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Original Research Article

Anaerobic co-digestion of food wastes and chicken dung in lab-scale two-stage system

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The depletion of natural energy resources (oil, gas) and increase in environmental pollution are the most important problems facing humanity. Waste from agricultural enterprises and the municipal sector is harmful to the natural environment. Nowadays one of the most efficient technologies for the processing of livestock manure is anaerobic digestion (AD). One of the main sources of raw materials in Uzbekistan, suitable for extracting biogas, are wastes of livestock-breeding and poultry farming, industrial organic wastes. Therefore, the aim of the work is to study the process of two-stage anaerobic co-fermentation, measure the production and composition of biogas. The laboratory set-up for two-stage co-anaerobic fermentation operated about 55 days. (1-st stage at 55 °C hydrolysis of a substrate containing 60,49gVS; 2-nd stage methane formation at 37°C; OLR_{MT}(organic loading rate) - 4.8 gCOD/dm^3/day; HRT (Hydraulic retention time)-14 days. Our results demonstrated wave-like dynamics. That are associated with the frequency of loading the substrate in hydrolyzer in the first stage and difference in the chemical composition of the first and second pressing hydrolyze. The results showed that most of the organic matter (65%) converted to biogas. The gas composition has been determined and its dynamics studied. The total methane yield in 14 days was 10950.73 ml. 181.03 ml of methane and 80.42 ml of hydrogen released from 1 g of organic waste. It shown that Uzbekistan has a big potential to produce biogas throw co-anaerobic digestion.

Keywords: Anaerobic digestion, co-fermentation, two stage AD, organic waste, biogas yield, biogas composition, chicken dung.

INTRODUCTION

The depletion of natural energy resources (oil, gas) and the increase in environmental pollution are the most important problems facing humanity.

The Central Asian countries, Uzbekistan particularly have three major challenges: ensuring food security, alleviating poverty and environmental protection (Beniwal and Warma, 2000). Waste from agricultural enterprises and the municipal sector is harmful to the natural environment. There are about 9500 large and medium scale farms in Uzbekistan (with livestock consisting of more 6.7 mil.heads of cattle, 12.4 mil. heads of sheep, 83.6 mil. heads of swine and, 21.1 mil. of poultry) (Toderich and Massino, 2008)Accordingly, the amount of organic waste increases from year to year.

Nowadays one of the most efficient technologies for the processing of livestock manure is anaerobic digestion (AD). Anaerobic conversion becomes desirable for sustainable management of biodegradable waste, fuel production, and leads to a low carbon footprint (Jain et al., 2015).

The AD carried out by a set of microbial communities that transforms organic compounds into substances collectively called biogas (Zamanzadeh et al., 2017). Biogas mainly consists of methane (55-75% CH_4) and carbon dioxide (25-45% CO_2) it also contains nitrogen, hydrogen, and hydrogen sulfide (Demirbas et al., 2016).

AD occurs in four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The two-stage AD system consists of the hydrolysis followed by methanogenesis (Li and Yang, 2016). Methanogens are key microorganisms for CH_4 production during AD (Muthudineshkumar and Anand, 2019).

Also, AD is a complex system that depends on different process conditions (type and structure of organic matter, particle size, temperature, pH, sewage properties, volatile fatty acid, organic loading rate, hydraulic retention time and carbon-nitrogen ratio, etc.) and microorganisms (Abdelgadir et al., 2014).

Recent studies suggest that the use of substrate mixtures (co-fermentation) for the synthesis of biogas provides an opportunity to significantly increase the generation of biogas in the process of methanogenesis of various wastes. Over the past decade, extensive research has been conducted on waste co-digestion especially for agricultural waste with high ammonium such as animal manure, and it has been well established that the better C/N(carbon to nitrogen ratio) and buffer capacities of the co-substrates helped improved treatment efficiencies and enhanced the maximum OLR (organic loading rate) (Gao et al., 2020).

Different researchers studied the two-stage anaerobic co-digestion (AcoD) process and demonstrated its potential advantages compared to a single stage (Hagos et al., 2017).

Major organic substrates used in anaerobic co-digestion with animal manure include food-waste, industrial-waste, and sewage sludge (Tufaner and Avşar, 2016). The indication that co-digestion of animal manure with other feedstock results in higher methane yields, which is a direct economic benefit, compared to anaerobic mono-digestion of animal manure (Ma et al., 2020).It is necessary to notice that the main sources of raw materials in Uzbekistan, suitable for extracting biogas, are wastes of livestock-breeding and poultry farming, municipal solid wastes, food wastes, industrial organic wastes, municipal sewage and residues of crops. The most feasible source is wastes (manure) of the stable livestock breeding industry. However, biogas technologies need for a scientific research.

Therefore, the aim of the work was to study the process of two-stage anaerobic co-fermentation, measure the production and composition of gas when decomposing the substrate of food waste and chicken manure.

MATERIALS AND METHODS

Experimental setup

The study conducted on a laboratory setup for two-stage anaerobic fermentation.The stages proceeded in different reactors: HR hydrolyser reactor (loaded periodically) and MT methane tank reactor (continuous loading mode).

Hydrolysers reactors (HR) are glass vessels with a total volume of 1 dm^3 and an effective volume of 0.4 dm^3 (4 reactors). The working volume of the methane tank (MT) is 2.2 dm^3 (2 reactors). The total volume of each MT-3 dm^3. The reactors placed in thermostats. The hydrolysis of substrates at the 1-st stage carried out at 55ºC (thermophilic condition).

For the HR 60.49 gVS, 28.8g of manure and 66.42 g of randomly generated kitchen waste (vegetables and fruit) taken on 250ml of anaerobic sludge. On the 7th day of fermentation, the liquid fraction of hydrolysate (HR1) used as a substrate for methane tanks (stage 2). HR was loaded with fresh anaerobic sludge to continue the decomposition of organic matter. Similar actions carried out on the 14th day with the resulting HR2 hydrolysates. The second stage of fermentation occurs at 37°C (mesophilic conditions). After the first and second pressing, the hydrolysate was fed to the methane tank in a constant loading mode, 60 ml of hydrolysate daily (respectively, 60 ml of anaerobic sludge from MT was withdrawn daily), thus, HRT, the time of liquid retention in the methane tank was 14 days. The loading rate for methane tanks (OLR_{MT}) is 4.8 gCOD/dm^3/day.

Measurement of biogas and statistical processing

Biogas collected from methanogenic reactors in a 3-liter gasholder. The measurements performed every day. The composition analyzed using an Optima 7 - biogas gas analyzer (MRUGmbH, Germany). When determining the gas composition, the analyzer connected to the pipe, and measurements made prior to stabilizing the readings.

Statistical processing of the obtained data, graphs performed and built in Microsoft Office Excel 2012. The figures and tables show the arithmetic mean values of a typical experiment and their standard square errors.

Physico-chemical analysis

The mineral component content (ash) and total carbon content determined by the standard gravimetric method. Porcelain cups weighed in sequence after drying in ШС-80-01 (Russia) (at 105°C) and burning in a LOIP muffle furnace LF-5/11-G2 (Russia) (at 605°C) (Vorobyova& Makarenko, 2005). To determine the COD (water-soluble components) carried out by the method of high-temperature combustion (at 1200°C) on QuickCOD-lab - 02E1717 or QuickTOCNPO – 01D1417 (LAR Process Analysers Company, Germany).

Determination of ammonium nitrogen in aqueous extracts and supernatant from bioreactors and hydrolyzers measured out using an automatic device Ammonitor Ion-Anaiyser (LAR Process Analysers Company, Germany) and using an ion-selective electrode "Elite-051" narH-ionomer Expert-001.3 (Russia).

Table 1. Chemical characteristics of substrates for fermentation

Substrates	Fruit / Vegetable Waste	Chicken dung	Ratio
	Water-soluble forms (mg/g per dry matter)		
Mineral components	68.83±0.19	311.57±25.89	1:4.52
Organic compound.	931.17±0.19	688.43±25.89	1.35:1
Total organic carbon	446.74±21.17	351.29±29.53	1.27:1
Total nitrogen	20.97±0.23	82.56±1.17	1:3.94
Total phosphorus	0.05±0.07	0.28±0.40	1:5.6
Organic carbon	164.11±0.68	117.99±8.54	1.39:1
COD	410.27±1.71	294.97±21.36	1.39:1
Na^+	2.35 ±0.17	1.73 ±0.10	1.36:1
K^+	20.56 ±0.51	6.6 ±0.1	3.12:1
Ca^{2+}	0.40 ±0.17	4.3 ±0.5	1:10.75
Cl^-	6.59 ±1.45	148.1 ±13.0	1:11.39
SO_4^{2-}	0	66.57 ±0.35	
NO_3^-	3.633 ±0.176	49.47±1.63	1:13.62
$N\text{-}NH_4^+$	0.225 ±0.034	6.03 ±0.41	1:26.80

Table 2. Physical and chemical parameters of the liquid fraction in HR-reactors at the 1st stage of the anaerobic fermentation process (37°C)

Parameters	After 7-day hydrolysis (HR1)	After 14-day hydrolysis (HR2)
pH	5.42+0.15	6.71+1.38
NH_4^+, mg/l	756.09+87.83	615.66+58.18
TN, mg/l	2479.20+187.52	1365+76.70
TP, mg/l	319.20+4.44	294.87+3.20
TOC, mg/l	25336.33+196.89	14221.00+866.06
COD, mg/l	64595.58+656.61	35552.28+216.15
TS, g/l	63.34+3.17	37.85+3.06
TVS, g/l	44.19+2.88	23.16+2.78

RESULTS AND DISCUSSION

Characterization of food waste, and chicken dung

Substrate for fermentation was a mixture of chicken manure, fruit and vegetables waste, in a ratio of 1:4 based on gVS (volatile solids). They dried at room temperature in the laboratory, crushed to a state of 1-2 cm.

The organic carbon, COD of soluble organic components, the content of individual anions and cations, and the total content of phosphorus and nitrogen of chosen substrates were determined (Table 1).

As can be seen selected substrates differ significantly from each other in their component composition. Chicken manure is high in total nitrogen, low in organic carbon and high in chlorine and ammonia nitrogen.

Characteristics of MR and HR

The process of hydrolysis and methanogenesis in reactors monitored by measuring the following parameters: pH, TS (total amount of solids), TVS (total amount of organic matter), COD, TOC (total organic carbon) and NH_4^+, TN (total nitrogen), TP (total phosphorus) (Table 2). They are limiting factors in the biological transformation processes of organic matter (Bernal et al., 2009).

Indicators of the efficiency of the process of destruction of organic matter during fermentation reduction of dry matter (Raposo et al., 2009), ammonium, organic matter, the volume of biogas released and the proportion of methane, and pH. As can be seen, all parameters reduced over the experiments.

Reduced pH indicates the need to extract hydrolysis fluid and add a new portion of active sludge. Then the pH increases from 5.42 to 6.71. From which we can assume completion of hydrolysis.

The results of the contents of TS, TVS, COD, and TOC decreased almost twice after the first spin.

The change in TS was significantly greater than in other studies of the two-stage anaerobic co-digestion in (Náthia-Neves et al., 2018a; Dareioti et al., 2014; Gao et al., 2015) where the decrease in TS was about 40 percent. However, the TVS in our study was less than that of Náthia-Neves et al. (2018b); Jabeenet al. (2015);Yenigün et al. (2013a)

COD decreased by 55% (Figure 2c, which is less than that of Mamimin et al. (2015) and higher than the Náthia-Neves

Table 3. Main characteristics of anaerobic sludge in methane tanks (MT) at the 2-nd stage of the process of anaerobic fermentation (methanogenesis) (55°C)

pH	7.51+0.09
TS,g/l	25.78+1.67
TVS,g/l	14.40+0.88
TOC,mg/l	2824.82+445.33
COD,mg/l	7925.99+850.57
NH_4^+, mg/l	481.71+55.57
TN,mg/l	1071.20+48.10
TP,mg/l	234.35+27.05

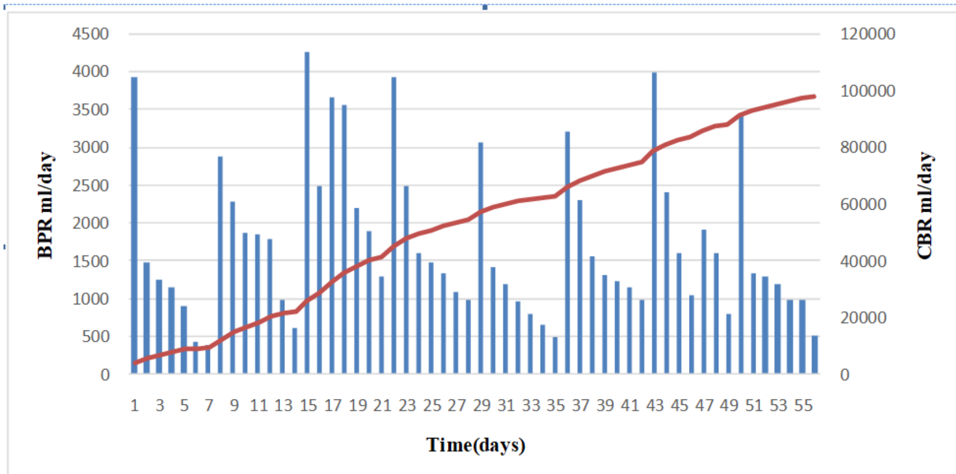


Figure 1: Biogas production rate (BPR) and cumulative biogas production (CBP) in the methanogenic reactor in 2-stage co-AD of chicken dung and food waste

et al. (2018c). We can notice a directly proportional decrease between COD (55%) and TOC (56%).

The content of ammonium nitrogen (mg/l N-NH_3) did not exceed the inhibitory level of the methanogenic microorganism, similar to the study by Yenigün et al. (2013b) where it is noted that the initial decrease in its concentration.

The results of the measurements showed that during the experiment, the state of the sediment in methane was stable and good, microbiological associations reproduced at a normal rate, and organic matter effectively transformed into methane (Table 3).

Cumulative biogas production and biogas production rate (CBP and BPR)

The laboratory set-up for two-stage co-anaerobic fermentation operated about 55 days. 1-st stage at 55 °C hydrolysis of a substrate containing 60,49gVS; 2-nd stage methane formation at 37^{0}C; OLR_{MT} - 4.8 gCOD/dm^3/day; HRT-14 days.

Our results demonstrated wave-like dynamics of biogas in the methanogenic reactor (Figure 1). That are associated with the frequency of loading the substrate in hydrolyzer in the first stage and difference in the chemical composition of the first and second pressing hydrolyze (Table 2).

Since the methanogenic reactor fed with hydrolyzed matter from the HR reactor, and the HR reactor fed with a substrate from chicken dung and food waste.

Biogas production was high in the first days of the process. During fermentation, the amount of organic substances converted to biogas decreases. As a result, MT receives less nutritious food for methanogens and there is a decrease for biogas. The total amount of biogas formed during the entire experiment was about 94 liters. The maximum amount of biogas released per day was 4280 ml.

As can be noticed we have sections with an identical wave pattern that take 14 days, which is how long it took to decompose each portion of the substrate, i.e. the entire process consisted of separate 14-day cycles "from loading to loading" of the substrate into hydrolysers.

One 14-day cycle of biogas production and its composition

In the first 24 hours after the reactors were loaded in HR with substrates, the largest amount of biogas emitted(Figure 2). For 6 days, almost the same amount of biogas released and its output decreases.

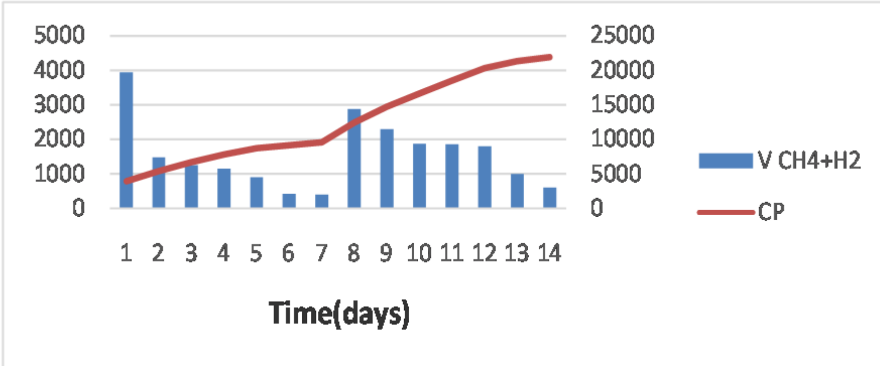
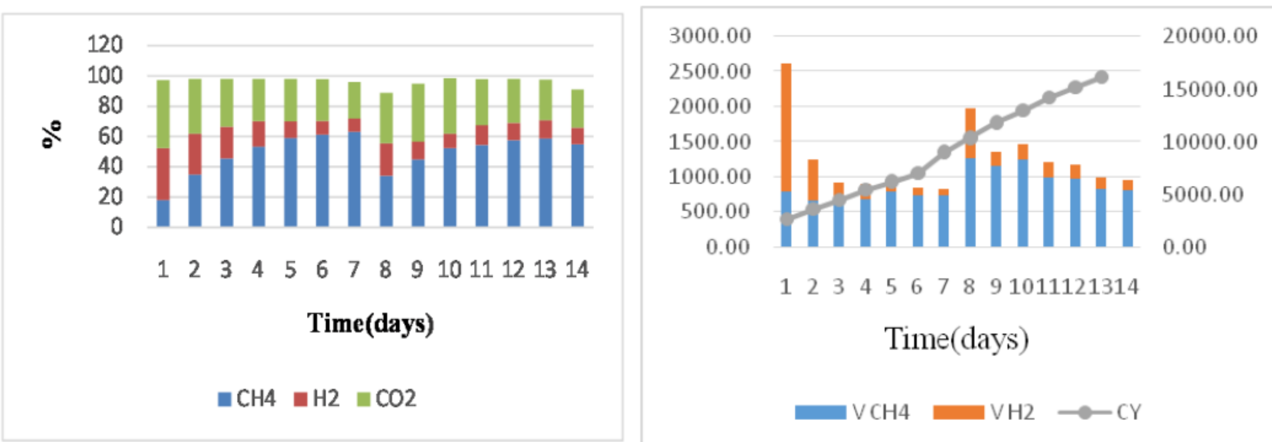


Figure 2. Methane and hydrogen production rate and cumulative production (CP) for one cycle of co-AD of substrates in two-stage mode



a *b*

Figure 3: Characteristics of **14-day cycle** of anaerobic decomposition of a substrate in a 2-stage set-up: a) Kinetics of methane (CH_4), carbon dioxide (CO_2), hydrogen (H_2) production during co-AD and b) Volumes of methane and hydrogen with a cumulative yield (CY)

From the results of the first stage of the fermentation process, it can be seen that the biogas in MR in the early days contains mainly hydrogen 1834, 38ml (26.98-34.53%) and carbon dioxide 2489, 97ml (36.30-44.84%) (Figure 3a).

Most of the biogas produced from the CO_2 and H_2 released from the hydrolysis reactor, but the purpose of this reactor was not to produce high biogas yields, but to produce hydrolysate, which will be used as a substrate for methanogens to produce a higher methane content. The experimentally observed jumps in the rate of methane production can be explained by the fact that hydrolysates after different pressing (I, II) have a different chemical composition and differ in the content of nutrients in them (Figure 3b).

During the second stage of anaerobic fermentation, the output of CO_2 and H_2 from the hydrolysis reactor to the methane tank decreases, and CH_4 concentration in the biogas begins to increase. As a result, the maximum amount of methane 1354.56 ml (58.89-61.20%) and carbon dioxide 1339 ml (28.24-27.71%) released by component composition. Similar results of gas output depending on the fermentation stage observed in Saad et al. (2019).

The gradual decrease in the amount of hydrogen and carbon dioxide released is related to the reduction in the amount of organic carbon in the decomposed substrate.

However, the experiment showed that the content of methane in the biogas synthesized in the methane tank increases, even if only gas supplied to the methane tank from the 1-st stage reactor, without loading the hydrolysate itself. Which confirms the presence of two types of methanogens in the methane tank: bacteria that can form methane from CO_2 and H_2, and a group of methane archaea that synthesize methane from acetic acid.

Those the main gases consisting of biogas were methane (42%), carbon dioxide (37%), hydrogen (18%), H_2S and others (3%) (Figure 4b.). The formation of H_2S was

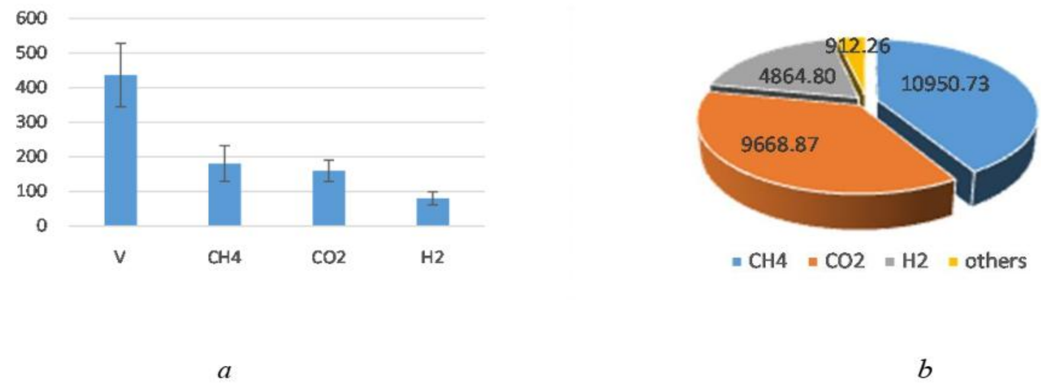


Figure 4: Biogas yield and composition for 14 days of co-anaerobic digestion in 2-stage system: a) The volume of 1gVS of biogas and its components and b) total biogas components yield from 60.49 gVS

probably related to protein hydrolysis.To reduce the proportion of it in methane-tank, $FeCl_3$ solution was added periodically (final concentration in MR 10 ppm).

The results showed that most of the organic matter (65%) converted into biogas. It was found that from 95.22 g of dry matter, 26396.67 ml of biogas is released, which is 436.38 ml from one gram of organic matter (Figure 4a.)

Anaerobic biodegradation of solid organic waste investigated. The kinetics of the processes of biodegradation of substrates and the extent of their transformation into biogas have been studied. Assembling gas streams in two-stage hydrolysis and the barbotage of the gas generated in the hydrolysers (Stage I) contribute to the stabilization of the methanogenesis process (Stage II). The gas composition has been determined and its dynamics studied. The total methane yield in 14 days was 10950.73 ml, which is 181.03 ml per gram of organic matter.181.03 ml of methane and 80.42 ml of hydrogen released from 1 g of organic waste. It is shown that Uzbekistan has a big potential to produce biogas throw co-anaerobic digestion.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the paper.

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