Selection of Most Promising Substrates for Biogas Production

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Abstract –Laboratory equipment AMTS-II was used for anaerobic batch regime testing of the methane generating potential of different organic matter: raw sewage sludge from wastewater treatment plant, glycerol from biodiesel production, fish farming residues and their blends. Twenty days were sufficient to indicate proper substrate compositions. The tests performed in this study enableto avoid useless and time consuming stationary experiments and to select promising options. The results of the tests indicate, that the methane generation potentials for the studied matter were the following: 140…230 m^3/Mg (Mg – mega gram, ton) for raw sludge, 300…310m^3/Mg for glycerol and 260 m^3/Mg for fish residues. After these tests continuous anaerobic degradations in laboratory reactors were carried out. The objective was to find out how toenhance biogas productivity of anaerobic reactors which are located by waste water treatment plants and are employed for excess sludge stabilisation. This objective can be achieved by the addition of waste residues: crude glycerol from biodiesel production and residues from fishery. The addition of glycerol in the amount of 2–5% by weight causes the enhancement of methane production of about 250–400%. At the same time, the increase of total solids percentage concentration in the outgoing sludge is ten or more times less. The content of methane in biogas is higher in the case of admixed substrate.

Keywords -Anaerobic testing, biogas enhancement, raw sewage sludge, glycerol, fish farming residue

I. INTRODUCTION

THE objective of the article is to explain how to use waste components: crude glycerol from biodiesel production and fish residues from fishery in an anaerobic degradation process of excess sludge from waste water treatment plants (WWTP). Also to explain the options do it by best way. Nowadays the possibilities for biogas production as an alternative energy source are becoming more important [1], and from a practical viewpoint determining the capabilities of different organic materials to produce biogas are vital. Research in this area is quite time-consuming and frequently the research environment is not adequate for the expected outcomes. Therefore the research was conceived to be carried through in two stages. The objective of the first stage was thetesting of promising substrates. The second stage was dedicated to the research on how much biogas could be effectively produced using the chosen substrates. The stage was carried through in a continuous regime and the knowledge acquired in the first stage was taken into consideration.

II. Problem Formulations and Methods

A. First stage

The preliminary testing of different compositions in various organic components to determine more appropriate variants are time saving for the whole investigative process. For this purpose, the AMPTS –II (Automatic Methane Potential Test System) device is ideal. The device has 15 testing units and up to 400 ml or grams of degradable material (liquid or pulps) can be hermetically placed into each unit. The units can be thermostatically managed from 5 to 90 ^oC, with temperatures of 35–55 ^oC are ideal for the anaerobic tests. The device is equipped with a mixer, stirring the solution at programmed mixing intervals. Carbon dioxide is eliminated from the evolved biogas by alkaline solution(3M NaOH) and the quantity of pure methane is determined by the device itself.Almost complete removal of CO_2 was successfully achieved using 2% Glycerol additives at normal operating conditions at an equal gas to liquid volumetric flow ratesusing 0.5M NaOHsolution[2].

In our practice, the following suspensions or pulps were used: a) pure inoculum, b) mixtures of inoculum and raw wastewater sewage sludge, c) blend of inoculum and glycerol from biodiesel production, d) blends of inoculum sewage sludge and glycerol, e) mixtures of inoculum fish residues, f) mixtures of inoculum, raw sewage sludge and fish residues. The targets of the experiments are presented in the table I. Among these variants, inoculum has three parallel units and other variants have 2 parallels. The data presented in tables II–VII represent the averages of the parallels.

The inoculum was the sewage sludge received from Tallinn wastewater treatment plant, where it was anaerobically treated in mesophilic conditions (35–38 ^{o}C) over the course of 20 days. This sludge or inoculum was used in the tests processes at temperatures of 38 or 55 ^{o}C. It was possible to anaerobically treat the inoculum in a laboratory at a temperature of 55 ^{o}C over the course of 15 days. This was regarded as an adaptation for the thermophilic test conditions and was used once (see set no. 1). In other cases, the use of inoculum was direct, which meant that if the test temperature was 55 ^{o}C then the inoculum adaptation was absent. When the test temperature was 38 ^{o}C, the direct use of inoculum was regarded as an adapted process.

The raw sewage sludge was also received from Tallinn wastewater treatment plant. It was mixture of the preliminary sediment and the excess activated sludge, and the mixture was intended for treatment by mesophilicanaerobic process in the plant. Glycerol was obtained from biodiesel production in Paldiski.

Fish residues were received from fish farming tanks in Saaremaa. These were sediments that were formed by fish excrements and settled fish fodder.

Table I, Components under investigation: Inoculum (IN), Glycerol (GL), Sewage sludge (SS), Fish farming residue (F) and their blends

Tests set	Temperature in ^{o}C		Variants of the pulps
	In process	Inoculum prepared	
1	55	55	IN, GL, SS, IN+GL, IN+SS, IN+GL+SS
2	55	38	IN, GL, SS, IN+GL, IN+SS, IN+GL+SS
3	38	38	IN, GL, SS, IN+GL, IN+SS, IN+GL+SS
4	38	38	IN, IN+F, IN+SS+F

The serving of the test equipment took place every day and the capacity of the created methane was recorded. According to these data, the graphical presentation of the rate of methane production was possible, and process efficiency and its stabilisation became visible. It became evident that different degradable compositions behave differently and the duration of methane production is not equal. The tracking of tests lasted up to 42 days. At that time, gas production was finished everywhere and it became apparent that optimal time for some cases was shorter. We can see from figures 1–6, that the observing time of 20 days is sufficient, and longer monitoring periods are not necessary in future. This evidence is numerically outlined in table II.

Table II, Average percentage ratio of methane (CH_4) production in time vs ultimate production

Tests sets	Duration of CH_4 generation	
	10 days	20 days
1	88.83	97.13
2	77.94	96.80
3	90.67	96.16
4	92.93	99.82

B. Second stage

A series of continuous experiments were carried out in order to investigate the influence of glycerol concentration and fish residue on the process. One experiment was performedwith raw sludge obtained from Tallinn (WWTP). Other experiments were realised with sludge and additive mixtures, by weight: a) sludge 98% + glycerol 2%, b) sludge 95% + glycerol 5%, c) sludge 98% + fish residue 2%. Glycerol was obtained from the local pilot plant of biodiesel in Estonia (Viljandi). Fishery residues were obtained from the salmon treatment department of Kakumäe fishery near Tallinn, and they were mainly derived from fatty salmon skins and intestines. Digesters with an inner working mass of 1.6, 4.5 and 5 kg were constructed of fibreglass. These were sealed with rubber stoppers and equipped with clamped tubes for influent/effluent. The temperature in the reactors was maintained by water jackets surrounding them, in the case of inner reactive mass of 1.6 and 4.5 kg. The reactor with the inner mass of 5 kg was surrounded by an electric heating pad. The digesters were maintained at a mesophilic temperature (below 40 ^{o}C and above 35 ^{o}C), which was mainly around 36–38 ^{o}C in the presence of two bacteria species:

- Bacillus cellulosaemethanicus, responsible for methane formation and
- Bacillus cellulosaehidrogenicus, responsible for hydrogen formation [wwai-07].

With the help of anaerobic fermentation, the microorganism decomposes the organic matter, releasing metabolites as carbon dioxide and methane [3].Mixing was performed with magnetic spinners. That was done every morning before and after feeding. Biogas was collected into a gas clock filled with water and from the level of water the amount of biogas was determined. The reactors were operated in the draw-and-fill mode (on a daily basis) with a retention time of 40 to 20 days. Initially, the reactors were inoculated with anaerobic sludge originating from Tallinn

WWTP. Itrepresents the mixture of raw sludge and contents of reactors. Sewage sludge and its mixtures with glycerol were inserted by syringe. The mixture of sludge and fish residue was added through a tube on top of the reactor. The sludge and fish residue was stored in a refrigerator at +4 to +6 ^{o}C until use. The most important parameters to be considered during the anaerobic fermentation process are temperature and pH. Both have a relivantimpact on thedevelopmentprocess[4]. The pH was measured by a pH meter (Denver Instrument, UP-5). Optimum value pH is situated between 6,8 and 7,6 [3]. Everyday sludge removal from the digester took place before feeding the reactor. A gas sample was taken and measured every morning. At first, the amount of gas was determined in the gas clock and then the gas components (CH_4, CO_2, O_2, H_2S and NH_3) were evaluated with biogas analyser (Gas Data GFM416 Biogas Analyser). Once a week, the following was measured: total (TS) and volatile (VS) solids, volatile fatty acids (VFA) and alkalinity (Alk) in the input and output material of the reactors. The carbon/nitrogen ratio is a measure of the relative amount of organic carbon and nitrogen present in the feedstock. The optimum C/N ratio is between 20-30, with most sources citing 25 as the ideal level. A low C/N ratio, or too much nitrogen, can cause ammonia to accumulate which would lead to pH values above 8.5 [5].

III. PROBLEM SOLUTIONS

A. *First stage*

1. *Set no.1*

These tests were carried out at a temperature of 55 ^{o}C and inoculum adaptation[6] was realised at the same temperature. The objective of the investigation was to examine glycerol and its blends with sewage sludge. A summary of the test and results are presented in table III. The highest calculated yield of methane per total dry solids gives glycerol. This is followed by mixtures of glycerol and sewage sludge. It is known that glycerol in high concentrations inhibits anaerobic degradation [7], [8]. Therefore, a detailed investigation is needed to explain the proper concentrations and the relationships between sewage sludge and glycerol. When there is a lack of sewage sludge, the addition of glycerol can not only compensate but also even increase methane generation [9], [10].

The graph curves in Fig. 1 show that the methane production period is different for each component. In the figure, Nml means normal milliliter of the specified operating conditions, where the temperature is 20 ° C (273.16 ^{o}K) and pressure of 1 atm (101325 Pa).

However, after 20 days it is practically finished and the following generation of methane in some variants is negligible.

The lowest methane production has inoculum because it has previously been through an active anaerobic degradation process and has lost most of its degradable matter. The highest methane production of the pulps show sewage sludge but its dry matter concentration is 2.4–2.5 times higher than adequate concentrations of glycerol-sewage sludge mixtures.

2. *Set no. 2*

The process is similar to the above described procedures except that inoculum adaptation for 55 ^{o}C was not used. A summary of the test is presented in table IV. The table shows that the same principal trends or inferences revealed in table III are valid here, but the numerical values of methane production per dry solids have a tendency to decline. Obviously, this is caused by the difference in temperature between inoculum preparation and the process undertaken. The inoculum formed in mesophilic conditions and it must work in thermophilic conditions. The picture of graph curves in Fig. 4 is very uneven with single peaks. The cause is obviously the same; mesophilicmicroflora has to be rearranged to thermophile conditions. Nevertheless, the process was stabilised and practically finished after 20 days.

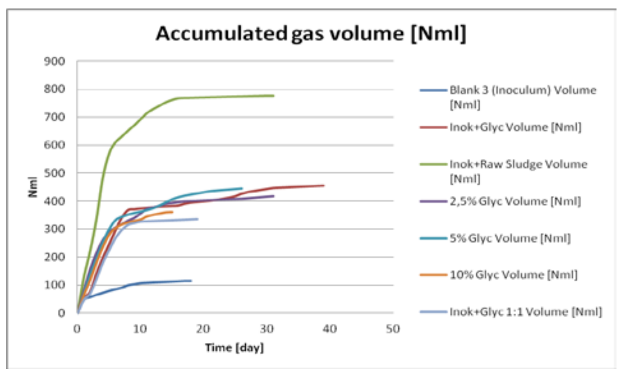

Fig.1, Cumulative methane generation (test set 1)

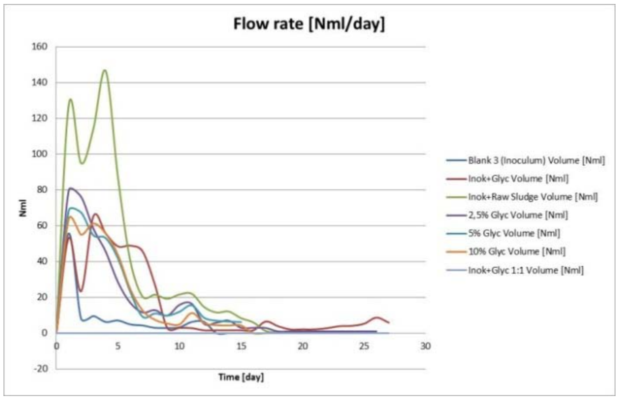

Fig.2, Daily methane generation intensity (test set 1)

3. Set no. 3

The structure of the tests set is the same as the two previous sets and the only difference is in temperature management. The data are presented in table V.

The table shows the result when the process and inoculum preparation took place in mesophilic (38 ^{o}C) conditions. Largely, the trends and inferences are similar to the two previous test sets. The difference is that the numerical values of the results are mainly placed between them. They are less from the first batch because the process temperature was lower and they are higher from the second batch because the temperature conflict was absent in this. Graphs curves are not presented, as they did not have notable differences.

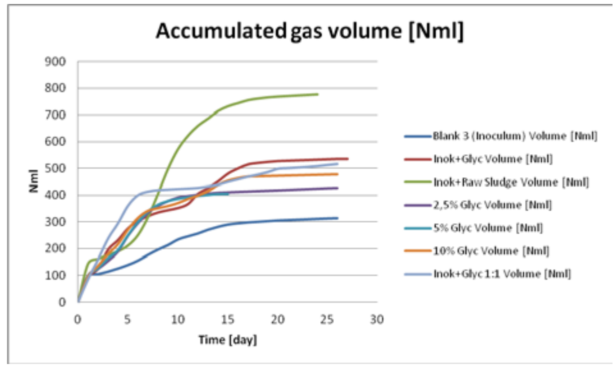

Fig. 3, Cumulative methane generation (test set 2)

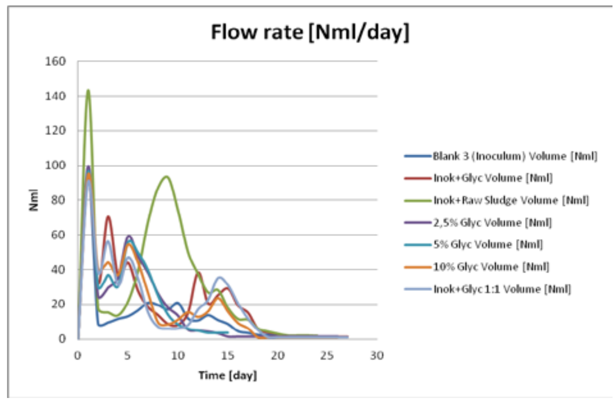

Fig. 4, Daily methane generation intensity (test set 2)

Table III, Characteristics and outcomes from tests set no. 1 (process and IN preparing by 55 ^{o}C)

Tests	Dry components in pulps kg/m^3					Production of methane			
	Inoculum	Glycerol	Sewage sludge	Total solids (TS)	Volatile solids (VS)	For pulps m^3/m^3		Per dry solids m^3/Mg	
						Blend	Substrate	Blend	Substrate
IN	22.50			22.50	12.05	0.273		12.13	
IN+GL	22.40	2.64		25.10	14.43	1.101	0.829	43.86	314.30
IN+SS	18.84		6.92	25.80	15.14	1.851	1.622	71.70	234.40
IN+GL+SS	21.48	1.02	1.89	24.40	13.81	0.972	0.711	39.82	232.50
IN+GL+SS	21.38	1.48	1.34	24.60	13.98	1.052	0.788	42.76	279.60
IN+GL+SS	22.02	1.92	0.82	24.80	14.15	0.979	0.712	39.50	259.50

Table IV, Characteristics and outcomes from tests set no. 2 (process 55 and IN preparing by 38 °C)

Tests	Dry components in pulps kg/m^3					Production of methane			
	Inoculum	Glycerol	Sewage sludge	Total solids (TS)	Volatile solids (VS)	For pulps m^3/m^3		Per dry solids m^3/Mg	
						Blend	Substrate	Blend	Substrate
IN	23.60			23.60	13.60	0.767		32.50	
IN+GL	23.50	3.02		26.52	16.31	1.311	0.547	49.44	181.050
IN+SS	19.39		9.01	28.40	16.78	1.893	1.270	66.65	140.954
IN+GL+SS	22.38	1.15	2.54	26.07	15.53	1.062	0.342	40.74	92.683
IN+GL+SS	22.73	1.67	1.79	26.19	15.73	0.962	0.227	36.70	65.607
IN+GL+SS	23.03	2.05	1.09	26.17	15.90	1.124	0.376	42.95	119.745

4. Set no. 4

It was previously was known that different fish farming wastes can be anaerobically treated [11], [12].These tests were carried out in conditions similar to the set 3, but the objective of the investigation was to determine the potential of methane productivity of fish farming residues and their mixtures with raw sewage sludge.The data are presented in table VI.

The data show that the potential of methane production from fish farming residues is placed between glycerol and raw sewage. Comparing with glycerol, their possible or presumable process inhibition is less or is absent entirely, and further tests are needed to explain this fully. The test graphs of the set are striking by their very smooth curves; the single post peaks are absent entirely.

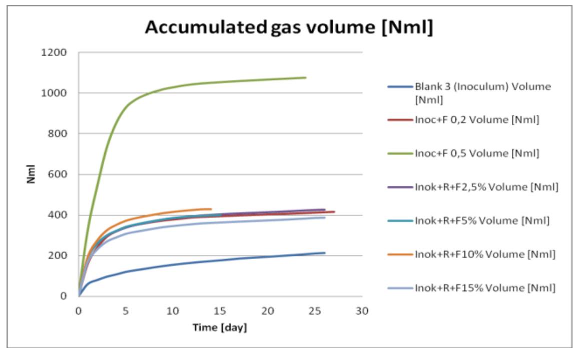

Fig. 5, Cumulative methane generation (test set 4)

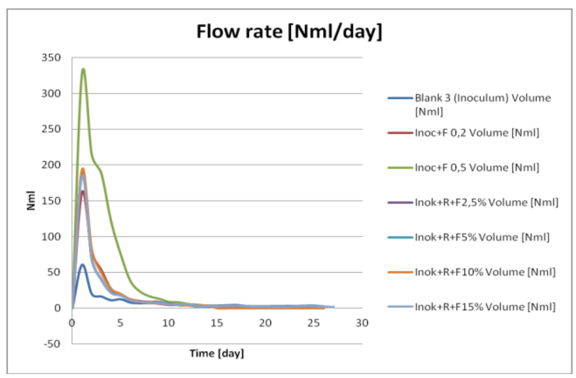

Fig. 6, Daily methane generation intensity(test set 4)

Table V, Characteristics and outcomes from tests set no. 3 (process and IN preparing by 38 °C)

Tests	Dry components in pulps kg/m^3					Production of methane			
	Inoculum	Glycerol	Sewage sludge	Total solids (TS)	Volatile solids (VS)	For pulps m^3/m^3		Per dry solids m^3/Mg	
						Blend	Substrate	Blend	Substrate
IN	23.50			23.5	14.13	0.556		23.64	
IN+GL	23.42	3.11		26.53	16.87	1.505	0.951	56.86	305.70
IN+SS	18.21		7.85	26.06	16.43	1.577	1.147	56.88	140.10
IN+GL+SS	22.06	1.37	2.09	25.52	15.83	1.238	0.717	48.50	207.20
IN+GL+SS	22.50	1.90	1.41	25.81	16.05	1.390	0.857	53.84	259.00
IN+GL+SS	22.88	2.35	0.83	26.06	16.24	1.337	0.795	51.81	250.10

Table VI, Characteristics and outcomes from tests set no. 4 (process and IN preparing by 38 ^oC)

Tests	Dry components in pulps kg/m^3					Production of methane			
	Inoculum	Sewage sludge	Fish	Total solids (TS)	Volatile solids (VS)	For pulps m^3/m^3		Per dry solids m^3/Mg	
						Blend	Subs-trate	Blend	Subs-trate
IN	24.40			24.40	12.92	0.487		19.96	
IN+F 0.2	21.99		3.23	25.22	13.98	1.272	0.833	50.44	257.895
IN+F 0.5	19.16		7.03	26.19	15.22	2.242	1.860	85.62	264.552
IN+SS+F35%	21.88	2.255	1.18	25.32	13.90	1.012	0.575	39.97	167.312
IN+SS+F50%	21.91	1.716	1.67	25.29	13.92	1.123	0.685	44.40	202.304
IN+SS+F75%	21.95	0.843	2.46	25.25	13.95	0.861	0.423	34.10	128.005
IN+SS+F90%	21.98	0.334	2.93	25.23	13.97	1.174	0.735	46.53	225.536

5. *Single substrate influence*

The nature of pulps or slurries single components are presented in table VII, whereby the essential data are juxtaposed against methane productivity, which is calculated from an adequate test sets and revealed as yield per dry (water free) solids.

The conspicuous connections between dry matter and some other component content and methane production were not revealed. Therefore, the determining factors are temperature, a proper inoculum forming temperature, the nature of substrate and concentrations, and the relations of components in the mixture.

Table VII, Tests components (CO) and their ability to produce methane

Tests set	CO	TS %	VS %	COD* g/L	P_{total} g/L	N_{-NH4} g/L	CH_4 m^3/Mg
1	IN	2.25	1.2	22.7	0.78	1.37	12.13
2	IN	2.36	1.4	21.7	0.76	0.76	32.50
3	IN	2.35	1.4	21.0	0.67	0.66	23.64
4	IN	2.44	1.3	29.2	0.77	0.87	19.96
1	SS	4.26	3.1	53.7	0.81	0.38	234.4
2	SS	5.06	3.2	53.4	0.82	0.14	141.0
3	SS	3.49	2.4	30.6	0.61	0.18	140.1
4	SS	3.36	2.2	36.2	0.63	0.42	
1	GL	89.4	91	1284	2.5	0.19	314.30
2	GL	89.5	91	1284	2.5	-	181.05
3	GL	89.5	91	1284	2.5		305.70
4	F	89.5	91	1284	2.5		261.20

*COD - chemical oxygen demand

B. *Second stage*

All tests began with a 40 day retention time with the aim to reduce it to 20 days. At the same time, the amount of methane production from digestion matter and the percentage of methane in biogas were measured. Table VIII belowgives the average values of several analyses of substrate used in the experiments. It shows that a small amount of additives may enhance solid concentration by as much as 2.5 times because additive water concentration was very low, i.e. 10.5% in glycerol and 48.2% in fish residue. Among these experiments, raw sludge digestion without an additive (Table IX and X) was specified as the standard process. The results obtained in the presence of additives were evaluated and compared with standard process values. The experiments described below reached a stable level on the ninth to twelfth day and on that day the observation of the experiment began. The decision to begin was visually cognitive and based on graphs depicting the biogas and methane production with time. The experiments with 100% sludge and its mixture with glycerol were started on the same calendar day and finished by 82 days. The experiment with the fish additives started later and its effected duration was 29 days (total 55 days). Data were mainly grouped by retention time. To reduce the numerical amount of the data and make them more comprehensive, the average results were evaluated for each group (Tables IX, and X)

Table VIII, Average computational concentration of different substrates used in experiments

Substrate	Total solids (TS), g/L			Volatile solids (VS), g/L		
	Sludge	Additive	Admixture	Sludge	Additive	Admixture
Sludge 100%	30.85			21.36		
Sludge 98% + glycerol 2%	30.23	17.90	48.13	20.93	16.30	37.23
Sludge 95% + glycerol 5%	29.29	44.75	74.05	20.29	40.75	61.04
Sludge 98% + waste fish 2%	29.99	10.38	40.37	20.27	9.85	30.12

Table IX, Data from single waste sludge digestion by reactor volume 1.7 litres

Days considered	Retention time, days	Volume load TS, kg/m^3	Input, g/L		Output, g/L		Organic removal input-output, g/L	
			TS	VS	TS	VS	ΔTS	ΔVS
9–21	40	0.89	35.40	26.63	22.38	14.05	13.03	12.63
22–30	35	1.01	35.39	26.62	22.16	13.23	13.23	13.39
31–41	30	1.09	32.64	24.17	22.33	13.82	10.31	10.35
42–55	25	1.05	26.20	16.25				
56–82	20	1.60	32.03	22.38	21.86	13.71	10.16	8.66
Average		1.23	31.97	22.69	22.10	13.73	11.24	10.50

Table X, Continue of the table IX

Retention time, days	Tempe-rature, °C	Methane yield		Methane contents in biogas, %	Solid removal, %	
		Per volume, L/m^3	TS removed, L/Δkg		ΔTS	ΔVS
40	36.5	109.7	339.6	50.98	36.51	47.23
35	37.4	82.1	217.1	51.84	37.40	50.25
30	36.4	92.9	270.3	52.16	31.59	42.81
25	38.5	117.9		54.51		
20	37.9	171.5	337.24	57.59	31.75	38.68
Average	37.2	128	310.9	54.39	33.55	42.95

In these tables, the last row presents the weighted average values. Due to the absence of essential information on some values, the data about pH, alkalinity, volatile fatty acids and impurities (H_2S, NH_3) are not considered. Likewise, in tables IX and X, the data of other experiments were computed. These include: sludge with 2% glycerol (reactive mass 1.6 kg), sludge with 5% glycerol (reactive mass 5.0 kg) and sludge with 2% fish residues (reactive mass 4.5 kg).

Detailed tables about the mixtures are not presented and only the last rows presenting weighted averages are shown in tables 11 and 12. The bracketed values are minimums and maximums considering the weighted average.

Table XI, The summarised data of the experiments on the level of weighted means

Substrate	Days considered	Retentio n time, d	TS input, g/L	VS input, g/L	TS output, g/L	VS output, g/L	ΔTS, g/L	ΔVS, g/L
Sludge 100%	73	27.6	32.0 (26.2–35.4)	22.7 (16.3–26.6)	22.1 (21.9–22.4)	13.7 (13.2–14.1)	11.2 (10.2–13.2)	10.5 (8.7–13.4)
Sludge 98% + glycerol 2%	69	31.0	49.3 (44.9–52.8)	38.8 (34.6–42.4)	24.6 (23.0–30.7)	13.3 (9.5–17.9)	24.7 (21.7–29.6)	24.6 (16.2–27.9)
Sludge 95% + glycerol 5%	70	35	64.0 (58.2–77.3)	58.6 (48.8–64.1)	27.0 (23.5–32.3)	15.1 (10.8–19.0)	44.5 (34.4–53.8)	43.7 (38.0–50.7)
Sludge 98% + fish 2%	29	35.7	43.0 (40.4–46.8)	32.4 (30.2–34.8)	23.8 (21.5–24.6)	14.0 (12.8–15.0)	20.8 (18.9–22.6)	18.4 (17.4–19.9)

Visual examination of tables IX and X and unrevealed tables present the main drift:
1. Decreasing the retention time increases the volume loading, and the methane production per volume unit of the reactor. Here, the volume of the reactor means the volume of the reacting mass in the reactor.
2. It is evident that organic matter removal in anaerobic digestion mainly takes place via the volatile organic matter and therefore the percentage removal of volatile solids as bio digestible is higher than total solids.
3. In the same experiment, the concentration values of input, output and removed organics vary around the average or median and they may be considered as stable.

Summarising the results of tables XI and XII points towards the following conclusions:
1. Admixed sludge has a higher volume load and higher concentration numbers.
2. The difference between the input output concentrations are more directly interconnected with the volume load and the concentration of output solids is influenced less.
3. Anaerobic digestion of admixed sludge produces biogas with a higher methane concentration.
4. A higher volume load gives a higher methane yield, but the yield per removed organics varies around a mean value.
5. Methane production is increased by additives more than the remaining solid residue in outgoing sludge or pulp.
6. The admixture from fishery has a higher potential to increase methane productivity than glycerol addition.

Table XII, Continue of the table XI

Substrate	Methane yield		Methane contents in biogas, %	Solid removal, %	
	Per volume, L/m^3	Per removed TS, L/Δkg		ΔTS	ΔVS
Sludge 100%	128 (82–172)	310.9 (217–340)	54. (51–57.6)	33.6 (31.6–37.4)	43 (38.7–50.3)
Sludge + 2% glycerol	323 (269–537)	381.9 (338–455)	61.4 (60.1–62.7)	50.1 (41.9–56.3)	66 (65.1–75.1)
Sludge + 5% glycerol	488.6 (234.9–705.3)	386.1 (273.1–530.4)	59.3 (57–61.6)	62 (54.7–69.6)	74.3 (68.1–77.9)
Sludge + 2% fish residues	369.4 (328.9–419.5)	627.7 (582.6–686.2)	63.5 (62.4–64.9)	48.5 (46.7–50.7)	56.8 (55.8–57.7)

Table XIII was derived on the basis of tables XI and XII. It compares the influence of additives to methane productivity. Methane production increased up to about 400% without a remarkable increase of residue solids in output sludge. This shows how to use existing anaerobic facilities of wastewater treatment plants for the production of alternative and green energy.

Table XIII, Comparison of weighted mean results (in brackets) against single sludge digestion

Substrate	Detention time in days	Percentage relations		
		TS load per reactor volume	Solids residue after treatment	CH_4 productivity per reactor volume
Raw sludge 100%	40–20	100 (1.23)	100 (22.1)	100 (128)
Sludge + 2% glycerol	40–20	164 (2.02)	111.3 (24.59)	252 (323)
Sludge+ 5% glycerol	40-20	173.1 (2.12)	122.1 (26.99)	382 (488.6)
Sludge + 2% fish residues	40–30	99 (1.22)	107.9 (23.84)	288.6 (369.4)

I. Comparison of results of the first and the second stage

The first stage tests indicated that inoculum preparation and substrate degradation should be carried through at the same temperature. Therefore a temperature of a round 37-38 ^{o}C was used. The inoculated sludge was received from Tallinn WWTP where the same mesophilic temperature was used. The first stage showed that glycerol and fish residues may be regarded as good substrate for anaerobic digestion. Comparing the measured data from both stages demonstrates that the forecast second stage data is inadequate. The processes in the batch regime and the continuous regime are different and obviously a more detailed evaluation of first stage is needed.

IV. Conclusions

1) AMTS II is possible for indicating of suitable composition in anaerobic stationary processes**:**

   (a) The sufficient testing period is 20 days.

   (b) The test results are significantly influenced by a difference between inoculum preparation and process temperatures. Generally, this influence deteriorates methane generation. It is important that the temperatures would be equal.

   (c) In the lack of raw sewage sludge, as a main substrate for the anaerobic reactors by wastewater treatment plants, additional substrates (waste glycerol, fish farming residues) can be used.

   (d) Methane productivity is significantly influenced by the nature of substrate concentrations and their compositional relations.

   (e) The approximate calculation of potential methane production per total dry solids (m^3/Mg) for single components can be revealed as: a) glycerol 300-310 m^3/Mg, b) raw sewage sludge of wastewater treatment plants 140 – 230 m^3/Mg, c) residues from fish farming pools 260 m^3/Mg
2) The yield of methane production in continuous feeding anaerobicreactors can be efficiently enhanced by adding glycerol or fishery residues. Methane concentration in the biogas is also higher.
3) Both additives are industrial waste. Their utilisation is an environmentally desirable process. By adding waste glycerol 2–5% by weight, the methane productivity per volume of the reactor increased around 250–400% and by adding fish residue 2% by weight, the methane productivity per volume of the reactor increased about 290%.
4) The increase of methane production by additives is more than ten times higher than the increase of solid residues in the outgoing sludge.

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