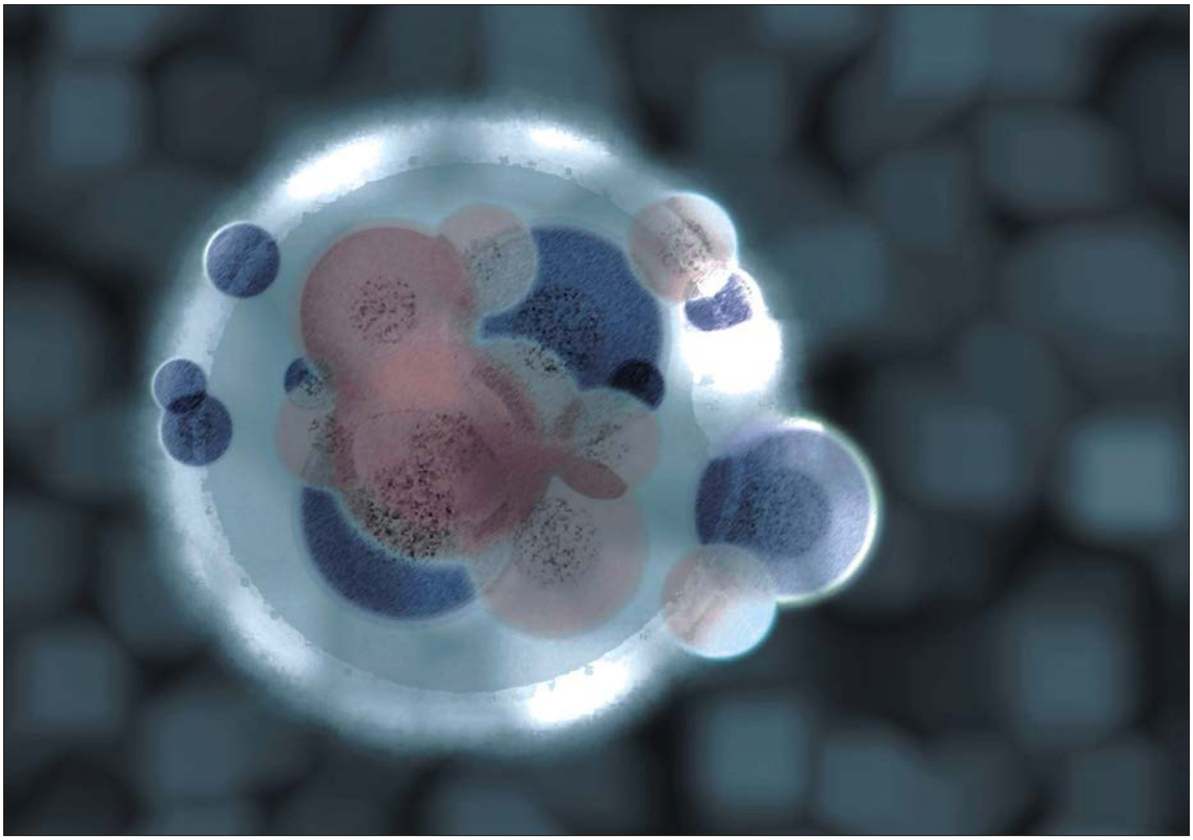

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Performance of Leach-bed Reactor with Immobilization of Microorganisms in Terms of Methane Production Kinetics

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Abstract: In this study, the performance of leach-bed with immobilization device reactor during dry anaerobic fermentation of solid cattle manure was investigated. Laboratory experiments using 50 L bioreactor were performed in batch operated mode at the temperature of 40±0.2°C (mesophilic condition). Immobilization device was positioned at the bottom of the bioreactor as a layer of polyethylene packing rings. Each 2nd run was initiated by inoculating with anaerobically digested liquor from previous run. The performance of the reactor was analyzed in terms of the kinetic constants of methane production. To describe methane production rate kinetics was used Gaussian equation (time to produce maximal methane production (t_0) and a (Nm^3 (kg oDM)$^{-1}$ day^{-1}) and b (day) constants) and was calculated Gompertz kinetic parameters of methane production (methane production potential (P), maximum methane production rate (R_m) and minimum time to produce methane (λ). Time to produce peak of methane yield (t_0) were 7.49±1.15 days and 4.82±0.11 days in the 1st and 2nd run. Kinetic constants P, R_m and λ were indicated in values 0.159; 0.015 and 1.61 for 1st run and 0.154; 0.021 and 0.7 for 2nd run, respectively. Overall P, R_m and λ to show reactor performance were 0.156 Nm^3 (kg oDM)$^{-1}$, 0.018 Nm^3 (kgoDM)$^{-1}$ day^{-1}; 1.16 days, respectively. Time to produce 95% methane production potential was 14.31 days, calculated effective anaerobic digestion time was 13.15 days. Recirculation of the fermentation medium and immobilization of microorganisms in polymeric carriers in the reactor makes it possible to initiate methanogenesis in 1-2 days and reduce the hydrolytic retention time due to biofilm formation.

Key words: Anaerobic digestion, methane production kinetics, cattle manure, immobilization of microorganisms, methanogenic bacteria, leach-bed reactor, biofilm

INTRODUCTION

Continuous and intensive livestock development in Kazakhstan has led to an increase uncontrolled dumping and storage of livestock and poultry wastes in the environment causing serious problems of environmental and social issues in the areas of livestock and poultry farms, therefore the rational processing of agricultural waste using anaerobic digestion technologies has become an important issue.

Anaerobic Digestion (AD) is a widely used for treatment of organic waste for biogas production. Final product of the bioconversion of organic substrates 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 and traces of other gases (Martins das Neves *et al*., 2009) can be used as an alternative renewable energy source. 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 (Budiyono *et al*., 2009). Anaerobic fermentation is microbiological and biochemical process which occur with the participation of several group of microorganisms of hydrolyzing, acidogenic, acetogenic and methanogenic bacteria, creating the syntrophic relationship and characterized by a series of biochemical transformation (Deublein and Steinhauser, 2008; Weiland, 2010; Seadi *et al*., 2008).

Numerous studies had been conducted by several researchers in order to increase and stabilize biogas yield, optimize anaerobic biogas technologies. Biogas production was improved by co-digestion of various substrate combinations to get balanced composition of nutrients (Fantozzi and Buratti, 2009; Crolla *et al*., 2011; Li *et al*., 2011; Lazor *et al*., 2010; Kryvoruchko *et al*.,

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2004). An effort to improve methane yield was carried out by increasing the inoculums content, which provides with active microbial consorsium of methanogenic bacteria (Budiyono *et al*., 2010a; Reimers, 2007; Szucs *et al*., 2012; Hansen *et al*., 2004). Another way to achieve optimization of biogas production is pretreatment of organic waste by solid-liquid separating (Lo *et al*., 1983), pretreatment with chemicals, physical thermal pretreatment and enzymatic with adding microorganisms, because the specific organisms may produce the necessary enzymes (Ward *et al*., 2008). Enhancement bacterial nutritional requirements by adding trace elements (Lebuhn *et al*., 2009; Lemmer *et al*., 2011), improvement mixing systems to increase contact between substrate and bacterial cells by modifying CSTR processes (Kaparaju *et al*., 2009; Fantozzi and Buratti, 2009; Normak *et al*., 2012), using leach-bed reactors and sequencing batch reactors for dry fermentation (Weiland, 2010; Pullammanappallil *et al*., 2005; Li *et al*., 2011; Zhang *et al*., 2000) was investigated by various researchers. Recent developments in the design of bioreactors have focused on retention of the active microorganisms in the reactor. These designs are based on the trends of the bacteria development, involved and attached to inert surfaces, forming biofilm (immobilized microflore). This system can provide with improved stability and control of the process for treatment of agricultural and industrial wastes (Wilkie and Colleran, 1989).

The aim of this work was to investigate the effectiveness of a single-stage leach-bed with immobilization of microorganisms process for treatment of solid cattle manure and evaluate the performance of the process in terms of methane production kinetics.

MATERIALS AND METHODS

Feedstock and sample preparation: The cattle manure used in research was taken from livestock farm of the University of Hohenheim for dry anaerobic digestion in leach-bed reactor with immobilization device. For each run was utilized 3 kg of cattle manure and 19 L of inoculum taken from the reactor (volume of 400 L), working continuously in biogas laboratory of the University of Hohenheim. The inoculum was used as liquor for the microbial initiation of single-phase leach-bed process with immobilization of microorganisms. Fresh Material (FM) of manure samples were tested in triplicate for Dry Matter (DM), Organic Dry Matter (oDM), ash and moisture content, because they are main indicators to evaluate biogas yield amount and degree of organic matter decomposition. These indicators were determined according to APHA (1995).

Table 1: Results of the analysis of cattle manure

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

Characteristics of feedstock are given in Table 1. The dry matter content (DM) of cattle manure as received was 25.93±0.64% and 84.07±1.73% solids were Organic Dry Matter (oDM). 4.12±0.36% of dry matter in manure was ash. Moisture content of manure was 74.07%. Fermented cattle slurry was used as a seed material (inoculum) and liquor for recycling was also analyzed to determine DM, oDM content. The 99.38% of fermented slurry was water, 40.47 ±0.66% oDM, 0.37±0.004% was ash.

Lab-scale anaerobic reactor: The lab-scale reactor in the form of a vertical cylinder with a working volume of 50 L was constructed by modifying the leach-bed reactor and fixed-bed reactor for microorganism immobilization. The height and the internal diameter of the reactor were 0.7 and 0.3 m, respectively. The bioreactor is equipped with immobilization device as a layer of polyethylene packing rings (inert material) which is positioned at the bottom of the bioreactor. Bed height of immobilization device was 20 cm. Three holes have been provided in the upper part of the lid, one for gas outlet, the second for the circulation of the filtrate and the third for sample removal. The outlet at the bottom of the reactor was reserved for a drain. Reactor scheme is shown in Fig. 1.

Bioreactor system consists of heating, recycling and gas measuring systems (Fig. 1). Reactor operated at a temperature of 40±0.2°C, which was provided by circulating hot water and the heating unit. The biogas production was measured using a drum-type gas meter of RITTER TG 1/5 with a liquid gate, working on the principle of water displacement (Ritter, Bochum-Langendreer, Germany). The content of the biogas (methane CH_4, carbon dioxide, CO_2, oxygen O_2 in percentage (%), hydrogen sulfide H_2S in amounts (ppm) was determined by a gas analyzer INCA dedicated to measuring biogas, biomethane, gas from organic waste and wastewater (UNION Instruments GmbH, Germany). Gas analyzer was calibrated with a standard gas with a methane content of 60.7% (v).

Experimental procedures: 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

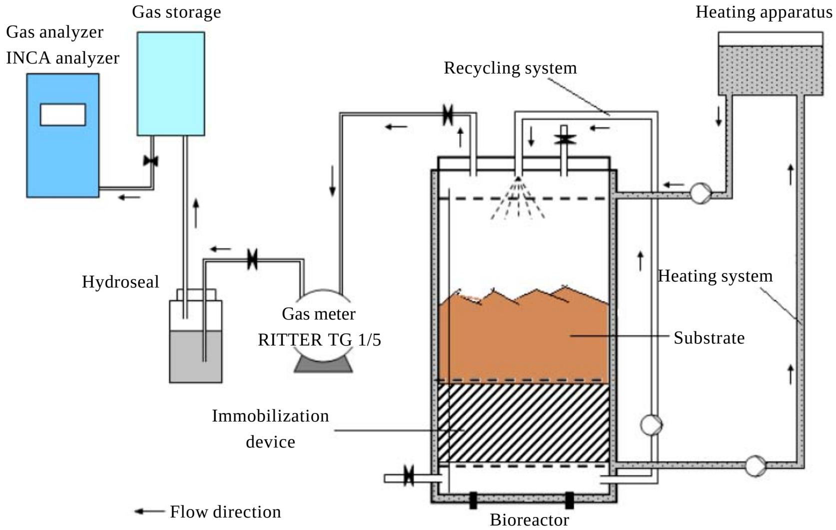

Fig. 1: System of lab-scale leach-bed reactor with immobilization of microorganisms

process of anaerobic fermentation lasted 28 days (Run 1) and 21 days (Run 2) of hydrolytic retention time (HRT) on mesophilic mode (at a temperature of 40±0.2°C). The experiments were performed in triplicate of 2 runs.

At first runs the reactor was inoculated with 19 L (0.019 m^3) of inoculum, taken from the reactor (volume of 400 L), working continuously in biogas laboratory of the University of Hohenheim, where was fermented cow manure. Then, 3 kg of cattle manure was loaded into from top of the reactor. The liquid filtrate was continuously recirculated by pump 15 min every 2 h during the whole fermentation cycle. Circulation flows toward the top of the reactor ("downflow"system) and the liquid is sprayed onto the surface of the solid fraction (Fig. 1). At the same time, passing through the solid substrate, the liquid fermentation medium enriched with nutrients and the process of methane fermentation and decomposition of organic matter by two groups of microorganisms (acidogenic and methanogens) takes place. Due to the circulation of the fermented substrate in anaerobic metabolism of microorganism, contact frequency between bacterial cells and immobilization device of the bioreactor will be increased, homogeneous media for further development and activity of the biofilm will be formed, process of dispersion of the substrate and nutrients in the biofilm will be improved. After the decline of the biogas production in first experimental run, the reactor was opened and the next batch of cattle manure (3 kg) loaded from top. In the second run inoculum was not changed or added extra, i.e., the second run was initiated with liquor from the first run.

Table 2: Quantitative characterization of feedstock

Parameters	Run 1	Run 2
Wet weight (kg)	3	3
Dry matter (kg)	0.778	0.789
Volatile solids (kg)	0.654	0.671
Inoculum added (l)	19	-
Digestion period (days)	28	21

Table 2 shows the quantitative features of cattle manure (based on the wet, dry matter and volatile solids) for loading. Reactor was loaded with 3 kg of cattle manure, which contains 0.778-0.789 kg of DM, 0.654-671 kg of oDM and used 19 L of fermented slurry.

Data from the analysis of the biogas content and the date, time, reactor temperature, air pressure, in which was taken measure, were recorded to determine the biogas production based on standard conditions Nm^3 (kg oDM)$^{-1}$: 273 K and 101 325 Pa 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 methane production to Gompertz and Gaussian equation. Analyses of the experimental results and calculations were performed in MS-Excel using the 'Solver' feature by non-linear regression.

Assuming that methane production rates and microbial kinetic growth of methanogens and its decay would follow the normal distribution over the course of digestion period, the Gaussian equation could be applied to simulate biogas production rates including ascending and descending limb. Gaussian equation is presented below (Lo *et al*., 2010):

$$y = a \times \exp\left(-0.5\left(\frac{(t-t_o)}{b}\right)^2\right) \quad (1)$$

In this equation, y is the biogas or methane production rate (Nm^3 (kg oDM)$^{-1}$) at time t, t is the time (day) over the digestion period. a (Nm^3 (kg oDM)$^{-1}$ day^{-1}) and b (day) are constants and t_0 is the time (day) where the peak (maximal) methane production rates occurred.

Cumulative methane production was simulated using a modified Gompertz equation (Lay *et al.*, 1998; Koppar and Pullammanappallil, 2008; Lo *et al.*, 2010; Budiyono *et al.*, 2010b; Xie, 2012). 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:

$$M = P \times \exp\left\{-\exp\left[\frac{R_m \times e}{P}(\lambda - t) + 1\right]\right\} \quad (2)$$

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).

RESULTS

Investigation of cumulative methane production: The research was carried out in triplication of two runs. The cumulative volume of methane production was observed during 28 days in the 1st run and 21 days in the 2nd run in term of methane production per total oDM added in standard condition (Nm^3 (kg oDM)$^{-1}$) as depicted in Fig. 2.

Figure 2 shows that, methane production rate tend to obey sigmoid function (S curve) as generally occurred in batch growth curve. All runs showed rapid production of methane from first 2 days and the subsequent exponential methane production till the 10th and the 15th day, respectively, in the 1st and 2nd run.

The maximum methane production in the 1st run was between days 4 and 14, when exponential growth of methanogenic bacteria took place. The curve of cumulative production for the 1st run gave volume of 0.148 Nm^3 (kg oDM)$^{-1}$ methane.

The 2nd run showed a rapid initiation of methanogenesis from the 1st day, due to the high concentration of methanogenic bacteria and their improved metabolism, the amount of formed methane was increased. The total methane yield was 0.150 Nm^3 (kg oDM)$^{-1}$ at the end of 21-day hydrolytic retention time. From Fig. 2 also can seen that the line

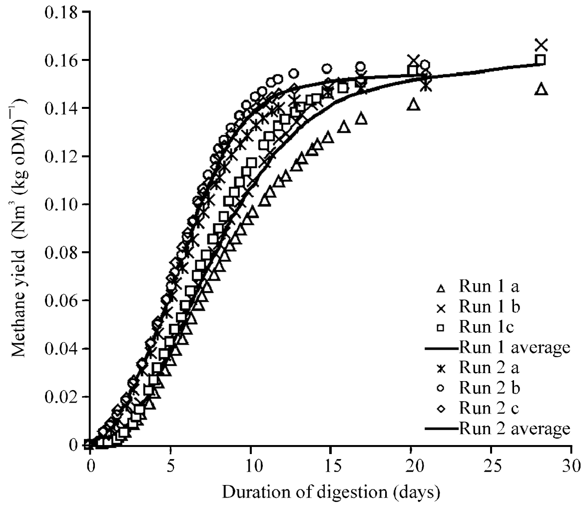

Fig. 2: Cumulative methane production of cattle manure for Run 1 and 2 with triplication

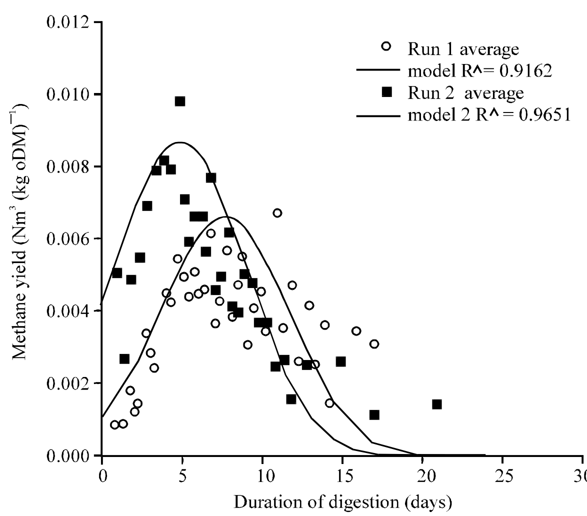

Fig. 3: Gaussian plots of methane production rate for Run 1 and 2 in average

slope of the 2nd run curve is sharper than the line of the 1st run, indicating higher methane production rate in the 2nd run.

Simulation of methane production rate: For simulation of methane production rate was used the Gaussian equation (equation 1), which includes ascending and descending limb of daily methane production. This Gaussian equation was used by Lo *et al.* (2010) and they reported that the Gaussian plots of biogas yield rate gave the best results than linear and exponential plots in the simulation of biogas production rate in anaerobic fermentation of municipal solid waste at different organic loading rates.

Table 3: Kinetic Gaussian parameters for methane production rate of cattle manure in the reactor with immobilization device

Runs	Maximum methane production rate (Nm^3 (kg oDM)$^{-1}$day^{-1})	Kinetic parameters t_0 (days)	a (Nm^3 (kg oDM)$^{-1}$ day^{-1})	b (days)
1	0.006±0.0006	7.49±1.15	0.0068±0.0012	3.90±0.19
2	0.009±0.0006	4.82±0.11	0.0087±0.0006	4.02±0.29

Table 4: Gompertz and experimental parameters of methane production from cattle manure

Parameters	Run 1	Run 2
Cumulative methane production, (Nm^3 (kg oDM)$^{-1}$) (experimental)	0.158±0.009	0.154±0.004
P (Nm^3 (kg oDM)$^{-1}$)	0.159±0.009	0.154±0.004
R_m (Nm^3 (kg oDM)$^{-1}$ day^{-1})	0.015±0.002	0.021±0.002
λ (days)	1.61±0.098	0.70±0.1
T95 (days)	17±1.65	11.62±0.46
Effective period to produce methane (days)	15.39±1.70	10.97±0.47

In Fig. 3 is shown daily methane production rate with simulation for each runs in average for three repetitions. From Fig. 3 can be seen that methane production limbs, demonstrating very slow methane yield at the beginning and the end period of fermentation time. During 10 days from the 4th till the 14th day, methane generation was more than 0.004 Nm^3 of methane per kg of oDM in a day for the 1st run as seen in Fig. 3. The maximum methane yield was 0.009 Nm^3 (kg oDM)$^{-1}$ on the 5th day in the 2nd run, thereafter, the methane production was gradually reduced to 0.002 Nm^3 (kg oDM)$^{-1}$ at the end of anaerobic digestion.

Determination coefficient R^2 for all experiments was 0.8627-0.9764, in average for 1st run was 0.9162 and 0.9651 for 2nd run, showing a good simulation. Kinetic parameters of the Gaussian plot are given in Table 3.

By Gaussian kinetic model of methane production rate for the 1st run, the time to achieve the maximum methane yield of 0.006 Nm^3 (kg oDM)$^{-1}$ day^{-1} (standard deviation = 0.0006 Nm^3 (kg oDM)$^{-1}$ day^{-1}) (t_0) in average for three experiments was 7.49±1.15 days. Constant associated with the time a was equal to 3.90±0.19 days and kinetic parameter b was 0.0068 with a deviation of 0.0012 Nm^3 (kg oDM)$^{-1}$ day^{-1}.

Table 3 shows that in the 2nd run t_0 was equal to 4.82±0.11 days to achieve the maximum yield of methane of 0.009 Nm^3 (kg oDM)$^{-1}$ day^{-1} as shown in Gaussian plot. The constants a and b show 0.0087±0.0006 Nm^3 (kg oDM)$^{-1}$ day^{-1} and 4.02±0.29 days, respectively.

Gompertz kinetic parameters: Analytical quantitative parameters of methanogenic bacteria growth curve were analyzed using a modified Gompertz equation, where the production of methane is considered as a function of methanogenic bacteria growth to estimate and describe anaerobic digestion. Cumulative methane production was fit into the equation and calculated values of the kinetic parameters of lag time λ, the maximum methane production rate R_m and the potential yield of methane P. Gompertz parameters were successfully used in the works of Lay *et al.* (1998); Hegde and Pullammanappallil (2007), Budiyono *et al.* (2010a) and Xie (2012) for evaluation of the performance of the digesters and for description of the methanogenesis process.

The results of kinetic methane production are shown in Table 4 and Fig. 4.

From Table 4 and Fig. 4 can be seen that the potential yield of methane (P) was 0.159 Nm^3 (kg oDM)$^{-1}$ (standard error = 0.005, standard deviation = 0.0099) in the 1st run, in the 2nd run of 0.154 Nm^3 (kg oDM)$^{-1}$ (standard error = 0.002, standard deviation = 0.004) in average for three experiments. Duration of the lag-phase is one of the most important factors for determining the effectiveness of anaerobic fermentation. Duration of the lag-phase was 1.61±0.098 days in the 1st run with a standard error of 0.057 reduced to 0.70±0.1 days in the 2nd run (standard error = 0.058). The maximum methane production rate (R_m) for the 1st run was 0.015 Nm^3 (kg oDM)$^{-1}$ day^{-1} (standard error = 0.0009, standard deviation = 0.002) increased to 0.021 Nm^3 (kg oDM)$^{-1}$ day^{-1} in the 2nd run (standard error = 0.0009, standard deviation = 0.002) as shown in Table 4.

Figure 4 shows that the average lag-phase period of methanogenesis was faster for 0.7 days with high rate (0,021 Nm^3 (kg oDM)$^{-1}$day^{-1}) in the 2nd run than in the 1st run due to enrichment by methanogens of reactor with immobilization device. In the modeling of the cumulative methane yield, the modified Gompertz plots showed R^2 0.9889 and 0.9861, respectively, for the 1st and 2nd run in average. The standard error was 0.006 (1st run) and 0.007 (2nd run).

One of the key parameters of the performance of anaerobic digestion is duration of fermentation, which indicates the biodegradability and the treatment rate of the substrate. So, the duration of the fermentation was investigated to estimate the overall duration of the anaerobic fermentation. Since, the cumulative methane production curve only asymptotically approaches methane yield, the reactor will take infinite time to produce 100% potential of methane. Therefore, the 95% value was arbitrarily was chosen as technical digestion time (T95) like in the research of Koppar and Pullammanappallil (2008). The technical digestion time, described with T95, is defined as the time needed to produce 95% of the maximum methane production. After subtracting the lag time (λ) from T95, the effective methane production period will be calculated (Xie, 2012).

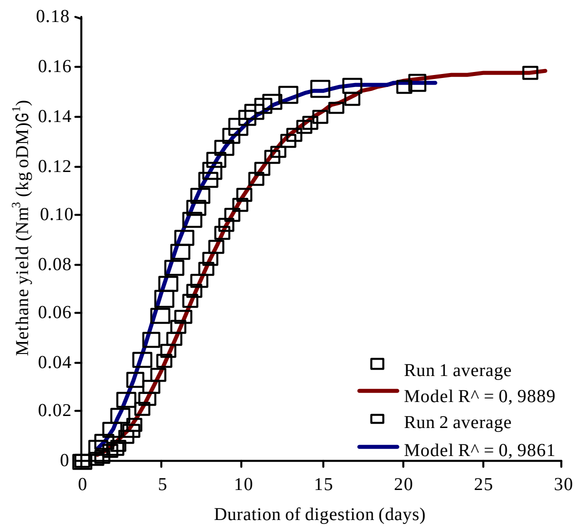

Fig. 4: Comparison of experimental and calculated data for Run 1 and 2, using the kinetic model of Gompertz equation

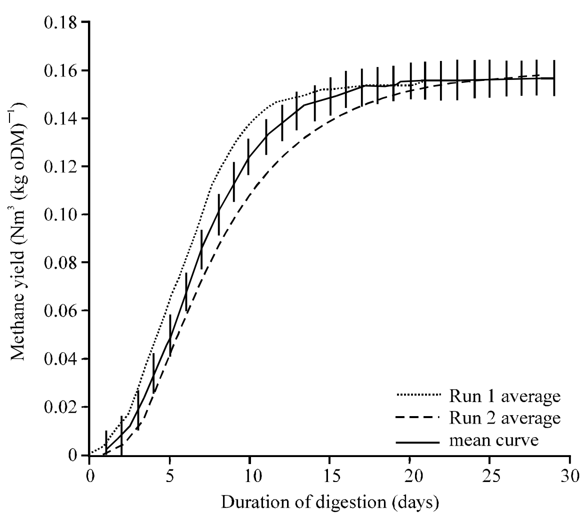

Fig. 5: Methane production in leach-bed with immobilization device reactor

According to the modified Gompertz equation, T95 simulated was 17±1.65 days for the 1st run, it was reduced to 11.62±0.46 days for the 2nd run. After subtracting the lag time, the effective biogas production periods lasted 15.39±1.70 and 10.97±0.47 days for the 1st and the 2nd run, respectively.

By the averaging all runs and experimental triplications was received the average methane potential yield -0.156 Nm^3 (kg oDM)$^{-1}$ with a standard deviation of 0.0066 Nm^3 (kg oDM)$^{-1}$ and a standard error of 0.0038 Nm^3 (kg oDM)$^{-1}$. Mean lag-phase time (λ) was 1.16±0.0257 days (standard error = 0.015 days). Average maximum methane production rate (R_m) was 0.018 Nm^3 (kg oDM)$^{-1}$ day^{-1} with a standard deviation of 0.0013 Nm^3 (kg oDM)$^{-1}$ day^{-1} and an error 0,0008 Nm^3 (kg oDM)$^{-1}$ day^{-1}. According to the final kinetic constants R_m, λ and P, the Gompertz equation was evaluated for leach-bed reactor with the immobilization device Eq. 3:

$$M = 0.156 \times \left\{ -\exp\left[\frac{0.018 \times e}{0.156}(1.16 - t) + 1 \right] \right\} \quad (3)$$

Methane production for all experiments was simulated with a level of reliability of 95% via the average final Gompertz parameters in order to illustrate performance of leach-bed with immobilization device reactor as shown in Fig. 5. 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.

DISCUSSION

Cumulative methane production rate of cattle manure during anaerobic digestion in leach-bed reactor with immobilization device, which was designed as modification of fixed-bed and leach-bed reactor for dry fermentation tends to sigmoid function as generally occurred in batch growth curve of methanogenic bacteria (Budiyono *et al*., 2010a,b). It is clearly seen in both two runs (Fig. 2 and 4). Daily methane production rate, simulated by Gaussian equation including ascending and descending limb indicated low methane production at the beginning and the end period of anaerobic digestion. 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 a,b).

As for every living being, the life cycle of methanogenic bacteria cultures is characterized by various phases of growth, regarding discontinuous batch processes: Lag phase, acceleration phase, exponential phase, retardation phase, stationary phase and phase of decline (Gerber and Span, 2008). In lag-phase, bacteria adaptation to their environment takes place and they grow relatively slowly and maximum rate of cell division is seen in the exponential phase. In the stationary phase of bacterial growth, access to essential nutrients becomes a limiting factor and a balance between cell growth with division and cell death process is created, then medium is depleted, toxic products of metabolism are accumulated. It is shown by reduced rates of propagation and termination of increasing number of cells. In the death phase (decline, lysis) is taken logarithmic death, turning into a period of decreasing rate of dying bacteria.

From Fig. 2, 3 and 4 can be clearly understandable through permanent changing concentrations of nutrients and inhibitors occurred small lag time for continuous adaption of methanogens to the medium, which took around λ = 1.61±0.098 days during the 1st run, in the 2nd run it was shortened to 0.7±0.1 days due to recirculation of filtrate from 1st run and immobilized microorganism on supporting materials inside bioreactor as biofilm, so lag period (λ) was reduced approximately 2.5 times in the 2nd run. According to the research of kinetic parameters of Budiyono *et al*. (2010a,b), lag-phase duration (λ) was 4.46±1.31 days for cattle manure digestion with rumen fluid during batch lab-scale experiments at 38.5°C. It is seen that, adaptation period of methanogenic bacteria in leach-bed reactor with immobilization device was significantly reduced result in improvement of anaerobic fermentation.

The real growth of methanogenic bacteria takes place primarily at the exponential phase, which lasted around 10 days (4th until 14th day) in the 1st run and 7 days (2nd until 9th days) in the 2nd run. During exponential growth phase, daily methane production achieved maximum methane yield on 7.49±1.15 and 4.82±0.11 days (t_0) in the 1st and 2nd run, respectively, according to Gaussian plot. From the Gompertz equation, maximum methane production rate (R_m) was calculated as 0.015±0.002 Nm^3 (kg oDM)$^{-1}$ day^{-1} and 0.021±0.002 Nm^3 (kg oDM)$^{-1}$ day^{-1} for the 1st and 2nd run. These values describe specific growth rate of methanogenic bacteria. After 14 days and 18 days of anaerobic digestion, methane production tends to decrease, which indicates next microbial growth phase-stationary phase, where the number of cells remains constant, but a lot of cell activities keep on going, such as energy consumption due to metabolism or biosynthetic processes (Gerber and Span, 2008). From Fig. 4 is shown that potential methane yield (P) was not overtopped final cumulative methane production in both runs, which means finished anaerobic digestion.

In the 1st run , it took approximately 17 days to reach 95% methane yield potential, the duration was further reduced to 11 days in the 2nd run. This shows that introducing leachate from a previously digested material inoculated the fresh feedstock with appropriate microorganisms to carry on the digestion process and biofilm on supporting material stabilized anaerobic digestion result in improved kinetics of digestion.

The overall performance of leach-bed reactor with immobilization device described with adaptation of methanogenic bacteria occurred on average 1.16 days, after 3 days observed exponential phase, where methanogenic bacteria produce methane extensively with average maximum methane production rate of 0.018 Nm^3 (kg oDM)$^{-1}$ every day, describing the maximum specific growth rate of bacteria. After 12.5 days of hydrolytic retention time, methane generation stably reduced, forming around 0.003-0.002 Nm^3 (kg oDM)$^{-1}$ methane. This period is described as a retardation phase, following stationary phase, where bacteria produce methane in average 0.002-0.001 Nm^3 (kg oDM)$^{-1}$ day^{-1}. The final mean duration to achieve 95% methane potential was 14.31 days (standard deviation = 1.05 days: standard error = 0.61 days). Effective anaerobic digestion time equal to 13.15 days. The technical digestion time (T95) and effective anaerobic digestion can be used as a guideline in design of the Hydraulic Retention Time (HRT) and solid Retention Time (SRT) for anaerobic digesters.

CONCLUSION

Dry fermentation of cattle manure contained 25.93% DM was conducted using single stage, batch, leach-bed with immobilization device reactor. Average cumulative methane yield was 0.156 Nm^3 (kg oDM)$^{-1}$. Duration of lag-phase was equal to 1.61 and 0.7 days for the 1st and 2nd run, respectively, final initiation of methanogenesis started on average at 1.16 days. The maximum methane production rate (R_m) was 0.018 Nm^3 (kg oDM)$^{-1}$ in a day with potential methane yield (P) of 0,156 Nm^3 (kg oDM)$^{-1}$ during an average 24.5 days of HRT. In conclusion, reactor with immobilization device allows intensifying the methanogenesis process, providing with the active microbial biomass (biofilm).

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