ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 12 (6): 236-244, 2013
ISSN 1682-296X / DOI: 10.3923/biotech.2013.236.244

Dry Fermentation of Agricultural Waste in the Modified Leach-bed Reactor with Immobilization of Microorganisms

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Abstract: Anaerobic dry fermentation of cattle manure was investigated to evaluate an efficiency of the single-stage leach-bed with immobilization device process. The experiments were performed in batch-operation mode at the temperature of 40±0.2°C in triplication of two runs on bioreactor with working volume of 50 L. The reactor was equipped with immobilization device positioned at the bottom as a layer of polyethylene packing rings. Each 2nd run was initiated by inoculating with anaerobically digested cattle slurry from previous run. The performance of the reactor was analyzed in terms of the biogas and methane production and the methane production kinetics. According to the results, the average cumulative methane yield was 0.156 $Nm^3(kg\ oDM)^{-1}$. The kinetic parameters of methane production, i.e., methane production potential (P), maximum methane production rate (R_m) and minimum time to produce methane (λ) were indicated as 0.156 $Nm^3(kg\ oDM)^{-1}$, 0.018 $Nm^3(kg\ oDM)^{-1}day^{-1}$ and 1.16 days, respectively. Time to produce 95% of methane production potential was 14.31 days, it gave 13.15 days for effective anaerobic digestion time. Immobilization of methanogens on the supporting material and recirculation system facilitated improvement of methane production kinetics.

Key words: Biogas, methane, agricultural wastes, cattle manure, immobilization of microorganisms, methanogens, biofilm

INTRODUCTION

The agro-industrial oriented economy of Kazakhstan requires continuous improvement of agricultural production and stable growth tendency in the number of livestock and poultry has been seen in the last 7-8 years (according to statistics for 2011 from FAOSTAT, there are 6.2 million head of cattle, 1.3 million pigs, 1.5 million horses, 15.1 million sheep, 32.6 million chicken). Continuous and intensive livestock development in Kazakhstan has led to an increase of uncontrolled dumping and storage on average 22.1 million tons (by dry weight) of livestock and poultry wastes in the environment every year causing serious problems of environmental and social issues in the areas of livestock and poultry farms. Nowadays, anaerobic digestion processes for agricultural wastes are world widely utilized to reduce above mentioned problems and it has become an alternative way of renewable energy production (Kothari *et al*., 2011; Weiland, 2010; Luna-DelRisco *et al*., 2011).

Anaerobic Digestion (AD) process has substantial environmental and economical advantages, such as clean energy production in the form of biogas (Dubrovskis *et al*., 2008), odor control and pathogen reduction during organic waste treatment (Wilkie, 2003), also minimization environmental impact from waste emissions and maximization fertilizer mineral content (Wilkie, 2003; Budiyono *et al*., 2009; Weiland, 2010).

Ward *et al.* (2008) reviewed different types of currently used bioreactors (basic apparatus for the anaerobic digestion of biomass) depending on the type of processed material and divided them into three types: batch, one stage and two-stage (or multi-stage) continuously fed systems. The batch reactors are considered as the simplest one which are just filled with raw materials and retained for a period and emptied. Another type is a one-stage continuously fed system, where all the biochemical reactions proceed in one reactor. And the most complex of them is two-stage or even multi-stage continuously fed systems, where hydrolysis/acidification and atcetogenesis/methanogenesis processes are separated (Ward *et al.*, 2008). Two bioreactor types for solid waste are also well known. "Wet" fermentation, where the total value of solids up to 16% and "dry" is the fermentation of substrate which contains 22-40% of total solids. Furthermore, semi-dry reactor that is between "wet" and "dry" reactors was described by Mata-Alvarez (2002) and Ward *et al.* (2008).

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Numerous studies had been done in order to improve biogas production technologies through anaerobic digestion of organic wastes (Ward *et al*., 2008; Nasir *et al*., 2012). Optimization of biogas production was achieved by modification of bioreactors to design high-rate systems. Recently, for the treatment of solid organic waste are applied leach-bed batch processes operated in a single-stage or dual-stage mode depending on the characteristics of the feedstock. This leach-bed process has been successfully used for the fermentation of municipal solid waste (Koppar and Pullammanappallil, 2008), for the conversion of plant substrate (corn, rye, grass) (Zielonka *et al*., 2010). Koppar and Pullammanappallil (2008) claim that this technology eliminates some points of process: shredding of feedstock, mixing or agitation of digester contents, necessity for expensive high-pressure vessels. This process was operated on two-stage mode for treatment of municipal solid wastes, yard waste, water hyacinth and sorghum by Chynoweth *et al.* (1992), Chugh *et al.* (1999) and Koppar and Pullammanappallil (2008). Hegde and Pullammanappallil (2007) modified this process to a single-stage design by flooding the fresh biomass bed with liquid drained from the previous digestion of the feedstock. Solid-phase digestion of agricultural substrates (horse dung) was researched in discontinuously operated leach-bed reactors by Kusch *et al.* (2008). Recently developed high-rate bioreactors focused on retention of the active microorganisms by involving and attaching them on the inert surfaces (packing materials) to reach immobilization of microflore in the form of slimy layer (biofilm) in the reactor. These designs can provide with improved stability and control of the process for treatment of agricultural and industrial wastes (Wilkie, 2000), furthermore immobilization of microorganisms in anaerobic fixed-bed bioreactors can reduce hydraulic retention time from a few hours to several days and prevent washout of microbial biomass (Wilkie, 2000; Deublein and Steinhauser, 2008).

The this present study we investigate and evaluate the performance of the modified single-stage leach-bed with immobilized microorganisms process for treatment of solid cattle manure, concentrating on the biogas production and biogas composition, also in terms of its methane production kinetics.

MATERIALS AND METHODS

Feedstock: Cattle manure was provided by livestock farm of the University of Hohenheim. For each experiments and runs were required 3 kg of cattle manure and 19 L of inoculum. The inoculum was taken from the 400 L running continuously reactor in biogas laboratory of the University of Hohenheim. The inoculum was fermented cattle slurry that was used as leachate for the microbial initiation of single-phase leach-bed process with immobilization of microorganisms and for recirculation system. Fresh Manure (FM) as received and inoculum samples were analyzed in triplicate for its Dry Matter (DM) and moisture content by heating at 105°C and Organic Dry Matter (oDM) and ash content by burning at 505°C for 12 h according to standard methods of APHA (1995). The feedstock samples were characterized as shown in Table 1. Accordance with results of tested substrate analysis, DM content of cattle manure was 25.93 and 84.07% of solids were oDM.

Table 1: Characterizations of the tested substrates used in anaerobic digestion[a]

Parameters (%)	Cattle manure	Inoculum (digested cattle slurry)
DM (in FM)	25.93±0.64	0.62±0.003
oDM (in DM)	84.07±1.73	40.47±0.66
Ash (in FM)	4.12±0.36	0.37±0.004
Moisture content	74.07	99.38

[a]Data are expressed as the Mean±SD of three replicate samples

Experimental set-up and operation: Bioreactor with a working volume of 50 L had been constructed by modifying leach-bed reactor and fixed-bed reactor. The reactor in the form of vertical cylinder was 30 cm in diameter and 70 cm in height. It was equipped with immobilization device positioned at the bottom of the bioreactor which was a layer of polyethylene packing rings. Bed height of immobilization device was 20 cm. Schematic diagram of modified leach-bed reactor is shown in Fig. 1.

The experiments were conducted in the biogas laboratory of the State Institute of Agricultural Engineering and Bioenergy of the University of Hohenheim (Stuttgart, Germany). The reactors were maintained at 40±0.2°C and operated at a batch mode until biogas production was detected. Anaerobic digestion was performed in two runs, lasted 28 days for Run 1 and 21 days for Run 2 of Hydraulic Retention Time (HRT) in three replicate indicated as Experiments.

Reactor was inoculated with 19 L (0.019 m^3) of fermented cattle slurry with DM content of 0.62±0.003% and oDM content of 40.47±0.66% (Table 1) unto the top of the immobilization device and on top of that device was loaded 3 kg of solid cattle manure. The leachate (fermented cattle slurry) continuously recirculated by pump 15 min every 2 h during the whole fermentation cycle. Circulation flows toward the upper part of the reactor ("downflow" system). Leachate was sprayed onto the surface of the solid fraction causing gradual enrichment of fermentation medium with nutrients resulted

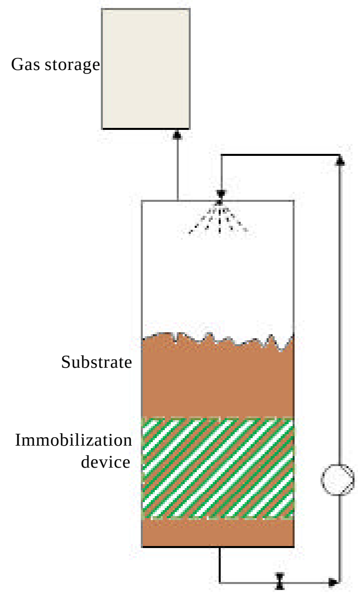

Fig. 1: Lab-scale set-up for dry digestion of cattle manure

Table 2: Quantitative characterization of feedstock

Parameters	Run 1	Run 2
Wet weight (kg)	3.000	3.000
Dry matter (kg)	0.778	0.789
Organic dry matter (kg)	0.654	0.671
Inoculum added (L)	19.000	-

in improvement of methane fermentation and decomposition of organic matter by methanogenic microorganisms and dispersion of the substrate and nutrients in the biofilm. Furthermore, contact frequency between cells and bacteria in immobilization device is increased in the bioreactor. After the decline of the biogas production in the 1st experimental run, the reactor was opened and the next batch of cattle manure (3 kg) was loaded from top. In Run 2 inoculum was not changed or added extra, i.e., Run 2 was initiated with leachate from the 1st run. In Table 2 are given quantities of loaded cattle manure.

Data analysis: The values of pH in the reactor were measured 2 times in a week with a WTW 330 handheld pH meter (WTW, Weilheim, Germany) by analyzing leachate sampled from the reactor. Biogas production was measured by a RITTER TG 1/5 drum type gas meter (Ritter, Bochum-Langendreer, Germany). The content of the biogas was analyzed for methane CH_4, carbon dioxide CO_2, oxygen O_2 (in (%)) and hydrogen sulfide H_2S (ppm) quantities with a INCA gas analyzer dedicated to measuring biogas, biomethane, gas from organic waste and wastewater (UNION Instruments GmbH, Germany). The gas analyzer was calibrated with standard gas having a methane content of 60.7% (v). Data taken at measurements (reactor temperature, air pressure, date and time) were recorded for biogas and methane production based on norm conditions (Nm^3(kg oDM)$^{-1}$): 273 K and 101 325 Pa in accordance with Ludington (2006).

Reactor performance was evaluated by a modified Gompertz equation closest to fundamental for biogas production in batch system (Budiyono *et al*., 2010). This equation describes the total methane production in batch reactors, assuming that methane production is a function of the methanogenic bacteria growth (Koppar and Pullammanappallil, 2008; Lo *et al*., 2010; Budiyono *et al*., 2010; Xie, 2012). The modified Gompertz equation is presented below (Eq. 1):

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

where, M is the cumulative methane production (Nm^3(kg oDM)$^{-1}$), P is the methane production potential (Nm^3(kg oDM)$^{-1}$), R_m is the maximum methane production rate (Nm^3(kg oDM)$^{-1}$day^{-1}), λ is the duration of lag phase (days), t is the cumulative time for methane production (days), e is equal to 2.718282. All Gompertz parameters M, λ and P were determined using non linear regression. Regression models were completed by the 'Solver' feature in MS-Excel.

RESULTS

Biogas generation and composition: Experimental reactors were tested during a period of 28 days in Run 1 and 21 days in Run 2 to assess the dry fermentation of cattle manure on a modified leach-bed with immobilization device reactors and evaluate the effectiveness of the process by Gompertz kinetic parameters. Biogas production was depicted by biogas volume and methane content. Cumulative biogas and methane production were determined by summing daily biogas and methane yield, respectively. All the experimental repetitions of 2 runs showed similar findings. To describe the features of the cumulative biogas and methane yield was selected Experiment 1. Results of daily and cumulative biogas production from Experiment 1 are shown in Fig. 2 and 3. The rapid initial biogas production was due to readily biodegradable organic matter and presence of high content of the methanogens as depicted in Fig. 2. The biogas generation started after inoculating, kept increasing until reaching the peak and then began to decline (Li *et al*., 2011). Run 1 showed the maximum biogas production on day 4 in the amount of

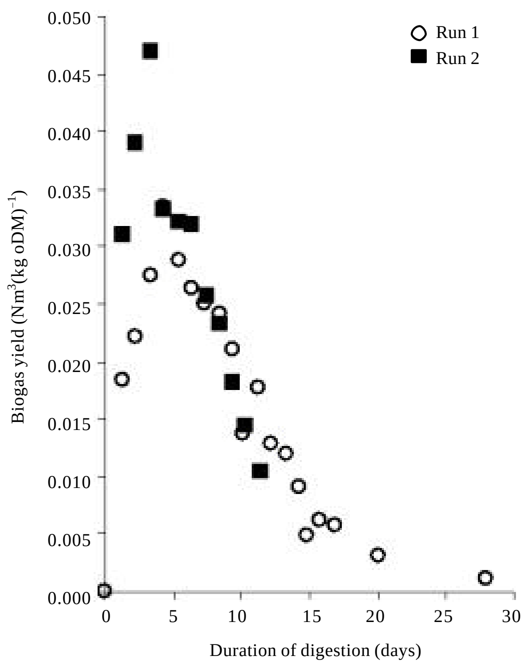

Fig. 2: Daily biogas production (experiment 1)

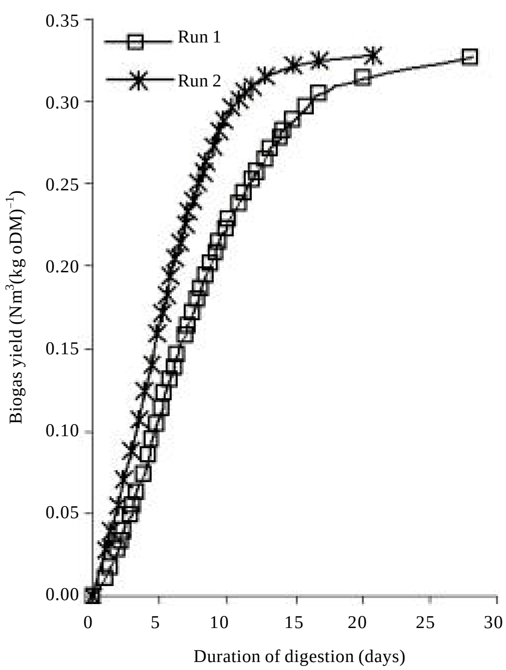

Fig. 3: Cumulative biogas production (experiment 1)

0.032 $Nm^3(kg\ oDM)^{-1}$ and daily biogas generation was observed more than 0.018 $Nm^3(kg\ oDM)^{-1}$ between days 2 and 10, reduced to less than 0.005 $Nm^3(kg\ oDM)^{-1}$ after day 19. The cumulative biogas yield was indicated as 0.327 $Nm^3(kg\ oDM)^{-1}$ at the end of Run 1. Biogas

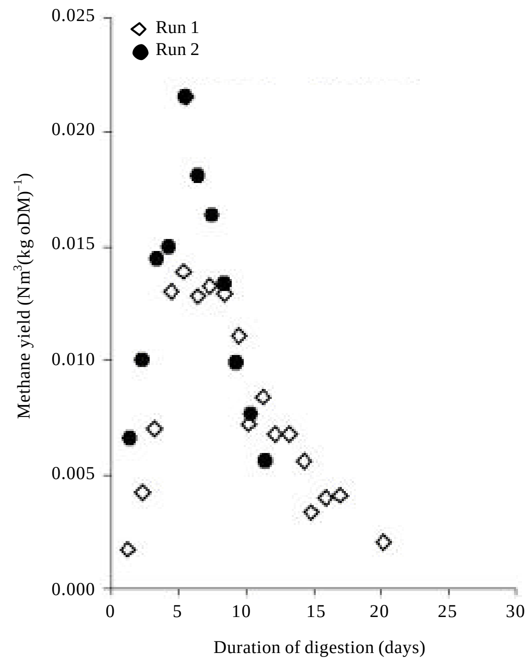

Fig. 4: Daily methane production (experiment 1)

production started faster in Run 2, i.e., peak biogas generation (0.047 $Nm^3(kg\ oDM)^{-1}$) was achieved up to day 3 of the experiment demonstrating the maximum degree of biogas formation till days 9 (daily production more 0.02 $Nm^3(kg\ oDM)^{-1}$) and intensive metabolism of microorganisms, since reactor was enriched with methanogens because of biofilm formation and use of fermented leachate from previous run. The cumulative biogas production was 0.328 $Nm^3(kg\ oDM)^{-1}$ at the end of 21-day of HRT. There were not observed several peaks during the digestion process in both two runs as reported by Li *et al.* (2011).

The process of methane formation is described in Fig. 4 and 5. In all runs are seen rapid methane productions after days 2 and 1, respectively in Runs 1 and 2. The methane obtained the peak value quickly on day 5 (0.014 $Nm^3(kg\ oDM)^{-1}$) and methane yield was more than 0.007 Nm^3 methane kg^{-1} of oDM every day between days 3 and 14 in Run 1, then production was decreased slowly to 0.0001 $Nm^3(kg\ oDM)^{-1}$. The curve of cumulative methane production gave volume of 0.148 $Nm^3(kg\ oDM)^{-1}$. Run 2 showed a rapid initiation of methanogenesis from the early days and the maximum methane yield was 0.022 $Nm^3(kg\ oDM)^{-1}$, 1.6 times more than in Run 1 due to the high content of methane-producing bacteria resulted in immobilization of microorganisms on the supporting materials. The total methane yield was 0.150 $Nm^3(kg\ oDM)^{-1}$ at the end of the digestion process.

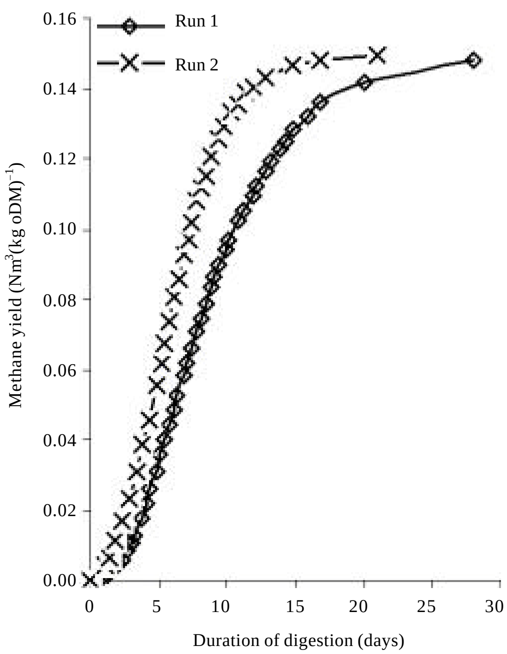

Fig. 5: Cumulative methane production (experiment 1)

The biogas quality for experiment 1 is given in Fig. 6. As depicted, 0 the initial percentage of methane in the biogas has increased and exceeded 26.5% after 3 days of start-up and 50% on day 5 and kept it up to the end of Run 1 presenting stable phase of the digestion. The methane content reached 35% on 2 days and increased sharply to 66% on day 7 as a peak percentage of methane in Run 2. But the maximal methane percentage in Run 1 achieved one day later than in Run 2 on day 8 (56.1%). The percentage of carbon dioxide exceeded the methane percentage during 4 days. The maximum percentage of carbon dioxide was 53.4% on day 2 in Run 1 and 50% on day 3 in Run 2, those were followed by a gradual decrease to 37%. The percentage of carbon dioxide has stabilized in between 42-45% from day 6 and 5, respectively in Runs 1 and 2. High initial percentage of carbon dioxide and low methane percentage associated with the biochemical transformation of organic matter in anaerobic digestion. In the second stage of anaerobic digestion (acidogenesis) 70% of low molecular weight compounds, such as simple sugars, amino acids and fatty acids decomposed to acetate, carbon dioxide and hydrogen and the remaining 30% to Volatile Fatty Acids (VFA) and alcohols (Seadi *et al.*, 2008; Li *et al.*, 2011). In addition, the regeneration time of acidogenic bacteria (*Bacterioids*, *Clostridia*) is 24-36 h (Deublein and Steinhauser, 2008). The methane content increased intensively and the maximum percentage also achieved faster for 1 day earlier in Run 2, because Run 2 was initiated with leachate from Run 1 which contained a high concentration of microorganisms.

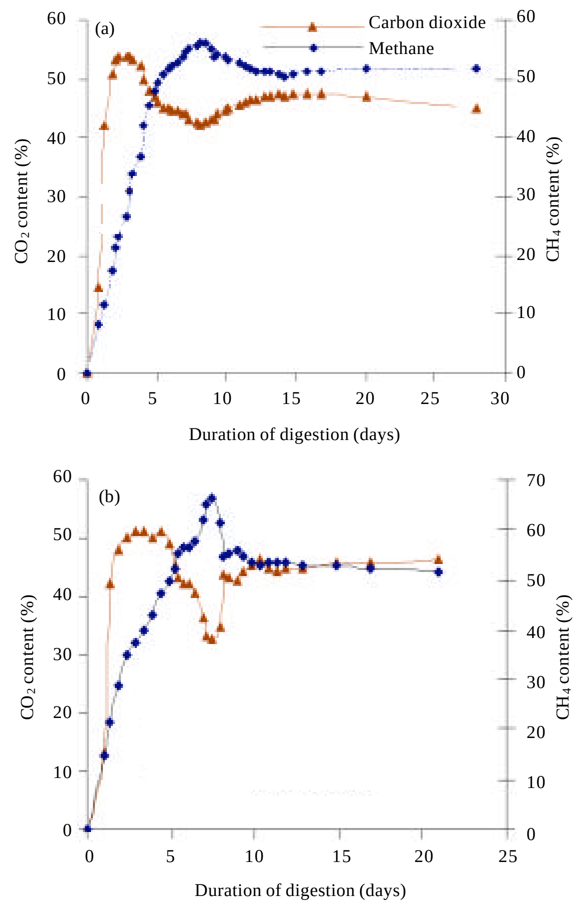

Fig. 6(a-b): Biogas content in percentage (experiment 1): (a) Run 1 and (b) Run 2

pH during anaerobic digestion: The pH changes during anaerobic fermentation are seen in Fig. 7. Due to the accumulation of VFAs by acidogenic bacteria, the pH value was initially around 7 in start up of runs. Since, easily digestible compounds of organic matter was hydrolyzed and transformed into fatty acids quickly. The pH began to increase gradually up to 7.5 as the VFAs were consumed by methanogens result in methane generation. Similar trends were observed by Li *et al.* (2011). It was ranged between 7 and 7.53 indicating normal and healthy anaerobic fermentation. Generally the methane formation occurs in a relatively narrow range of pH from about 6.5-8.5 with an optimal range between 7.0 and 8.0 (Weiland, 2010; Ahn *et al.*, 2010).

Gompertz kinetic parameters: Analytical quantitative parameters were analyzed using the modified Gompertz equation to study the performance of the reactor. The calculated values of the parameters of lag time λ, the maximum methane production rate R_m and the potential methane yield P are shown in Table 3. Gompertz parameters were successfully used in the works of Lay *et al*. (1998), Hegde and Pullammanappallil (2007), Budiyono *et al.* (2010) and Xie (2012) for evaluation of the performance of the digesters.

In Experiment 1, the potential methane yield (P) was indicated in the value of 0.148 $Nm^3(kg\ oDM)^{-1}$ for Run 1 and 0.150 $Nm^3(kg\ oDM)^{-1}$ for Run 2 with an average of 0.149 $Nm^3(kg\ oDM)^{-1}$. Duration of the lag-phase is one of the most important factors for determining the effectiveness of anaerobic fermentation (Xie, 2012). It was 1.67 days for Run 1, has been reduced to 0.7 days in Run 2, averaged to 1.14 days. The maximum methane production rate (R_m) is also essential indicator of anaerobic digestion, since it describes specific growth rate of methanogenic bacteria. R_m for Run 1 was 0.014 $Nm^3(kg\ oDM)^{-1}day^{-1}$, increased to 0.019 $Nm^3(kg\ oDM)^{-1}day^{-1}$ in Run 2. The average maximum methane production rate (R_m) for Experiment 1 was 0.017 $Nm^3(kg\ oDM)^{-1}day^{-1}$. Similar trends were observed in Experiments 2 and 3. Average potential yield of methane (P) was 0.163 and 0.157 $Nm^3(kg\ oDM)^{-1}$ for Experiments 2 and 3, respectively. The mean potential methane yield for all experiments and runs was 0.156 $Nm^3(kg\ oDM)^{-1}$ with a standard deviation of 0.0065 (standard error = 0.0038). The average lag time (λ) for Experiments 2 and 3 were 1.15 days and 1.19 days, respectively and final mean lag time (experiments 1, 2 and 3) was 1.16 days (SD = 0.026; standard error = 0.015). Average maximum methane production rate (R_m) for experiments 2 and 3 was 0.0185 $Nm^3(kg\ oDM)^{-1}day^{-1}$ and 0.019 $Nm^3(kg\ oDM)^{-1}day^{-1}$, respectively. Mean maximum methane production rate (experiments 1, 2 and 3) was 0.018 $Nm^3(kg\ oDM)^{-1}day^{-1}$ with a standard deviation of 0.0013 (standard error = 0.0008). According to calculated mean Gompertz kinetic parameters by the averaging all runs and experimental triplications was simulated Gompertz plot as presented in Fig. 8. The value of R^2 for the ultimate reliability of the basic values for all experiments and runs was 0.9775 with a standard error of 0.008.

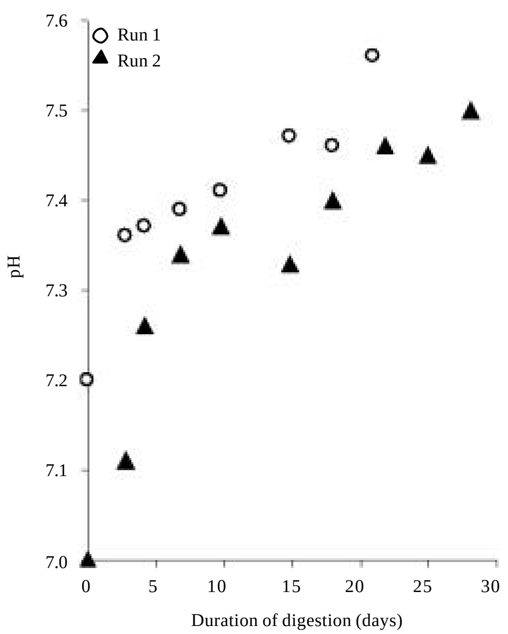

Fig. 7: Change of pH during the anaerobic fermentation (experiment 1)

Duration of fermentation is one of the key indicators for evaluation of the performance of anaerobic digestion because it describes the biodegradability and the treatment rate of the substrate (Xie, 2012). Since, the cumulative methane production curve only asymptotically approaches methane yield the reactor will take infinite time to produce 100% potential of methane (Koppar and Pullammanappallil, 2008). Therefore, the 95% value was arbitrarily was chosen as technical digestion time (T95). The technical digestion time described with T95 is defined as the time needed to produce 95% of the maximum methane production. And Xie (2012) reported that after subtracting the lag time (λ) from T95, the effective

Table 3: Overall performance of methane production

	Experiment 1		Experiment 2		Experiment 3			
Parameters	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Final range[a]	Standard error
M ($Nm^3(kg\ oDM)^{-1}$)[b]	0.148	0.150	0.166	0.158	0.160	0.154	0.156 ± 0.0066	0.0038
P ($Nm^3(kg\ oDM)^{-1}$)[c]	0.149	0.150	0.166	0.158	0.160	0.154	0.156 ± 0.0065	0.0038
R_m ($Nm^3(kg\ oDM)^{-1}day^{-1}$)[c]	0.014	0.019	0.015	0.022	0.017	0.021	0.018± 0.0013	0.0008
λ (days)[c]	1.67	0.7	1.5	0.8	1.67	0.6	1.16 ± 0.0257	0.015
T95% (days)[d]	17.95	11.9	17.95	11.87	15.1	11.09	14.31±1.0522	0.608

[a]Data are expressed as the Mean±SD of three experiments. [b]Experimental data. [c]Gompertz parameters (evaluated by fitting experimental data). [d]Technical digestion time

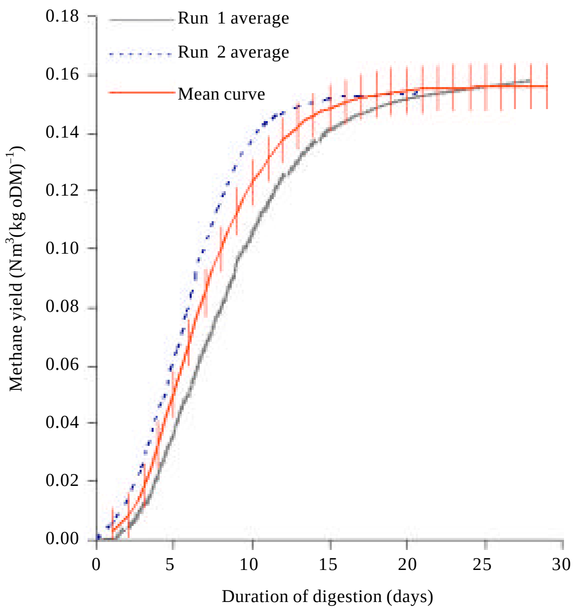

Fig. 8: Methane production for all triplicates of two runs

methane production period can be determined. According to the modified Gompertz equation, T95 simulated was 17±1.65 days for Run 1, it was reduced to 11.62±0.46 days for Run 2. After subtracting the lag time, the effective biogas production periods lasted 15.39±1.70 and 10.97±0.47 days for Runs 1 and 2, respectively.

DISCUSSION

The high initial generation of biogas and methane in all runs until day 3 and a temporary decrease in the production of biogas and methane between 3-4 days are represented by selective fermentation of rapidly biodegradable organic material as mentioned by Angelidaki and Sanders (2004). And an intensive biogas and methane formation from day 3 and a stable course of the process unto day 16 are described by growth curves of methanogenic bacteria which is characterized by various growth phases of lag phase, acceleration phase, exponential phase, retardation phase, stationary phase and phase of decline (Gerber and Span, 2008) and growth curve of methanogenic bacteria tends to sigmoid function as generally occurred in batch systems (Budiyono *et al*., 2010). Daily biogas and methane production indicated by low production rate at the beginning of anaerobic digestion and kept increasing until reaching the peak after that it began to decline. But several peaks during the digestion process were not noticed as in researches of Li *et al.* (2011). This is predicted due to the methane production rate in batch mode is directly corresponds to specific growth rate of methanogenic bacteria in the bioreactor (Budiyono *et al*., 2010). The real growth of methanogenic bacteria takes place primarily at the exponential phase which lasted around 10 days (day 4-14) in Run 1 and 7 days (day 2-9) in Run 2. Adaptation of methanogenic bacteria occurs on average 1.16 days in experiments with cattle manure in leach-bed reactor with immobilization device. The pH of the fermentation medium in the reactors ranged between on average 7.0 and 7.5, it corresponds to the normal and healthy anaerobic fermentation system of 6.5-8.5 (Deublein and Steinhauser, 2008). To estimate the overall duration of the anaerobic fermentation was used a time required to obtain 95% of the methane potential (Koppar and Pullammanappallil, 2008; Kaparaju *et al*., 2009). In our experiments, it took about 17.95 days in Run 1 to reach 95% of the methane potential, this duration was reduced to 6 days in Run 2. Those showed that using of filtrate from a previously digested material and immobilization of methanogens in bioreactor inoculates fresh feedstock with respective microflore of methanogenic microorganisms result in improved kinetics (Budiyono *et al*., 2010). Overall the mean duration to achieve 95% of the methane potential for all experiments was 14.31 days with a standard deviation of 1.05 days and calculated effective anaerobic digestion time was equal to 13.15 days. Determined technical digestion (T95) and effective anaerobic digestion time can be used as a guideline in design of the Hydraulic Retention Time (HRT) and Solid Retention Time (SRT) for anaerobic digesters of solid cattle manure in a biogas plants. Effectiveness of the dry fermentation for solid cattle manure was studied by Ahn *et al.* (2010) and Li *et al.* (2011) in a batch mode reactor during 63 days. Ahn *et al.* (2010) recorded potential m ethane production in the value of 0.028 $Nm^3(kg\ oDM)^{-1}$ at the temperature of 55°C while ultimate methane yield of 0.251 $Nm^3(kg\ oDM)^{-1}$ represented at the 35°C by Li *et al.* (2011).

CONCLUSION

In conclusion, dry fermentation of cattle manure with dry matter content of 25.93% in a single-stage, batch, leach-bed with immobilization device reactor without anaerobic pretreatment and mixing is feasible and stable process. Initiation of methane production started on average at 1.16 days, pH maintained between 7.0 and 7.5 suitable for methanogenesis. Average mean cumulative methane yield was 0.156 $Nm^3(kg\ oDM)^{-1}$. Immobilization of methanogens on the supporting materials (polyethylene packing rings) improved methane production kinetics. Kinetic parameters of anaerobic digestion, P, R_m and λ were found to be 0.156 $Nm^3(kg\ oDM)^{-1}$, 0.018 $Nm^3(kg\ oDM)^{-1}day^{-1}$ and 1.16 days on average for all experimental runs according to results of simulation by Gompertz equation.

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