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 Nm³(kg oDM)⁻¹. The kinetic parameters of methane production, i.e., methane production potential (P), maximum methane production rate (Rₙₐₜ) and minimum time to produce methane (λ) were indicated as 0.156 Nm³(kg oDM)⁻¹, 0.018 Nm³(kg oDM)⁻¹day⁻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.

Keywords: 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 chickens). 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; Budyono 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 acetogenesis/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 microflora 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.

**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 m³) of fermented cattle slurry with DM content of 0.62±0.003% and oDM content of 40.47±0.66% (Table 1) onto 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
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 (Nm³[(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):

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

where, \( M \) is the cumulative methane production (Nm³[(kg oDM)⁻¹]), \( P \) is the methane production potential (Nm³[(kg oDM)⁻¹]), \( R_m \) is the maximum methane production rate (Nm³[(kg oDM)⁻¹] day⁻¹), \( \lambda \) 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 \( M, \lambda \) 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...
0.032 Nm³/(kg oDM)⁻¹ and daily biogas generation was observed more than 0.018 Nm³/(kg oDM)⁻¹ between days 2 and 10, reduced to less than 0.005 Nm³/(kg oDM)⁻¹ after day 19. The cumulative biogas yield was indicated as 0.327 Nm³/(kg oDM)⁻¹ at the end of Run 1. Biogas production started faster in Run 2, i.e., peak biogas generation (0.047 Nm³/(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 Nm³/(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 Nm³/(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 Nm³/(kg oDM)⁻¹) and methane yield was more than 0.007 Nm³ methane kg⁻¹ of oDM every day between days 3 and 14 in Run 1, then production was decreased slowly to 0.0001 Nm³/(kg oDM)⁻¹. The curve of cumulative methane production gave volume of 0.148 Nm³/(kg oDM)⁻¹. Run 2 showed a rapid initiation of methanogenesis from the early days and the maximum methane yield was 0.022 Nm³/(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 Nm³/(kg oDM)⁻¹ at the end of the digestion process.
The biogas quality for experiment 1 is given in Fig. 6. As depicted, 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 (Sadiq et al., 2008; Li et al., 2011). In addition, the regeneration time of acidogenic bacteria (Bacteroides, Clostridia) is 24-36 h (Deubel 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.

**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 $\lambda$, 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 Pullamanappalli (2007), Badjiko 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)$^{-1}\text{day}^{-1}$, increased to 0.019 Nm$^3$(kg oDM)$^{-1}\text{day}^{-1}$ in Run 2. The average maximum methane production rate ($R_m$) for Experiment 1 was 0.017 Nm$^3$(kg oDM)$^{-1}\text{day}^{-1}$. 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 ($\lambda$) 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)$^{-1}\text{day}^{-1}$ and 0.019 Nm$^3$(kg oDM)$^{-1}\text{day}^{-1}$, respectively. Mean maximum methane production rate (experiments 1, 2 and 3) was 0.018 Nm$^3$(kg oDM)$^{-1}\text{day}^{-1}$ 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 value for all experiments and runs was 0.9775 with a standard error of 0.008.

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 substrate will take infinite time to produce 100% potential of methane (Koppar and Pullamanappalli, 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 ($\lambda$) from T95, the effective

![Fig. 7: Change of pH during the anaerobic fermentation (experiment 1)](image)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P$ (Nm$^3$(kg oDM)$^{-1}\text{day}^{-1}$)</td>
<td>0.148</td>
<td>0.150</td>
<td>0.166</td>
</tr>
<tr>
<td>$R_m$ (Nm$^3$(kg oDM)$^{-1}\text{day}^{-1}$)</td>
<td>0.014</td>
<td>0.019</td>
<td>0.015</td>
</tr>
<tr>
<td>$\lambda$ (days)</td>
<td>1.67</td>
<td>0.7</td>
<td>1.5</td>
</tr>
<tr>
<td>T95% (days)</td>
<td>17.95</td>
<td>11.9</td>
<td>17.95</td>
</tr>
</tbody>
</table>

*Data are expressed as the Mean±SD of three experiments. *Experimental data. *Gompertz parameters (evaluated by fitting experimental data). *Technical digestion time
methylene 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 until 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 (Kopper and Pullammanappillil, 2008; Kapanu 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 microflora 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 Alm et al. (2010) and Li et al. (2011) in a batch mode reactor during 63 days. Alm et al. (2010) recorded potential in ethane production in the value of 0.028 Nm³(kg oDM)⁻¹ at the temperature of 55°C while ultimate methane yield of 0.251 Nm³(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 Nm³(kg oDM)⁻¹. Immobilization of methanogens on the supporting materials (polyethylene packing rings) improved
methane production kinetics. Kinetic parameters of anaerobic digestion, \( P, R_\text{a} \) and \( \lambda \) 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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