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The Kinetics of Methane Production from Co-digestion of Cattle Manure

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Abstract: In this article, the kinetics of methane production from co-digestion of liquid manure from cattle with the addition of winemaking waste, food waste and biowaste was investigated in order to describe and evaluate methanogenesis in terms of growth curve of methanogenic bacteria. Experiments were carried out in «Hohenheim» biogas yield testing system at the temperature of 37°C. The cumulative methane yield was 0.330 ± 0.038 , 0.277 ± 0.041 , 0.148 ± 0.013 and 0.250 ± 0.025 m³ CH₄ per kg oDM in normal condition, respectively in mono-digestion and co-digestion of liquid manure from cattle with winemaking, food and biowaste. The kinetic Gompertz parameters of methane production (P-potential yield of methane, R_m-maximum methane production rate and λ-duration of lag phase) were analyzed. The highest potential methane yield (P) showed co-fermentation of liquid manure from cattle with biowaste 0.387 Nm³ (kg oDM)⁻¹, the highest methane production rate (R_m) was 0.022 ± 0.003 Nm³ (kg oDM)⁻¹ day⁻¹ for mono-digestion of cattle slurry, the lowest 0.006 Nm³ (kg oDM)⁻¹ day⁻¹ was obtained during co-digestion with food waste. Duration of lag phase (λ) was within 10.17-14.60 days for all samples. Additional, the duration of digestion to produce 95% of the potential methane yield and efficient methane production was determined.

Key words: Anaerobic digestion, methane production kinetics, cattle manure, co-digestion

INTRODUCTION

Methane fermentation is a complex microbiological and biochemical process in the absence of oxygen which is carried out by four groups of microorganisms-hydrolyzing, acidogenic, acetogenic and methanogenic bacteria, creating syntrophic relationship and requiring different environmental conditions (Weiland, 2010). During interconnected in series and parallel biochemical reactions, microorganisms affect to specific components of the substrate selectively performing their transformation only under certain conditions (Gujer and Zehnder, 1983; Liu *et al.*, 2009). Thus, the process is catalyzed by a consortium of microorganisms that convert the complex macromolecules into low molecular weight compounds (methane, carbon dioxide, water and ammonia) (Fantozzi and Buratti, 2009). The final product of an anaerobic fermentation is biogas which is composed of 48-65% methane, 36-41% carbon dioxide and 7% nitrogen, <1% oxygen, 32-169 ppm (parts per million) hydrogen sulfide (Martins das Neves *et al.*, 2009).

Anaerobic fermentation technology is widely used for agricultural waste, including cattle waste. Processing manure to produce biogas, fertilizer and other by-products solves the environmental protection problem, improving soil fertility, producing ecological clean energy (Budiyo *et al.*, 2010).

The types of substrates and the ratio of nutrients C:N:P are very important in the implementation of a balanced anaerobic digestion of biomass. All types of biomass can be used as substrates as they contain carbohydrates, proteins, fats, cellulose and hemicellulose as main components (Deublein and Steinhauser, 2008; Ward *et al.*, 2008; Weiland, 2010). The methane formation can have a significant impact of co-substrates content. Depending on the composition of the substrate, the intermediate degradation products can restrict and inhibit degradation. For example, the degradation of fats may lead to an increase of fatty acids that limited further degradation. In the decomposition of proteins, methane fermentation can be limited by formation of ammonia and hydrogen sulfide (Deublein and Steinhauser, 2008).

Currently, to increase biogas yield from the biomass fermentation is often practiced using waste in combination with other substrates (Alatrisme-Mondragon *et al.*, 2006; Labatut and Scott, 2008; Ward *et al.*, 2008; Liu *et al.*, 2009). Use as an additive of ruminant animal manure has become the most relevant area of research, since the cattle manure contains high levels of microorganisms capable of hydrolysing lignine cellulosic materials (Malik *et al.*, 1989; Deublein and Steinhauser, 2008; Ward *et al.*, 2008; Liu *et al.*, 2009). On this issue, numerous studies, especially on the issue of determining the potential release of methane from various organic wastes for the

evaluation and optimization of conditions for the production of biogas were carried out. Numerous models were developed that take into account the biological and physico-chemical basis of anaerobic fermentation and the growth kinetics of methanogenic microorganisms (Lay *et al.*, 1998; Gerber and Span, 2008; Koppa and Pullammanappallil, 2008; Lo *et al.*, 2010). In assessing the overall biogas production rate in the anaerobic reactors the limiting step performed by methanogenic stage, despite the fact that the methane-producing bacteria have a lower growth rate than the acid-forming bacteria. The kinetic parameters of methane production facilitate the understanding of the methanogenesis process and optimization of biogas plants.

MATERIALS AND METHODS

Feedstock and sample preparation: Studies of the potential biogas yield were conducted using agricultural waste of liquid manure from cattle (cattle slurry) with combination of other organic waste as food waste, biowaste and winemaking waste. Samples of agricultural wastes were taken from the farm of the University of Hohenheim. Food waste (leftovers) from the university canteen, winemaking waste (grape pulp) and biowaste from the laboratory of the university.

The main parameters for the evaluation of biogas yield and degree of decomposition of the organic substances (Fresh Matter (FM)) are dry matter content (DM), Organic Dry Matter (oDM), as well as ash and moisture content. These indicators are investigated according to APHA (1995), by drying at the 105°C for 12 h and burning at the 505°C for at least 6 h in oven.

The results of determination DM, oDM, ash and moisture content of tested substrates are shown in Table 1.

Winemaking waste and biowaste were used as dry solid substrates in which the average content of DM for

3 samples of substrates was higher than 90% (95.48±0.05 and 89.9%±0.05, respectively). Liquid manure from cattle contained 3.75±0.09% of DM. The content of DM in the food waste was 18.9±0.15% while the humidity was 81.06%.

In the substrates of winemaking wastes and food wastes was recorded the highest content of oDM in DM. Mineral content (ash) is less than 5% in cattle slurry, food waste and winemaking waste.

For mono-fermentation of cattle manure (sample 1) was used about 40 mL of the substrate and for co-fermentation of cattle manure (samples 2-4) with other wastes 30 mL of the substrate. Ration of tested substrates in a mixture was 70:30 by content of oDM. The complete characterization of samples is shown in Table 2.

Biogas yield testing system: Studies to test the biogas production from waste were carried out at 37°C in «Hohenheim» biogas yield testing system in the biogas laboratory of the University of Hohenheim, Germany. This biogas yield testing system consists of fermenters in the form of glass syringes (flasks for sampling) of 100 mL with 1/1 gradation and capillary extension (Fig. 1), fermentation chamber-incubator (Fig. 2) and gas transducer.

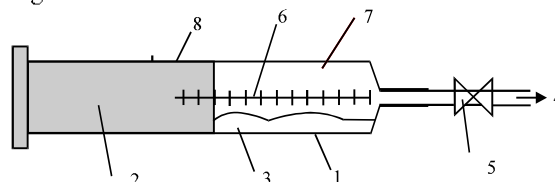


Fig. 1: Laboratory mini-reactor of «Hohenheim» biogas yield testing system, (Helffrich and Oechsner, 2003) (1) Glass syringe, (2) Stopper, (3) Substrate, (4) Hole for gas analysis, (5) Clamp for the tube, (6) Graduations, (7) Gas space, (8) Grease

Table 1: Results of the substrate analysis

Substrate samples	Parameters (%)			
	Dry matter in fresh material	Organic dry matter in dry matter	Ash in fresh material	Moisture content
Liquid manure from cattle	3.75±0.09	72.14±0.480	1.04±0.008	96.25
Wine making waste (grape pulp)	89.90±0.05	96.15±0.110	3.46±0.110	10.10
Food waste (leftovers)	18.94±0.15	91.20±0.023	1.67±0.009	81.06
Biowaste	95.48±0.05	84.12±0.400	15.16±0.390	4.52

Table 2: Characterization of experimental samples

Samples	Substrates	Fresh matter content (g)	Average content of dry matter (g)	Average content of organic dry matter (g)	Moisture content (%)	Ration of substrates by organic dry matter
1	Liquid cattle manure	40.2±0.295	1498±0.011	1080±0.008	96.27	
2	Liquid cattle manure + wine making waste	30.4±0.047	1479±0.002	1153±0.001	95.41	70:30
3	Liquid cattle manure + food waste	32.1±0.150	1483±0.018	1138±0.016	95.07	70:30
4	Liquid cattle manure + biowaste	30.5±0.184	1503±0.007	1130±0.005	95.14	70:30



Fig. 2: Fermentation chamber of «Hohenheim» biogas yield testing system

The methane content was measured by gas transducer AGM 10 (transducers of Europe GmbH, Germany) with a non-dispersive infrared (NDIR) detector capable of detecting methane content of the biogas in the range from 0 to 100%. Gas sensor was calibrated by the standard gas, containing 60.7% (v) methane. Incubator temperature, air pressure, date and time at which the measurements were made, were also recorded for analysis of biogas content at standard conditions (273 K and 101325 Pa) determined in accordance with Ludington (2006).

Simulation: Studies of the methane production kinetics for the description and evaluation of methanogenesis were carried out by fitting the experimental data of cumulative methane production to the Gompertz equation (Lay *et al.*, 1998; Koppa and Pullammanappallil, 2008; Lo *et al.*, 2010; Budiyo *et al.*, 2010; Xie, 2012). Analyses of the experimental results and calculations were performed in MS-Excel using the 'Solver' feature by non-linear regression. This equation describes the total methane production in batch reactors assuming that methane production is a function of methanogenic bacteria growth. The modified Gompertz equation is presented below:

$$M = P \times \exp\left\{-\exp\left[\frac{R_m \times (\lambda - t)}{P} + 1\right]\right\}$$

where, M is the cumulative methane production ($\text{Nm}^3 (\text{kg oDM})^{-1}$), P is the methane production potential ($\text{Nm}^3 (\text{kg oDM})^{-1}$), R_m is the maximum methane production rate ($\text{Nm}^3 (\text{kg oDM})^{-1} \text{day}^{-1}$), λ is the duration of lag phase (days) and t is the cumulative time for methane production (days).

RESULTS

The cumulative methane production analyses: According to the results of "Hohenheim" biogas yield testing system was obtained and calculated specific cumulative methane yield per kg of oDM in the normal condition (273 K and

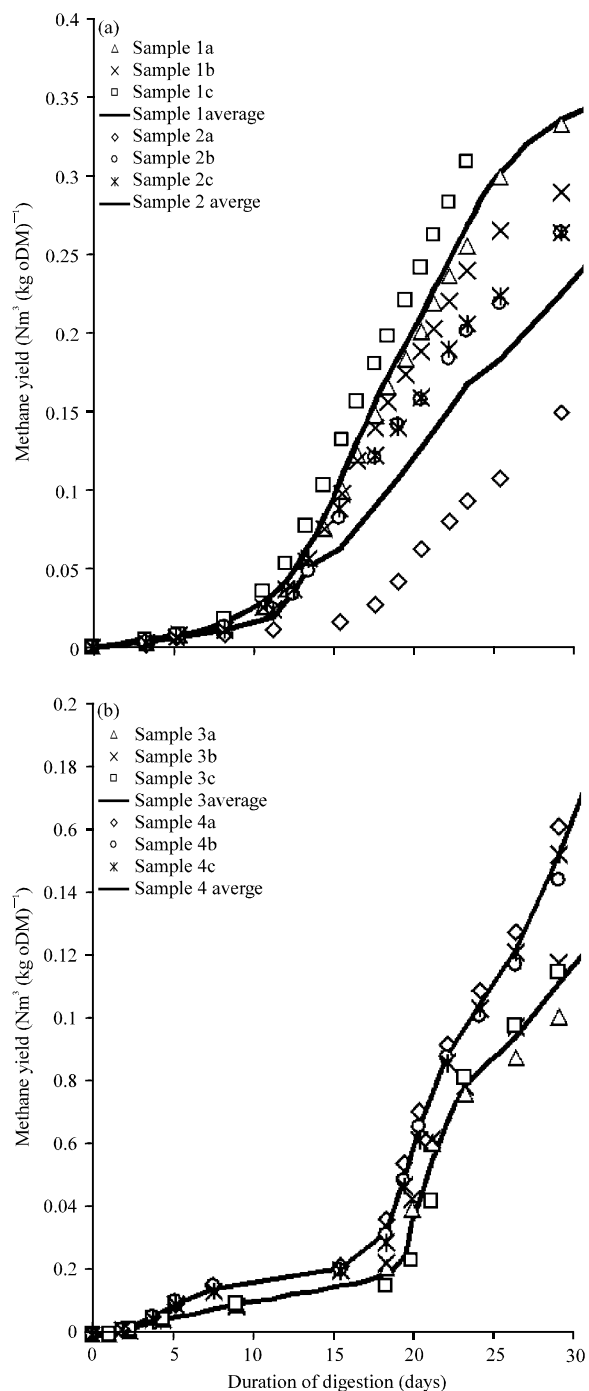


Fig. 3(a-b): Cumulative methane production, Average methane production for samples (a) 1 and 2 (with triplication a, b, c) and (b): 3 and 4 (with triplications a, b, c)

1013.25 Pa). Anaerobic fermentation lasted 35 days at the temperature of 37°C. The cumulative methane yield is described in Fig. 3. Methane production in sample 1 was stable, i.e., methanogenesis was gone according to the

microbial growth curve, forming approximately 0.03 Nm³ CH₄ per kg of oDM on the 10th day, 0.2 Nm³ CH₄ to the 20th day, at the end of the fermentation cycle 0.330±0.038 Nm³(kg oDM)⁻¹.

Intensive methane production was observed from the 11th day to 31st day in sample 2. Curve of the total methane yield gave the value of 0.277 Nm³ (kg oDM)⁻¹ with a standard deviation of 0.041 Nm³ (kg oDM)⁻¹.

In experiments with a mixture of liquid cattle manure with the addition of food waste and biowaste (samples 3 and 4) observed very late intensive methane production from the 19th day. Up to 20 days of HRT was formed only 0.03 Nm³ (kg oDM)⁻¹. And before the end of run was formed 0.148±0.013 and 0.250±0.025 Nm³ (kg oDM)⁻¹.

Kinetic parameters of methane production: Results of the analysis of the kinetic parameters P, R_m and λ are shown in Table 3 and Fig. 4. For three repetitions average ultimate methane yield (P) in the experiments with a mixture of liquid manure from cattle with winemaking waste (sample 2) was 0.338 Nm³ (kg oDM)⁻¹ (standard deviation of 0.013 Nm³ (kg oDM)⁻¹ methane), 0.288±0.015 and 0.387±0.011 Nm³ (kg oDM)⁻¹, respectively, for samples 3 and 4. For sample 1 (mono-fermentation of liquid manure from cattle) the potential methane production (P) was equal to 0.381±0.045 Nm³ (kg oDM)⁻¹, i.e. addition of bio-waste in the ratio of 70:30 increases the methane potential production but only to 0.006 Nm³ (kg oDM)⁻¹ methane. Adding winemaking wastes to cattle slurry reduces the methane yield to 0.043 Nm³ (kg oDM)⁻¹. The lowest potential methane yield was for sample 5 with the addition of food waste, where the parameter was 0.76 times less than in mono-fermentation of cattle slurry.

According to the Gompertz model, potential methane yield for samples 2-4 exceeded the cumulative experimental methane yield for 0.061 Nm³ (kg oDM)⁻¹ (18%), 0.140 Nm³ (kg oDM)⁻¹ (48.6%) and 0.137 Nm³ (kg oDM)⁻¹ (35.4 %) of methane.

Duration of lag phase (λ) for samples 2-4 was 11.58±2.96, 11.8±1.21 and 14.60±0.83 days, respectively. Minimum time to produce methane (λ) for all three mixtures longer than in sample 1 it was found extending of the lag phase to 1.41 day in sample 2, to 1.61 days in sample 3 and the lag phase was found longer to 4.43 days in sample 4, despite the high ultimate methane yield.

Maximum methane production rate (R_m) in samples 2-4 was lower than in sample 1. The maximum methane production rate (R_m) for sample 2 was equal to 0.014 Nm³ (kg oDM)⁻¹ day⁻¹ it was 0.64 times lower than in sample 1. Sample 3 showed the lowest value of the maximum methane production rate equal to 0.006±0.0004 Nm³ (kg oDM)⁻¹ day⁻¹ and hence, the

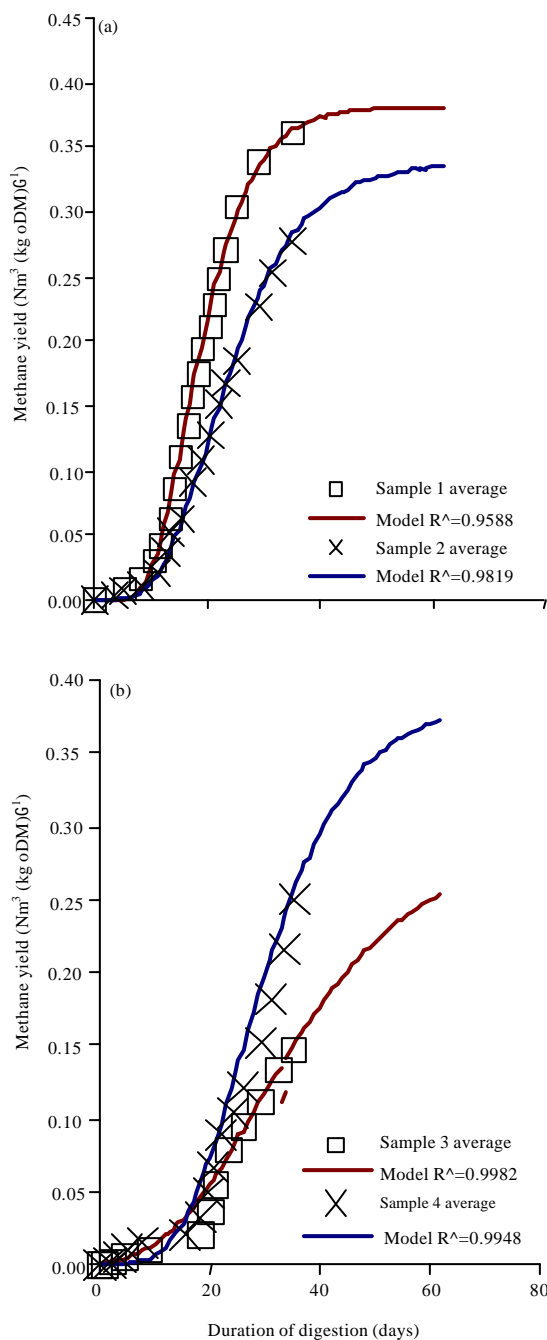


Fig. 4(a-b): Kinetic model of the Gompertz equation (on average), Results for samples (a) 1 and 2 and (b) 3 and 4

methane production was gone almost 4 times slower compared to sample 1. The highest methane generation rate in sample 4 was 0.013±0.002 Nm³ (kg oDM)⁻¹ day⁻¹, increased 2-fold compared with sample 3, reduced to 0.009 Nm³ (kg oDM)⁻¹ day⁻¹ than in sample 1.

Table 3: Results of the kinetic analysis of methane production (average data)

Sample	Cumulative methane yield, Nm ³ (kg oDM) ⁻¹	Gompertz parameters (model)			Duration to produce 95% potential methane yield (days)
		P, Nm ³ (kg oDM) ⁻¹	R _m , Nm ³ (kg oDM) ⁻¹ day ⁻¹	λ, Days	
1	0.330±0.038	0.381±0.045	0.022±0.0030	10.17±0.34	34.71±1.55
2	0.277±0.041	0.338±0.013	0.014±0.0020	11.58±2.96	46.35±6.82
3	0.148±0.013	0.288±0.015	0.006±0.0004	11.80±1.21	76.43±4.72
4	0.250±0.025	0.387±0.011	0.013±0.0020	14.60±0.83	58.70±5.78

The time required to reach 95% of methane production for sample 2 was 46.35±6.82 days. Time to reach 95% of methane production for sample 3 was 76.43 days with a standard deviation of 4.72 days, 30 days longer than for sample 2. Experiments with a mixture of liquid manure from cattle with biowaste showed 58.7±5.78 days for production 95 % methane potential. According to the Gompertz equation the calculated effective duration for methane production was 34.77, 64.63 and 44.1 days. These values indicate that the fermentation time of mixed substrates with liquid manure from cattle longer to 7.35, 37.09 and 16.56 days than mono-digestion of cattle slurry.

The kinetic models of the Gompertz equation for the sample of cattle slurry with the addition of winemaking wastes showed similarities in the plots with the experimental plot of the cumulative methane production. The value of the approximation reliability was equal to R² = 0.9928 on average with a standard error of 0.01406 it means that accuracy between the regression function and the actual value is equal to 99%.

As seen in Fig. 4, between the experimental and calculated plots in sample 3 was found deviation due to the instability of the fermentation process. The model of cumulative methane yield for sample 3 based on the Gompertz equation with the approximation reliability of R² = 0.9982 (standard error = 0.00208) showed the inhibition and volatility of methanogenesis from the 10th day to the 25th day. That was due to the addition of food waste to cattle slurry, since rapidly degradable organic substances (lactose, sucrose and fatty acids) prevailed in food waste which are rapidly degraded during the hydrolysis and acidogenesis, leading to intense accumulation of acids. High acid accumulation during methanogenesis leads to inhibition of process.

If compare the plots of experimental and calculated data obtained for sample 4 it is possible to find an uneven flow of methane production. Model of the methane yield for sample 4 showed the approximation reliability of R² = 0.9948, the standard error of 0.00635. Instability during fermentation is also due to pH changes in the medium, as there are easily degradable organic substrates, fruit and food waste in the biowaste.

DISCUSSION

In all samples with a mixture of food waste and biowaste was observed uneven production of methane

that was because the rapidly degradable organic substances content which led to increased ammonia content which is one of the major causes of digestion inhibiting.

According to models based on the calculated data of the kinetic model of Gompertz equation, can be observed asymptotic approximation of the cumulative methane production curve only in samples 1 and 2. Therefore, for samples 3 and 4 was modeled anaerobic digestion process on the basis of the kinetic parameters. So the duration of the fermentation for samples 2-4 in 35 days are not enough it means neediness in renew it.

Substrate degradation depends on the structure of its components. Sugars and starches are broken down very quickly having a simple structure and require only a short residence time in the fermenter. The more complex structure of the substrate, the longer is degrading. Cellulose and hemicellulose are well structured and decomposed slowly. Lignin is decomposed very bad by bacteria, because it shows strength even to acids. Therefore, mixture of liquid cattle manure with winemaking waste showed a slow daily production of methane and later initiation of methane formation, than methanogenesis of sample 1, due to the low concentration of bacteria and adaptation of methanogenic bacteria to the new environment. It is known, grape pomace consists of 37-39% peel (based on total weight), 15-34% pulp, 1.0-3.3% residual ridges, 23-39% seeds and contains 5.4-8.3% pectin by weight of dry matter (Ponomarev, 1997).

Biowaste contains vegetable waste, food waste, paper and other waste (Deublein and Steinhauser, 2008). Therefore, the composition of biowaste consists of easily and hardly decomposable organic compounds with up to 84% of oDM. And in the food waste are more easily decomposed substances (sucrose, fructose, fatty acids, etc.). In the first phases of methane generation of hydrolysis and acidogenesis, readily degradable substances quickly converted to the monomers, therefore the overall content of acid in the medium increases, thereby decreases pH. Reducing of the pH leads to the accumulation of acids and inhibition of methanogenesis. Hydrolyzing and acid-forming bacteria reach their optimum activity in the acidic medium level at the pH 4.5-6.3; acetic acid and methane forming bacteria can

only survive at neutral or slightly alkaline medium at the pH 6.8-8. The pH of the normal and healthy anaerobic fermentation system should be in the range 6.5-8.5 (Ahn *et al.*, 2010). For all bacteria, if the pH exceeds the optimum, their vital functions become slower which reduce formation of biogas.

Biogas and methane production in the experiments with the addition of food waste (leftovers) and biowaste lower than in other samples, due to the rapid hydrolysis and acidogenesis and composition of co-substrates. Initiation of methanogenesis inhibited and required further digestion of these substrates, as a result of the acid accumulation, i.e. longer than 35 days.

CONCLUSION

These experiments showed that the increase in methane yield depends on the biochemical processes of anaerobic fermentation step and composition of fermenting biomass. Methane formation is carried out with the participation of methanogenic *Archaea* (*Methanobacterium*, *Methanospirillum hungatii*, *Methanosarcina*) (Deublein and Steinhauser, 2008), forming methane from acetate and CO₂ on the 5-16th day of fermentation. Kinetic constants from the Gompertz equation for mono-digestion of liquid manure from cattle were 0.381 Nm³ (kg oDM)⁻¹ (P), 0.022 Nm³ (kg oDM)⁻¹ day⁻¹ (R_m) and 10.17 days (λ), for co-fermentation of cattle manure with winemaking waste were 0.338 Nm³ (kg oDM)⁻¹ (P), 0.014 Nm³ (kg oDM)⁻¹ day⁻¹ (R_m) and 11.58 days (λ). Potential methane yield was 0.288 and 0.387 Nm³ (kg oDM)⁻¹ for co-fermentation of cattle manure with food waste and biowaste, respectively. Co-fermentation of cattle slurry with food waste showed the lowest maximum methane production rate (0.006 Nm³ (kg oDM)⁻¹ day⁻¹) and it took 76.43 days to achieve 95% of the potential methane yield and 11.8 days for the lag phase. Lag phase duration was 14.6 days, the maximum methane production rate -0.013 Nm³ (kg oDM)⁻¹ day⁻¹ and time to achieve 95% of the methane potential-58.7 days in the co-fermentation of cattle slurry with biowaste.

REFERENCES

- APHA, 1995. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington DC.
- Ahn, H.K., M.C. Smith, S.L. Kondrad and J.W. White, 2010. Evaluation of biogas production potential by dry anaerobic digestion of switchgrass-animal manure mixtures. Applied Biochem. Biotechnol., 160: 965-975.
- Alatraste-Mondragon, F., P. Samar, H.H.J. Cox, B.K. Ahring and R. Iranpour, 2006. Anaerobic codigestion of municipal, farm and industrial organic wastes: A survey of recent literature. Water Environ. Res., 78: 607-636.
- Budiyono, B., I.N. Widiasta, S. Johari and Sunarso, 2010. The kinetic of biogas production rate from cattle manure in batch mode. Int. J. Chem. Biol. Eng., 3: 39-44.
- Deublein, D. and A. Steinhauser, 2008. Biogas from Waste and Renewable Resources. Wiley-Vch Verlag GmbH and Co. KGaA, Weinheim, Germany, ISBN:13-978-3-527-31841-4, Pages: 423.
- Fantozzi, F. and C. Buratti, 2009. Biogas production from different substrates in an experimental continuously stirred tank reactor anaerobic digester. Bioresour. Technol., 100: 5783-5789.
- Gerber, M. and R. Span, 2008. An analysis of available mathematical models for anaerobic digestion of organic substances for production of biogas. Proceedings of the International Gas Union Research Conference, October 8-10, 2008, Paris, France, pp: 1-30.
- Gujer, W. and A.J.B. Zehnder, 1983. Conversion processes in anaerobic digestion. Water Sci. Technol., 15: 127-167.
- Helffrich, D. and H. Oechsner, 2003. The Hohenheim biogas yield test: Comparison of different laboratory techniques for the digestion of biomass. Agrartechnische Forsch., 9: 27-30.
- Koppar, A. and P. Pullammanappallil, 2008. Single-stage, batch, leach-bed, thermophilic anaerobic digestion of spent sugar beet pulp. Bioresour. Technol., 99: 2831-2839.
- Labatut, R.A. and N.R. Scott, 2008. Experimental and predicted methane yields from the anaerobic co-digestion of animal manure with complex organic substrates. Proceedings of the Annual International Meeting on ASABE, Volume 8, June 29-July 2, 2008, Rhode Island, USA., pp: 19-19.
- Lay, J., Y. Li and T. Noike, 1998. Mathematical model for methane production from landfill bioreactor. J. Environ. Eng., 124: 730-736.
- Liu, Y., S.A. Miller and S.I. Safferman, 2009. Screening co-digestion of food waste water with manure for biogas production. Biofuels, Bioprod. Bioref., 3: 11-19.
- Lo, H.M., T.A. Kurniawan, M.E.T. Sillanpaa, T. Y. Pai and C.F. Chiang *et al.*, 2010. Modeling biogas production from organic fraction of MSW co-digested with MSWI ashes in anaerobic bioreactors. Bioresour. Technol., 101: 6329-6335.

- Ludington, D., 2006. Calculating the heating value of biogas. DLtech Inc. Ithaca, New York. http://syreen.gov.ny.archive/docs/File/Articles/from%20dr.abd%20alrhman%20alchyah/2/Heating_Value_of_Biogas.pdf
- Malik, R.K., P. Tauro and D.S. Dahiya, 1989. Effect of delignification pretreatment and selective enrichment on methane production from cattle waste. *Biotechnol. Bioeng.*, 33: 924-926.
- Martins das Neves, L.C., A. Converti and T.C.V. Penna, 2009. Biogas production: New trends for alternative energy sources in rural and urban zones. *Chem. Eng. Technol.*, 32: 1147-1153.
- Ponomarev, A.P., 1997. Grabe processing technologies. MACA, Moscow, pp: 115. (In Russ).
- Ward, A.J., P.J. Hobbs, P.J. Holliman and D.L. Jones, 2008. Optimisation of the anaerobic digestion of agricultural resources. *Bioresour. Technol.*, 99: 7928-7940.
- Weiland, P., 2010. Biogas production: Current state and perspectives. *Applied Microbiol. Biotechnol.*, 85: 849-860.
- Xie, S., 2012. Evaluation of biogas production from anaerobic digestion of pig manure and grass silage. Ph.D. Thesis, National University of Ireland.