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EVALUATION OF BIOMETHANE YIELDS FROM HIGH-ENERGY ORGANIC WASTE AND SEWAGE SLUDGE: A PILOT STUDY FOR A WASTEWATER TREATMENT PLANT

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Abstract

The article describes a pilot study on a wastewater treatment plant operating a biogas plant (2.793 MW). The authors of the experiment used organic waste material, including: chicken fat with feathers (FF), molasses (M), glycerol (GL), raw sewage sludge (SS) and digested sewage sludge as an inoculum. The parameters of raw and digested sludge were compared, e.g. changes in the concentrations of ammonium nitrogen (N-NH4+), alkalinities, chemical oxygen demand (COD) and light metal ions. Potential biodegradation pathways for the organic waste used in the experiments were also proposed. The proposed sequences of chemical reactions are a useful tool for further biochemical analyses and for the mathematical modeling of anaerobic digestion.
The results showed that fat with feathers was the most valuable high-energy substrate as it gave a cumulative methane yield of 822 m^3/mg VS (VS – volatile solids). There were comparable values of cumulative methane yield from molasses (350 m^3/mg VS) and glycerol (342 m^3/mg VS), whereas sewage sludge gave the lowest yield (246 m^3/mg VS).

Key words: anaerobic digestion, biodegradation pathways, biomethane efficiency, high-energy waste, sewage sludge

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1. Introduction

The volume of wastewater generated around the world has increased significantly in the last decade. Simultaneously, the amounts of sewage sludge (SS) have grown and accounted for 35-50% of the current operating costs of wastewater treatment plants (Wim, 2008). Sludge mainly contains water, organic matter, degradable particles, and living microorganisms (Li et al., 2017). The disposal of sewage sludge follows stabilization to control its biological activity, minimize the release of harmful chemicals into the environment and reduce its odor. Aerobic and anaerobic digestion as well as composting are the most environmentally-friendly biological treatment techniques, thanks to which it is possible to neutralize and dispose of sewage sludge (Kumar et al., 2017; Mata-Alvarez et al., 2000).

Anaerobic digestion (AD) is one of the most technically mature and cost-effective sewage sludge treatment methods (Bacenetti et al., 2013; Bacenetti and Fiala, 2015; Pilarska et al., 2019b). The most

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important potential advantages of stabilization are methane generated in the process, lower volume of sludge requiring ultimate disposal, and a high rate of pathogen destruction (Gyenge et al., 2018). However, sewage sludge usually contains low concentrations of solids, so biogas yields are low as well (Pilarska et al., 2018, 2019a). Moreover, there are various microbiological interferences in the course of anaerobic digestion of sewage sludge, largely caused by the toxic effect of chemical compounds, including heavy metals. Heavy metals tend to bind with proteins, thus changing the properties of the cytoplasmic membrane and inactivating enzymes (Dong et al., 2013). Anaerobic co-digestion (AcoD) of sewage sludge with other organic substances leads to higher levels of solids in sewage while reducing the effect of inhibitory factors. This option is feasible and yields high volumes of biogas, enables dilution of toxic substances and guarantees stability of the process (Sosnowski et al., 2008). Sewage sludge can be digested in biogas plants after being mixed with a variety of waste (Fersiz et al., 2017; Lafittle and Forster, 2000; Sami and Luostarinen, 2010; Pilarska et al., 2019b). The most frequent problems arising in the course of digestion of organic waste with SS result from the accumulation of volatile fatty acids (VFA) (Chen and Cheng, 2008; Pilarska et al., 2018) and lowered pH in the system (Aymerich et al., 2013), as well as from the buffer capacity of sewage sludge (Fonoll et al., 2015). The pH of the medium is an important indicator monitoring the biochemical process, as it is a major limiting factor of the growth of microorganisms. The optimum pH for most anaerobic microorganisms is 6.8-7.6, except for hydrolytic bacteria, where the optimum range is 5-7 (Chen and Cheng, 2008). Although it is a good solution in methane production, the disposal of high-energy fat waste needs improvement.

Glycerol, molasses, and chicken fat with feathers were high-energy substrates added to sewage sludge in the experiment described in this article. Both glycerol and molasses are ranked among the most valuable high-energy raw materials because of their chemical structure and biodegradability. The results of many studies show that glycerol can be applied successfully, but a control strategy is necessary to adjust the amount added, avoid the risk of organic overloading, and maintain a stable digestion process (Athanasoulia et al., 2014; Nghiem et al., 2014). There are few reports on the use of molasses as a feed in biogas plants, even though the material gives very promising results as a substrate and is readily available on the market (Fang et al., 2011). Therefore, it is worthwhile to pursue this line of research. As far as lipids and lipid-rich waste are concerned, they are not commonly used as substrates in anaerobic digesters because they inhibit anaerobic biocenosis and cause frequent problems in operation (Silvestre et al., 2011).

The aim of this study was to analyze and compare the yields of biogas and biomethane from selected substrates, including raw sewage sludge. Digested sewage sludge was used in the experiment as the inoculum. Anaerobic batch reactors were used for laboratory investigations conducted at controlled (mesophilic) temperatures and pH. The authors also formulated a hypothesis concerning the reasons for the acidification of glycerol (during its biodegradation) and fat, which contains glycerol. The sequences of chemical reactions developed by the authors and used in this experiment are also a useful tool for further research.

2. Material and methods

2.1. Substrates and inoculum

Sewage sludge samples were acquired from the wastewater treatment plant in Poznań. The plant is operated by Aquanet S.A., a leading water supplier in Poland. Digested sewage sludge, used as the inoculum, was obtained from Aquanet's 2.793 MW biogas plant, which produces biogas from sewage sludge. Fat with feathers was provided by a poultry processing plant producing Vienna sausages and pies from chicken meat. Molasses made from sugar beets was provided by a sugar plant. Glycerol was acquired from an agrorefinery producing higher fatty acid methyl esters. All the manufacturers were seated in the Wielkopolska province. Tables 1 and 2 show the characteristics of the substrates used in this study.

2.2. Biogas production setup and procedure

The digestion mixture ratios were determined following the VDI 4630 guideline concerning the digestion of organic materials, characterization of substrates, sampling, collection of material data, as well as digestion tests. The guideline describes the conditions of efficient biogas production, including assumptions concerning the inoculum for fermenters operated on sewage sludge. The authors followed the guideline and data in reference publications. The content of total solids (TS) in the batch was kept at a maximum level of 10% in order to guarantee adequate mass transfers. The C:N ratio of the mixtures ranged from 10 to 30. Before digestion the pH of the mixture ranged from 6.8 to 7.5 (Parkin and Owen, 1986). Table 3 shows the compositions of the mixture and their selected parameters.

The production of biogas and the analyses of the biogas and methane yield were made according to the German standard DIN 38 414-S8: Bestimmung des Faulverhaltens (S8) Schlamm und Sedimente (Beuth Verlag GmbH, Berlin 1985). The methane digestion process was carried out in a multichamber biofermenter.

There were 15 digestion chambers in the tests. The author described the principle of operation of the biofermenter in another publication (Pilarska, 2018).

Following the VDI 4630 guideline for specific substrates, the experiment was continued until the daily biogas yield was below 1% of the total amount of biogas generated. The retention times of the material in the fermenter depended on a number of

factors (including the substrate type and process stability) and were different for each type of the substrate used in the study.

Table 1. The characteristics of chicken fat with feathers (FF), molasses (M), glycerol (GL), and raw sewage sludge (SS) used in the experiment

Indicator	*Unit*	*FF*	*M*	*GL*
TS	%	27.46	80.33	89.28
VS	% TS	98.23	85.67	99.98
pH	–	5.34	8.64	7.59
Conductivity	mS/cm	19.8	23.3	20.9
C/N ratio	–	40	46	91
C	% TS	71.31	41.60	36.53
N	% TS	1.79	0.91	0.40
$N\text{-}NH_4^+$	% TS	1.56	0.25	0.10
P_{total}	% TS	0.15	0.05	0.02
Alkalinity	mg $CaCO_3/dm^3$	424	168	46
COD	mg/dm^3	2458	1289	884

Table 2. The characteristics of the inoculum and raw sewage sludge (SS) used in the experiment

Indicator	*Unit*	*Inoculum*	*SS*
TS	%	3.46	4.59
VS	% (of TS)	71.53	79.78
pH	–	7.95	6.47
Conductivity	mS/cm	26.6	18.4
C/N ratio	–	11	10
C	% TS	33.06	40.90
N	% TS	2.95	4.10
$N\text{-}NH_4^+$	% TS	2.48	0.35
P_{total}	% TS	2.66	2.29
Alkalinity	mg $CaCO_3/dm^3$	4150	847
VFA	mg/dm^3	140	3900
COD	mg/dm^3	1750	3190
Light metal ions			
Mg	% TS	0.72	0.53
Ca	% TS	2.86	1.97
K	mg/kg	414	343
Heavy metals			
Ni	mg/kg TS	64.1	68.4
Cd	mg/kg TS	2.5	2.0
Cu	mg/kg TS	460	475
Pb	mg/kg TS	40.8	55.6
Zn	mg/kg TS	1070	1078
Cr	mg/kg TS	97.5	99.8
Hg	mg/kg TS	0.35	0.61

Table 3. The ratios and selected parameters of the digestion mixtures

	Substrate (g)	*Inoculum (g)*	*Mixture pH*	*Mixture C/N ratio*	*Mixture TS (%)*
FF	50	950	7.33	12	4.67
M	20	980	7.40	12	5.07
GL	15	985	7.90	12	4.76
SS	500	500	7.09	11	4.03

2.3. Analysis of substrates

Most of the analytical methods applicable to the substrates and gas generated in the experiment were standardized or modified for the purposes of the laboratory at the Institute of Biosystems Engineering.

The essential parameters of the substrates and inoculum (Tables 1 and 2) were analyzed in accordance with applicable standards and procedures, as presented by the author in earlier publications (Pilarska, 2018).

2.4. Analysis of gas samples

The volumes of gas generated were measured once in 24 hours. The gas volumes of at least 1 dm^3 were analyzed qualitatively, initially once a day and then once in three days - as lower gas volumes were generated.

The content of methane, carbon dioxide, hydrogen sulfide, ammonia, and oxygen was measured by means of IR absorption sensors and an electrochemical sensor line. The gas concentration was measured with Mg-72 and Mg-73 measurement devices from Alter S.A. The gas analyzer measures gas concentrations within the following ranges: 0-100% CH_4, 0-100% CO_2, 0-25% O_2, 0-2,000 ppm H_2S and 0-1,000 ppm NH_3. The gas concentration was measured as frequently as possible because the measurement range was so wide. The gas monitoring system was calibrated once a week by means of calibrating mixtures (Air Products) at the following concentrations: 65% CH_4, 35% CO_2 (in a single mixture) as well as 500 ppm H_2S and 100 ppm NH_3. Synthetic air with an O_2 content of 20% was used for the calibration of O_2.

2.5. Calculation of cumulative biogas and methane

Having finished the quantitative and qualitative analyses of the gas generated, the biogas yield per unit was assessed, i.e., m^3/t of organic dry matter. The biogas yield from a particular substrate was calculated by subtracting the quantity of biogas generated from the inoculum. The ratio of gas generated from the seeding sludge for the batches in the reactors containing the substrate mixtures or the reference substrate was calculated using the following Eq. (1):

$$V_{IS(corr.)} = \frac{\Sigma V_{IS} m_{IS}}{m_M} \quad (1)$$

where: $V_{IS(corr.)}$ – the volume of gas released from the seeding sludge (ml_N); ΣV_{IS} – the total volume of gas in the test, (ml_N); m_{IS} – the mass of the seeding sludge used for the mixture (g); and m_M – the mass of the seeding sludge used as the control, (g) (Pilarska et al., 2017).

The specific volume of digestion gas (V_S) produced from the substrate vs test duration was calculated from reading to reading using Eq (2):

$$V_S = \frac{\Sigma V_n 10^4}{m w_T w_V} \quad (2)$$

where: V_S – the specific volume of gas produced referred to the mass of loss on ignition (l_N/kgGV); ΣV_N – the net volume of gas produced from the substrate in a specific test time (ml_N); m – the mass of the weighed-in substrate or reference substrate (g); w_T – the dry residue of the sample (%) (Pilarska et al., 2017).

3. Results

3.1. Fat with feathers, molasses and glycerol – characterization

The fat with feathers (FF) used in the experiment had a relatively low level of total solids TS (27.46%) and a high level of volatile solids VS (98.23%), which resulted in potentially good yields of methane (Table 1). The chemical oxygen demand (COD) is a measure of the relative content of organic and inorganic compounds. The COD of FF (including protein as a component of feathers, with a high content of sulfur) was 2,458 mg/dm^3. It was greater than the COD of molasses (M) and glycerol (GL). The pH of fat is acidic, as reported in reference publications (Zhu et al., 2011) and confirmed by the authors of this study (pH=5.34). The pH value of fat is determined by its chemical structure – fat is an ester of glycerol and fatty acids, mainly triacylglycerols (Gunstone, 2004).

Molasses and glycerol – the other substrates used in the study – had high levels of TS and VS, as shown in Table 1. The presence of mineralized inorganic matter in molasses, such as alkaline earth metal ions, is indicated by its high conductivity (23.3 mS/cm), as compared with other substrates, and by a rather high pH value (8.64). If the content of Ca^{2+} ions in molasses is high, which happens frequently, the ions may inhibit digestion (Ahn et al., 2006). This undesirable effect did not occur in the molasses used in this study, which met the Polish standard PN-68/R-64772. Refined glycerol was also used in our study, especially to avoid any process disturbances that may have been caused by the presence of contaminants in crude glycerol. The data in Table 1 show that the concentration of toxic N-NH_4^+ in fat was low (1.56%), and it was slightly less toxic than in glycerol and molasses.

3.2. Comparison of digested and raw sludge parameters

As can be seen in Table 2, the inoculum (digested sludge) and raw sludge used in the experiment had lower TS content than the other substrates. There were also relatively low values of the C:N ratio of the inoculum and raw sewage sludge (SS). Interestingly, the inoculum exhibited rather high conductivity (26.6 mS/cm). This fact pointed to the content of mineral components, which may have favored the development and metabolism of anaerobic bacteria (Pilarska et al., 2019b; Wan et al., 2011). The authors observed that the levels of potassium, magnesium, and calcium in the inoculum were higher than in the raw sewage sludge.

The digested sludge had low content of organic carbon (as compared with the raw sludge), which confirmed its presence in the digestion products (CH_4, CO_2). After digestion the total nitrogen content also decreased from 4.10% (in the SS) to 2.95% (in the inoculum). Conversely, the content of ammonium nitrogen, which is known to inhibit methane digestion, was noticeably higher after digestion: 2.48% in the inoculum and 0.35% in the raw SS. It was caused by the denitrification of NO_3^- to ammonium nitrogen N–NH_4^+ (partial denitrification) or molecular nitrogen N_2 (total denitrification) as well as by ammonification (Marcinelli, 1996).

Changes in alkalinity are also interesting in the context of nitrogen conversions and general control of the methane digestion process. The data in Table 2 show that the digestion of sewage sludge resulted in a nearly fivefold increase in the value of alkalinity, from 847 mg/dm^3 to 4,150 mg/dm^3. The nitrates reduction process resulted in the formation of HCO_3^- ions. In consequence, there was an increase in alkalinity, which depends on the content of carbonates and bicarbonates. During denitrification bacteria use protons and electrons from organic carbon compounds. Equation (3) below shows the formation of HCO_3^- ions in a nitrate reduction with an acetate:

$$5CH_3OO^- + 8NO_3^- + 3H^+ \rightarrow 10HCO_3^- + 4N_2 + 4H_2O \quad (3)$$

3.3. Biodegradation of substrates

The substrates which contain organic material are biologically processed by microorganisms during methane digestion. Anaerobic digestion consists of four phases: hydrolysis, acidogenesis, acetogenesis, and methanation (Deublein and Steinhauser, 2011). The main objective of the methane digestion process is to maintain pH within the optimum range of 6.8-7.5, so it is necessary to select suitable compositions of the substrate/inoculum digestion mixtures, as reported in Table 3. During the first 7 days of the experiment there were various changes in the pH values of the substrates, as shown in Fig. 1. The authors used their own experience (Pilarska et al., 2016; Pilarska et al., 2019b), the observed changes in pH as well as information in reference publications (Deublein and Steinhauser, 2011) to suggest the potential pathways of biodegradation of the organic waste material used in the experiment. They were described by means of Eqs. (4)-(41). Chicken fat with feathers was the most complex of the compounds decomposed in the experiment. The biodegradation process was considered using the example of stearic acid triglyceride, which is the most abundant in animal fat (Gunstone, 2004).

Fats are decomposed by hydrolysis to glycerol $C_3H_8O_3$ and higher carboxylic acids (stearic acid $C_{17}H_{35}COOH$ was assumed in our study), as shown in equation 4. The next step is the acidogenic phase (acidogenesis), which enables large molecules of organic compounds to be divided into smaller ones,

including: ethyl alcohol, 2-oxopropanoic acid, 1,4-butanedioic acid, 2-hydroxypropanoic acid, propan-2-one (Eqs. 5-9). During the acetogenic phase (acetogenesis) these compounds are decomposed into ethanoic acid (Eqs. 12-16). Ethanoic acid and other products of acidogenesis, i.e. methyl alcohol (Eq. 10) and methanoic acid (Eq. 11), are converted into the final products of anaerobic digestion: CH_4, CO_2, H_2 and other gases (Eqs. 17-21).

The decomposition of glycerol - both as a product of hydrolysis of fats (Eq. 4) and as an autonomous substrate used in the biogas production process – gives two intermediate products in the first phase (Eq. 22): glyceric aldehyde (2,3-dihydroxypropanal) and dihydroxyacetone (1,3-dihydroxypropanone) (Lui and Greeley, 2011).

Glyceric aldehyde is first produced as a result of the decomposition of glycerol. It provides methyl aldehyde (formaldehyde), methanoic acid (formic acid) and methyl alcohol in the acidogenic phase, see reaction - Eq. (23) (Pilarska et al., 2017). The sugar dihydroxyacetone (DHA) has three carbon atoms and remains stable within pH of 4-6. At higher pH DHA is decomposed and methyl alcohol is formed (Eq. 24). The formation of this compound may be accompanied by destabilization of the anaerobic digestion process. It is manifested by a rapid decrease in pH to 5.5, as shown in Fig. 1.

The effect disturbs the production of methane and biogas, as can be seen in the diagrams in Figs. 2 and 3. The restoration of the optimum conditions for methanogenic bacteria (pH=7) guarantees stability of the process (Eqs. 25-29). Glycerol, which is a component of fat and one of the intermediate products of the decomposition of fats in hydrolysis (Eq. 4), acidifies the medium in the process of biodegradation of FF (Fig. 2). Changes in pH are much less pronounced because fat has a higher buffer capacity. Previous reports emphasized only the adverse effect of VFA - mostly propionic acid, sometimes isobutyric acid (Nielsen et al., 2007; Noutsopoulos et al., 2013), although methyl aldehyde and methanoic acid may have a similar or stronger acidification effect.

Saccharose, the main component of molasses, was assumed to be the initial chemical compound for its biodegradation. During hydrolysis saccharose is decomposed into simpler sugars: glucose and fructose (Eq. 30), which are further decomposed into simpler compounds: ethyl alcohol and propionic acid (Eqs. 31, 32). The course of successive phases - until the final products of methanogenesis are obtained - is similar to the phases described for the other substrates.

Assuming that sewage sludge predominantly contains the waste products of digestion excreted from the human body, according to the data in reference publications (Honda et al., 2002) and in the authors' opinion, it is mostly composed of water and fiber. Cellulose and hemicellulose, $(C_6H_{10}O_5)_n$, were selected as the most representative components of SS. At the initial phase both of these compounds are decomposed into glucose and hexose ($C_6H_{12}O_6$). The successive paths of biodegradation of the resulting chemical compounds are known: the formation of ethanoic acid, ethyl alcohol and, finally, the gas mixture $CH_4 + CO_2$.

3.4. Cumulative biogas and methane yield. Process stability

The analysis of the yield of biogas and methane showed that the highest volumes were obtained from the fresh matter (FM) of the following substrates (in descending order): glycerol (biogas – 464 m^3/Mg FM, methane – 306 m^3/Mg FM), molasses (biogas – 448 m^3/Mg FM, methane – 241 m^3/Mg FM), chicken fat with feathers (biogas – 310 m^3/Mg FM, methane – 221 m^3/Mg FM) and sewage sludge (biogas – only 15.2 m^3/Mg FM, methane – 7.4 m^3/Mg FM, because of the very low value of TS), see Fig 4a. On the other hand, the measurement of biogas yields in terms of VS (see Fig. 4b) enables comparison of the results with forecasts based on chemical reaction stoichiometries. In terms of VS, the highest volumes of biogas and methane were obtained in the anaerobic digestion of FF (biogas – 1,149 m^3/Mg VS, methane – 822 m^3/Mg VS), as shown in Fig. 4b. This resulted from the highest number of C atoms per fat molecule. The diagram of the pH profiles of the substrates (Fig. 1) shows that the pH value of chicken fat with feathers decreased from 7.4 to 6.8 until Day 3. As a rule, the process of fat digestion in the experiment was relatively stable and its duration was short (26 days).

Molasses was the next most valuable high-energy substrate providing 650 m^3/Mg VS of biogas and 350 m^3/Mg VS of methane. The digestion of molasses proceeded without disturbances. After the first two days, when the pH decreased to 6.2 (Fig. 1), it increased to 7.2 in the next 9 days (on Day 9). Stable biogas production continued until Day 20, when the substrate was completely gasified, as confirmed by the shape of the curves in Figs. 3a, b. The cumulative yield of methane from glycerol (342 m^3/Mg VS) was similar to the yield obtained from molasses, whereas the cumulative biogas yield was much lower (521 m^3/Mg VS). As shown in Fig. 3a, b, the process of biogas production from glycerol became stable only after 20 days, after pH stabilized at 7.5. As described in Section 3.4, earlier stages of the process were considerably disturbed by rapid acidification of the medium (pH=5.5) between Days 3 and 7 of the digestion process (see the diagram in Fig. 1). On Day 8 the conditions were restored to enable the growth and activity of methanogenic bacteria. The acidification of the medium resulted in extended biogasification time of the GL sample to 38 days. Raw SS gave the lowest yields in terms of VS (biogas – 415 m^3/Mg VS, methane – 246 m^3/Mg VS). The results may largely depend on the chemical composition of wastewater, i.e. the compounds that inhibit AD, for instance heavy metals, disinfectants, antibiotics, which inactivate and destroy bacterial flora. There were no undesirable changes in pH during the anaerobic digestion of sewage sludge (Fig. 1) and the biogas production process remained stable (Fig. 3 a, b).

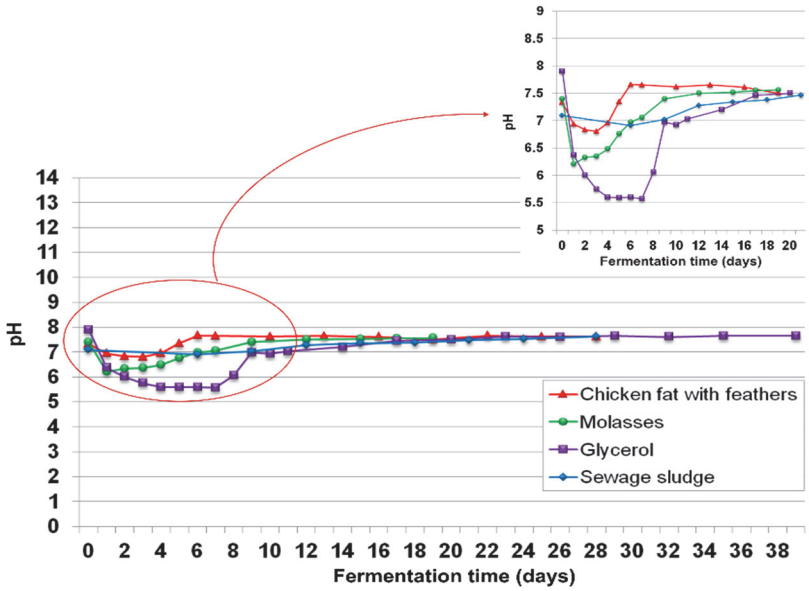


Fig. 1. pH variation profiles of digested substrates: FF, M, GL and SS

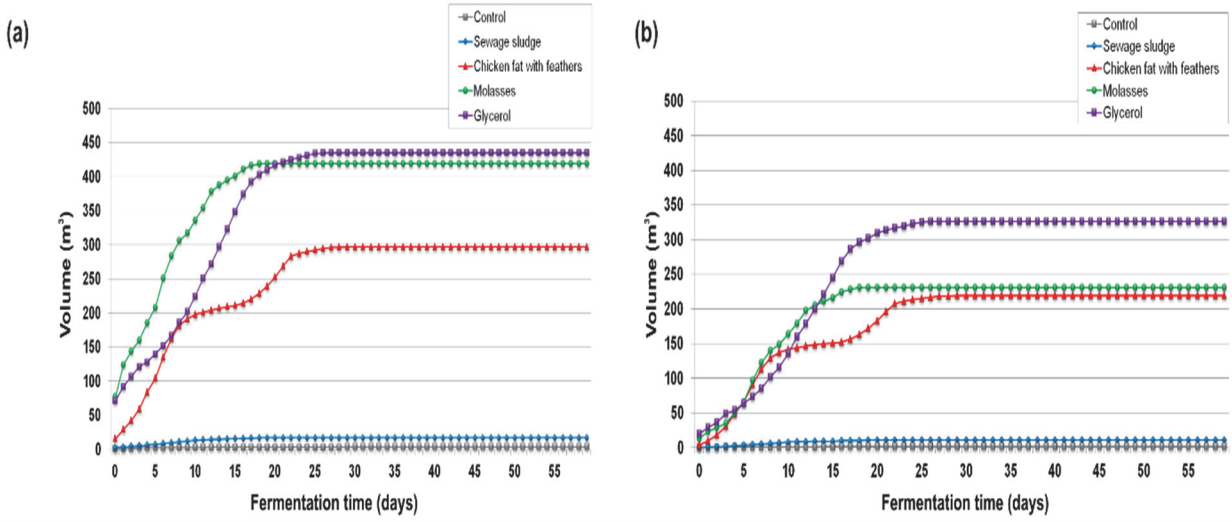


Fig. 2. Cumulative production of (a) biogas and (b) methane from fresh matter of FF, M, GL, SS and control

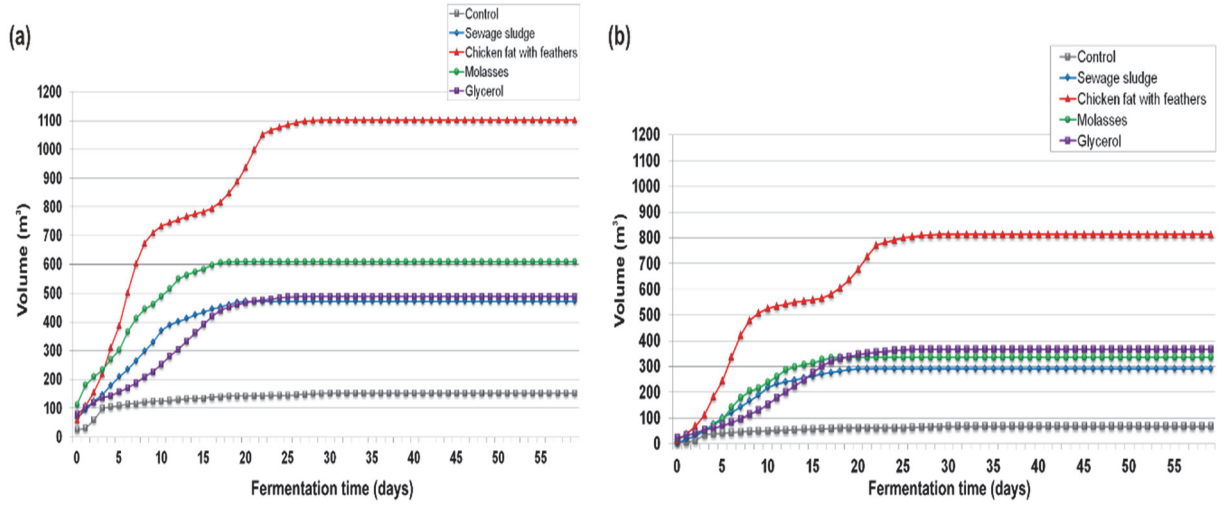
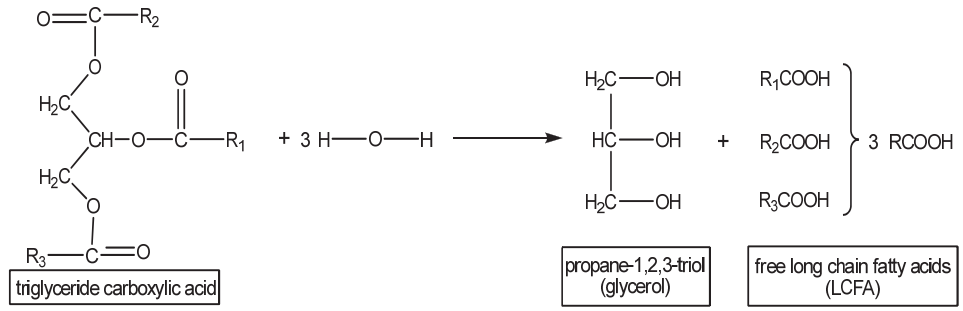


Fig. 3. Cumulative production of (a) biogas and (b) methane from volatile solids of FF, M, GL, SS and control

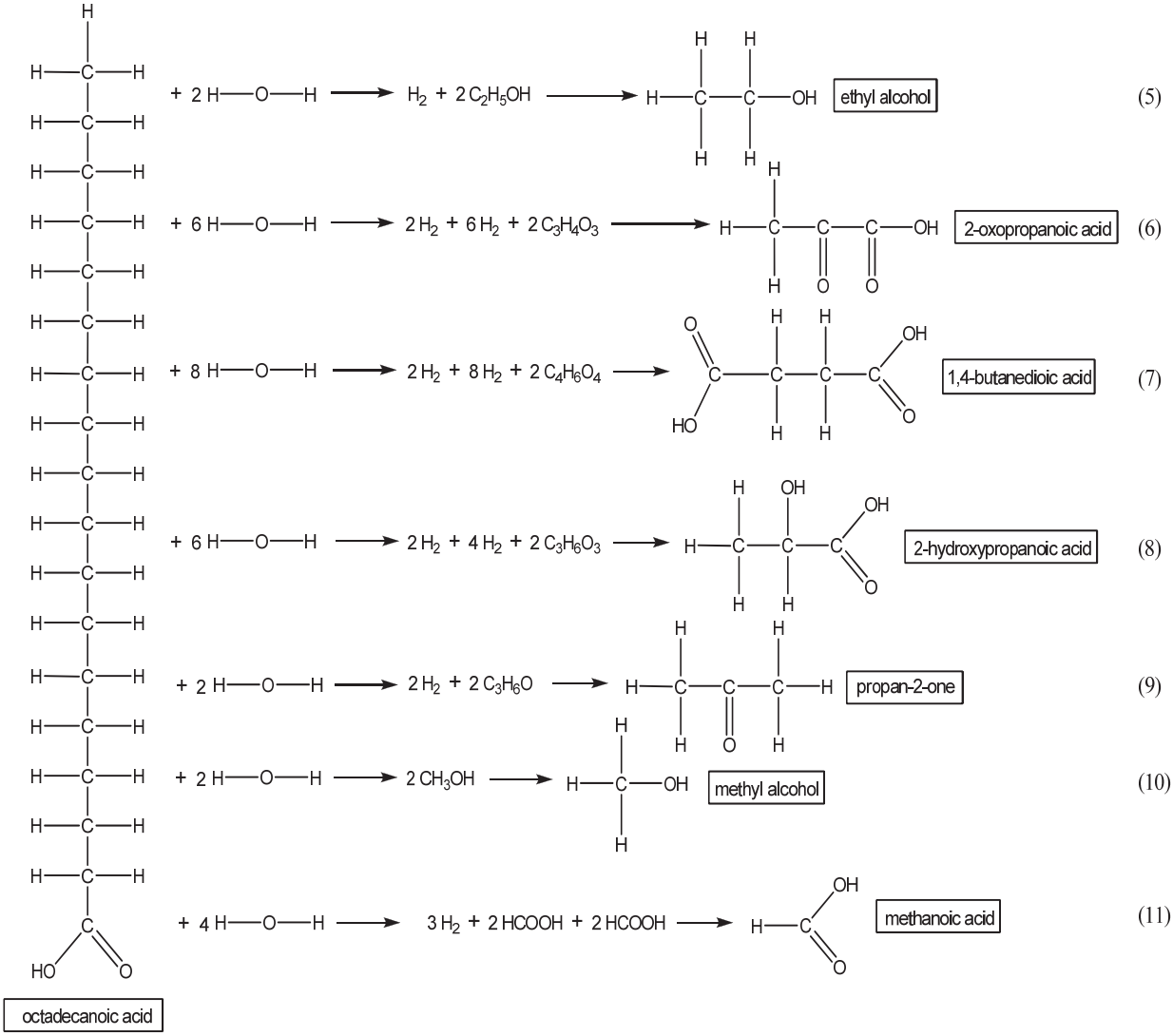
Hydrolysis



(4)

$R - C_nH_{2n+1}$

Acidogenic Phase



(5)

(6)

(7)

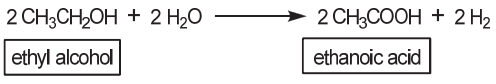
(8)

(9)

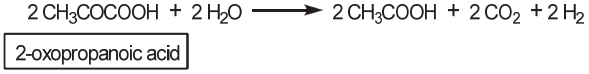
(10)

(11)

Acetogenic Phase



(12)



(13)

$$2\,(CH_2COOH)_2 + 2\,H_2O \longrightarrow 2\,CH_3COOH + CH_2(OH)COOH + 2\,H_2 + 2\,H_2O \quad (14)$$

ethane-1,2-dicarboxylic acid — 2-hydroxyethanoic acid

$$2\,CH_3CHOHCOOH + 2\,H_2O \longrightarrow 2\,CH_3COOH + 2\,HCO_3^- + 2\,H^+ + 2\,H_2 \quad (15)$$

2-hydroxypropanoic acid

$$2\,CH_3COCH_3 + 2\,H_2O \longrightarrow 2\,CH_3COOH + 2\,CH_4 \quad (16)$$

propan-2-one

Methanogenic Phase

$$CH_3COOH \longrightarrow CH_4 + CO_2 \quad (17)$$

ethanoic acid

$$4\,CH_3OH \longrightarrow \overbrace{3\,CH_4 + HCO_3^- + H^+ + H_2O}^{CO_2 + H_2O} \quad (18)$$

$$CH_3OH + H_2 \longrightarrow CH_4 + H_2O \quad (19)$$

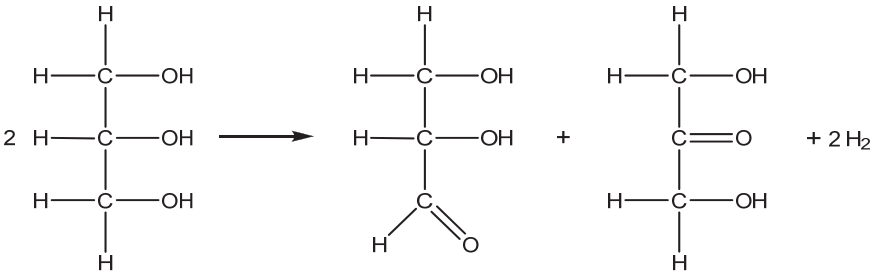
methyl alcohol

$$HCOOH + H_2O \longrightarrow CH_4 + 3\,HCO_3^- \quad (20)$$

$$4\,HCOOH \longrightarrow CH_4 + 3\,CO_2 + 2\,H_2O \quad (21)$$

methanoic acid

Hydrolysis



$$2 + 2\,H_2 \quad (22)$$

propane-1,2,3-triol (glycerol) — 2,3-dihydroxypropanal (glyceric aldehyde) — 1,3-dihydroxypropanone (dihydroxyacetone)

Acidogenic Phase

$$CH_2(OH)CH(OH)CHO + H_2O \longrightarrow HCHO + HCOOH + CH_3OH \quad (23)$$

2,3-dihydroxypropanal — methyl aldehyde — methanoic acid — methyl alcohol

$$CH_2(OH)COCH_2(OH) + H_2O \longrightarrow 2\,CH_3OH + CO_2 \quad (24)$$

1,3-dihydroxypropanone

Acetogenic Phase

$$2\,CO_2 + 4\,H_2 \longrightarrow CH_3COOH + 2\,H_2O \quad (25)$$

Methanogenic Phase

$$12\,CH_3OH \longrightarrow 9\,CH_4 + 3\,HCO_3^- + 3\,H^+ + 3\,H_2O \quad (26)$$

$$4\,HCOOH \longrightarrow CH_4 + 3\,CO_2 + 2\,H_2O \quad (27)$$

$$4\,H_2 + CO_2 \longrightarrow CH_4 + 2\,H_2O \quad (28)$$

$$CH_3COOH + H_2O \longrightarrow CH_4 + H_2CO_3 \quad (29)$$

Hydrolysis **Hydrolysis**

$$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6 \quad (30) \quad (30)$$

saccharose saccharose glucose fructose glucose fructose

Acidogenic Phase **Acidogenic Phase**

$$C_6H_{12}O_6 \rightarrow 2\,C_2H_5OH + 2\,CO_2 \quad (31) \quad (31)$$

$$C_6H_{12}O_6 + 2\,H_2 \rightarrow 2\,C_2H_5COOH + 2\,H_2O \quad (32) \quad (32)$$

glucose or fructose glucose or fructose

Acetogenic Phase **Acetogenic Phase**

$$C_2H_5COO^- + H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3\,H_2 \quad (33) \quad (33)$$

$$C_6H_{12}O_6 + 2\,H_2O \rightarrow 2\,CH_3COOH + 2\,CO_2 + 4\,H_2 \quad (34) \quad (34)$$

$$C_2H_5OH + 2\,H_2O \rightarrow CH_3COO^- + 2\,H_2 + H^+ \quad (35) \quad (35)$$

$$2\,HCO_3^- + 4\,H_2 + H^+ \rightarrow CH_3COO^- + 4\,H_2O \quad (36) \quad (36)$$

$$2\,CO_2 + 4\,H_2 \rightarrow CH_3COOH + 2\,H_2O \quad (37) \quad (37)$$

Methanogenic Phase **Methanogenic Phase**

$$2\,C_2H_5OH + CO_2 \rightarrow CH_4 + 2\,C_2H_5COOH \quad (38) \quad (38)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (39) \quad (39)$$

$$CH_3OH + H_2 \rightarrow CH_4 + H_2O \quad (40) \quad (40)$$

$$CO_2 + 4\,H_2 \rightarrow CH_4 + 2\,H_2O \quad (41) \quad (41)$$

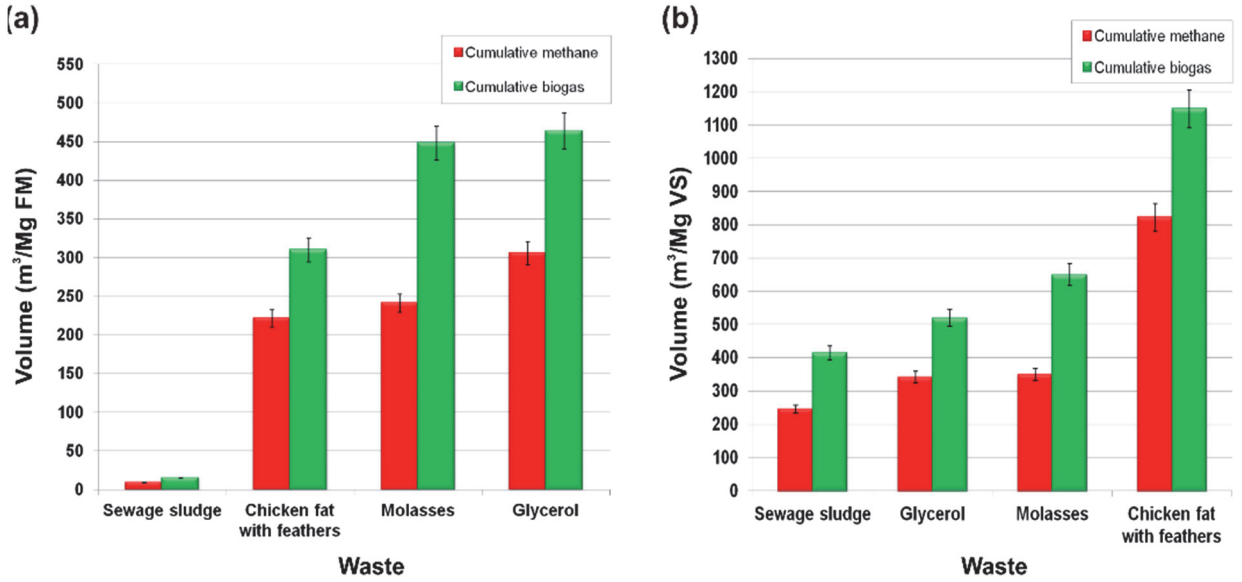


Fig. 4. Cumulative methane and biogas yield from Mg of (a) volatile solids and (b) fresh matter

4. Discussion

The experiments gave favorable results mainly due to the right inoculum, which was the digested sewage sludge. The inoculum was characterized by high buffer capacity, which was an advantage. The alkalinity of sewage sludge considerably influences its buffer capacity. The inoculum, which was the main component of the mixtures in our study, was highly alkaline (4,150 mg/dm^3). This fact may be particularly important for pH stability during digestion (Pilarska, 2018). In addition, the inoculum used in our study had very low content of volatile fatty acids (140 mg/dm^3), see Table 2. The content of volatile fatty acids (VFA) in the inoculum was reduced (comparing for SS – 3,900 mg/dm^3) as a result of digestion, during which organic carbon compounds as VFA were converted into methane and carbon dioxide (see: chemical

reactions, Section 3.3.). The COD value of digested sludge amounted to 1,750 mg/dm^3, whereas the COD value of raw sewage sludge was almost two times greater (3,190 mg/dm^3). These values were comparable to the data provided in reference publications (Zhu et al., 2011; Silvestre et al., 2011).

Additionally, our study indicated the levels of heavy metals in sewage sludge (Table 2). They are commonly found in municipal wastewater and sewage sludge, especially in large conurbations. According to Shrivastava and Benerjee (2004), the concentrations of heavy metals in sewage sludge in descending order look as follows: Zn> Cu> Cr> Ni> Pb> Cd, which was in line with the values measured in this study. Unlike most other toxic substances, heavy metals are not biodegradable, which was confirmed by their concentrations in the digested sludge and raw sludge. However, it is known that not all bacteria are so sensitive to heavy metals due to various immunity mechanisms (Dong et al., 2013). It is assumed that acidogenic species are more immune to heavy metals than methanogens. Deublein and Steinhauser (2011) listed the concentrations at which inhibition starts. At this stage of methanogenesis any permissible concentration of heavy metals in sewage sludge (also in the sewage sludge analyzed in our experiment) may inhibit methane production.

The comparison of the results of our study (Fig. 5) with data in reference publications showed that the yields of biogas and methane from fat with feathers (Wan et al., 2011; Silvestre et al., 2011) and from sewage sludge (Borowski et al., 2014; Zhu et., 2011; Sosnowski, 2008) were similar. There is little information about molasses as a substrate for biogas plants. Available reports indicate that renowned R&B centers still have to tackle the problem of digestion of molasses mixed with digested or raw sewage sludge. Fang et al. (2011) investigated yields resulting from the co-digestion of desugared molasses (DM) with cow manure. The researchers obtained 300 mL-CH_4/g VS-added (combined with 5% DM) using continuous stirred tank reactors (CSTR). The same authors investigated the effect of potassium and sodium contained in molasses as AD inhibitors. The yields obtained in an M/inoculum system in our study were higher than the values recorded in the publication quoted above.

Recently researchers have discussed the problem of destabilization or inhibition of anaerobic digestion with glycerol as the substrate. Studies focused on optimization of the substrate concentration and on technological issues. According to Fountoulakis et al. (2010), the addition of not more than 1% (v/v) of glycerol may improve the yield of biogas. Nghiem et al. (2014) proposed intermittent injection of low (0.63% v/v) and high (3% v/v) doses of glycerol and found that when the lower ones were used according to the procedure, they better improved biogas yields per volume of glycerol added (0.63% v/v). Another solution was proposed by Athanasoulia et al. (2014), who used a cascade of two anaerobic continuous stirred tank reactors (CSTR) fed with a mixture of SS with 0%, 2%, 3%, 4% (v/v) glycerol. The results showed the positive effect of glycerol at concentrations of 2% and 3%. When 4% of glycerol was added, the system failed due to overloading. In our study 1.5% glycerol was used (15 g/1000 g, Table 3) in batch experiments.

Yet, the acidification and extended times of methane digestion of the substrate were not avoided. The authors of this study used a similar concentration of glycerol (Pilarska et al., 2014) in a system composed of glycerol/sludge/pig manure (sludge without hygienization, obtained from another treatment plant than the SS used in this study) and recorded a good daily biogas yield (33.4 dm^3/d). In most studies the yields of anaerobic digestion of glycerol were lower or similar to those recorded in our experiment. However, recently Athanasoulia et al. (2014) succeeded in increasing the daily biogas production from 30 L/d to 114 L/d (CSTR process).

Our study showed the potential pathways of biodegradation of the materials used. According to the authors of this study, the proposed sequences of chemical reactions are also a useful tool for appropriate biochemical analyses and for the mathematical modeling of anaerobic digestion.

5. Conclusions

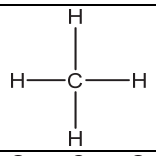
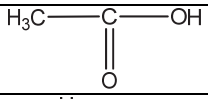
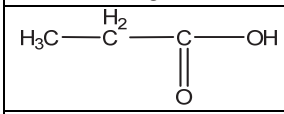
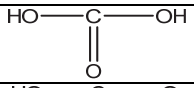
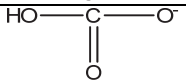
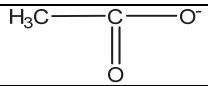
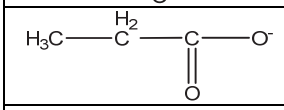
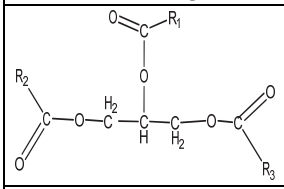
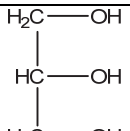
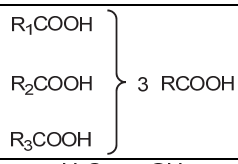
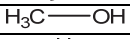
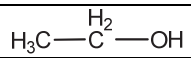
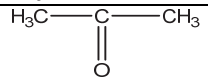
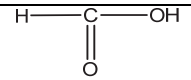
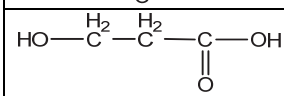
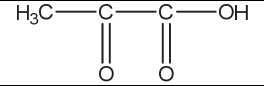
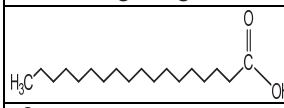
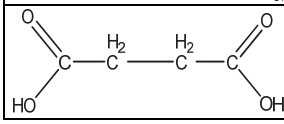
The comparison of the parameters of digested sludge and raw sludge revealed higher alkalinity due to the increased amount of HCO_3^- ions during the denitrification of the digested sludge, and a decrease in its COD, showing that AD reduced the content of organic compounds and inorganic contaminants.

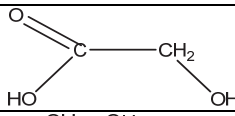
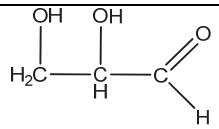
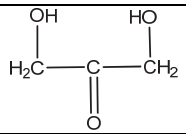
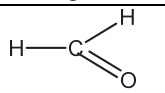
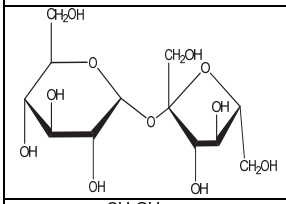
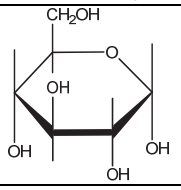
The destabilization of methane digestion of glycerol, accompanied by a rapid drop in pH to 5.5, was mainly attributed to the compounds formed at the glycerol hydrolysis phase: glyceric aldehyde and dihydroxyacetone, as well as products of their decomposition, i.e. methyl aldehyde and methanoic acid. As glycerol is a product of the biodegradation of fats, it is thought that the same compounds may acidify the medium in the anaerobic digestion of fat. The proposed chemical reactions are a useful tool for biochemical analyses and for the mathematical modeling of AD.

Fat with feathers appeared to be the most valuable high-energy substrate (cumulative methane 822 m^3/Mg VS) due to its chemical structure and stable AD. The biomethane yields from molasses and glycerol were comparable (350 m^3/Mg VS and 342 m^3/Mg VS, respectively). Sewage sludge gave the lowest result (246 m^3/Mg VS), which was caused by the presence of inhibitors in the raw SS. The cumulative biogas values for fat and sewage sludge were comparable with those reported in reference publications, and they were better for molasses and glycerol.

Annex

A table with the formulas and names of chemical compounds used in Eqs. (3)-(41).

Condensed formula	*Molecular formula*	*Name of compound*
	CH_4	methane
	CO_2	carbon dioxide
	$C_2H_4O_2$	ethanoic acid
	$C_3H_6O_2$	propionic acid (propanoic acid)
	H_2CO_3	carbonic acid
	HCO_3^-	hydrogen carbonate (bicarbonate)
	CH_3COO^-	acetate anion (acetate)
	$C_2H_5COO^-$	propanoate anion (propanoate)
	—	triglyceride carboxylic acid
	$C_3H_8O_3$	propane-1,2,3-triol (glycerol)
	—	free long chain fatty acids (LCFA)
	CH_4O	methyl alcohol
	C_2H_6O	ethyl alcohol
	C_3H_6O	2-propanone (acetone)
	CH_2O_2	methanoic acid (formic acid)
	$C_3H_6O_3$	3-hydroxypropionic acid (hydracrylic acid)
	$C_3H_4O_3$	2-oxopropanoic acid (pyruvic acid)
	$C_{18}H_{36}O_2$	octadecanoic acid (stearic acid)
	$C_4H_6O_4$	ethane-1,2-dicarboxylic acid (succinic acid)

Condensed formula	*Molecular formula*	*Name of compound*
	$C_2H_4O_3$	2-hydroxyethanoic acid (glycolic acid)
	$C_3H_6O_3$	2,3-dihydroxypropanal (glyceric aldehyde)
	$C_3H_6O_3$	1,3-dihydroxypropanone (dihydroxyacetone)
	CH_2O	methyl aldehyde
	$C_{12}H_{22}O_{11}$	sucrose
	$C_6H_{12}O_6$	glucose or fructose

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