
Co-digestion of Plant Residuals and Chicken Dung in Two-Stage Solid State Anaerobic System with Single and Multiple Hydrolysers

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Abstract: Solid-state anaerobic digestion has been widely used to treat various types of organic wastes. Generally, hydrolysis is considered as a rate-limiting step of anaerobic digestion. It tends to produce the higher concentration volatile fatty acids under a high organic loading rate. But, if the concentrations of fatty acids in the single digester are too high anaerobic digestion could be inhibited or failed. That's why division of whole digestion process into 2 stages (*Hydrolysis* and *Methanogenesis*) was developed, it makes possible to support high level of methane-production at the high loading rate. In 2-stage anaerobic system different process conditions (such as pH value or temperature) could be adjusted and optimized for the individual groups of microbes in certain degradation steps. Presented paper describes methane production in two-stage solid state anaerobic digestion of plant residuals and chicken dung using lab-scale system. The main objectives of the study were: (1) to examine the influence of temperature regime of hydrolysis stage on whole methane production; (2) to evaluate methane yield in anaerobic digestion of fruit/vegetable waste or hay biomass with a chicken dung; (3) to assess contribution of different stages the digestion into cumulative methane production. All substrates were air dried (at 25-27°C) and well grinded before experiments. Methanogenesis reactors were operated under mesophilic conditions; hydrolysis stages were under mesophilic or thermophilic temperature. Methane production rate and cumulative methane yield was measured separately in hydrolysis and methanogenesis reactors. Total digestion of the substrates at 55°C hydrolysis was in progress 15-17 days; at 37°C hydrolysis – 21-23 days. Methane yield in 2-stage solid state anaerobic digestion of dung/fruit-vegetables waste under 55°C-hydrolysis was 20% higher as against 37°C-hydrolysis (283.5 versus 236 ml CH₄/gVS); the digestion of dung/hay mixture gave 29% higher methane gas under 55°C-hydrolysis (233 versus 180 ml CH₄/gVS). It was revealed that the most of total methane gas in 2-stage SS-AD lab-system under ambient pressure was produced in the methanogenesis reactor. A wave-like methane production was observed in hydrolysis reactors, the maximum gas was released in the first day. Periodically, after each 5-7 days the production was stopped and liquid fraction of hydrolysate was withdrawn as a feedstock for methanogenesis reactor. This led to some interruption in the loading of methane-tank and considered as inconvenience for an operation under continuous mode. Integrating of multiple hydrolysis reactors into common system with strong order of loading and withdrawal provides more constant production of feedstock and maintains stable gas production. It is also suggested to accumulate hydrolysates of different withdrawals in one storage unit and mix them before dosed food supply into methanogenesis reactor.

Keywords: Two-stage SS-AD, Co-fermentation, Plant Residuals, Chicken Dung, Methane Production

1. Introduction

Effective technologies for organic waste processing are a big challenge for many developing countries around the world. Enormous amount of food waste, agricultural residuals and

municipal waste has been being produced constantly in global.

Uzbekistan is the first largest by population (about 33 mln) and the third spacious (448900 km²) country in Central Asia. Natural gas is dominating energy source in the structure of energy resources in the country, it makes up about 85% in manufacture of primary energy; and hydro energy (as

renewable energy) contributes 12.7% in the common energy stock [1]. Meanwhile, approximately 4-6 million tons of municipal solid waste (contained more than 40% of food waste and plant residuals) is generated in Uzbekistan annually, and only 10% of them (glass, metals, paper or plastic) are processed or recycled. In the capital of Uzbekistan, Tashkent city about 1.5 million tons of household waste is annually generated and taken out to the city disposal site of 59 hectares [1]. Additionally, more than 100 million m³ of organic waste is generated annually as a result of the vital activity of 8 million cattle and 15 million sheep and goats [2].

Anaerobic digestion (AD) is considered as effective methods for organic waste utilization and one of the promising sources of alternative energy. Moreover, possibility to use post-digestion matters as fertilizers is very important benefits from the technology. Uzbekistan has a big potential of biomass energy, according to preliminary calculations, the total potential of biogas in the country is estimated at 8.9 billion m³. In terms of calorific value, this corresponds to 6.5 billion m³ of natural gas, which is over 10% of the republic's annual need for energy resources [2]. However, AD-technology has not yet become commonly used in Uzbekistan.

It is known, that trophic relationships and interactions between different groups of microorganisms in anaerobic digester are essential to maintain different sequential steps of biomass decomposition - hydrolysis, acidogenesis, acetogenesis and methanogenesis. Methane production is under impact of such factors as chemical composition and disintegration rate of substrates, sudden change of pH, temperature, organic loading rate, etc. [3, 4]. It is clear, that overall efficiency of methanogenesis depends on activity and growth of microorganisms involved in the earlier phases of fermentation [5]. The main question is to create appropriate conditions for all so different groups of microorganisms.

Recently, solid-state anaerobic digestion (SS-AD) has been widely used to treat various types of organic wastes; the total solids content of SS-AD usually varies from 15 to 40% [6-8]. As compared with ordinary liquid-state AD, SS-AD has many advantages. For example, SS-AD requires less energy and water and can treat more organic solids; the digested residues can be easily handled without dewatering [9]. By the way, co-digestion of different substrates is popular in biogas production, because it offers more balanced feedstock for the enhancement of methane yield [10, 11]. However, in practice, the operation of SS-AD is often difficult to guarantee [12]. Despite the large number of existing studies on SS-AD and the rising interest in this technology, very few investigations have been performed on its stability [9, 13].

It should be reminded, that hydrolysis is a rate-limiting step of anaerobic digestion of organic waste. It tends to produce the higher concentration volatile fatty acids (VFAs) under a high OLR. But, if the concentrations of fatty acids in the single digester are too high anaerobic digestion could be inhibited or failed [4].

That's why division of whole AD process into 2 stages

(called *Hydrolysis* and *Methanogenesis*) was developed, it has advantages over single-stage process, especially if we consider solid state AD. Two-stage solid-state anaerobic digestion (2-stage SS-AD) makes possible to support high level of methane-production at high OLR. In common single stage digesters, pH ranges between 6.5 and 8 and it is one of the process variables which is hardly adjustable; process values cannot be adapted when all four degradation steps (hydrolysis, acidogenesis, acetogenesis and methanogenesis) take place in a single digester [14]. It is known, that hydrolysis or acid forming bacteria are more robust to any changes and can operate in a wider range of conditions (pH, temperature, OLR, etc.) than methanogens [15]. In 2-stage anaerobic system different process conditions (such as pH value or temperature) could be adjusted and optimized for the individual groups of microbes in certain degradation steps; and their rates can be changed without any damages for other groups of microorganisms, and methanogens particularly.

Recently, 2-stage SS-AD systems have been being analyzed and used intensively. Current studies are mostly focused on seeking and adjustment of substrates for co-digestion, pretreatment of substrates, selection of appropriate pressure and temperature regimes, or development of membrane filtration system for liquid phase of hydrolysis (acidogenic) reactors [16-20, 14].

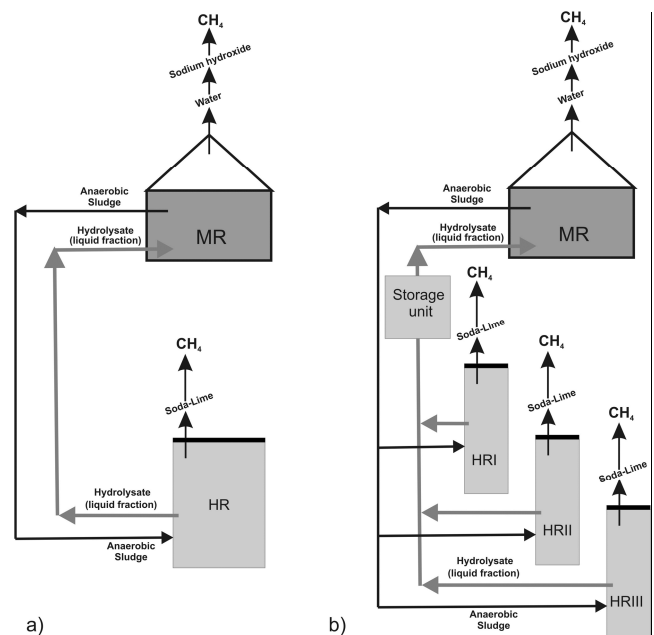


Figure 1. Layout of the experimental lab-scale installation for 2-stage high solid stage anaerobic digestion.

(a) 2-stage SS-AD lab-system with single hydrolysis reactor; (b) lab-system with multiple integrated hydrolysis reactors; MR – methanogenesis reactor, HRI, HRII, HRIII - hydrolysers, Storage unit – vessel for liquid hydrolysate accumulation, Soda-Lime and Sodium hydroxide (5% water solution) – sorbents to eliminate CO₂ from biogas.

Presented paper describes methane production in 2-stage SS-AD of different plant residuals with chicken dung under lab-scale conditions. The aims of the study: (1) to examine the

influence of temperature regime of hydrolysis stage on whole methane production in 2-stage SS-AD; (2) to evaluate methane yield in 2-stage SS-AD of fruit/vegetable waste (FVW) and hay biomass with a chicken dung; (3) to assess contribution of different stages of SS-AD into cumulative methane production.

2. Materials and Methods

2.1. Inoculum and Laboratory System for Anaerobic Digestion

Inoculum of anaerobic sludge (AS) for the study was obtained from Municipal Waste Water Treatment plant ("Salar" Station, Tashkent city) and Institute of Microbiology of Academy of Science of the Republic of Uzbekistan.

Two-stage SS-AD was studied in the lab-scale system. Principal scheme of experimental installation is presented in figure 1.

2.2. Operational Parameters

The first set of the study was performed under operational parameters described below:

- Methanogenesis reactors (MR) and hydrolysers (HR) were operated under *mesophilic temperature* (37°C).
- Batch mode reactors on a laboratory scale were adopted in this study for hydrolysis. The total volume of each HR was 1 L, 6 items in total; working volume – 250 ml. Air dried and well grinded substrates (36 g VS) were put into each HR (OLR_{HR} 144 gVS/l). Then, 250 ml of inoculum (from methane-tank) was added per HR (17-19% of DM per reactor).
- Total time of hydrolysis in each HR was about 21 days. During that period hydrolysates were withdrawn triply: 1st withdrawal was made after 7 days of hydrolysis, 2nd withdrawal - after 14 days, and 3rd withdrawal - after 21 days. Hydrolysates were wrung out (pressed out) using 2 layer-nylon textile to separate solid fraction from liquid. MRs were fed by liquid hydrolysis effluent. New portion of anaerobic sludge from MRs was added into HRs in place of liquid hydrolysate. After 21-23 days hydrolysates (liquid and solid fractions) were removed from reactors; and each HR was loaded by new substrate and sludge. So, the process was cyclic.
- Working volume of methanogenesis reactor (MR) – 2000 ml (2 items for 2 replication); total volume of each – 3000 ml.
- Average OLR_{MR} 3.0-4.7 gCOD/L, 200 ml of hydrolysate 2 times in a week.
- Magnetic stirring of MRs.
- System was operated at ambient pressure.
- Gas yield measurement, feeding of MTs, hydrolysate withdrawing and adding of new portion of inoculate from MRs into HRs was manual.
- Produced biogas was purified by consecutive transmission (bubbling) through distill water and sodium hydroxide solution (NaOH, 5%) – in case of

MRs; or by passing through Soda-Lime pellets (Sigma-Aldrich) – in case of HRs. Methane gas yield was measured using water columns in measuring cylinders.

The second set of experiments was done under some different operational parameters:

- MRs were operated under *mesophilic temperature* (37°C) and HRs worked in *thermophilic environment* (55°C).
- Methanogenesis reactors (MRs) were operated in *continuous mode*. Average OLR_{MR} was 1.27-2.63 gCOD/L, 100 ml of liquid hydrolysate every day. HRT_{MR} – 20 days.
- HRs were started up and loaded in *strong consecutive order* – one time in each 2 days.
- Time of hydrolysis was about 15 days*. Liquid fractions of hydrolysates were withdrawn regularly, on 5th, 10th and 15th days of the process. They were accumulated and kept in storage vessel before using them as feed solution. Hydrolysis liquid and solid fractions were completely removed from the HR after 15 days, and each HR was loaded by new substrate and sludge. The process was cyclic.

Substrates for co-digestion: (1) chicken dung / fruit-vegetable kitchen waste (FVW), 36 g VS/250 mL, 1:8 in terms of VS, (2) chicken dung / hay (dry grass), 36 g VS/250 mL, 1:8 in terms of VS. All substrates were air dried (at 25-27°C) and well grinded before experiments.

2.3. Operational Control

For operational control some parameters of HRs and MRs such as pH, TS (total solids), TVS (total volatile solids), NH_4^+ (ammonium), TN (total nitrogen), TP (total phosphorus), COD (chemical oxygen demand), TOC (total organic carbon) were monitored. The concentrations of TS and TVS were measured by drying (105°C, 24 h) and volatilization (600°C, 1 h) in melt pots [21]. Value of pH was measured using a pH/ion meter (pH-sensor "ЭСК-10601/7", "Expert-001.3", Russia) in accordance with standard electrometric methods of measurement.

TN, TP, TOC and COD of MRs and HRs were determined using "QuickTNPC" device (LAR Process Analyser Company, Germany). NH_4^+ ion concentrations were detected using ammonium selective electrode (Metrohm, Switzerland) using "Ammonitor" device (LAR Process Analyser Company, Germany) [22].

2.4. Data Analysis

Statistical analysis of the data was done with OriginPro 8.0. The data are presented as the mean \pm standard deviation for continuous variables and as numbers or percentages for categorical variables. Parametric data were compared using the independent *t*-test. One-way analysis of variance (ANOVA) was performed to compare parametric data (significant at $P=0.05$).

3. Results and Discussion

3.1. Two-stage Anaerobic Digestion with Single Hydrolyser at 37°C

The first set of experiments on 2-stage SS-AD was carried out using lab-scale mesophilic hydrolysers (HRs) loaded with mixed substrates (chicken dung with some plant residuals) up to 17-19%DM; methane-tanks operated in semi-continuous mode under mesophilic temperature, and fed by hydrolysis effluent (liquid fraction). Methane production was measured in HRs and MRs separately. Obtained results are shown in figure 2.

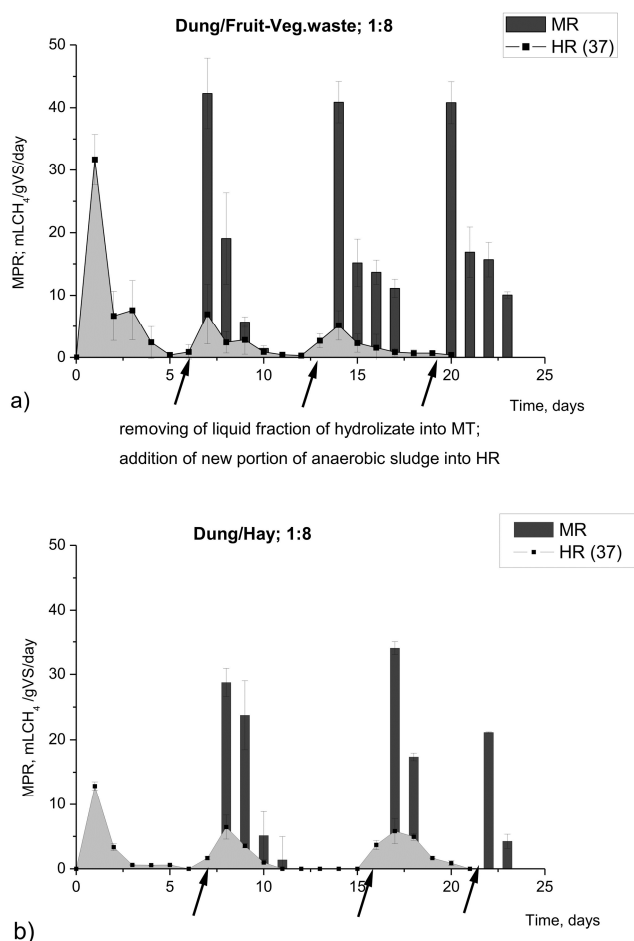


Figure 2. Methane production rate in 2-stage SS-AD at 37°C hydrolysis.

(a) Dung and Fruit/vegetable waste mixture (1:8 on basis of VS) and (b) Dung and Hay mixture (1:8 on base of VS), 37°C – hydrolysis and methanogenesis.

It should be noted that hydrolysis of different biomass at a temperature of 37°C was in progress about 21-23 days. As the decomposition of organic substrates and the saturation of HR-contents by various volatile fatty acids, pH-value of hydrolysate was fallen down from 7.8-8.0 in the beginning to 5.1-5.3 pH after 6-7 days approximately; and methanogen activity in HRs was decreased, methane production stopped to almost zero. At the moment liquid fraction of the hydrolysate was withdrawn (about 200 ml) and HR with partly disintegrated biomass was charged by new dose of mesophilic

sludge from MR (200 ml).

It should be stressed that microbe association of mesophilic anaerobic sludge is very divers. It is well known that several groups of microorganisms work interactively to converse complex organic fractions into such final products as CH_4 , CO_2 , H_2 , H_2O and some others. Besides, bacterial biomass is growing gradually (at appropriate conditions). The main function of Hydrolysis reactor is hydrolysis and acidogenesis of substrates. Particulate matters of substrates are converted there into dissolved compounds by excreted enzymes of various hydrolytic bacteria. It is usually quite slow process which depends on many factors - temperature, size of particles, pH, chemical composition of substrates, volatile fatty acids content etc. After that phase, soluble products are digested inside cells of acidogenetic species of microorganisms and excreted by them as volatile fatty acids, lactic acid, carbon dioxide, hydrogen, ammonia, hydrogen sulfide, nitrogen oxides and etc. As a result, hydrolysate is acidified and methane-forming organisms are inhibited [23]. So, methane gas is complementary product of the first stage of SS-AD process; it can be registered while methanogens are active.

That is why, a wave-like methane production with few peaks in response to fresh anaerobic sludge additions into hydrolysis reactors was observed. At that, methane tanks were fed by the hydrolysate liquids. It was usually 2 times in a week; and almost complete conversion of organic fractions from hydrolysate into methane gas took 3-4 days. So, methane production in MRs also demonstrated a wave-like character and was not stable.

Sampling tests of anaerobic sludge from MRs revealed some unwanted changes in chemical composition (table 1).

Table 1. Main chemical characteristics of anaerobic sludge (liquid fraction) in methane-tanks operated in semi-continuous mode (fed by 200 mL hydrolysate at once each 4-5 days) with 37°C hydrolysis.

Parameters	After 25 days of operation	After 55 days of operation
pH	8.00±0.02	8.00±0.28
NH_4^+ , mg/l	604.40±35.95	928.37±36.82
N-NH_4^+ , mg/l	471.43±28.04	724.13±28.72
TN, mg/l	1125.30±184.20	1336.75±218.85
TP, mg/l	47.61±8.40	57.10±4.94
TOC, mg/l	1827.98±32.53	1949.50±66.97
COD, mg/l	4601.23±159.24	4977.00±173.95
TS, g/l	15.6±0.09	20.5±0.14
TVS, g/l	9.63±0.14	11.83±0.05

For instance, the tendency of accumulation of nitrogen in MRs was determined; ammonium ion concentration increased from 604.40 to 928.37 mg/l during 55 days of operation and the same continued in the sequel. Evidently, it was the contribution of chicken dung disintegration as a substrate with high nitrogen content (water soluble nitrogen compounds (SolN) in chicken dung was about 82.6±1.16 mg/gDM; N-NH_4^+ was 6.03±0.41 mg/gDM). It is known, that ammonium concentration can be limiting factor for methanogens [23]. Therefore, MR's sludge was diluted by tap water from time to time to control the situation that ammonium concentration did not came up to critical (inhibiting) values there.

3.2. Two-stage Anaerobic Digestion with Multiple HRs System at 55°C

Then 2-stage SS-AD of the same substrates was studied as described above in chapter 2, methane tanks in lab-scale installation was operated in continuous mode, they were fed by liquid hydrolysates from HRs, operated under thermophilic temperature (HRT=20 days, OLR was about 1.27-2.63 g/l COD, 100 ml of hydrolysate every day). Dynamic of methane-production at AD of mixture of Dung/FVW in each HR and MR is presented in figure 3.

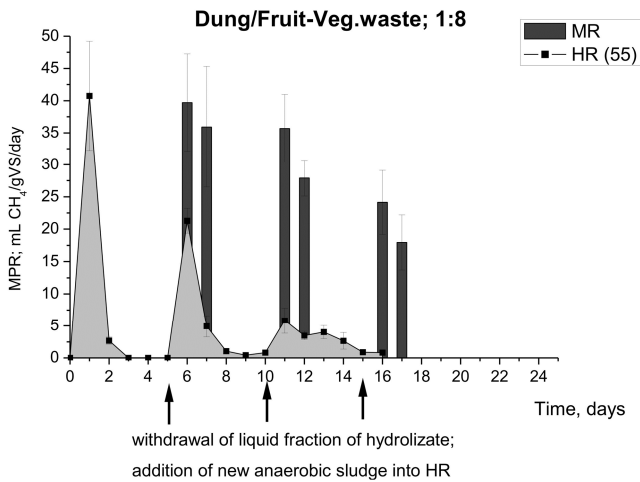


Figure 3. Methane production rate in 2-stage SS-AD at 55°C hydrolysis.

Substrate: Dung and Fruit/vegetable waste mixture (1:8 on basis of VS), 55°C – hydrolysis and 37°C methanogenesis; HR – hydrolysis reactor, MR – methane-tank.

It was predictable, that hydrolysis time of substrates under higher temperature takes less time – 14-17 days (at 55°C) instead of 21-23 days (at 37°C).

Unlike previous experiments, multiple HRs were used as integral cascade: each 3 hydrolyzers served 1 methane tank, they were started up, loaded and withdrawn in strong order to

provide stable work of MR and comparative sustain methane-production (figure 4).

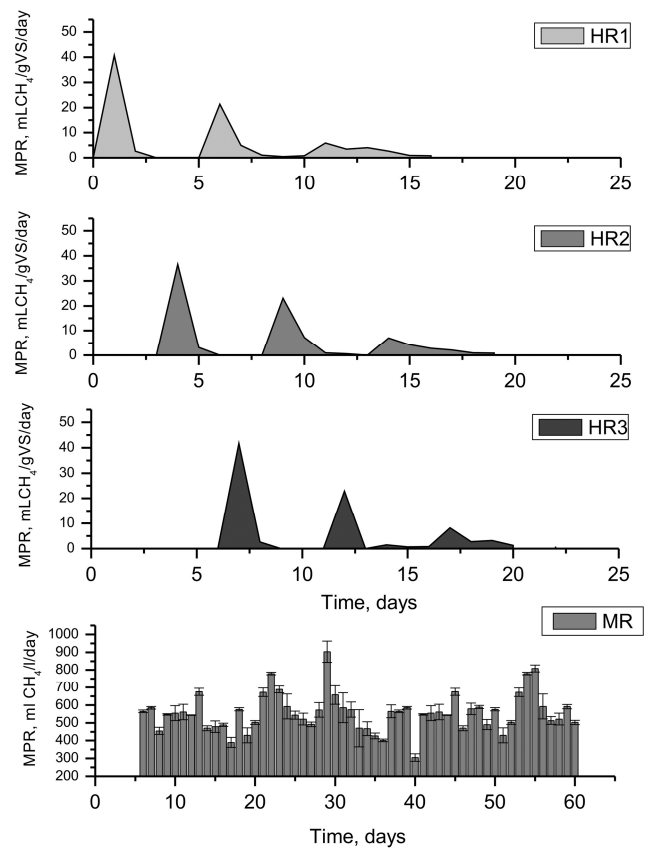


Figure 4. Methane production rate in 2-stage SS-AD with multiple hydrolysis reactors.

Substrate: Dung and Fruit/vegetable waste mixture (1:8 on base of VS), 55°C – hydrolysis and 37°C methanogenesis; HR1, HR2, HR3 are hydrolysis reactors bonded in a common system; MR – methane-tank

Average chemical characteristics of HRs and MRs are shown in tables 2 and 3.

Table 2. Main chemical indicators of anaerobic sludge in methane-tanks operated in continuous mode (100 mL hydrolysate/ day) with 55°C hydrolysis.

Parameters of liquid fraction	Before operation	After 15 days of operation	After 30 days of operation
pH	7.52±0.21	7.31±0.04	7.48±0.04
NH ₄ ⁺ , mg/l	573.00±39.6	534.00±21.92	578.25±21.57
N-NH ₄ ⁺ , mg/l	446.94±30.89	416.52±17.10	451.04±16.82
TN, mg/l	627.20±15.27	804.78±51.45	981.75±160.16
TP, mg/l	28.75±0.21	22.85±3.72	39.50±0.71
TOC, mg/l	1901.20±0.21	3102.75±62.18	5654.75±49.14
COD, mg/l	4753.40±0.85	7756.63±155.53	8886.00±123.04
TS, g/l	21.40±0.10	23.98±0.22	28.45±0.54
TVS, g/l	13.03±0.05	14.33±0.52	17.23±2.27

Table 3. Main characteristics of liquids in hydrolysis reactors at 55°C (substrate - Dung/FVW, 1:8 on the basis of VS).

Parameters	1st withdrawal (3-5 days of hydrolysis)	2nd withdrawal (8-10 days of hydrolysis)	3rd (final) withdrawal (14-15 days of hydrolysis)
pH	5.11±0.28	5.18±0.26	5.38±0.21
NH ₄ ⁺ , mg/l	693.94±111.96	441.96±99.45	440.41±91.29
N-NH ₄ ⁺ , mg/l	541.27±87.33	344.73±77.57	343.52±71.21
TN, mg/l	2325.20±112.74	1000.50±182.02	906.00±85.85
TP, mg/l	67.60±22.40	69.00±7.16	80.00±15.93

Parameters	1st withdrawal (3-5 days of hydrolysis)	2nd withdrawal (8-10 days of hydrolysis)	3rd (final) withdrawal (14-15 days of hydrolysis)
TOC, mg/l	25508.75±507.11	10674.88±493.10	9729.13±591.95
COD, mg/l	63773.25±1017.45	26686.06±1233.87	24323.43±1479.64
TS, g/l	54.49±3.27	24.12±0.64	21.19±2.67
TVS, g/l	40.50±2.93	15.46±0.08	14.57±2.17

It is significant that concentration of some chemical things in hydrolysates which were withdrawn after 5, 10 and 15 days of operation at 55°C were different, 1st withdrawal was the most saturated (more full of nutritional chemicals) in comparison with 2nd and 3rd ones. For instance, in case of loading of HRs by Dung/FVW (39 gVS/250 ml; 1:8) COD was about 64 g/l in the 1st withdrawal, then 27 and 23g/l; with loading by Hay (25 gVS/250 ml) COD measured in 1st, 2nd and 3rd hydrolysate withdrawal was about 37, 28 and 20 g/l accordingly; with loading by FVW (25 g VS/250 ml) they were about 52, 14, 9.6 g/l respectively. That is why methane production from the first portion of hydrolysate was every time the highest.

This fact should be taken into consideration for intelligent planning of operational regimes at 2-stage SS-AD (loading and start up time of HRs particularly) to provide sustainable methane production rate. It is also recommended to accumulate hydrolysates of different withdrawals in one storage unit and mix them before dosed food supply into MRs. Methane production rates in methanogenesis reactors under two operational regimes are shown in figure 5.

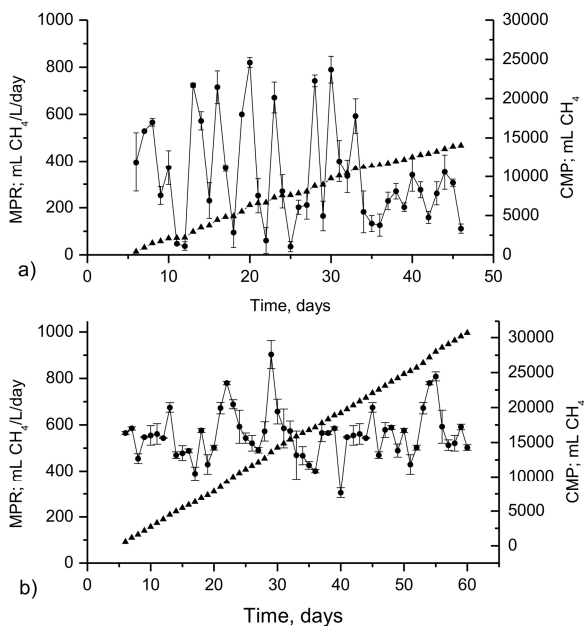


Figure 5. Methane production rate (MPR) and cumulative methane production (CMP) in methane-tanks fed by liquid hydrolysate in 2-stage SS-AD, semi-continuous (a) and continuous (b) mode.

(a) HR work volume= 250 mL, 36 gVS of substrate; DM content is about 17%, 37°C; MR work volume = 2 L; OLR=3.0-4.7 gCOD/L 2 times in a week (200 mL hydrolysate at once), 37°C; (b) HR work volume= 250 mL, 36 gVS of substrate; DM content in HRs is about 17%. MR work volume = 2 L; OLR = 1.27-2.63 gCOD/L/day (100 mL hydrolysate/day); HRT=20 days; 55°C for hydrolysis and 37°C for metnanogenesis.

Substrates: chicken dung with hay (1:8 on the basis of VS); chicken dung with fruit/vegetable waste (1:8 in terms of gVS); pure hay; and pure fruit-vegetables waste.

For example, total methane yield in 2-stage SS-AD of Dung/FVW at 55°C-hydrolysis was 20% higher than at 37°C-hydrolysis (283,5 versus 236 mL CH₄/gVS); total methane production in result of AD of Dung/Hay mixture at the same conditions was 29% higher (233 versus 180 mL CH₄/gVS).

Evidently, that operation with integrated system of hydrolysis reactors is more preferable, because production of methane gas is more stable and MPR-fluctuations are less. The observed dynamics of methane productions in HRs and MRs in 2-stage SS-AD of different substrates at 37°C and 55°C-hydrolysis were similar, but cumulative methane yields varied. Total methane production at 55°C hydrolysis was higher in comparison with 37°C hydrolysis (figure 6). It corresponds to all substrates under the study.

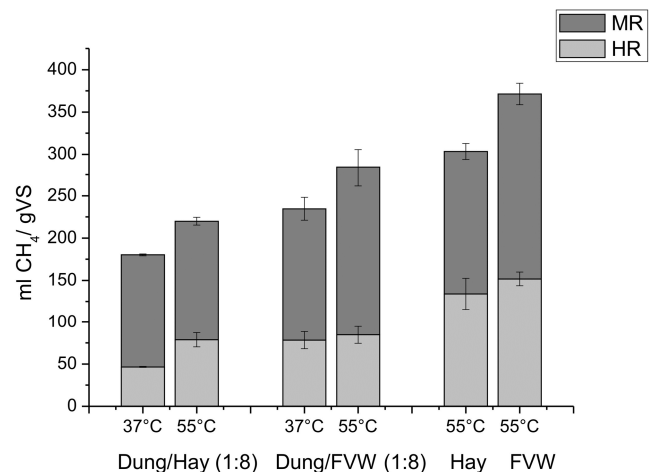


Figure 6. Methane yield in 2-stage high solid AD under 37°C and 55°C hydrolysis.

It is important to note that digestion of pure substrates (plant residuals) gave more methane gas than their mixture with small amount of chicken dung. So, total methane production in AD of pure hay or fruit and vegetable waste was about 30% more in comparison with mixture with dung. We propose that it is connected with inhibiting effect of some chemical compounds of chicken dung on methanogens, such as; high nitrogen compounds concentrations and unexpected content of some medical things or minerals. Rough calculation has shown that about 57% VS of hay and 88% VS of fruit-vegetable waste can be converted in biogas at 2-stage SS-AD with 55°C hydrolysis.

It was revealed that the most of total methane gas in 2-stage SS-AD lab-system under ambient pressure was produced in

the methanogenesis reactor (figure 6): about 60,5% and 70,2% of cumulative methane yield at 55°C hydrolysis was measured in MRs at AD of mixture substrate of dung/hay and dung/fruit-vegetable waste accordingly; and 66% and 74% with 37°C hydrolysis. Approximately 56% of methane was produced in MRs at AD of pure hay at 55°C hydrolysis and about 59% at AD of FVW (55°C hydrolysis). At the same time contribution of hydrolysis reactors to methane production is more sensitive to different external impacts (for instance, to temperature regime, the degree of grinding of the substrates or presence of some chemical compounds). It confirms the fact that hydrolysis is limiting (key) stage of whole anaerobic digestion of different organic substrates; and its effectiveness determines common progress of methane production.

4. Conclusions

Recently, two-stage solid state anaerobic digestion systems have been being used more often for processing of different types of organic waste. They demonstrate number advantages in compare with ordinary liquid-state fermentation and anaerobic systems with single digester, because they require less energy and water, are able to treat more organic solids, and the digested residues can be easily handled as fertilizers without dewatering.

The study has shown that methane gas is produced in both stages of the 2-stage SS-AD system – in hydrolysis and methanogenesis reactors. It is preferable to use methanogenesis reactors under mesophilic conditions, because mesophilic anaerobic sludge is recognized as very divers association of interacted microorganisms with different functional features, which provides anaerobic digestion of organic matters step by step and can be used as appropriate inoculum for hydrolysis reactors operated under any temperatures. In the same time, it better to use hydrolysis reactors under thermophilic conditions. Total digestion of the substrates at 55°C hydrolysis usually takes 15-17 days; at 37°C hydrolysis – 21-23 days. Methane yield in 2-stage solid state anaerobic digestion of dung/fruit-vegetables waste under 55°C-hydrolysis was 20% higher as against 37°C-hydrolysis (283.5 versus 236 ml CH₄/gVS); the digestion of dung/hay mixture gave 29% higher methane gas under 55°C-hydrolysis (233 versus 180 ml CH₄/gVS). It was revealed that the most of total methane gas in 2-stage SS-AD lab-system under ambient pressure was produced in the methanogenesis reactor.

A wave-like methane production is observed in hydrolysis reactor, the maximum gas is usually released in the first day. Generally, hydrolysis is recognized as a rate-limiting step of anaerobic digestion. It tends to produce the high concentration volatile fatty acids under a high organic loading. As the decomposition of organic substrates and the saturation of hydrolysis reactors contents by various volatile fatty acids, pH-value of hydrolysate is fallen down from 7.8-8.0 to 4.7-5.3 pH after 6-7 days approximately; and methanogen activity is decreased, methane production stopped to almost zero; and liquid fraction of hydrolysate should be withdrawn as a feedstock for methanogenesis reactor. Obviously, this leads to some

interruption in the loading of methane-tank and considered as inconvenience for an operation under continuous mode.

Integration of few hydrolysis reactors into common integrated system with strong order of loading and withdrawal of hydrolysate is recommended for a constant production of feedstock (liquid hydrolysate) for methanogenesis reactor and stable methane production. It is also suggested to accumulate hydrolysates of different withdrawals in one storage unit and mix them before dosed food supply into methanogenesis reactor.

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