVS); dan 14.75 days, respectively. The future work will be carried out to study the dynamics of biogas production if both the rumen inoculums and manure are fed in the continuous system.

Keywords—rumen fluid, inoculums, anaerobic digestion, biogas production.

I. INTRODUCTION

ENERGY is one of the most important factors to global prosperity. The dependence on fossil fuels as primary energy source has lead to global climate change, environmental degradation, and human health problems. In the year 2040, the world predicted will have 9–10 billion people and must be provided with energy and materials [1]. Moreover, the recent rise in oil and natural gas prices may drive the current economy toward alternative energy sources such as biogas.

Anaerobic digestion (AD) is a technology widely used for treatment of organic waste for biogas production. AD that utilizes manure for biogas production is one of the most promising uses of biomass wastes because it provides a source of energy while simultaneously resolving ecological and

agrochemical issues. The anaerobic fermentation of manure for biogas production does not reduce its value as a fertilizer supplement, as available nitrogen and other substances remain in the treated sludge [2].

Numerous studies had been conducted by several researchers in order to optimize biogas yield in AD. An effort to improve biomass conversion efficiency and biogas yield conducted by several researchers i.e by improving contact between bacteria and substrate using stirring [3]-[5]; immobilizing microbe using fixed film reactor [6]-[7] as well as Anaerobic Sequencing Batch Reactor (ASBR) [8]; improving substrate composition by co-digesting with others substrate [4],[9]-[10]; and controlling ammonia inhibition [11]. In addition, an effort to optimize biogas yield also has been done by using two continuously stirred tank reactors (CSTR) in series [12]-[13]; selectively retaining the solids within the reactor by holding mixing prior to effluent removal [14]; pretreatment of manure by separating solids from digested material in order to improve biodegradability and accessibility [15]-[17]; and improving bacterial nutritional requirement [18]-[19].

In contrast with other researchers mentioned before, an effort to improve methane yield was carried out by increasing the inoculums content in biodigester [20]-[24]. Several results from these study i.e inoculums are substantially relevant in biogas production rate [20]; amount of methane produced seemed proportional to the initial cattle manure as inoculums [21]; a strong influence of the bovine rumen fluid inoculums on anaerobic biostabilization of fermentable organic fraction of municipal solid waste [23]; and the higher percentage of inoculums gave the higher production of biogas [24]. However, almost all of AD studied before, inoculums used were dominated by digested sludge from anaerobic digester. In addition, to our best knowledge, so far there is no academic literature available on presenting the mathematical description concerning the effect of inoculums content to biogas production rate. This study will focus on the use of rumen fluid as inoculums in anaerobic digestion of cattle manure. The aim of the current work was to assess the effect of rumen fluid concentration to biogas production rate. Furthermore, kinetic parameters from mathematical model concerning biogas production rate in batch mode of biodigester also will be studied and presented in this paper.

Budiyono, S. Johari, and Sunarso is with the Doctor Program in Animal Agriculture, Post Graduate Program, Diponegoro University, Jl. Imam Bardjo, SH No. 5 Semarang, Central Java – Indonesia (e-mail: budhi.66@googlemail.com)

I N. Widiassa is with the Department of Chemical Engineering, Faculty of Engineering, Diponegoro University, Jl. Prof. Sudarto, SH No. 1, Semarang - Central Java - Indonesia

II. MATERIALS AND METHODS

A. Sample preparation

The cattle manures and rumen fluids used in this research were taken randomly from slaughterhouse located on Semarang city. The fresh raw manure was collected from animal holding pen unit while rumen was collected from evisceration unit. Rumen fluid was prepared as follows: rumen content is poured to 100 L tank and added 25 liter tap water. Solid content then be separated from slurry by filter cloth. To assure that solid content in solution are dominated by bacteria, solution obtained then be filtered by 10 micron cartridge filter. Before using, all of raw manure collected is homogenized by mixing with propeller mixer. Raw manure and rumen fluid sample was analyzed its dry matter (DM) and volatile solid (VS) content by mean heating at 105 and 600 °C, respectively. DM and VS content of fresh cattle manure and rumen fluid are presented in Table 1.

TABLE I
DM AND VS CHARACTERISTICS OF FRESH CATTLE MANURE AND RUMEN FLUID

Parameter	Unit	Fresh manure	Rumen fluid
DM	%	22.75 ± 0.53	1.71 ± 0.03
VM	%	19.49 ± 0.13	1.50 ± 0.01

B. Experimental apparatus set up.

A series laboratory test of 400 ml biodigester was operated in batch system. The main experiment apparatus consists of biodigester and biogas measurement. Biodigester were made from polyethylene bottle plugged with tightly rubber plug and was equipped with valve for biogas measurement. The temperature of biodigester was maintained at certain value thermostatically controlled electrically heated water bath. Biogas formed was measured by 'liquid displacement method' as also has been used by [25]. The schematic diagram of experimental laboratory set up as shown in Figure 1.

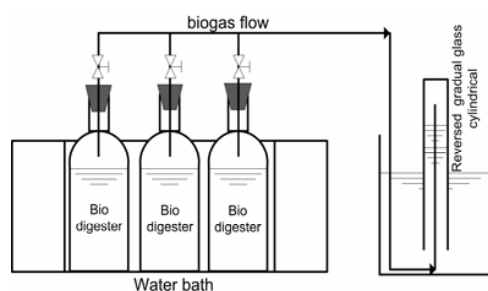


Fig. 1. Schematic diagram of series laboratory batch assessment of anaerobic digestion

C. Experimental design.

The influence of rumen fluid inoculums to biogas production rate was studied by comparing substrate mixed with and without rumen fluid as inoculums fed to the biodigester. Fixed 100 gram raw cattle manure were mixed with 100 ml of rumen fluid and fed to 400 ml anaerobic biodigester. In addition, 100 grams of the raw cattle manure were mixed with 100 ml of tap water and fed to the same anaerobic biodigester was used as control. The cattle manure to rumen fluid (MR) weight ratio of 1:1 was choose due to the optimum weight ratio of 1:1 according to Balsam [32]. Operating temperature was varied at room temperature and 38.5 °C. The biodigester performance was measured with respect to cumulative volume of biogas produced after corrected to standard pressure (760 mm Hg) and temperature 0 °C. All of treatment was carried out by triplication.

D. The experimental procedures.

MR with certain ratio as research variables was fed to biodigester and homogenized with mixer propeller. CO₂ gas was bubbled to biodigester to assure that biodigester in anaerobic condition. Biogas formed was measured every two days and stopped after biogas was insignificantly produced. The similar procedure was performed in three replications

E. Data analysis.

Significance difference between treatments was determined statistically by Duncan Multiple Range Test (DMRT). Observation data concerning biogas volume as a time function was used for kinetic study of biogas production. Rate constants of biogas production were determined using non linear regression.

F. Model development for biogas production kinetic in batch mode

Biogas production kinetic in was studied by developing the equation closest to fundamental for biogas production in batch system. By assuming biogas production rate in batch condition is correspond to specific growth rate of methanogenic bacteria in the biodigester, biogas production rate predicted will obey modified Gompertz equation [26] as follows:

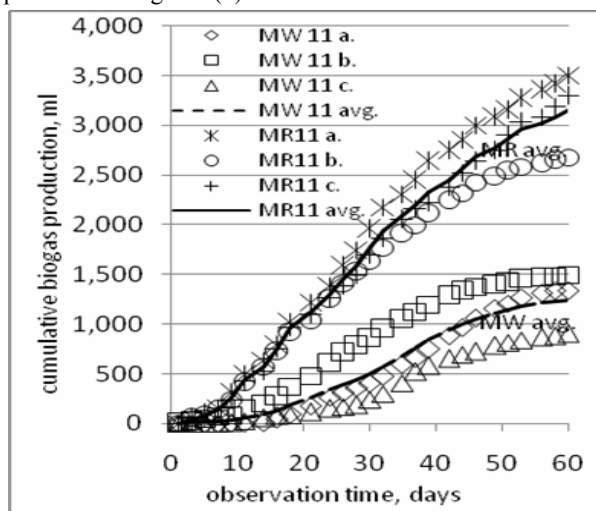
$$P = A \cdot \exp \left\{ - \exp \left[\frac{Ue}{A} (\lambda - t) + 1 \right] \right\} \quad (1)$$

In these equation, P is cumulative of specific biogas production, ml/gVS; A is biogas production potential, ml; U is maximum biogas production rate (ml/gVS.day); λ lag phase period (minimum time to produce biogas), day; and t cumulative time for biogas production, day. A, λ , and U constants can be determined using non linear regression. From the above equation, kinetic constant of biogas production rate will be expressed by U constant. The higher U exhibits the higher biogas production rate.

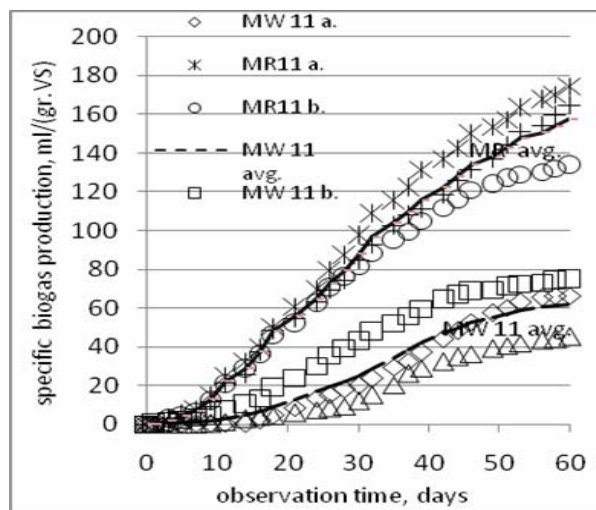
III. RESULTS AND DISCUSSIONS

A. The influence of rumen fluid to cumulative biogas production

This research step was directed to study either the effect of liquid rumen to cumulative biogas production is significant or not. The substrate consists of 100 gram manure and 100 ml rumen (MR 11) was fed to the digester and compared to substrate of manure and water in equal weight ratio (MW 11). The research was carried out in triplication. The cumulative volume of biogas production was observed during 60 days as depicted in Figure 2(a). In other term, the cumulative biogas production per total VS added (specific biogas production) is presented in Figure 2(b).



(a)



(b)

Fig. 2. The influence of rumen fluid to biogas production; average from three bloc research with triplication; room temperature

Fig. 2 shows that, in general, biogas production rate tend to obey sigmoid function (S curve) as generally occurred in batch growth curve (this is especially more clearly for MW 11 sample). Biogas production is very slow at the beginning and

the end period of observation. This is predicted due to the biogas production rate in batch condition is directly corresponds to specific growth rate of methanogenic bacteria in the biodigester [26]. In the around of the first 12 days observation, biogas production is very low or indeed do not formed yet due to the lag phase of microbial growth. In the range of 12 to 50 days observation, biogas production is significantly increase due to exponential growth of microorganisms. After 50 days observation, especially for manure without rumen fluid (MW 11), biogas production tend to decrease and this is predicted tend due to stationary phase of microbial growth.

From Fig. 2 (a) and (b) also can be seen that after 60 days observation still there is the tendency to increase biogas production and don't stop yet especially for manure mixed with rumen fluid (MR 11). This is predicted that the carbons contained by all waste constituents are not equally degraded or converted to biogas through anaerobic digestion. According to Richard [27] and Wilkie [28], anaerobic bacteria do not or very slow degrade lignin and some other hydrocarbons. In other word, the higher lignin content will lower biodegradability of waste. Animal manure such as waste used in this study include lignocellulosic rich materials, so anaerobically degradation also rather unoptimum [11].

Figure 2(a) and (b) also shows that, generally, substrates consist of manure and rumen (MR11) exhibit higher biogas production than substrates contain manure and water (MW11). In other terms, specific biogas production per gram VS added (Fig. 2.b) of MR11 is higher than MW11. The same behaviour is also shown in average biogas production curve. In the 60 days observation, average biogas production observed from MW11 and MR11 substrates were around 60 and 160 ml/(gVS). This result shows that the presence of liquid rumen in feed cause cumulative biogas production more than twice fold in compare to feed without liquid rumen. In other term, the substrates contain manure are statistically gave the significant effect to biogas production ($P < 0.05$). This is suggest that high concentration of anaerobic bacteria content in liquid rumen works effectively to degrade organic substrate from manure. According to Aurora [29], rumen of the ruminant animals contains the highly anaerobic bacteria dominated by cellulolytic bacteria able to biodegrade cellulose material from manure. This is agree with other results of researcher before that amount of biogas produced seemed proportional to the initial inoculums [21] and the bovine rumen fluid inoculums had a strong effect on anaerobic biostabilization of fermentable organic fraction of municipal solid waste [23]; as well as the higher percentage of inoculums gave the higher production of biogas [24].

From Fig. 2 also can be seen that the line slope of MR11 curve is sharper than MW11 line. The implication is that, biogas production rate (ml/gVS.day) of MR11 is higher than MW11. This indicated that the addition of liquid rumen to feed will increase biogas production rate in compare to feed without liquid rumen. Similar with this results, inoculums are substantially relevant in process kinetics of biogas production [20]. Finally, the most important finding from this research can be drawn the conclusion that the liquid rumen seeded to biodigester has significant effect to cummulative biogas

production and biogas production rate. Mathematically, the discussion concerning the effect of inoculums to kinetics constant of biogas production rate will be presented in the further section.

B. The influence of rumen fluid to the kinetic of biogas production

As stated before, biogas production kinetic in was studied by developing the equation closest to fundamental for biogas production in batch system. Kinetic constants of U, A, and λ , from equation (1) can be determined using non linear regression. In this study, data obtained from the research before was solved numerically using non linear regression. Kinetic constants obtained are completely presented in Table 2. By plotting experimental data and simulation of the modified Gompertz equation (equation 1) will be obtained the graph as depicted in Fig. 3.

TABLE II
KINETIC CONSTANS OF BIOGAS PRODUCTION RATE

Treatments	U, (ml/gVS.d)	A, ml/(gVS)	λ , days
MR 11	3.89 ± 0.28	172.51 ± 6.64	7.25 ± 1.65
MW 11	1.74 ± 0.13	73.81 ± 4.01	14.75 ± 2.87

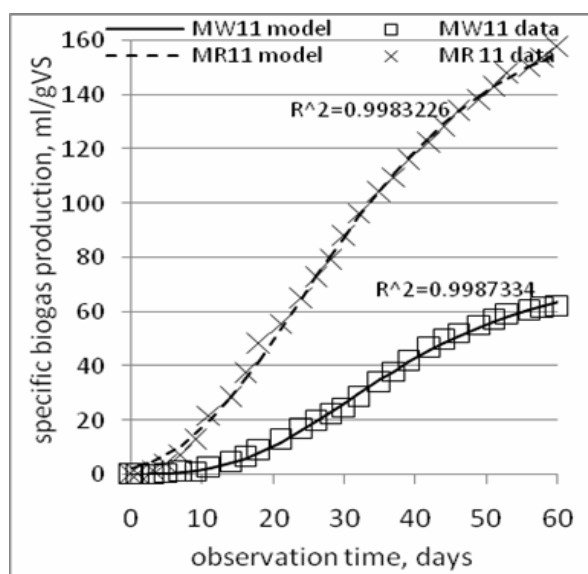


Fig. 3. Comparison between data and calculation using kinetic model of Gompertz equation

From Table 2 and Figure 3 can be seen that the rumen fluid inoculated to biodigester cause significant effect to constant of biogas production rate. With the rumen fluid inoculums (MR11), gave the kinetic parameters of biogas production i.e biogas production rate constants (U), maximum biogas production (A), and minimum time to produce biogas (λ) are 3.89 ml/(gVS.day); 172.51 (ml/gVS); dan 7.25 days,

respectively. While the substrate without rumen fluid (MW11) gave the kinetic parameters U, A, and λ are 1.74 ml/(gVS.day); 73.81 (ml/gVS); dan 14.75 days, respectively. This results imply that rumen fluid inoculums caused biogas production rate and efficiency increase more than two times in compare to manure substrate without rumen fluid. Rumen fluid caused biogas production rate (U) increase from 1.74 to 3.89 ml/(gVS.d) and the biogas produced will increase from 73.81 to 172.51 ml/(gVS). Moreover, rumen fluid reduced the minimum time to produce biogas (λ) from 14.75 to 7.25 days. The ultimate question is why substrate with rumen fluid caused significant effect on biogas production? Several reasons may be able to be discussed as follows: (1). Rumen fluid contains high anaerobic bacteria either quantity and species in compare to anaerobic bacteria content in manure neat. Higher quantity and species of anaerobic bacteria content in rumen fluid enable to degrade more kind of substrate content in manure; and (2). The carbons contained by all of waste constituents (manure) are not equally degraded or converted to biogas through anaerobic digestion. Finally, the most important finding from this research can be drawn the conclusion that, mathematically, the rumen fluid seeded to biodigester has significant effect to cumulative biogas production and biogas production rate. All of these data are very important information due to design biogas plant in the implementation step. The further research, kinetic constant was studied at room temperature and 38.5 °C

C. The influence of temperature to kinetic constants

This research step was directed to study the influence of temperature to biogas production kinetic. The research was carried at room temperature and 38.5 °C and in 30 L volume of biodigester. The temperature of 38.5 °C was selected due to the fact that the rumen condition on animal ruminants is \pm 38.5 °C. The substrate consists of 12 kg manure and 12 L rumen fluid (MR11) was fed to the biodigester and compared to manure substrate and water in equal weight ratio (MW11). The cumulative volume of biogas production was observed during 80 days observation as depicted in Figure 4. The experimental data obtained then is analyzed using non linear regression for determining kinetic constants as presented in Table 3. By plotting experimental data and simulation of the modified Gompertz equation (equation 1) will be obtained the graph as depicted in Fig. 4.

Table 3 and Fig. 4 show that either at room temperature as well as at 38.5 °C, substrate contains rumen fluid (MR11) consistently exhibits higher biogas production rate than substrate without rumen fluid (MW11). In addition, either substrate with and without rumen fluid, anaerobic digestion at 38.5 °C exhibit higher biogas production rate than at room temperature. Increasing temperature from room temperature to 38.5 °C caused biogas production rate increase from 4.19 to 9.49 (ml/gVS.d) (MR11). Moreover, increasing temperature from room temperature to 38.5 °C has also caused biogas production rate increase from 1.84 to 5.68 (ml/gVS.d) (MW11). In term of maximum biogas production (A), increasing temperature from room temperature to 38.5 °C caused maximum biogas production MR11 and MW11 from 194.23 to 418.26 and 58.16 to 136.60 ml/(gVS), respectively.

This is because due to general rule that temperature is a very important operational parameter in an AD processes. The optimum temperature for all of bacteria groups is the range of 35-40 °C [30]-[31]. In addition, the 38.5 °C is predicted as optimum temperature of bacteria due to the fact that the rumen condition on animal ruminants is 38.5 °C. The temperature difference of ± 2.5 °C can cause the decrease of the rumen

microbial activity. This is agree with the overview conducted by Balsam [32], that temperature variation around 2,5 °C can inhibit bacterial growth rate of methane former. However, the further intensive study is needed to access the optimum temperature of anaerobic digestion especially for AD using rumen fluid as inoculum.

TABLE III THE COMPARATION OF KINETIC CONSTANTS AT ROOM TEMPERATURE AND 38.5 °C

Treatments	U, (ml/gVS.d)	A, ml/(gVS)	λ , days
MW11 - room temperature	1.84 ± 0.16	58.16 ± 15.89	13.75 ± 2.66
MR11 - room temperature	4.19 ± 0.20	194.23 ± 14.55	6.85 ± 1.55
MW11 - 38.5 °C	5.68 ± 0.67	136.60 ± 9.21	9.07 ± 1.56
MR 11 - 38.5 °C	9.49 ± 1.45	418.26 ± 25.67	4.46 ± 1.31

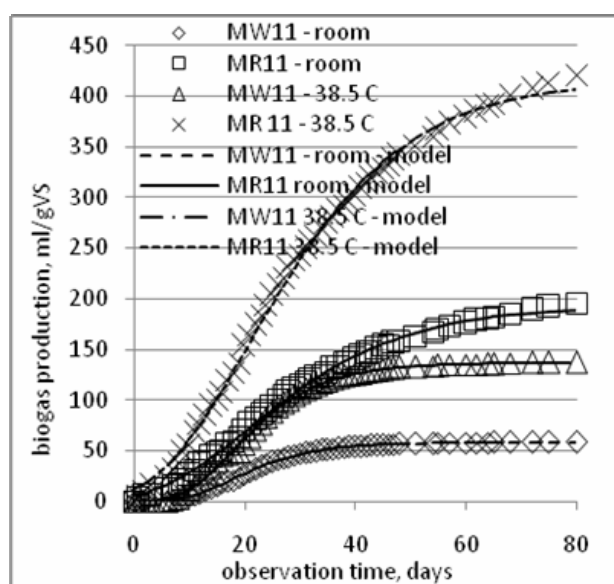


Fig. 4. Comparison of biogas production kinetic at room temperature and 38.5 °C, volume of biodigester 30 L

IV. CONCLUSIONS

Biogas production kinetic was studied by performing a series laboratory experiment using rumen fluid of animal ruminant as inoculums. The most important finding from this research is that the liquid rumen seeded to biodigester has significant effect to cumulative biogas production and biogas production rate. Rumen fluid inoculums caused biogas production rate and efficiency increase two to three times in compare to manure substrate without rumen fluid. With the rumen fluid inoculums, gave the kinetic parameters of biogas production i.e biogas production rate constants (U), maximum biogas production (A), and minimum time to produce biogas (λ) are 4.3931 ml/(gVS.day); 194.4 (ml/gVS); dan 4.4 days, respectively. While the substrate without rumen fluid gave the kinetic parameters U, A, and λ are 2.59 ml/(gVS.day); 58.16

(ml/gVS); dan 9,4 days, respectively. Either at room temperature as well as at 38.5 °C, substrate contains rumen fluid (MR11) consistently exhibits higher biogas production rate than substrate without rumen fluid (MW11). The temperature of 38.5 °C is predicted as optimum temperature of bacteria due to the fact that the rumen condition on animal ruminants is 38.5 °C. However, the further intensive study is needed to access the optimum temperature of anaerobic digestion especially for AD using rumen fluid as inoculum. The effect of liquid rumen concentration to biogas production will need to be studied in the next step research. In addition, the future work will be carried out to study the dynamics of biogas production if both the rumen inoculums and manure are fed in the continuous system.

REFERENCES

- [1]. Okkerse, C. and H. V. Bekkum.. From fossil to green. *Green Chem.*, 1999, 1: 107-114
- [2]. Alvarez, R. and G. Lide'n. The effect of temperature variation on biomethanation at high altitude, *Biores. Technol.* 2008, 99: 7278-7284
- [3]. Krylova, N.I., R.E. Khabibouline, R.P. Naumova, and M.A. Nagel, M.A. The influence of ammonium and methods for removal during the anaerobic treatment of poultry manure. *J. Chem. Technol. Biotechnol.* 1997, 70 (1): 99-105
- [4]. Callaghan, F.J., D.A.J. Wase, K. Thayanithy, and C.F. Forster. Co-digestion of waste organic solids - batch studies. *Bioresource Technol.* 1999, 67 (2), 117-122.
- [5]. Karim, K., R. Hoffmann, K. T. Klassonb, M.H. Al-Dahhan. Anaerobic digestion of animal waste: Effect of mode of mixing, *Water Research*, 2005, 39: 3597-3606
- [6]. Lo, K.V., A. J. Whitehead, P. H. Liao, N. R. Bulley. Methane production from screened dairy manure using a fixed-film reactor. *Agricultural Wastes*, 1984, 9(3): 175-188
- [7]. Vartak, D.R., C. R. Engler, M. J. McFarland, and S. C. Ricke. Attached-film media performance in psychrophilic anaerobic treatment of dairy cattle wastewater, *Bioresource Technology*, 1997, 62: 79-84
- [8]. Ndegwa, P.M., D.W. Hamilton, J.A. Lalman, and H.J. Cumba. Effects of cycle-frequency and temperature on the performance of anaerobic sequencing batch reactors (ASBRs) treating swine waste, *Bioresource Technology*, 2008, 99: 1972-1980
- [9]. Gelegenis, J., D. Georgakakis, I. Angelidaki, V. Mavris. Optimization of biogas production by co-digesting whey with diluted poultry manure. *Renewable Energy*, 2007, 32(13): 2147-2160
- [10]. Lehtomäki, A., S. Huttunen, J.A. Rintala. Laboratory investigations on co-digestion of energy crops and crop residues with cattle manure for

- methane production: Effect of crop to manure ratio, *Resources, Conservation and Recycling*, 2007, 51: 591–609
- [11]. Nielsen, H.B. and I. Angelidaki. Strategies for optimizing recovery of the biogas process following ammonia inhibition. *Bioresource Technology*. 2008, 99(17):7995-8001
- [12]. Boe, K. and Angelidaki, I. Serial CSTR digester configuration for improving biogas production from manure, *Water Research*, 2009, 43(1): 166-172
- [13]. Kaparaju, P., L. Ellegaard, I. Angelidaki. 2009. Optimisation of biogas production from manure through serial digestion: Lab-scale and pilot-scale studies, *Biores. Technol.* 100: 701–709
- [14]. Kaparaju, P., Buendía, I., Ellegaard, L., Angelidaki, I., Effects of mixing on methane production during thermophilic anaerobic digestion of manure: lab-scale and pilot-scale studies. *Bioresour. Technol.* 2008., 99: 4919–4928
- [15]. Liao, P.H., K.V. Lo, S.T. Chieng. Effect of liquid—solids separation on biogas production from dairy manure. *Energy in Agriculture*, 1984, 3: 61-69
- [16]. Kaparaju, P. and Angelidaki, I., 2008. Effect of temperature and microbial activity on passive separation of digested cattle manure. *Bioresour. Technol.* 99: 1345–1352
- [17]. Møller, H.B., A.M. Nielsen, R. Nakakubo, H.J. Olsen. Process performance of biogas digesters incorporating pre-separated manure, *Livestock Science*, 2008, 112: 217–223
- [18]. Kayhanian, M. and D. Rich. Pilot-scale high solids thermophilic anaerobic digestion of municipal solid waste with an emphasis on nutrient requirements, *Biomass and Bioenergy*, 1995, 8(6): 433-444.
- [19]. Demirci, G. G. and G. N. Demirel. Effect of initial COD concentration, nutrient addition, temperature and microbial acclimation on anaerobic treatability of broiler and cattle manure, *Bioresource Technology*, 2004, 93: 109–117
- [20]. Luengo, P.L. and J. M. Alvarez. 1988. Influence of temperature, buffer, composition and straw particle length on the anaerobic digestion of wheat straw-pig manure mixtures. *Resources, Conservation and Recycling*, 1(1): 27-37
- [21]. Castillo, R.T., P.L. Luengo, and J.M. Alvarez. 1995. Temperature effect on anaerobic of bedding manure in a one phase system at different inoculum concentration, *Agriculture, Ecosystems and Environment*, 54:55-66
- [22]. Sans, C., J. Mata-Alvarez, F. Cecchi, P. Pavan and A. Bassetti. Acidogenic fermentation of organic urban wastes in a plug-flow reactor under thermophilic conditions. *Bioresource Technology*, 1995, 54(2): 105-110
- [23]. Lopes, W. S., V. D. Leite, and S. Prasad. Influence of inoculum on performance of anaerobic reactors for treating municipal solid waste. *Bioresource Technology*, 2004, 94(3): 261-266
- [24]. Forster-Cameiro, T., M. Pérez and L. I. Romero. Influence of total solid and inoculum contents on performance of anaerobic reactors treating food waste. *Bioresource Technology*, 2008, 99(15): 6994-7002.
- [25]. Yetilmezsoy, K and S. Sakar. Development of empirical models for performance evaluation of UASB reactors treating poultry manure wastewater under different operational conditions, *J. Hazardous Materials*, 2008, 153: 532–543
- [26]. Nopharatana, A., P. C. Pullammanappallil, W. P. Clarke. Kinetics and dynamic modeling of batch anaerobic digestion of municipal solid waste in a stirred reactor, *Waste Management*, 2007, 27: 595–603
- [27]. Richard, T. The effect of lignin on biodegradability. Cornell Composting, Cornell Waste Management Institute, In <http://www.cfe.cornell.edu/compost/calc/lignin.html>. 1996.
- [28]. Wilkie, A.C. Anaerobic digestion of dairy manure: design and process consideration. in *Dairy Manure Management : Treatment, Handling, and Community Relations*, Natural Resource, Agriculture, and Engineering Service, Cornell University, Ithaca. 2005. pp. 301-312
- [29]. Aurora, S.P. Microbial Digestion in Ruminants. Indian Council of Agricultural Research, New Delhi, 1983.
- [30]. Wenxiu, T. and W. Mengjie. Experiment and research on a mesophilic anaerobic digester with dairy cattle manure in northern china. *Biomass*. 1989, 20(1-2): 41-52
- [31]. Anonimously. Biogas production process. (<http://zorg-biogas.com/library/biogas-production-process>). Downloaded on August 7, 2009, 0.800
- [32]. Balsam, J. Anaerobic digestion of animal wastes: factors to consider. ATTRA-national sustainable agriculture information service. United States Department of Agriculture's, USA, 2002