Effect of Rotten Butter Shock Load on Anaerobic Digestion of Chicken Manure

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ABSTRACT

Anaerobic digestion is a popular method for improving fertilizing properties, but there is no report on the effect of shock load with butter on anaerobic digestion of chicken manure. Therefore, this study aimed to investigate the anaerobic digestion of chicken manure with butter addition. The volatile suspended solid (VSS) was set at 20g VSS/L with different butter additions from 0 to 60 g VSS/L and different oxygen flow rate (OFR) from 0 to 2.5 mL/h. The results showed that ammonia ranged from 0.072 g/L to 0.082 g/L, while the volatile acids ranged from 425 mg/L to 325 mg/L. The volatile organic acid was significantly influenced by a change in OFR compared to ammonia, while a correlation between hydrogen and hydrogen sulfide was observed. The results showed that the highest hydrogen and methane production was obtained at butter addition of 30 g VSS/L with OFR 1.4 mL/h with volumes of 78 mL and 25 L respectively. In addition, hydrogen sulfide emissions induced rapid growth with increase in butter concentration.

Keywords: Anaerobic digestion; butter; chicken manure; dark fermentation

INTRODUCTION

The annual chicken manure production in Poland is approximately 105.5×10^6 Mg (Domaszewicz *et al.*, 2016), and represents a large part of wastes, which needs proper utilization and management for reuse. Chicken manure is rich in ammonia and water content (Suchowska-Kisielewicz, 2016), it is denser compared to cow dung and therefore possesses more volatile acids and phosphate. Meanwhile, anaerobic digestion (AD) of chicken manure has great potential as an economical substrate compared to pig manure. Pig breeding apart from meat yields no profits (Theuerl *et al.*, 2019), conversely, poultry also produces eggs (from living), which is remarkable when methane production is not profitable enough (Fagbohungbe *et al.*, 2019). Furthermore, Alfa *et al.* (2014), reported that chicken manure produces less biogas than cow dung but more than lemongrass, hence, it is a potential source of methane and hydrogen but is inhibited by large volumes of ammonia in the anaerobic digestion (AD) process (Alsouleman *et al.*, 2016;). Chicken manure is usually pretreated by the bird gastric system with other plant grains and insects (Chodová & Tůmová, 2020), meanwhile, anaerobic digestion is a popular method for improving its fertilizing properties (Ogundijo *et al.*, 2017). Billen *et al.* (2015) proposed an approach for the production of electricity from manure and the several methods involved include dilution of water chemicals and thermal reduction of ammonia. Also, Lami *et al.* (2016) tested the thermal and alkaline (CaO) pretreatment of chicken manure mixed with orange peels in ratio 3:1 and obtained up to 75% thermal pretreatment reduction of the substrate and 1091.67 mL of biogas at 80 °C

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while the CaO pretreatment obtained 1124mL biogas. Anjumet *et al*. (2016) used thermal treatment at 60 °C, while Janczak *et al*. (2017) added biochar to reduce ammonia emissions. Furthermore, Abouleinen *et al*. (2009) found that a high level of ammonia ranging from 8 g^{-N} kg^{-1} CM to 14 g^{-N} kg^{-1}CM did not interfere with the anaerobic digestion process. In the dark fermentation (DF), fat as glycerol is considered as a material that has great potential to produce hydrogen (Słupek *et al*., 2019), but with particularly isolated bacteria (Trchounian & Trchounian, 2015). Ammonia emission and shifting process to basic conditions block the potential of chicken manure to produce hydrogen by DF in the acidophilic process (Tang *et al*., 2008). Another modification is the use of shock load for enhancing biogas production, meanwhile, food wastes industries produce fats as a kind of wastes, when fat wastes are provided into the sewage waste biogas plants, there is an immense problem of foaming. However, there are no previous reports on the effect of shock load with butter for chicken manure. The butter is an example of daily fat wastes, that often becomes rancid and is easily wasted due to the short expiry date and regular overproduction. The fats digestion usually reaches high efficiency through AD (García and Cammarota, 2019). Therefore, there is a need to determine the mixture of substrates related to fats and AD availability. Fat wastes have been reportedly improved in a ratio of 1:8 digestion of cow manure (Sandriaty *et al*., 2018). Bacteria (also shock load) usually inhibit methane production (Bundhoo *et al*., 2015). Another problem with the process is hydrogen sulfide emission, specifically for chicken manure (Khoshnevisan *et al*., 2017). However, there are no reported dependencies on hydrogen sulfide with other gases present in biogas plants. The observance of biogas components dependence is crucial to eliminate or limit hydrogen sulfide emission. A study examined butter addition as a stressing agent by shock-loading in wheat straw (Kaparaju *et al*., 2009). There is a need to also consider the effect of micro-aeration on the mixed substrate, similar to monosubstrate (Sołowski *et al*., 2019) given that it is beneficial for anaerobic digestion (Pokorna-Krayzelova *et al*., 2018), and dark fermentation (Sołowski *et al*., 2019). However, there are no reports on the influence of micro-aeration on denitrification. Volatile acid control by micro-aeration had been carried out on monosubstrate (Nguyen and Khanal, 2018). Therefore, there is a need to determine the effect of the mixture either towards AD predominantly or rather to DF. In this study, the relationships between microaeration with AD to denitrification and volatile acid reduction were tested. Meanwhile, volatile acids are byproducts that increase the profitability of biogas plants (Atasoy and Cetecioglu, 2020), and are easily formed in dark fermentation compared to anaerobic digestion (Hitit *et al*., 2017). This study also aims to identify when the shock load shifts the process for hydrogen or methane production digestion. In addition, it compared the influence of vegetal fat (butter) addition from 10 g VSS/L to 60 g VSS/L on chicken manure concentration of 20 g VSS/L to the anaerobic digestion process.

MATERIALS AND METHODS

Materials

The inoculum was collected from an agriculture biogas plant in a mesophilic range of temperature, mainly on maize silage, stored for about two weeks to minimize biogas production, and sieved before use to remove large particles.

The chicken manure was collected from one of the broiler poultry farms located in the Pomeranian district, Poland. The bedding for poultry farming was wheat straw and the broilers were fed with corn seeds. After the collection, the substrate was stored at -18 °C, kept at ambient temperature a day before the experiment, and was then applied for methane fermentation.

Table 1. Characteristics of inoculum and substrates

Material	pH	TS [%FM]	VSS [%TS]
Inoculum	8.2	1.09±0.03	45.35±1.03
Chicken manure 20 g VSS/L	7.8	3.10±0.03	51.00±1.06
Chicken manure 20 g VSS/L and 10 g VSS/L of butter	7.9	2.60±0.02	47.00±1.20
Chicken manure 20 g VSS/L and 20 g VSS/L of butter	7.8	2.70±0.02	49.00±1.11
Chicken manure 20 g VSS/L and 30 g VSS/L of butter	7.8	3.4± 0.02	51.20±1.03
Chicken manure 20 g VSS/L and 40 g VSS/L of butter	7.83	5.7± 0.02	53.00±1.11
Chicken manure 20 g VSS/L and 45 g VSS/L of butter	7.8	1.8± 0.02	47.00±1.14
Chicken manure 20 g VSS/L and 60 g VSS/L of butter	7.9	5.7± 0.02	52.00±1.11

The fat used was rotten butter from the restaurant '3 Smaki' in Gdańsk with concentrations from 0 to 60 g VSS/L added with 20 g VSS/L of manure as shown in table 1. The samples were centrifuged (5000 rpm for 10 minutes) to separate liquid and solid phase. A solid fraction of pretreated chicken manure (P-CM) was employed during experiments.

The amount of fresh matter (FM) for the inoculum and substrate total solids (TS) [%FM] and volatile solids (VSS) [%TS] were determined according to Standard Methods (Moriarty, 2013;) (Table 1).

Measurements for each substrate and inoculum are needed to determine the right amounts of VTS in each fermentor and biogas efficiency production of the substrates in the units m^3/Mg FM, TS, and VSS.Ammonia cuvette tests (Hach, UK) were used to determine mg NH_4^- N/L, meanwhile, samples of liquids were filtered before the analyses with 0.45 µm membrane syringe-filter (Pureland). The error of measurement was±0.01 mL

To determine the concentration of volatile organic acid, the FOS Nordmann-method was used (Nordmann, 1977). 100 mL of the sample was centrifuged (5000 rpm for 10 minutes) and. 20 mL of supernatant was titrated to pH=4.4 (FOS) with sulfuric acid (0.1 M) (Chemland, 98%) with continuous stirring. The error of measurement was ±0.01 mL.

Equipment

The experiments were carried out in the Laboratory of Biomass Energy Transformation at the Szewalski Institute of Fluid–Flow Machinery, Polish Academy of Science in Gdańsk (Poland), while the procedures were performed according to the NREL norms for biogas production (Moriarty, 2013). Methane fermentations were carried out in 2000 mL glass reactors with a working load of 1200 mL and the tested substrates were placed in the reactors with inoculum. After closing, the reactors were purged with nitrogen for 5 minutes to remove oxygen and then micro-aerated twice per day using a syringe of 25 mL volume with error ± 0.1 mL and pressure of 1.29 atm, while the oxygen flow rates (OFR) ranged between 0 mL/h to 2.5 mL/h, Furthermore, the reactors were placed in a water bath with a temperature of 38±2°C to create appropriate conditions for mesophilic fermentation with a pH between 8.1 and 8.3 adjusted by solution of 0.1 M HCl (Chemland, 34%), and 0.1M NaOH (Chemland 100%). The process temperature was established at 38°C in line with the Darżyno plant and some glycerol fermentations (Toledo-Alarcón*et al.*, 2017). The biogas was collected in each digester in a cylindrical vessel filled with barrier liquid to prevent solubility. This system works on the principle of connected vessels, meanwhile, all the experiments were implemented in triplicate and the set-up is shown in Figure 1.

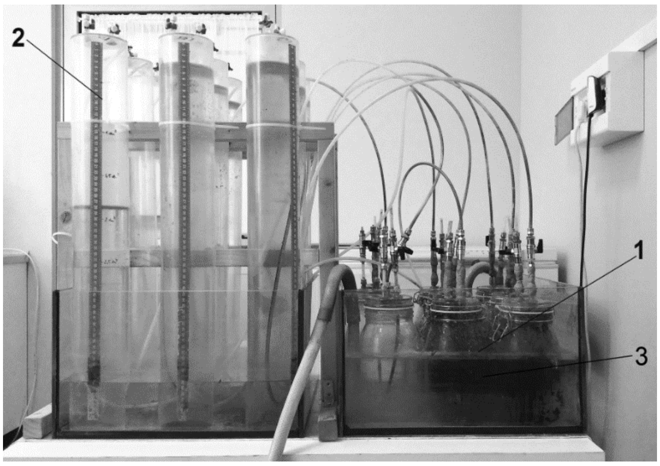

Figure 1. Fermentation set-up used in the experiment: 1. glass reactors, 2. cylindrical vessel for collecting biogas, 3. water bath chamber under mesophilic conditions (38±2 °C)

Batch experiments were continued until daily biogas production was less than 1% of the total. Furthermore, volumes of measured biogas were normalized to standard conditions (0° Cand 1.013 bar) using Equation (1) where: V_s is the volume of measured gas at standard temperature and pressure, V_m is the volume of measured gas at ambient conditions, T_m is ambient temperature, T_s is a standard temperature, and P_s is standard pressure.

$$V_s = \frac{V_m \cdot T_s \cdot P_m}{T_m \cdot P_s} \quad (1)$$

The qualitative and quantitative assessments of the gases were performed in two stages. First, the gas was assessed using a portable biogas analyzer (GA5000, Geotech), with a minimum volume of 0.45 dm^3. The analyzer used was ATEXII2G Ex ib IIAT1 Gb (Ta= -10 °C to +50 °C), IECEx, CSA quality, and UKAS ISO 17025 calibration certificate. Biogas measurements were carried out every day at the same time with an accuracy of ±0.0001 dm^3. The equipment was used to measure CH_4, CO_2, O_2, H_2, and H_2S in the ranges of 0-100%, 0-100%, 0-25%, 0-1000 ppm, and 0-5000 ppm, respectively. Meanwhile, during the second stage, when hydrogen concentration was above 1000 ppm, the gas was assessed using a gas chromatograph (GC) GC SRI 8060, with a thermal conductivity detector (SRI) and argon as a carrier (gas flow rate was 0.6mL/h). A Silco packed column Restek® with characteristics of 2m/2mm ID 1/8" OD Silica was used, while the detector temperature ranged between 46 °C and 196 °C. Furthermore, the oven was set at a temperature of 23 °C to 200 °C, while the injection temperature

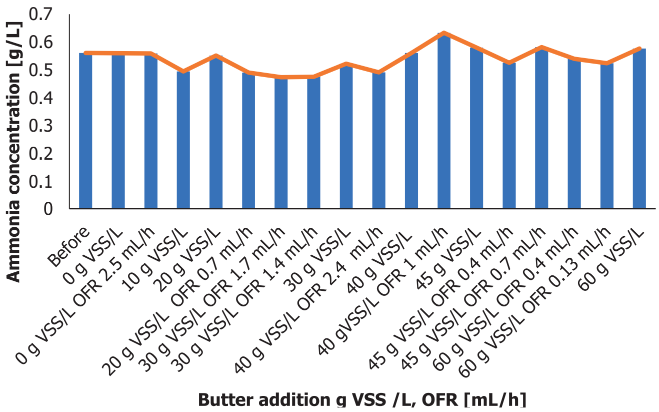

Figure 2. Ammonia concentration before fermentation and after fermentation at different concentrations of butter and micro-aeration rates

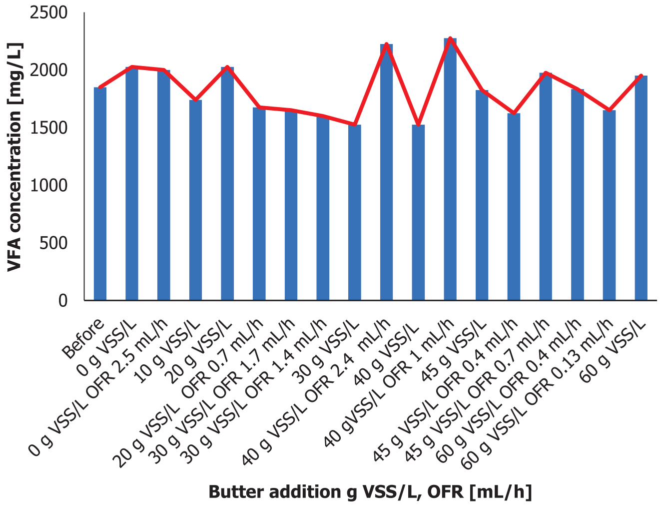

Figure 3. Volatile fatty acids (VFA) concentration before and after fermentation at different concentrations of butter and micro-aeration rates

(splitless mode) was 45 °C. Calibrations of the devices were performed twice a week, meanwhile, oxygen and nitrogen were assumed as ballast gases and were not considered as biogas.

RESULT AND DISCUSSION

Ammonia Production

Figure 3 shows that the ammonia change between butter addition from 0 g VSS/L and 60 g VSS/L were significant, but minimal in 0 g VSS/L and OFR 0 mL/h, while two times higher decrease was found at OFR 2.5 mL/h. Furthermore, a reduction was observed in the range of butter addition from 0 g VSS/L to 40 g VSS/L. From this concentration, ammonia reduction or increase depended on micro-aeration, for the butter concentration of 20 g VSS/L to 30 g VSS/L, microaeration improved denitrification, meanwhile, anaerobic conditions were better at 40 g VSS/L compared to OFR 2.4 mL/h microaeration. In the 40 g VSS/L butter and OFR 1 mL/h treatment, there was a significant increase in ammonia, meanwhile, for 45 g VSS/L butter, only OFR 0.4 mL/h caused a reduction of ammonia. In contrast, for butter addition of 60 g VSS/L, microaeration improved reduction while anaerobic condition caused an increase. The butter addition 10 g VSS/L to 30 g VSS/L in this study was more efficient compared to (Budych-Gorzna *et al.*, 2016).

Volatile Organic Acids

The volatile acid reduction which occurred in the range of butter addition of 20 g VSS/L with OFR 0.7 mL/h up to 40 g VSS/L was strictly anaerobic. Similarly, 60 g VSS/L under microaerated conditions decreased volatile organic acid, while anaerobic conditions increased this value. In the strictly anaerobic conditions with 60 g VSS/L, 45 g VSS/L, and 20 g VSS/L, the volatile acid concentration increased, but there was a reduction in the 10 g/L. Furthermore, in the other strictly anaerobic cases (OFR 0 mL/h), the concentration of volatile acids decreased. Therefore, the increase or decrease in VOC depends on micro-aeration and the mass of added butter. The most significant concentration of volatile organic acids adjustment (butyric, propionic, and acetic acids) occurred at 40 g VSS/L. These findings in line with dark fermentation reports suggest a significant increase in hydrogen production (Dreschke *et al.*, 2018). However, Figure 4 with 9 show that the results obtained were not consistent with the principal rule. With butter addition of 30 g VSS/L, micro-aeration increased, while volatile acid concentration decreased, meanwhile, hydrogen production was higher in OFR 1.4 mL/h compared to 1.7 mL/h. This shows that micro-aeration decreased volatile acids production in higher range of OFR compared to hydrogen production. Therefore, it is a control parameter in the case of organic acid production such as in butyric acid fermentation (Atasoy *et al.*, 2018).

Methane Production

The differences between methane productions from 0 g VSS/L to 60 g VSS/L were significant as indicated by Figures 4A and 4B which show accumulated methane volumes up to 3.7 L. Microaeration correlated with an increase in the growth of initial concentration and enhanced accumulated methane production as observed in samples of 0 to 40 g VSS/L in line with a previous study (Sołowski *et al.*, 2020). The limiting load of butter,

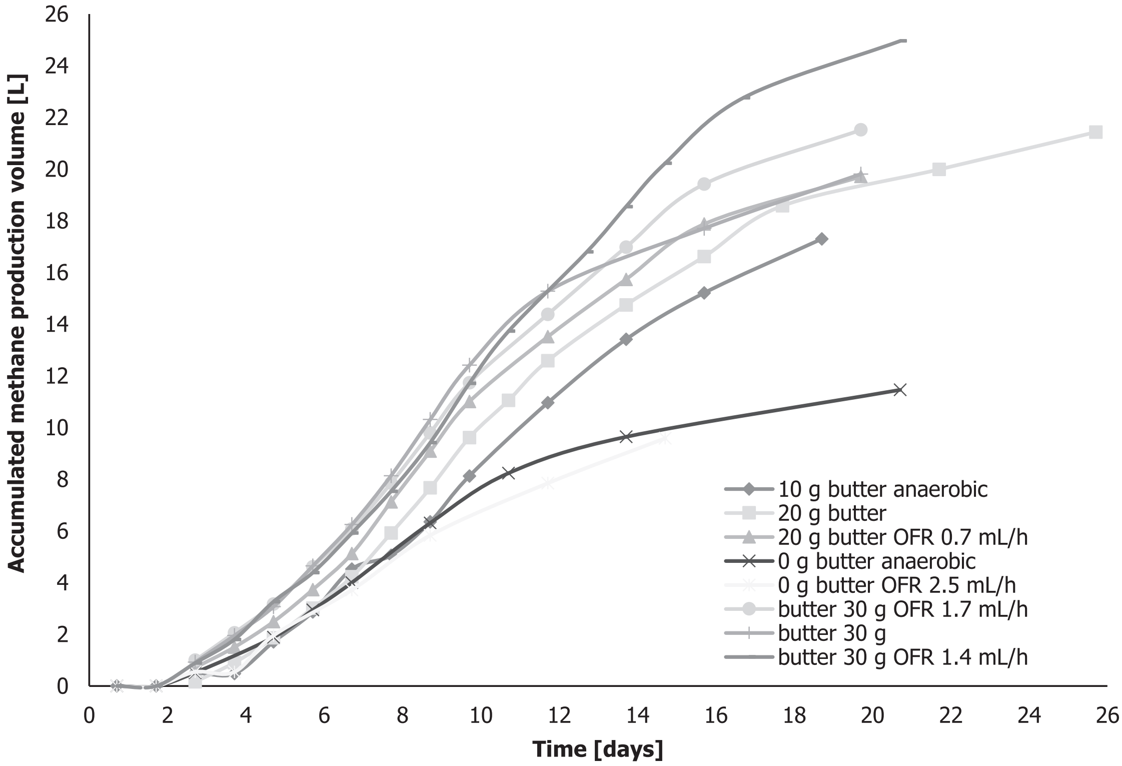

Figure 4A. Accumulated methane production from chicken manure of 20 g VSS/L from different butter loads g VSS/L and oxygen flow rates

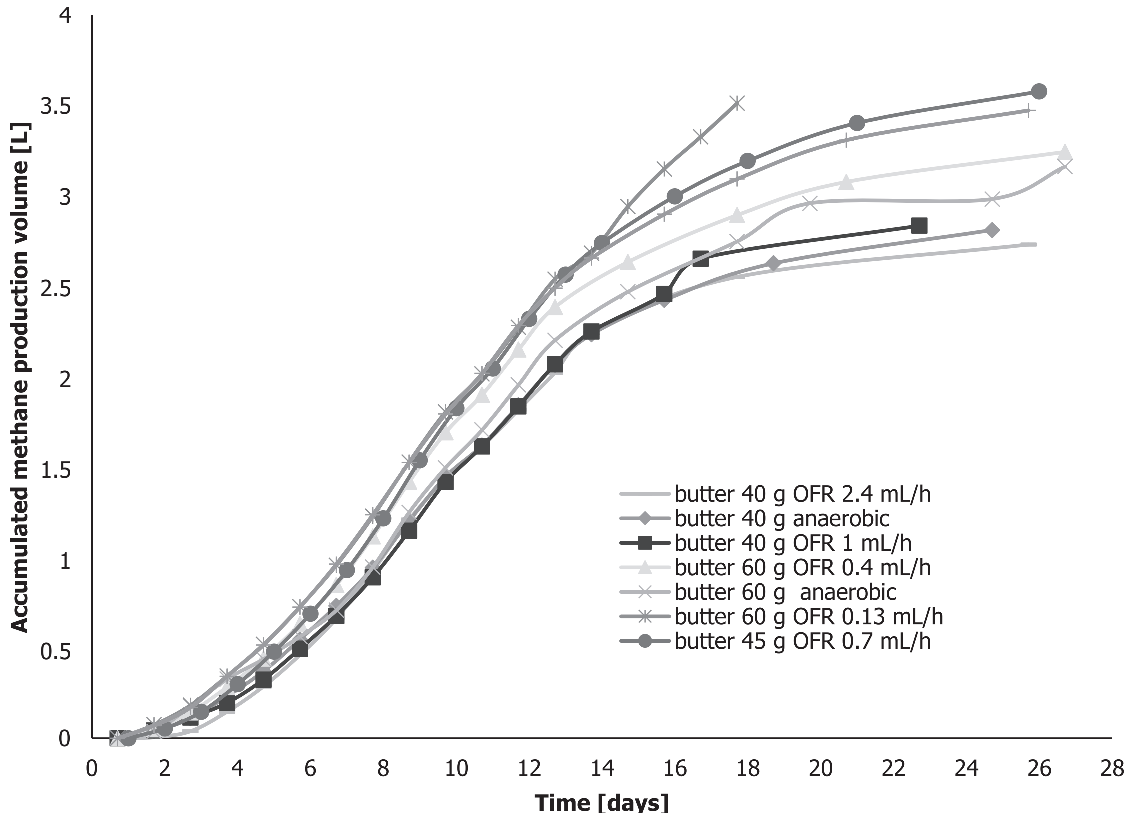

Figure 4B. Accumulated methane production from chicken manure of 20 g VSS/L from different butter loads g VSS/L and oxygen flow rates

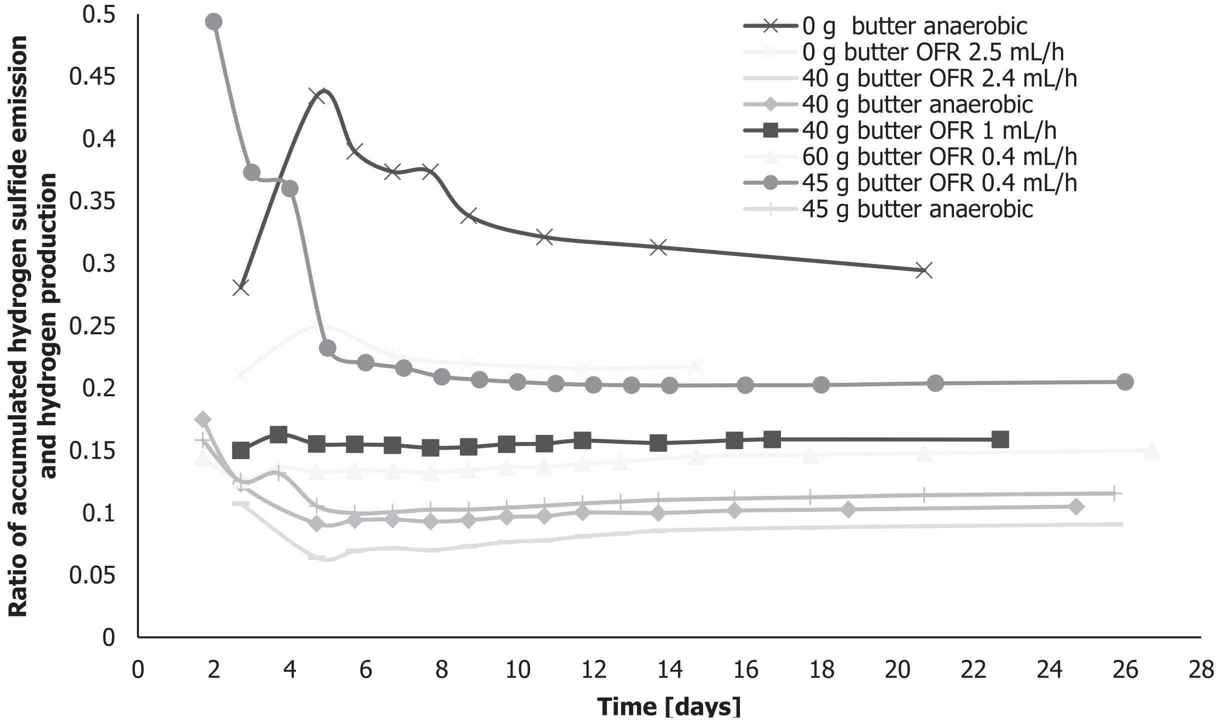

Figure 5A. The ratio of accumulated hydrogen sulfide emission and hydrogen production from chicken manure at different butter loads and oxygen flow rates

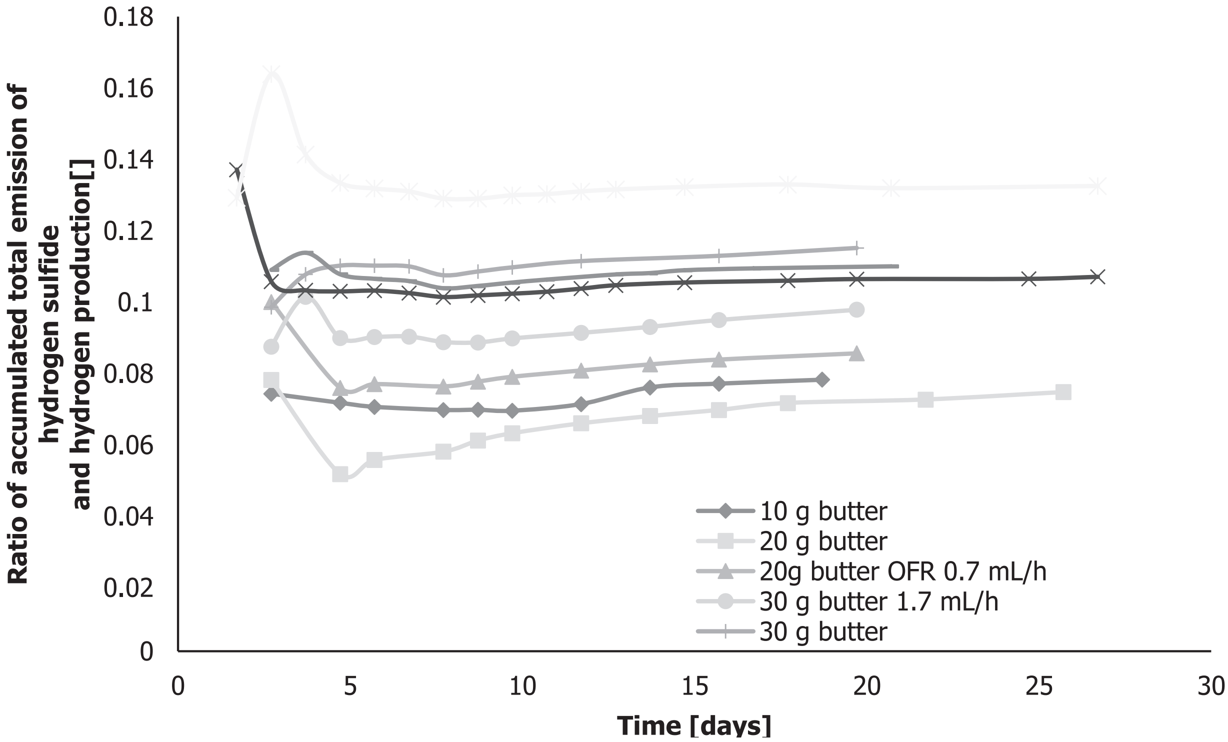

Figure 5B. The ratio of accumulated total emission of hydrogen sulfide and hydrogen production from chicken manure at different butter loads and oxygen flow rates

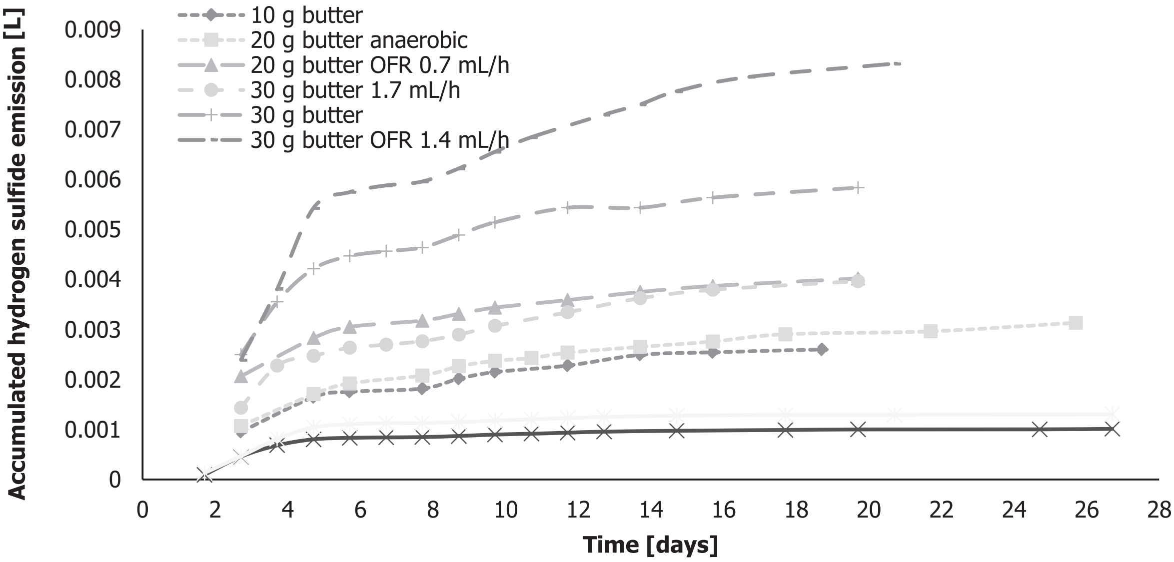

Figure 6A. Accumulated hydrogen sulfide emission from chicken manure different butter loads and oxygen flow rates

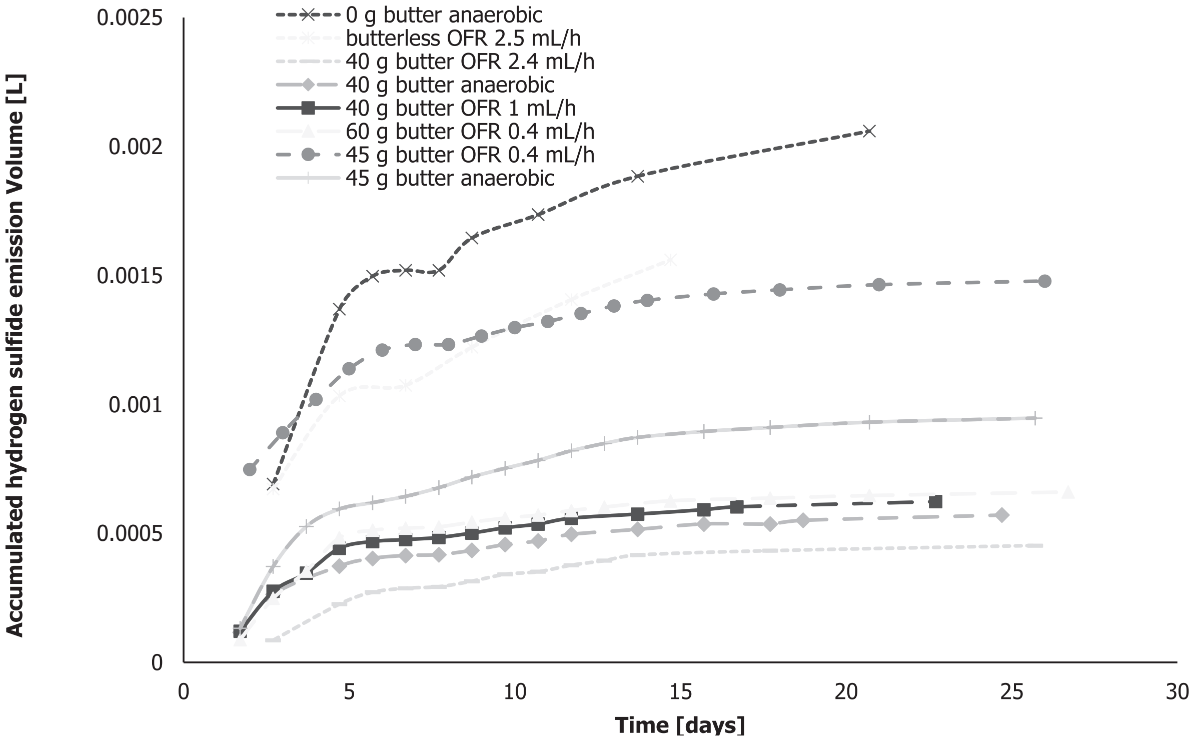

Figure 6B. Accumulated hydrogen sulfide emission from chicken manure of 20g VSS/L at different butter loads g VSS/L and oxygen flow rates.

which improved the efficiency of methane production was determined as 30 g VSS/L. At the first range namely butter concentration between 10 g VSS/L and 30 g VSS/L (Figure 4A), methane production increased compared to 0 g VSS/L chicken manure anaerobic digestion. Furthermore, methane production decreased rapidly above the addition of 30 g VSS/L, almost 4 times higher than the value at 60g VSS/L (Figure 4B), 3 to 8 times higher than the highest volume at 60 g VSS/L (anaerobic and OFR 0.13 mL/h), 10 times at 40 g VSS/L

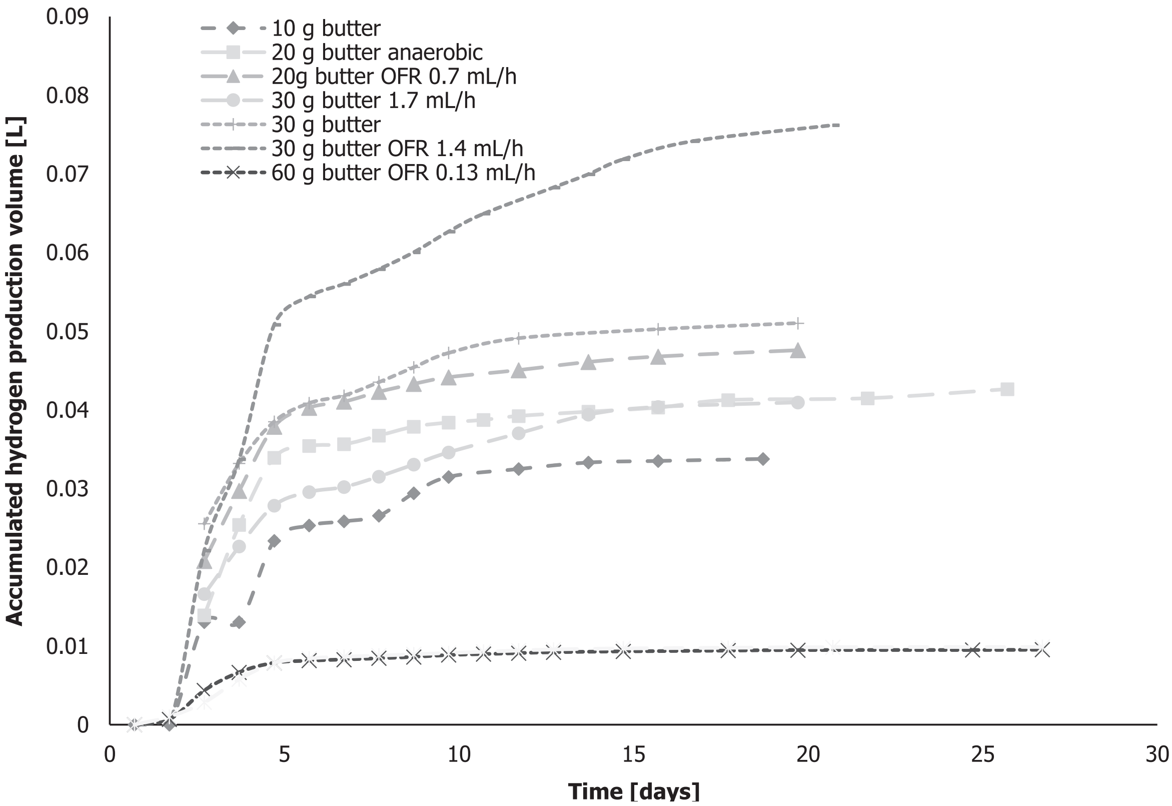

Figure 7A. Accumulated hydrogen production from chicken manure of 20 g VSS/L from different butter loads g VSS/L and oxygen flow rates

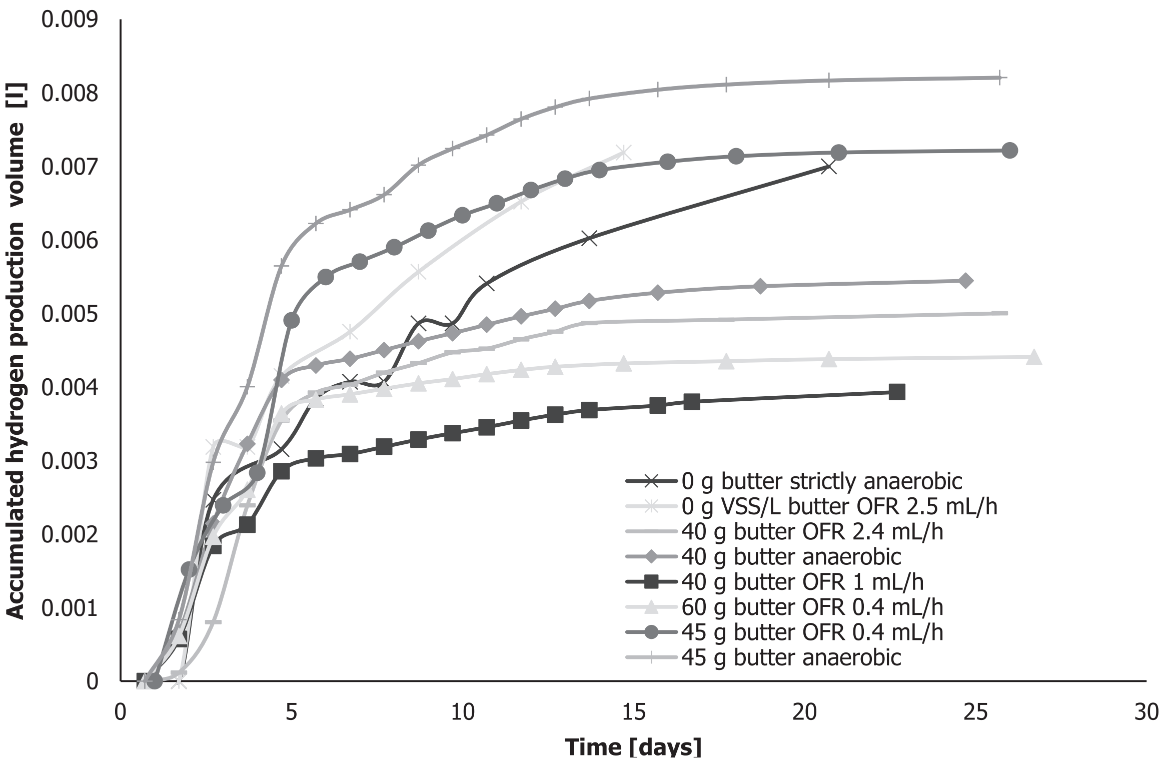

Figure 7B. Accumulated hydrogen production from chicken manure of 20g VSS /L from different butter loads g VSS/L and oxygen flow rates.

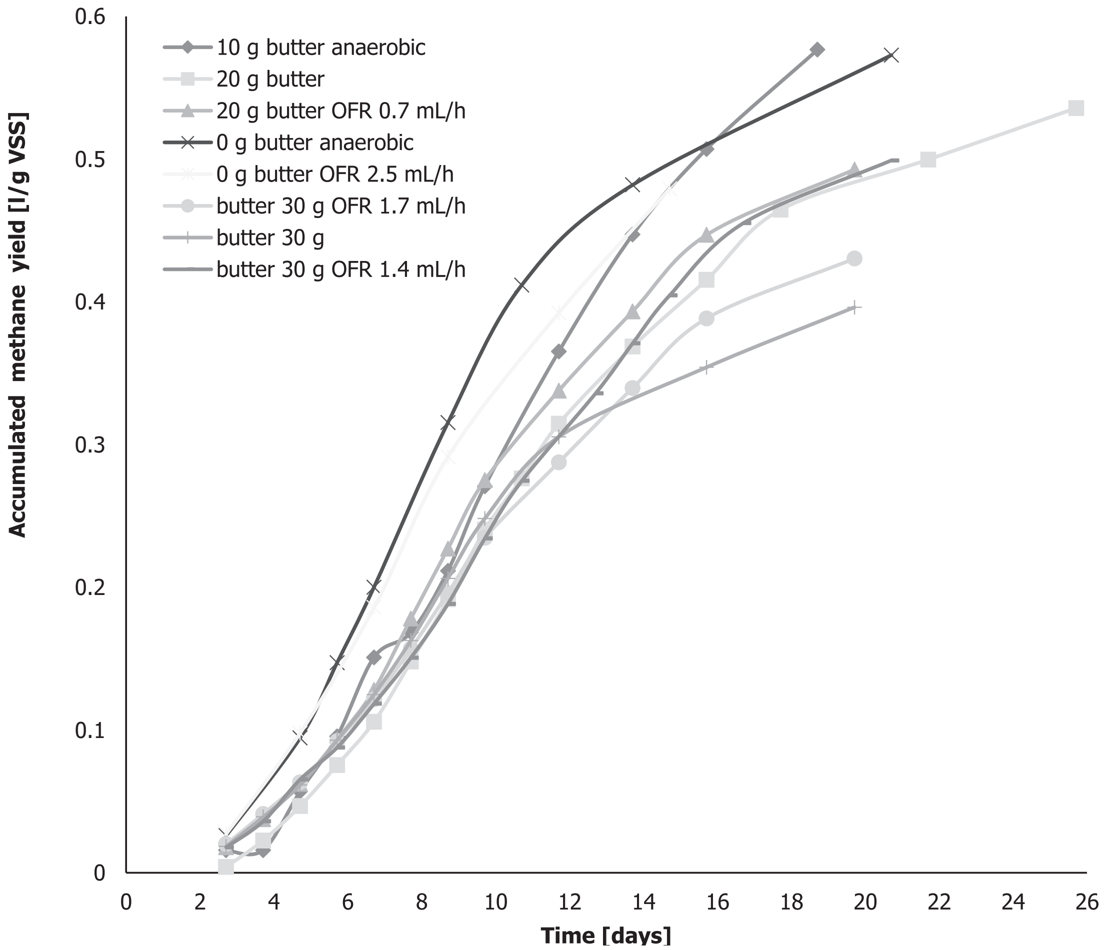

Figure 8A. Methane Yield from chicken manure of 20 g VSS/L from different butter loads g VSS/L and oxygen flow rates.

and OFR 2.4 mL/h, up to 250 times at 60 g VSS/L and OFR 0.4 mL/h. Moreover, 20 g VSS/L butter addition in strictly anaerobic conditions had 6 times higher biogas production similar to methane volume with 30 g VSS/L and OFR 1.7 mL/h. The high methane production at OFR 1.4 mL/h butter 30 g VSS/L (25 L of methane) correlated with the reduction of volatile acids and ammonia. This was due to the high conversion of volatile acids to methane, while the process is enhanced by the reduction of ammonia (Andriani, *et al*. 2014). In the 40g VSS/L and 1mL/h, methane production decreased due to an increase in ammonia concentration. The addition of butter under strictly anaerobic conditions prolonged methane production by supplying more substrate but did not improve yield as shown Table 2.

Hydrogen Production Versus Hydrogen Sulfide Emission

The gas analyzer showed the presence of hydrogen, methane, carbon dioxide, oxygen, nitrogen, and hydrogen sulfide. The hydrogen sulfide emission was lower than hydrogen production at a stable level under strict anaerobic condition at 0 g VSS/L (Figure 6A and 7A), but increased with hydrogen production enhancement and the ratio was then stabilized after 5 days. After this period, the total hydrogen/hydrogen sulfide (Figures 5A and 5 B) ratio did not change due to micro-aeration and butter addition. Furthermore, the ratio was twice lower in butter addition from 10 g VSS/L to 30 g VSS/L compared to 0 g VSS/L and 40 g VSS/L with OFR 1 mL/h, meanwhile, this was caused by an increase in volatile acids concentration (figure 3). At 40 g VSS/L, the smallest ratio was observed with a higher increase in ammonia (Figure 2). This showed that efficient hydrogen production correlates with a low-level of volatile acids and ammonia.

Figures 6 to 7 show that the decrease in volatile organic acid in the butter addition of 30 g VSS/L, was higher than hydrogen production. These results showed optimum values of butter addition (10g VSS/L - 30 g

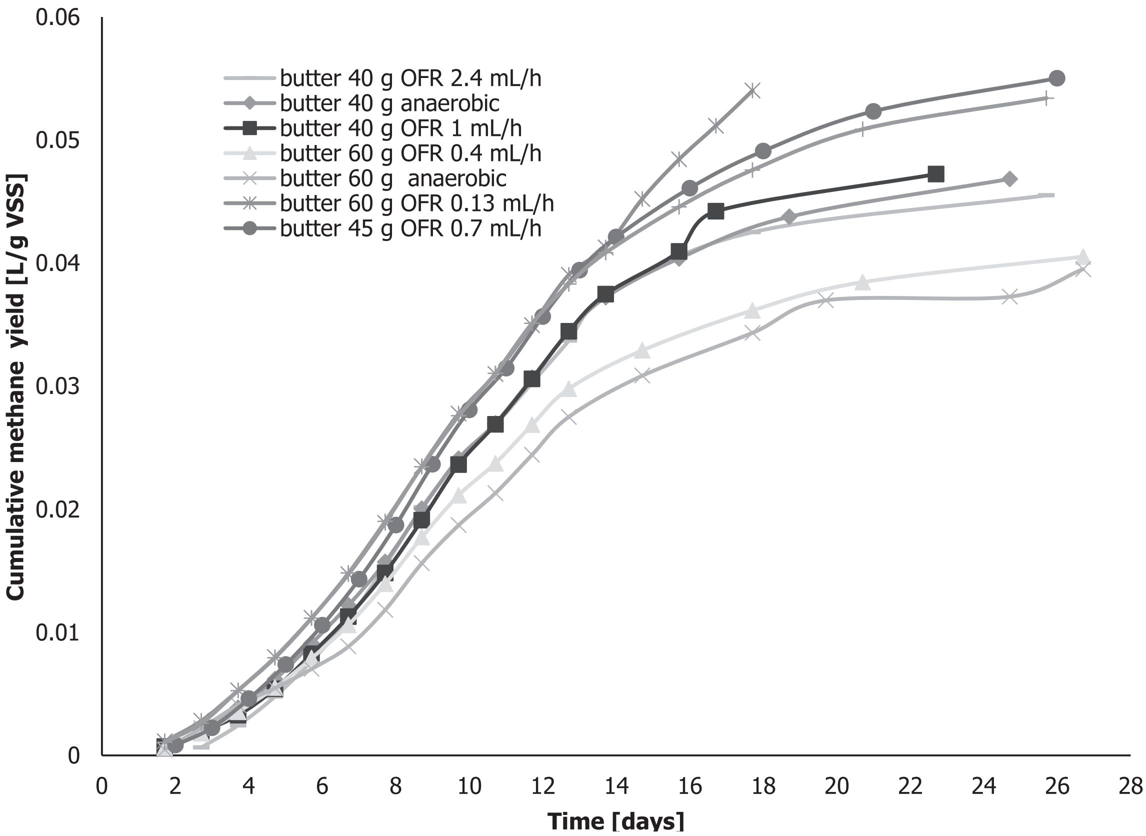

Figure 8B. Methane yield from chicken manure of 20 g VSS/L ats different butter loads g VSS/L and oxygen flow rates

VSS/L) to manure, with high hydrogen and methane production followed by ammonia and volatile acids reduction. The hydrogen production was slow up to 60 g VSS/L, where a slight increase was observed along with methane. At 30 g VSS/L and OFR 1.4 mL/h, the biogas mixture contained 60 to 74 % methane, and 0.01 % to 3 % of hydrogen, while other treatments contained 60 to 70 % methane and 0.5 to 3 % of hydrogen. The hydrogen obtained is not converted in acetogenesis from the acidogenesis (DF) part. Based on the results, hydrogen production from chicken manure with or without butter correlates (Figure 7A and 7B) with hydrogen sulfide emission (Figure 6A and 6B).

A previous study showed that bacteria during hydrogen production decompose fats, sugars, and proteins (Edwiges *et al.*, 2018). The protein was produced from the bacterial fermentation of chicken manure, while the hydrogen sulfide was obtained from the decomposition of proteins containing methionine and cysteine amino acids. Furthermore, the growth trend of hydrogen sulfide was identical to that of hydrogen production. The butter addition from 0 g VSS/L to 45 g VSS/L caused an increase in hydrogen sulfide emission. At 40 g VSS/L, the hydrogen sulfide emission increased with significant changes in the volatile acids and ammonia concentration due to the decomposed proteins. Compared to 45 g VSS/L, the addition of 60 g VSS/L in micro-aeration cases showed an increase in the accumulated hydrogen production. In addition, hydrogen sulfide emission grew with an increase in the added butter, but the ratios between accumulated hydrogen and hydrogen sulfide were still stable. These trends were not observed in previous publications of dark fermentation (Mechery, *et al*, 2019) or anaerobic digestion (Gallipoli *et al*., 2020). This is because the majority of samples from AD and DF were only analyzed by gas chromatography (GC). In GC, both gases are observed periodically, but dependencies are not assumed. Simultaneous determination of hydrogen and hydrogen sulfide needs to combine different techniques of GC analysis.

Biogas Component

The overall discussion of results involving three biogas components is presented in Table 2. Based on the results, the trends of accumulated hydrogen and hydrogen sulfide volume differed significantly from methane This is shown by the high cumulative hydrogen and methane production which did not match with gas component yield. The methane yield decreased significantly with butter addition to chicken manure above 30 g VSS/L (Table 2). Meanwhile, the highest methane yield obtained in this study was smaller compared to (Budych-Gorzna *et al.*, 2016) but higher than fat wastes in (Rafieenia *et al.*, 2017). Furthermore, butter addition up to 30 g VSS/L was the limit after which methane yields significantly decreased 10 times compared to 0 g VSS/L. At this concentration, hydrogen production increases and then rapidly declines. There was no observed shock load in wheat straw (Karapaju *et al.*, 2009) but a rapid decrease of hydrogen yield was observed above this concentration. Moreover, methane yield slightly increased at 45 g VSS/L but then decreased rapidly. The micro-aeration growth reduced methane yield in most cases except at 45 g VSS/L with OFR 0.4 mL/h, while the most significant change in the hydrogen sulfide to hydrogen ratio occurred in the first 5 days with unstable growth of both components. This was then followed by a shift in the process from hydrogen to higher methane production (also called hydrogenotrophic AD) (Liu *et al.*, 2020). These results show that volatile acids and ammonia reduction stimulate hydrogen yield and inhibit the emission of hydrogen sulfide. The butter addition increased the decomposition of proteins, which led to higher emission of hydrogen sulfide. Although the methane production decreased, the overall yield increased up to 30 g VSS/L. With 30 g VSS/L and OFR 1.4 mL/h, the growth was similar for 16 days, this incidence of accumulated hydrogen is unusual with most cases of production (Yuan e*t al.*, 2019). Furthermore, the hydrogen sulfide emission trends were similar to hydrogen production but were 10 or more times lower. There was an increase with butter addition from 0 to 60 g VSS/L, while the optimal conditions for hydrogen production were 30 g VSS/L butter addition with OFR 1.4 mL/h. The methane and hydrogen production was 10 days longer than observed for wheat straw and sour cabbage (Sołowski *et al.*, 2020), while the micro-aeration reduced hydrogen sulfide emission (Pokorna-Krayzelova *et al.*, 2018). As shown in Table 2, the length of biogas production depends on the ratio of butter and chicken manure. For samples treated with only chicken manure, the biogas production lasted 15 days, meanwhile, with 20 g VSS and OFR 0.7, the duration was 25 days. In the butter addition from 0 to 30 g VSS/L the digestion occurred for 19 days, meanwhile, at 30 g VSS/L, the chicken manure to butter ratio of (2:3) and 1:2 (40 g VSS/L) prolonged the process to 21 and 23 days respectively. In addition, the 5 g VSS/L, and 45 g VSS/L treatment (ratio manure 1:2.5), enhanced the process to 26 days, while the 15 g VSS/L and 45 g VSS/L (ratio manure 1:2.5), reduced the process to 25 days. Methane Yields were compared in Figure 8A and 8B.

Figure 8A shows that the methane yield ranged from 396 mL CH_4/g VSS to 577 mL CH_4/g VSS and the highest was found in the butter addition 0 g VSS/L. These results were higher than: cow manure with food wastes (butter mixture, palm oil, meat, and margarine) with a ratio of 1:8 and 208.93 mL CH_4/g VSS (Sandriaty *et al.*, 2018); lipid waste 1.67 g VSS/L (tuna 7.5 %, butter 22.3%, apple 27%, banana 27 %, chicken breast 7.5%, bread 1.5%, pasta 1.5%, and minestrone soup 5.5%) of 257 mL CH_4 /g VSS; as well as protein waste 1.67 g VSS/L (tuna 31.1%, butter 5.5%, apple 7.85%, banana 7.85%, chicken breast 31.1%, bread 3.2 %, pasta 3.2%, minestrone soup 10.2%) of 350 mL CH_4 /g VSS (Rafieenia *et al.*, 2017). However, these results were lower than in the case of chicken manure with waste activated sludge 1:1 of 880 mL CH_4 /g VSS Budych-Gorzna *et al.*, 2016). Hydrogen yields ranged from 0.36 mL H_2 /g VSS to 1.54 H_2 mL/g VSS in reverse order compared to methane yields.

The hydrogen yields obtained in this study were higher than glycerol 15 gVSS/L -0.002 mL H_2/g VSS (Paillet *et al.*, 2019) but less than lipid waste 1.67 g VSS/L (tuna 7.5 % butter 22.3%, apple 27%, banana 27%, chicken breast 7.5%, bread 1.5%, pasta 1.5%, minestrone soup 5.5%) of 27.93 mL H_2 / g VSS, and protein waste 1.67 g VSS/L (tuna 31.1 % butter 5.5%, apple 7.85%, banana 7.85%, chicken breast 31.1%, bread 3.2 %, pasta 3.2%, minestrone soup 10.2%) 8.02 mL H_2/g VSS (Rafieenia *et al.*, 2017).

CONCLUSIONS

The addition of butter improves anaerobic digestion in concentration from 10 g VSS/L to 30 g VSS/L by increasing the accumulation of gases namely hydrogen and methane. The optimal and limit concentration for methane and hydrogen production ranges from 30 g VSS/L to 20 g VSS/L. Above this concentration, only an increase in hydrogen sulfide emission was observed with a decrease in methane and hydrogen production except for butter addition 60 g VSS/L. Furthermore, the optimal condition for the anaerobic digestion of the substrates was 20 g VSS/L of chicken manure with 30 g VSS/L butter, and oxygen flow rate 1.7 mL/h which produced 25 L of methane and 78 mL of hydrogen. This study showed that hydrogen sulfide emission is dependent on hydrogen production. Hydrogen sulfide emission increased with the addition of butter, while micro-aeration reduced ammonia and volatile organic acids appearance but improved the hydrogen and methane production. Moreover, butter addition up to 30 g VSS/L increased hydrogen yield along with hydrogen sulfide emission. Shock load using butter for chicken manure did not shift the process from methane to hydrogen production but lowered methane yield. The shift to hydrogen was observed with butter addition up to 30 g VSS/L but this phenomenon require further studies.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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