THESIS ON CIVIL ENGINEERING F67

Determining Biogas Yield from Industrial Biodegradable Waste

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TTÜ PRESS

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Declaration:
I hereby declare that this doctoral thesis, my original investigation and achievement, which is being submitted for doctoral degree at Tallinn University of Technology, has not been submitted for any academic degree.

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ARGO KUUSIK

TTÜ KIRJASTUS

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LIST OF ORIGINAL PUBLICATIONS THAT CONSTITUTE THE THESIS AND AUTHORS

The thesis is based on five academic publications, which are referred to in the text as Paper I, Paper II, Paper III, Paper IV and Paper V. Papers I to V are indexed by the ISI Web of Science:

Paper I: Kuusik, Argo, Loigu, E., Kuusik, A., Sokk, O. (2013). Possibility of Enhancing Methane Productivity in Anaerobic Reactors in the Treatment of Excess Sludge from Wastewater Treatment Plants. International Journal of Science and Engineering Investigations, 2 (12), 33-36.

Paper II: Kuusik, Argo, Kuusik, A., Loigu, E., Sokk, O., Pachel, K. (2013). Selection of Most Promising Substrates for Biogas Production. International Journal of Energy and Environment, 7 (3), 115-124.

Paper III: Kuusik, Argo, Kuusik, A., Pachel, K., Loigu, E., Sokk, O. (2013). Generalised Integration of Solid Waste Treatment Practices to Enhance Methane Productivity, Generate Suspension Fertiliser and Upgrade Biogas. European Scientific Journal, 9 (36), 14-30.

Paper IV: Kuusik, Argo, Pachel, K., Kuusik, A., Loigu, E. (2014). Anaerobic co-digestion of sewage sludge with fish farming waste. In: 9th International Conference on Environmental Engineering: Water Engineering (1-8). Vilnius, Lithuania: VGTU Press “Technika”.

Paper V: Kuusik, Argo, Pachel, K., Kuusik, A., Loigu, E. (2017). The possible agricultural use of digestate. Proceeding of the Estonian Academy of Security Sciences, 66 (1), 64-74.

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I	ARK	ARK	ARK	ARK, EL, AK, OS	ARK
II	ARK	ARK	ARK	ARK, AK, EL, OS, KP	ARK
III	OS	ARK, OS	ARK, OS	ARK, AK, KP, EL, OS	ARK, OS
IV	ARK	ARK	ARK	ARK, KP, AK, EL	ARK
V	ARK	ARK	ARK	ARK, KP, AK, EL	ARK

ARK – Argo Kuusik
AK – Aare Kuusik
EL – Enn Loigu
KP – Karin Pachel
OS – Olev Sokk

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Kuusik, A., Pachel, K., **Kuusik, Argo**, Loigu, E., Tang, W. Z. (2014). Reverse osmosis and nanofiltration of biologically treated leachate. Environmental Technology, 35 (19), 2416-2426.

Kuusik, A., Pachel, K., **Kuusik, Argo**, Loigu, E. (2014). Landfill runoff water and landfill leachate discharge and treatment. In: 9th International Conference Environmental Engineering: Water Engineering: Selected Papers (1-6). Vilnius, Lithuania: VGTU Press "Technika".

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Kuusik, A.; Loigu, E.; Sokk, O.; **Kuusik, Argo**. (2012). Enhancement of Methane Productivity of Anaerobic Reactors of Wastewater Treatment Plants. World Academy of Science, Engineering and Technology (Issue 65): WASET 2012 Tokyo, Japan International Conference, 29-30 May 2012. WASET, 1191−1193.

ABBREVIATIONS

AD	anaerobic digestion
AMPTS II	automatic Methane Potential Test System II
B	yeast from brewery
BG	biogas
BMP	biomethane potential
BOD_7	biochemical oxygen demand
BOD_L	ultimate BOD of the influent sludge (mg O_2/l)
C	carbon
$CaCO_3$	calcium carbonate
Cd	cadmium
CH_4	methane
CHP	combined heating and power
CO_2	carbon dioxide
COD	chemical oxygen demand
COM	compost
Cr	chromium
Cu	copper
CW	catering waste
d	day
DE	distilled water
DM	dry matter
DS	dry solids
EBA	estonian Biogas Association
FI	fish offal
Fish2	baltic herring
GL	glycerol
H_2	hydrogen
H_2S	hydrogen sulfide
HCO_3	bicarbonate
Hg	mercury
HRT	hydraulic retention time
Inoc	inoculum (Blank)
K-JSS	Kohtla-Järve sewage sludge
KITC	kitchen waste
LCFA	long-chain fatty acids
LE	*Lemna*
LEA	leachate
LWW	landfill wastewater

M	molar
mln	million
MPP	methane production potential
MSW	municipal solid waste
N	nitrogen
N_2	nitrogen gas
NaOH	sodium hydroxide
NF	not found
NH_3	ammonia (gas) nitrogen
NH_4+	ammonium (ion) nitrogen
Ni	nickel
Nml	normalized (1.0 standard atmospheric pressure, temperature 0 or 20 ^oC and zero moisture content) milliliter
Nm^3	normalized cubic meter
NT	not tested
Nw	not working (inhibated)
oDM	organic dry matter
OH	hydroxide
OIL	cooking oil
OLR	organic loading rate
P	phosphorus
p	atmospheric pressure
Pb	lead
R	the gas constant (R = 0.082 atm L/mol K)
RO	reverse osmosis
S	sulphur
SFP	sludge from Fish farm
SRT	solids retention time
SS	sewage sludge
T	temperature
TN	total nitrogen: organic nitrogen + NH_3 + NH_4^+ + NO_2^- + NO_3^-
TK	total potassium
TP	total phosphorus
TUT	Tallinn University of Technology
TOC	total organic carbon
TS	total solids
TV	drinking water from the tap
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acids

VOC	volatile organic compounds
VOLR	volumetric organic loading rate
VS	volatile solids
VSS	volatile suspended solids
W	whey
WWTP	wastewater treatment plant
Zn	zinc

1. INTRODUCTION

The continuously increasing use of renewable energy sources (biogas, wind energy, solar panels) over the past few years is a sign of the more effective application of knowledge, wider availability of new technologies and the reduction in prices for equipment.

The production of biogas from biodegradable waste and sediments is one option in stabilising biodegradable materials, as a result of which thermal and electric energy are produced in addition to biogas. The remaining digestate from the production of biogas can also be used for composting or as a fertiliser in agriculture. Different biodegradable wastes have different potential for biogas production, and some of them need pre-treatment before they can be used in the production of biogas. Most frequently, the established biogas stations are designed for the fermentation of certain types of material, and they are in most cases built in the immediate vicinity of the production places (piggeries, cattle sheds, wastewater treatment plants, etc.). In recent times, efforts have been made to look for solutions and possibilities for co-fermentation, in which case two or more biodegradable materials are used simultaneously in the production of biogas. All this requires research and precise management of the operations of a biogas station.

In this study, the potential for producing biogas from biodegradable materials originating from different Estonian industries has been examined, both in separate fermentation and co-fermentation processes. Furthermore, it has been studied whether the produced digestate is safe enough and can be used as a fertiliser in agriculture.

2. BACKGROUND

The conversion of organic material from solid wastes to methane containing gases can be accomplished in a number of ways, including hydrogasification, pyrolysis and anaerobic digestion [1].

Anaerobic digestion (AD) (methane fermentation) is the process in which specialised anaerobic microorganisms break down the biodegradable material in an oxygen-free environment to produce biogas composed primarily of methane (50-70%) and carbon dioxide (20-30%) and stable organic nutrient-rich digestate [1, 2, 3].

The AD of solid waste and wastewater sludge has long been used to stabilise organic wastes prior to final disposal of these wastes. Among the benefits to be realised from such treatment are [1]:

- a reduction in the organic content of the sludge
- improved sludge dewaterability
- destruction of most pathogens
- generation of a potentially valuable by-product (methane)
- volume reduction.

Not only is interest in anaerobic processes being generated because of their waste treatment potential, but the potential for generating methane from waste materials takes on added significance and can lead to efficient resource recovery of waste [4]. Since the methane is a significant greenhouse gas, anaerobic digestion has higher control over the methane production and contributes to lower the carbon footprint of the food waste management in the way that the fugitive emissions are lower than then the emissions in the cases of the landfilling and aerobic composting [4, 5].

2.1. Biological aspects of methane fermentation

Methane fermentation is a complex process that can be divided into four phases of degradation – hydrolysis, acidogenesis, acetogenesis and methanation – according to the main process of decomposition within the given phase (Figure 2.1) [6, 7].

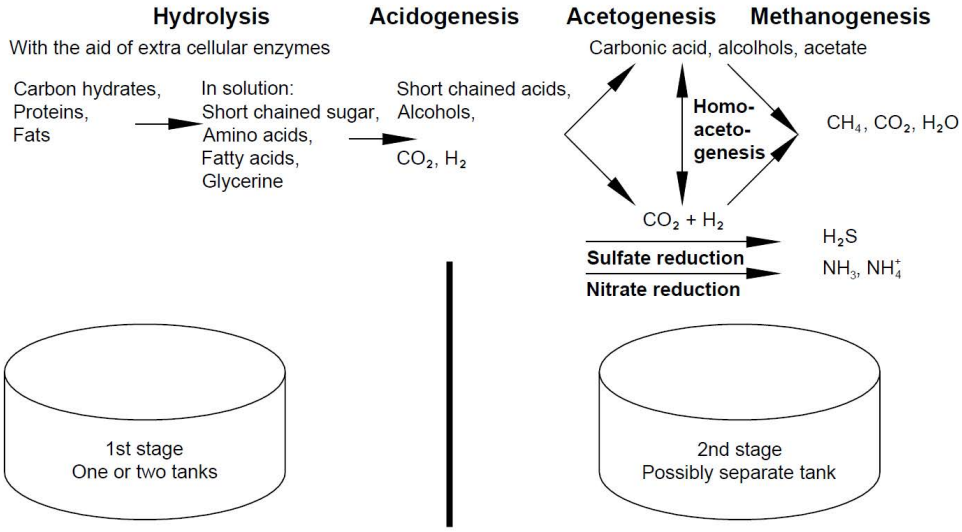

Figure 2.1 Biochemistry of methane gas production [6, PAPER V]

The individual phases are carried out by different groups of microorganisms, which partly stand in syntrophic interrelation and place different requirements on the environment.
In principle, methane formation follows an exponential equation. The course of biogas production (the biogas yield: V_{BR} [m^3/d]) can be theoretically described by the following equation, where C_1 and C_2 are constants [6].

$$V_{BR} = C_1 \times (1\text{-}e^{-C_2 \times t_{BR}}) \quad (1)$$

The methane generation rate can be estimated from the kinetic equations (e.q. 8 and 9) developed for the ADs [8, 1].

2.2. Methane fermentation phases

The first and second phases of degradation as well as the third and fourth phases are linked closely to each other. Therefore, one can effectively accomplish the process in two stages (Figure 2.1). In both stages, the rates of degradation must be equal in size. [6]
If the first stage runs too fast, the CO_2 portion in the biogas increases, the acid concentration rises and the pH value drops below 7.0. Acidic fermentation is then also carried out in the second stage. If the second stage runs too fast, methane production is reduced. There are still many bacteria from the first stage in the substrate. [9]
The bacteria of the second stage must be inoculated. With biologically difficultly degradable products, the hydrolytic stage limits the rate of degradation. In the second stage, the acetogenesis possibly limits the rate of decomposition. [6]

In the first phase (the **hydrolysis**), undissolved compounds, like cellulose, proteins and fats, are cracked into monomers (water-soluble fragments) by exoenzymes (hydrolase) of facultative and obligatorily anaerobic bacteria [7, 10]. In fact, the covalent bonds are split in a chemical reaction with water [11].

The monomers formed in the hydrolytic phase are taken up by different facultative and obligatorily anaerobic bacteria and are degraded in the second – **acidogenic phase** – to short-chain organic acids, alcohols, hydrogen and carbon dioxide [6, 7, 12]

The products from the acidogenic phase serve as substrate for other bacteria, those of the **acetogenic phase**. The acetogenic reactions are endergonic. Acetogenic bacteria are obligatory H_2 producers. The acetate formation by the oxidation of long-chain fatty acids runs on its own and is thus only thermodynamically possible with very low hydrogen partial pressure. [6]
The acetogenic phase limits the rate of degradation in the final stage. From the quantity and composition of the biogas, a conclusion can be drawn about the activity of the acetogenic bacteria. [6, 7]

In the fourth stage (**methanogenic phase**), methane formation takes place under strictly anaerobic conditions. The methanogenesis is the final stage of anaerobic digestion. In this stage, the hydrogen and acetic acid formed by acid producers will be converted into methane and carbon dioxide, which are the major constituents of biogas. This reaction is categorically exergonic. As follows from the description of the methanogenic microorganisms, all methanogenic species do not degrade all substrates. [6, 7]

2.3. Physico-chemical factors influencing biogas production

Biomass composition depends primarily on the source: agricultural, municipal or industrial wastes. Chemical composition analyses play an important role when estimating biogas or methane yield. [2]
The nutritional requirements of anaerobic bacteria are extremely important to supply the basic cellular building material for growth and to be able to synthetize the enzymes and co-factors from metabolic reactions. [2] To obtain proper breakdown of the organic compounds, several conditions must be fulfilled [13].
Particle size can also influence the rate of anaerobic digestion, as it affects the surface area for the biodegradation of biomass material [2]. Mshandete et al. (2006) found that decreasing particle size from 100 mm to 2 mm will improve fiber degradation, and therefore high methane yield will be achieved. Environment (temperature and pH) is also important in anaerobic digestion [2].

2.3.1. Parameter: temperature

Temperature has an important effect on the physicochemical properties of the components found in the digestion substrate. The optimum temperature of anaerobic digestion depends on the digester type and substrate type. It also influences the growth rate and metabolism of microorganisms and hence the population dynamics in the anaerobic reactor [8].
The microorganisms participating in the process of anaerobic digestion (especially methanogenic ones), are divided into three large categories:

- cryophiles (Psychrophiles), operating at temperatures from 12 to 24°C, digestion characteristic area under cryophilic regime;
- mesophiles, operating at temperatures between 22-40°C, characteristic area for mesophilic regime digestion;
- thermophiles, operating at temperatures between 50 – 60°C, characteristic area for thermophilic regime digestion [14]

The rate of hydrolysis, generally, is also increased with increasing temperature [15, 16].

The temperature shows two optima for acidifying bacteria; a smooth one at about 32-42 °C for mesophilic microorganisms and a sharp one at 48-55 °C for thermophilic microorganisms (Figure 2.2) [1, 6, 7,].
Most of the methanogenic microorganisms belong to the mesophiles; only a few are thermophilic [6, 7]. A few others are even able to produce methane at low temperatures (0.6-1.2 °C), such as on the surface of permafrost soils. In laboratory tests, methane formation could also be proven with temperatures below freezing, i.e. down to −3 °C [6].

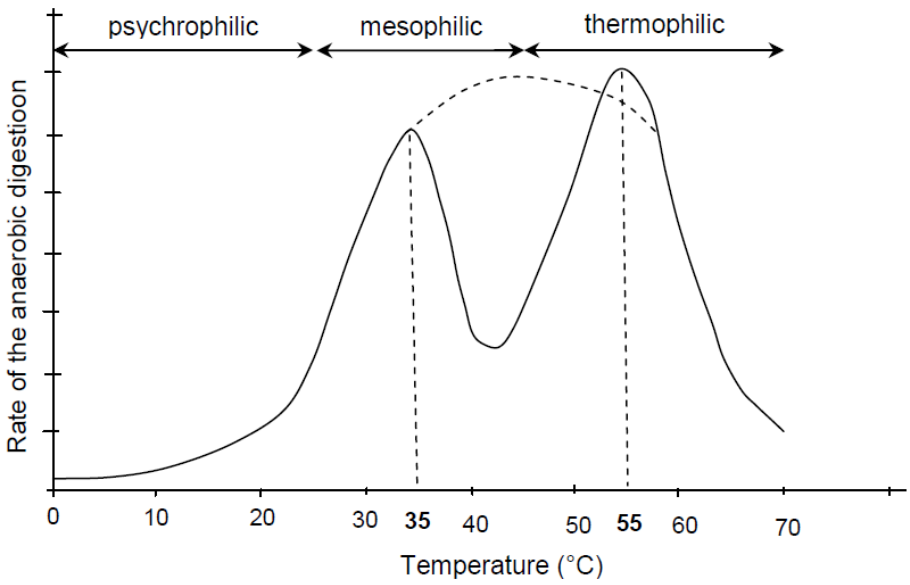

Figure 2.2 Influence of temperature on the rate of anaerobic digestion process [17, 18, 19]

As a rule of thumb, the biological activity doubles for every 10 °C increase in temperature within the optimal temperature range [20]. Acetotrophic methanogens are one of the most sensitive groups to increasing temperatures [8]. Thermophilic methanogens are more temperature-sensitive than the mesophiles. Even small variations in temperature cause a substantial decrease in activity. An increasing temperature has several benefits including increasing solubility of the organic compounds, enhanced biological and chemical reaction rates, and an increasing death rate of pathogens (thermophilic conditions) [8].
However, the application of high temperatures (thermophilic conditions) has counteracting effects: there will be an increase in the fraction of free ammonia, which plays an inhibiting role for the microorganisms; however, the increasing acid constant pK_a of the VFA will make the process more susceptible to inhibition [8, 21]. Control is thus a very sensitive issue for thermophilic compared to mesophilic digestion.
Therefore, the temperature variations should be kept exactly within a range of +/− 1-2 °C/day [20]. Otherwise, gas losses of up to 30% have to be taken in consideration [6]. Temperatures in the range of 40-45 °C are particularly critical for mesophilics because they lose their activity irreversibly in that range [6].
Under mesophilic operating conditions, the inhibition of ammonium is reduced because of the lower content of inhibiting free ammonia. In general, it has to be mentioned that the energy balance is better in the mesophilic range than in the thermophilic range [6].

The thermophilic mode of operation results in about a 50% higher rate of degradation, and, with fat-containing materials in particular, a better microbial availability of the substrates and thus a higher biogas yield [6]. Pathogenic and phytopathogenic germs are inactivated by higher process temperatures, so that special hygienic procedures are not necessary when using a temperature > 55 °C and a material retention time of > 23 h [6].

2.3.2. Parameter: pH

Each group of microorganisms has a different optimum pH range. Methanogenic archaea are extremely sensitive to pH [8, 22]. Anaerobes can be grouped into two separate pH groups: acidogens and methanogens. The optimum is 5.5-6.5 for acidogens and 7.8-8.2 for the methanogens [20]. Optimum pH for the combined cultures ranges from 6.7-7.5 with neutral pH bending the ideal [8, 20]. Therefore, it is important to adjust the pH-value in the second stage higher than that in the first stage of a two-stage biogas plant. Only archaeal genus *Methanosarcina* is able to withstand lower pH values (pH = 6.5 and below) [6].
If the pH value sinks below pH = 6.5, then the production of organic acids leads to a further decrease of the pH value by the hydrolytic bacteria and possibly to cessation of the fermentation. In the reality, the pH-value is held within the neutral range by natural procedures in the fermenter [4]. Two buffering systems ensure this.
A too strong acidification is avoided by the carbon dioxide/hydrogen carbonate/carbonate buffer system. During the fermentation, CO_2 is continuously evolved and escapes into air. With a falling pH value, more CO_2 is dissolved in the substrate as uncharged molecules. With a rising pH value, the dissolved CO_2 forms carbonic acid, which ionises. Thus, hydrogen ions are liberated. [6]

$$CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + 2CO_3^{2-} \qquad (2)$$

At pH = 4, all CO_2 is as free molecules; at pH = 13, all CO_2 is dissolved in the form of carbonate in the substrate. The centre around which the pH value swings with this system is at pH = 6.5. At a concentration of 2.5-5 g/L, hydrogen carbonate gives a particularly strong buffering. [6]
Too weak acidification is avoided by the ammonia-ammonium buffer system. With a falling pH value, ammonium ions are formed with release of hydroxylions. With a rising pH value, more free ammonia molecules are formed and will be toxic to the methanogenic archaea. [6]

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \qquad (3)$$

$$NH_3 + H^+ \leftrightarrow NH_4^+ \qquad (4)$$

The centre around which the pH value swings with this system is at pH = 10.
Both buffering systems can be overloaded by a feed of quite rapidly acidifying waste water or organic material by toxic substances, by a decrease in temperature or by a too high volume load in the bioreactor; e.g., by feeding waste water out of a starch processing plant, which incurs the possibility of acetic acid toxification [6].

The drastic drop in methanogenic activity at pH 8.0 and above could be due to a shift of NH_4^+ to a more toxic unionised form NH_3 [20].
In anaerobic treatment process, the drop in pH is often caused by the accumulation of VFAs and/or the excessive generation of carbon dioxide [20].
The system pH is controlled by the CO_2 concentration in the gas phase and the HCO_3-alkalinity of the liquid phase. If the CO_2 concentration in the gas phase remains constant, the possible addition of HCO_3-alkalinity can increase the digester pH. A buffering capacity of 70meq $CaCO_3$/l or a molar ratio of at least 1.4:1 of bicarbonate/VFA should be maintained for a stable and well buffered digestion process, although it has been shown that the stability of the ratio in particular is of prime importance, and not so much its level. [8]
One of the first options to resolve the problem is to reduce the volumetric organic loading rate (VOLR) to the point where the accumulated VFAs are allowed to be consumed faster than produced. Once the excess VFAs are exhausted, the pH of the reactor will return to a normal operating range and the methanogens begin to rejuvenate. [8, 20]

2.3.3. Parameter: nutrients (C/N/P - ratio)

The C/N/P-ratio of the substrate should be in the range of 16 : 1-25 : 1. However, this is only an indication, as nitrogen can also be bound in lignin structures. [7]
The need for nutrients is very low due to the fact that not much biomass is developed with the anaerobic process, so that for methane formation even a nutrient ratio C : N : P : S of 500-1000 : 15-20 : 5 : 3 and/or an organic matter ratio of COD : N : P : S = 800 : 5 : 1 : 0.5 is sufficient [7].
Substrates with a too low C/N ratio lead to increased ammonia production and inhibition of methane production [23]. A too high C/N ratio means a lack of nitrogen, from which there are negative consequences for protein formation and result in the energy and structural material metabolism of the microorganisms. A balanced composition is absolutely necessary. [6] According to reference a ideal C:N ratio for anaerobic digestion is between 25 and 30 [24].

In addition to nitrogen and phosphorus, several other trace nutrients are identified as essential for anaerobic microorganisms. Ni is particularly important because it is a structural constituent of factor F430, which is found only in methanogenic archaea [20]. Co is also important because it is the structural constituent of vitamin B_{12}, which catalyses the methanogenesis [20].

2.3.4. Parameter: inhibitors

When planning and operating a biogas plant, it has to be borne in mind that some compounds that are formed as products of the metabolism of the anaerobic degradation, even to a limited extent, inhibit the biocenosis and can even be toxic at higher concentrations. [6]
Inhibition of the overall anaerobic digestion process by ammonia is a common occurrence during the digestion of feedstocks with naturally high ammonia concentrations such as manure [25].
The toxicity of anaerobic processes is mediated by the substances present in the influent waste stream or through byproducts of the metabolic activities of the microorganisms. Ammonia, heavy metals, halogenated compounds, cyanide and phenol are examples of the former, while ammonia, sulphide and long-chain fatty acids (LCFAs) belong to the latter group [20].
The inhibition depends on the concentration of the inhibitors, the composition of the substrate and the adaptation of the bacteria to the inhibitor. Anaerobic bacteria need a low concentration of the inhibitors as trace elements. [6]

Oxygen

The importance of oxygen concentration varies greatly for the different microbial communities that comprise the biogas process. Some of the organisms, such as those that produce methane, are very sensitive to oxygen (the inhibition begins at 0.1 mg L/O_2) and die if they come in contact with air [6, 21]. Others can survive quite low concentrations of oxygen, while others grow better if oxygen is present. The free radicals of oxygen are strong oxidising agents that can destroy cells by oxidizing various cell components. Microorganisms that can live in the presence of oxygen have different defence systems, that is, various enzymes that can protect the cell against oxidation by oxygen [21]. The organisms that are sensitive to oxygen do not have this enzymatic defence system and are destroyed in the presence of air. Microorganisms are usually divided into different groups depending on their relationship with oxygen. Both strict anaerobes and so-called facultative aerobes are found in the biogas process. Strict anaerobes only grow in the absence of oxygen. This group includes the methane-producing organisms. On the other hand, facultative aerobes grow in both the presence and absence of oxygen [21]. This group includes numerous fermentative microorganisms. In the presence of oxygen,

they can grow by aerobic respiration, but then they switch to fermentation when oxygen is depleted. This means that a temporary air leakage to a biogas process need not be a problem because there are microorganisms that can rapidly consume the incoming oxygen [21].

Short- and Long-chain fatty acids

The level of VFA is an indicator of the health of an anaerobic treatment system. During anaerobic digestion, complex organic matter is hydrolysed and fermented into low-molecular-weight compounds, including short-chain fatty acids, such as acetate, propionate and butyrate as intermediary products, which may act as potential inhibitors of bacteria in anaerobic digestion [2, 20]. In a healthy anaerobic system, the VFA concentration in the effluent is relatively low and usually in the range of 50-250 mg/L [20]. When the symbiotic relationship between acidogens and methanogens breaks down, VFA accumulates. Studies suggest that VFA concentrations exceeding 2,000 mg/L inhibit methanogens, but higher acetic or butyric acid at concentrations exceeding 10,000 mg/L inhibits methane formation at neutral pH [20].

Wastewater and sludge from edible oil refinery (glycerol), slaughterhouse, wool scouring, meat packing, restaurants and dairy processing contain high concentrations of lipids. Lipids are an important organic component of waste in the anaerobic process. They generate the highest theoretical amount of methane when compared to other components [20].
LCFAs are produced by the hydrolysis of lipids such as fats, oils and greases during anaerobic treatment [20].
The mechanisms associated with LCFAs toxicity are caused by adsorption onto the cell wall/membrane and interference with the transport. In addition, inhibition by LCFAs will reduce anaerobic biomass granulation and granule flotation, along with impaired syntrophic interaction between microbial groups. [2, 20]

Ammonium (NH_4+) and ammonia (NH_3)

Ammonium (NH_4^+) and free ammonia (NH_3) are the two most predominant forms of inorganic nitrogen present. It has been indicated that free ammonia is the most toxic of both, due to the fact that it can pass through the cell membrane and into the cell, causing proton imbalance and potassium deficiency [8, 26]. The free ammonia concentration mainly depends on three parameters: total ammonia concentration, temperature and pH [8]. An increased temperature has a positive effect on the microbial growth rate but it also results in a higher (free) ammonia concentration [26]. It is found that thermophilic digestion is more

easily inhibited than mesophilic digestion. An increase in pH would result in a higher toxicity level due to the shift to a higher ratio of free to ionised ammonia [26]. The resulting instability of the process often leads to an increase in the amount of VFA, which again leads to a decrease in pH and consequently to a lower free ammonia concentration: the process remains stable but the methane yield is reduced [26].

$$NH_4^+ \leftrightarrow NH_3 + H^+ \qquad (5)\ [2, 20]$$

In anaerobic systems, ammonia nitrogen (NH_3-N) concentrations of 50-200 mg/L are considered to be stimulatory, while concentrations of 1500-3000 mg/L are inhibitory at a pH level over 7.4. Concentrations above 3,000 mg/L are considered to be very toxic for anaerobic bacteria. The microorganisms most affected by ammonia inhibition are methanogens. [2]

Heavy metals

Industrial contributions are the primary source of heavy metals in urban wastewater and account for up to 50% of the total metal content in sewage sludge. Industrial contaminants include zinc, copper, chromium, nickel, cadmium and lead. [8]

The presence of heavy metals can often cause difficulties in the nitrification/denitrification step of the wastewater treatment processes due to inhibition and may hamper the sludge disposal by land application, but sodium, potassium, calcium and magnesium can also lead to disturbances in biogas plants [6].

During the anaerobic digestion of biomass, heavy metals take part in several physico-chemical reactions, in which the three main ones are [2]:

1) precipitation as sulphide, carbonate and hydroxides,
2) sorption to the solid fraction, either biomass or inert particulate matter,
3) formation of complexes in solution

To estimate whether heavy metals stimulate or inhibit the process, an evaluation on the total metal concentration, chemical forms of the metals and factors such as pH and redox potential have to be taken into account. Zayed and Winter (2000) have found that methanogens are more inhibited than acidogens when exposed to heavy metals [2].

Heavy metals are only toxic to anaerobic bacteria in their soluble form.

2.4. Substrates

In general, all types of biomass can be used as substrates as long as they contain carbohydrates, proteins, fats, cellulose and hemicellulose as their main components [6].

The practically attainable methane yield depends on many factors, such as composition, grain size and proportions of the assigned substrates; on the microbial degradability of the biomass, the content of dry matter and organic dry matter; and the relationship of the nutrients to each other [6, 27]. Also, the parameters of the technology of fermentation are of importance, e.g., the number of stages, the temperature, the residence time of the substrate in the bioreactor, the kind and frequency of the mixing of the substrate, and the quantity and frequency of the substrate addition [27].
These parameters must also be analysed in a laboratory test and in a pilot plant before the construction of a production plant [28]. In a first simple fermenting test, the basic degradability of a substrate, the graph of the degradation and the biogas yield have to be determined. Sometimes, the maximum recommendable volume load and the changes of the concentrations of certain materials have to be measured [27].
Methanogenic microorganisms have a long regeneration time in general. To avoid washing out from the reactor, hydraulic residence times must be at least 10-15 days with reactor systems that do not have the facilities for retaining and returning biomass [27].

Most agricultural biogas plants are used to fermenting liquid manure, nowadays quite often combined with co-substrates to increase the biogas yield [6, 7, 27].
Different types of biowaste accumulate over the year depending on the season.
The composition of the residual waste (waste generated by households) depends on the location of the household [28]. Waste from shops or trade can also be considered residual waste because of its very similar composition [27].
In the central congested areas of cities (the location of multi-storey housing), the biowaste is poor in structure and quite pasty [28]. This waste includes leftovers, spoiled food, market waste and different industrial wastes [6, 27].
In the outskirts of a town or in rural settlements, the biowaste is fairly rich in structure and fibrous; therefore, it is well suited for composting [28].

Until the 1990s, residual waste (i.e. household waste) was discarded on landfills, by default [4]. The biological components of the waste were degraded quite slowly and the fermentation process took about 20-40 years [7, 28]. The landfill gas produced during the process was gathered by using horizontal drainages and gas pits for disposal [6, 7]. About 12-300 m^3 of landfill gas was produced in total per Mg of residual waste. However, it contained quite a high level of toxic and

corrosive organic components, so that damage to combined heating and power units (CHPs) often resulted [27].

The anaerobic degradation of sewage sludge is called stabilisation, digestion or sewage sludge fermentation.
The sewage sludge from the primary clarifier (primary sludge) and from the final clarification basin (excess sludge) is segregated with pumps, dehydrated after sedimentation and stabilised by forming biogas [28]. With a second dehydration step and a mechanical coagulation, it is concentrated of up to 30% of dry matter [28]. The material composition of the sewage gas depends on both the origin and composition of the waste water and on the mode of operation of the sewage plant [27].
The dry sludge is used agriculturally as fertiliser [7]. Nowadays, it is also quite often burned, e.g., in an incineration plant together with residual waste [7, 27].

The content of organic matter is increased by co-substrates added to the substrate: hence, the yield of biogas. From an economic point of view, it is only profitable, however, if the materials are sourced from a location within a distance of 15-20 km [6, 7].
In general, the content of dry matter (liquid substrates and co-substrates) in the substrate should be below 12% to ensure the functionality of standard pumps and a proper mixing in the bioreactor, which is important for an efficient transformation process [6].
The importance of co-digestion over a single digestion process includes increment in biogas production, dilution of toxic compounds, and improvement in the buffer capacity, nutrients balance which includes supply of carbon to nitrogen ratio, micro and macro nutrients; and stabilization of pH [28].
However, the addition of co-substrates poses a higher hygienic risk. If the residue from the fermentation process is to be used as fertiliser for agricultural areas, the co-substrates should meet national requirements and should not pose any hazard from exposure, e.g., they must be free of pathogens [27].

2.5. Anaerobic digestion in Estonia: current status

According to Directive 2009/28/EC, which mandates the levels of renewable energy use, Estonia has assumed the obligation to increase the share of renewable energies in gross final energy consumption to 25 percent by 2020 in comparison to the reference year of 2005. According to the national action plan, this means that the share of renewable sources should be 38.4 per cent for thermal energy, 17.6 per cent for electricity and 10 per cent for transportation by 2020. Altogether, the share of renewable energy used annually should reach

8,325 GWh, constituting 25 per cent of the final energy consumption by 2020. [29].
According to Luna del Risco (2011), Estonia has great potential for the production of biogas using manures, sewage sludge, herbal biomass and organic residues [2]. There are about 288 thousand hectares of abandoned agricultural land in Estonia suitable for the cultivation of energy crops, and 128 thousand hectares of semi-natural grasslands [2].
The Estonian Biogas Association (EBA) estimates that the actual potential for economically feasible biogas production is around 500 million Nm^3 per year (data for 2012), which could result in the production of 300 million Nm^3 of biomethane, containing 98 percent of methane, per year. In 2010, 13.13 million Nm^3 of biogas was produced, constituting 2.6 per cent of the actually utilised biogas potential [30]. By the end of 2015, the production capacity was 10.56 MW. In 2014, 42.84 GWh of electric energy was produced from biogas, while the respective amount was already 49.79 GWh in 2015 [29].

In Estonia, the technically and economically feasible potential for biogas production with shares from the substrates listed below and the presumable deadlines for their utilisation are, as follows and in table 2.1 and 2.2 [31]:
1. From 15 per cent (2020) to 25 per cent (2050) from the hay mowed from semi-natural habitats for nature conservation purposes.
2. From 20 per cent (2020) to 50 per cent (2050) from the silage received from unused agricultural lands (productivity 15 t/ha, the yield of biogas 155 Nm^3/t).
3. According to the Estonian Rural Development plan for 2014-2020, it is recommended to use 5 per cent of utilised agricultural areas for growing energy cultures (5 per cent of 1,078,330 hectares is 53,917 ha, (the assumed productivity is 15 t/ha, the yield of biogas 155 Nm/t).
4. 50 per cent of the forming sewage sludge will be used for the production of biogas.
5. It is possible to use 60 per cent of all manure and slurry for the production of biogas.
6. 80% of the collected biowaste sorted by type (from food industry, kitchen and canteen waste).

Table 2.1 The potential for using biogas as an energy resource in Estonia [31]

Biomethane substrates	**2010**	**2020**	**2030**	**2040**	**2050**
Semi-natural habitats, GWh	0	87	70	80	145
Unused land, GWh	0	891	1 500	2 000	2 227
Farmlands, GWh	0	338	400	500	677
Slurry and manure, GWh	15	150	441	441	441
Biowaste, GWh	0	40	109	109	109
Industrial waste, GWh	0	33	79	79	79
Sewage sludge, GWh	17	11	30	30	30
SUM, GWh	**32**	**1 550**	**2 630**	**3 240**	**3 708**
TOTAL, PJ	0.12	5.58	9.47	11.66	13.35

Table 2.2 The estimated potential of biogas in Estonia [32]

Substrate	Resource	The theo-retical amount of biogas, mln Nm^3	Realistically available (90%), mln Nm^3	Rapidly available %	BG (60% CH_4)	Biomethane potential (98% CH_4), mln Nm^3	GWh el year (8,200 with working hours)	nominal power, MW
Biomass from unused lands	177,385 ha	412	371	100%	371.2	222.7	792.2	90.4
Energy crops (5% of agri-cultural land)	53,917 ha	125	113	100%	112.8	67.7	241	27.5
Biomass from semi-natural habitats	100,000 ha	107	96	25%	24.1	14.5	51	5.9
Cattle	163,135 tk	97	87	72%	62.7	37.6	134	15.3
Pigs	360,990 tk	12	10	65%	6.8	4.1	15	1.7
Other agri-cultural residues	32,124 t	5	4	90%	4.0	2.4	9	0.9
Bio-degradable waste from the food industry	42,667 t	21	19	80%	15.4	9.2	33	3.7
Biowaste	24,000 t	4	4	80%	2.9	1.7	6	0.7
Sewage sludge	466,974 t	7	6	80%	5.0	3.0	11	1.2
Waste from industry	25,000 t	15	13	100%	13.1	7.9	35	4.0
TOTAL	--	805	725	--	618	371	1,326	151

Altogether, there are 18 operating biogas stations in Estonia; 5 of them are agricultural biogas stations, 7 are waste water and industrial waste water treatment facilities and 6 are serving as production units for landfill gas [30].

As of 1 March 2015, biogas is produced from agricultural raw material by Valjala Seakasvatuse OÜ (OÜ Saare Economics Jööri), Aravete Biogaas OÜ, Oisu Biogaas OÜ, Vinni Biogaas OÜ and Tartu Biogaas OÜ in Estonia. There are three

fermentation stations for industrial waste water: OÜ Eastman, Salutaguse Pärmitehas and AS Estonian Cell. The waste water treatment plants are AS Tallinna Vesi, AS Narva Vesi, AS Tartu Vesi and AS Kuressaare Veevärk. The producers of the landfill gas are Väätsa prügila AS, Paikre OÜ (Raba landfill, Pärnu), Baltic Energy Partners OÜ (Pääsküla landfill), Tallinna Prügilagaas OÜ (Jõelähtme landfill), AS Uikala Prügila (Uikala landfill) and AS Doranova (Aardlapalu landfill). [30, 32, 33]

Table 2.3 Production of electricity and heat from biogas in Estonia in 2014 [32, 33]

	Biogas plant	**Installed electric power MWel 2014. Year**	**electricity production [MWh] 2013.year**	**electricity production [MWh] 2014.year**
Biogas plants that run on agricultural inputs	Jööri	0.35	1,247	1,125
	Aravete	2	7,587	7,935
	Oisu	1.2	4,941	7,639
	Ilmatsalu	1.5	The station had not started production	4,077
	Vinni	1.36	3,351	8,221
	SUM:	**6.41**	**17,126**	**28,997**
Industrial wastewater treatment plants	OÜ Eastman	not known	not known	not known
	Salutaguse yeast factory	not known	not known	not known
	AS Estonian Cell	start-up	start-up	start-up
	SUM:			
Wastewater treatment plants	Tallinna Vesi AS	not known	not known	not known
	Narva Vesi AS	not known	not known	not known
	Kuressaare Veevärk	0.1	not known	not known
	Tartu Vesi	0.3	The station had not started production	
	SUM:	0.4		
Landfills	Paikre OÜ	0.15	1,097	874
	Pääsküla landfill	0.86	3,835	2,774
	Jõelähtme landfill	1.94	9,977	8,632
	Aardlapalu landfill	0.4	production of electricity from landfill began on 1 June 2014	1,500
	Uikala landfill	Application to *KIK		
	SUM:	**3.35**	**14,909**	**13,780**
TOTAL	**SUM:**	**10.16**	**32,035**	**42,777**

*KIK – Environmental Investment Centre

The listed institutions in table 2.3 use biogas for the production of heat and electric energy. In Aardlapalu landfill, Tartu, preparations are underway to use landfill gas as a motor fuel for compressed gas buses in the city of Tartu. A biogas reactor of Tartu Veevärk (waterworks) and several smaller biogas stations are under construction [30]. In 2013, biogas stations in Vinni and Oisu were launched, which use cow and pig slurry and manure as raw materials [30]. In 2015, a 0.4 MW combined heat and power station of Uikala landfill was launched. The majority of biogas stations have significantly increased their annual production, and process optimisation allows for progressively approaching the maximum production capacity of the biogas stations [29].
The biogas reactor of AS Estonian Cell in Kunda is the biggest in Europe. The wastewater of this reactor has a high concentration of organic matter, a suitable temperature (over 38 °C) and only a small amount of toxic compounds. The produced gas will be used in the plant's own dry kilns. [34]

Baltic Energy Partners OÜ has been in charge of the harnessing of Pääsküla Landfill (located in Tallinn) since 1994. Biogas is collected and distributed for the supply of heat and electricity to the local heating network and the national grid. Since closure of the landfill, biogas yield was estimated at 5 million m^3. [2, 30]

Tallinna Prügilagaas OÜ Tallinn Waste Recycling Centre collects the landfill gas produced in the deposit area and delivers it to OÜ Tallinna Prügilagaas, which produces electricity and a small amount of heat from it.
Vertical gas collection wells, which are connected to the compression station by plastic pipes, are used for collecting the biogas. The gas will be used in a gas engine, which is equipped with a gas burner to avoid sudden emissions due to engine stoppage. The combined heat and power station was completed in February 2010, and its electric power is 1.9 MW. [35]

Tallinna Vesi AS has been recovering the biogas produced from the biodegradation of sewage sludge from Paljassaare Waste Water Treatment Plant since 1993. It is estimated that average biogas production is 2.8 million m^3 per year with an energy content of 13.1 GWh. The company uses the biogas to supply the energy demands for running the facility. [2]

Saare Economics OÜ works with a farm scale biogas digester built in 2004. The biomass used to feed the digester is pig slurry. The facility is located in Jööri Village in Saare County and collects its raw material from 8 pig farms located on Saaremaa Island. Approximate biogas production is estimated to be 2.4 million m^3 per year, with an electricity and heat capacity of 350 kWel and 420 kWth per year. The reactor digestate is used by local farmers as a composting additive and fertiliser. [2]

Salutaguse Pärmitehas AS is a food industry company specialised in the development of yeasts. Biogas is produced from the residues of food processing and used solely for heat production.

There are also other sources of biogas in Estonia from landfills. However, biogas is not being collected and used for energetic purposes; instead it is being burnt in a flare. [2]

Vinni Biogaas OÜ Vinni biogas station was completed in 2013. The electric power of the biogas station is about 1.36 MW and the thermal energy output is about 1.41 MW. The annual estimated electricity production of the biogas station is 9.6 GWh and the annual thermal energy production about 9.6 GWh. In 2015, the annual production was 7,939 MWh. [36]

Oisu Biogaas OÜ Oisu biogas station was completed in 2013. The electric power of the biogas station is about 1.2 MW and the thermal energy output is about 1.2 MW. The annual estimated electricity production is 8.4 GWh and the annual thermal energy production about 8.4 GWh. In 2015, the annual production was 8,203 MWh. [37]

In 2009-2012, **Aravete Biogaas OÜ** developed a biogas project in Ambla rural municipality near Aravete village. The station was completed in the summer of 2012, and the main raw material for the biogas station is cow manure, which comes from the cattle sheds of Aravete Agro. In addition, raw materials from the surrounding agricultural and food industry enterprises are also used. The station's output is 2.0 MW. [30]

In 2015, **Estonian Cell AS** produced 5,013,488 m³ of biogas. In regard to methane content, the company replaced more than 30 per cent of natural gas with biogas, produced in-house every month throughout the second half of 2015. The methane content in the produced biogas is over 75 per cent, which allows biogas to be used as a replacement for natural gas in the production process of mechanical pulp. According to the company’s estimate, 5 million cubic metres of natural gas can already be replaced with in-house produced biogas in 2016. The energy value of the biogas produced per year is around 50 GWh. The company has been the biggest biogas producer in Estonia since 2014. [38]

3. AIMS AND OBJECTIVES OF THE STUDY

The aim of the thesis is to give an overview on methane production and its kinetics from industrial substrates from Estonia and an investigation of digestate as a fertiliser for agricultural purposes. The analysed substrates in this study were chosen according to availability in Estonia.
The main objectives of this thesis were to:

- evaluate the biochemical methane potential from industrial substrates (glycerol, compost from landfill, fish farm sludge, wastewater sewage sludge, catering waste, kitchen waste, brewery yeast, whey, cooking oil, etc.) (**PAPERS I-IV**)
- increase methane and biogas production by co-digestion of sewage sludge and different substrates (**PAPERS I-IV**)
- evaluate the digestates obtained from laboratory-scale experiments of the anaerobic co-digestion of different organic wastes for agricultural use (**PAPER V**).

4. METHODOLOGY AND EXPERIMENTS

The study of AD based on biodegradable waste was carried out using three different experimental devices: six laboratory scale reactors, one Armfield W8 anaerobic digester and one Automatic Methane Potential Test System (AMPTS) II.

4.1. Biochemical Methane Potential (BMP) test [*Paper V, 39*]

Methane production potential (MPP) tests were conducted with Automatic Methane Potential Test System II (AMPTS II). The instrument setup can be divided into three units A, B, and C, as it can be seen on photo 4.1.

In the *Sample Incubation Unit* (unit A), up to 15 vials containing small amounts of a sample with anaerobic inoculum are incubated at a desired temperature. The media in each vial is mixed by a slow rotating agitator. Biogas is then continuously produced, a parameter which is used to estimate the biomethanation activity inside each vial.

In the *CO_2-absorbing Unit* (unit B), the biogas produced in each vial passes through an individual vial containing an alkaline solution. Several acid gas fractions, such as CO_2 and H_2S, are retained by a chemical interaction with NaOH, only allowing CH_4 to pass through to the biomethane Gas Volume Measuring Device. A pH indicator is added to each vial to control the acid binding capacity of the solution.

In the *Gas Volume Measuring Device* (unit C), the volume of CH_4 gas released from unit B is measured using a wet gas flow measuring device with a multi-flow cell arrangement (15 cells). This measuring device works according to the principle of liquid displacement & buoyancy and can monitor ultra low gas flows; a digital pulse is generated when a defined volume of gas flows through the device. An integrated embedded data acquisition system is used to record, display and analyse the results.

The *UPS (Uninterruptible power supply Unit* (unit D) was added to avoid a power cut and loss of data.

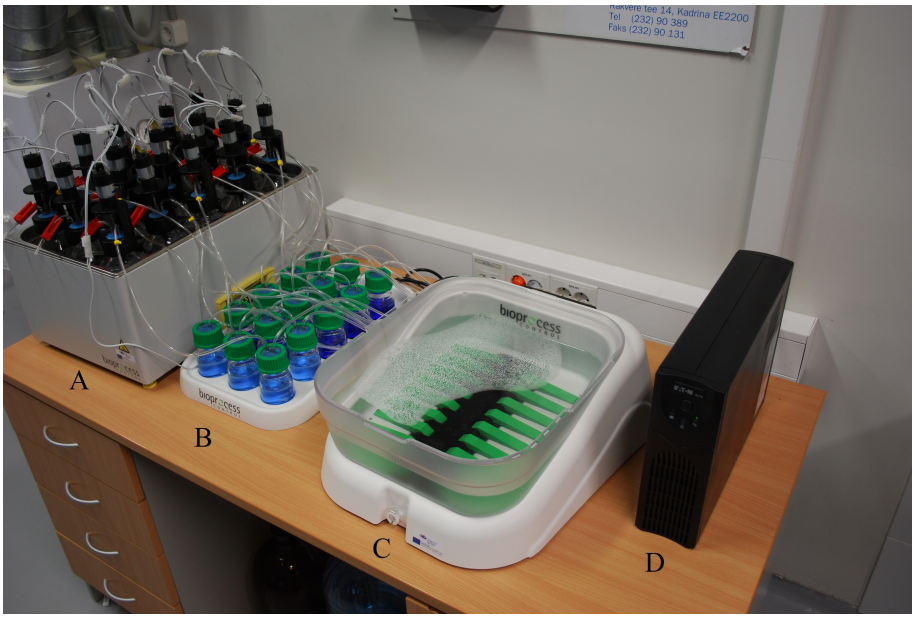

Photo 4.1 AMPTS II

4.2. One-stage test [*Paper V*]

The laboratory scale reactors with working volumes of 5 litres were constructed using fiberglass. The digesters were sealed with rubber stoppers and tube clamps containing an influent/effluent port to allow the injection of wastes. A water jacket and electric heating pad around the digester were used to maintain the temperature of the digesters, while magnetic spinners were used for mixing. Described one-stage reactor scheme in on figure 4.1 and photo of it on photo 4.2.

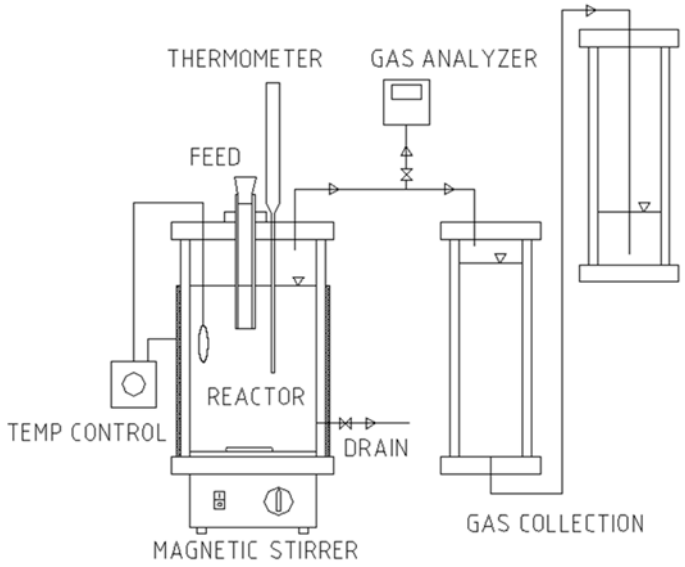

Figure 4.1 One-stage reactor scheme

Gas samples from continuous experiments were taken by portable biogas analyser Gas Data GFM416 Biogas Analyser.

Photo 4.2 One-stage reactor

4.3. Inoculum and substrate [*Paper V*]

Initially, the laboratory scale reactors were inoculated with anaerobic sludge (+38 °C) from the municipal wastewater plant biogas station of the city of Tallinn. Other substrates for laboratory tests and their origin are outlined in Table 5.1.

The chemical composition of the inoculum was, as follows: total solids (TS) 5.77 g/l, volatile solids (VS) 12.9 g/l. The inoculum was stored in 10 litre tanks and sieved through a 2 mm mesh. The chemical characterisation of the inoculum on total solids (TS) and volatile solids (VS) were measured each time before test set-up.

The substrates analysed in this thesis were collected from different Estonian industries between 2012 and 2015. A total of 12 different substrates were studied according to their availability in Estonia: wastewater sewage sludge (Tallinn WWTP, Kohtla-Järve WWTP), fish farming sludge (salmon breeding pool), catering and kitchen waste, glycerol (biodiesel factory), brewery residues (yeast), compost (Tallinn landfill), landfill leachate retentate (Väätsa landfill), dairy (whey), fish factory waste (fish skin, fish offal) and *Lemna* (duckweed).

The specific chemical parameters studied for each group of substrates are presented in Table 5.1. For homogenisation solid substrates as compost, fish factory waste, catering and kitchen waste samples milled to reach 1 mm and stored in fridges. All other samples were used without any treatment.

4.4. Experimental procedure [*Papers I-V*]

The BMP test was performed with AMPTS II. AMPTS II follows the same measuring principles as conventional methane potential tests, which make the analysis results fully comparable with standard methods. Sample material was mixed in 400 ml amounts in 500 ml serum bottle reactors. Each reactor contained the individual materials, nutrient medium and inoculum. In these experiments, a substrate-to-inoculum ratio of 0.2 and 0.5 was used. The serum bottles were immediately sealed with tube clamps after blow out with nitrogen (2 min). The bottles were placed into the incubation unit (+38 ± 0.2 °C) and mixed for 60 seconds with a 2 min pause at 24 h over 42 days by a slow rotating agitator. The produced biogas in each reactor was directed through an individual vial containing a 3 M alkali solution (NaOH). Gases such as CO_2 and H_2S were removed by chemical reactions, and CH_4 was the only gas that passed through unchanged. From the carbon dioxide absorption unit, the gas was directed to a flow cell array. All experiments were carried out in duplicate. With the AMPTS

II, both the gas volume measurements and data logging are fully automatic during the long incubation period. Experimental data were calculated and generated into a standard data sheet. The methane production from inoculum was determined in blank tests to which no substrate was added. The digestate products collection was performed at the end of the test.

One-stage digester first inoculated with inoculum from Tallinn WWTP biogas plant II reactor sludge. The temperature in digester was kept constant at 38°C. Feeding the digester with substrate began on the second day. Digester mixing was performed every morning before and after feeding and using the timer once every hour for 15 min. Biogas was collected through the displacement of water in gas clocks. The reactors were operated in draw and fill mode (on a daily basis) and the reactors were fed daily with 250 g of organic waste substrates with a hydraulic retention time of 30 to 20 days. The organic loading rate was up to 2 kgVS/(m^3*day). The digestate collection for chemical analyses was performed in the middle and at the end of the test.

The Armfield W8 anaerobic digester reactors jointly operated in draw and fill mode (on a daily basis) at a mesophilic temperature of 38 °C, and the reactors were fed daily with 250 g of organic waste substrates with a hydraulic retention time of 30 to 20 days. Digester mixing was performed every morning before and after feeding and using the timer once every hour for 15 min with a peristaltic pump. Biogas was collected through the displacement of water in gas clocks. The organic loading rate was up to 2 kgVS/(m^3*day). The digestate collection for chemical analyses was performed in the middle and at the end of the test.

4.5. Analytical methods [*Papers I-V*]

Substrates and digestates were analysed for pH. The optimum value for pH is between 6.8 and 7.6. The pH was measured by an electrode (Denver Instrument, UP-5), while TS and VS, total and soluble chemical oxygen demand (COD), total nitrogen (TN), ammonium nitrogen (NH_4+-N), total potassium (TK) and total phosphorus (TP) were determined according to standard methods.

The metal content (Cd, Cr, Cu, Hg, Ni, Pb and Zn) was evaluated in digestates to examine the chemical hazard related to their use as fertilisers. The results of bacterial pathogen (*Salmonella* spp) contamination were expressed as the presence or absence of pathogens.
The analysis of substrate and digestate samples from laboratory experiments was carried out in accredited laboratories in Estonia (Water Quality Laboratory at Tallinn University of

Technology and Agricultural Research Centre at Saku, which are authorised according to EN ISO/IEC 17025).
Biogas production was measured fully automatically in AMPTS II and by the displacement of water in one-stage and Armfield W8 gas bells. Both biogas samples (CH_4, CO_2, O_2, H_2S and NH_3) from one stage and Armfield W8 continuous experiments were taken with a biogas analyser (Gas Data GFM416 Biogas Analyser).

4.6. Calculations

Theoretical BMP
The methods described below are designed to easily determine the methane productivity of a specific substrate from its COD characterisation, elemental composition or organic fraction composition in order to obtain reliable results quickly and attain an economic advantage. These methods are applied by considering that all the organic material is degraded; therefore, a proper adjustment of this value is necessary by using the biodegradability obtained from the experimental BMP tests. The methane potential is expressed as ml CH_4 at standard temperature and pressure conditions per amount of organic material added (VS). [40]

Chemical oxygen demand (COD)
The maximum methane potential can be calculated from the amount of material and the COD concentration of the test using Eq. (8), assuming that this equation is valid for any substance or product [40]. This equation gives the theoretical value of methane at laboratory conditions [40]:

$$BMP_{thCOD} = \frac{n_{CH_4} RT}{pVS_{added}} \qquad (6)$$

where BMP_{thCOD} is the theoretical production at laboratory conditions, R is the gas constant (R = 0.082 atm L/mol K), T is the temperature of the glass bottle (308 K), p is the atmospheric pressure (1 atm), VS_{added} (g) are the volatile solids of the substrate and n_{CH4} is the amount of molecular methane (mol) determined from Eq. (7) [40]

$$n_{CH_4} = \frac{\mathrm{COD}}{64\left(\frac{\mathrm{g}}{\mathrm{mol}}\right)} \qquad (7)$$

Gas production

Digester gas contains about 65-70% methane, 30-35% carbon dioxide and trace amounts of nitrogen, hydrogen, hydrogen sulphide and water vapour [6, 7, 8]. It has a relative density of around 0.86. With an average concentration of 65% methane, the heating value is approximately 21-25 MJ/m^3, about 30-40% lower than the heating value of 37.3 MJ/m^3 for natural gas [8].

The methane generation rate can be estimated from the kinetic equations developed for the ADs:

$$P_x = \frac{YES_o}{1+k_d\theta_c} \quad (8)$$

$$V = 0.35m^3/kg\{[ES_o] - 1.42(P_x)\} \quad (9)$$

Where [8]:

P_x is the net mass of cell produced (kg/d)

Y the yield coefficient (g/g), for municipal sludge: 0.04–0.1 mg VSS/mg BOD utilised

E the efficiency of waste utilisation (0.6–0.9)

S_o the ultimate BOD_L of the influent sludge (kg/d)

k_d the endogenous coefficient (d^{-1}). For municipal sludge: 0.02–0.04 d^{-1},

Θ_c the mean cell residence time (d), equal to the solids retention time (SRT)

V the volume of methane produced (m^3/d)

0.35 the theoretical conversion factor for the amount of methane produced from the conversion of 1 kg BOD

1.42 the conversion factor for cellular material into BOD

If the biochemical methane potential (BMP) of a substrate is evaluated, the most interesting parameter to evaluate is the amount of gas produced per gram of VS added [39].

Use a mean value of the three blanks when withdrawing the gas production from the inoculum.

$$BMP = \frac{V_S - V_B\frac{m_{IS}}{m_{IB}}}{m_{VS,sS}} \quad (10)$$

BMP - is the normalised volume of methane produced per gram of VS of substrate added (Nl/gVS)

V_S- is the accumulated volume of methane produced from the reactor with the sample

(i.e., inoculum and substrate)

V_B - is the mean value of the accumulated volume of methane produced by the three blanks (i.e., inoculum)

m_{IS} - is the total amount of inoculum in the sample

m_{IB} - is the total amount of inoculum in the blank

$m_{VS,sS}$ - is the amount of organic material (i.e., volatile solids) of the substrate contained in the sample bottle [39].

The degradation profile vs. time can also be plotted [41]. If only one substrate is used, the accumulated volume can be plotted directly. If several substrates with different inoculum to substrate ratios should be compared, calculate the methane potential at every time step and compare the volume produced per gram of VS instead [41].

Hydraulic retention time (HRT) [d]

$$\text{HRT [d]} = \frac{V_{cd}\ [m^3]}{V_{fs}\left[\frac{m^3}{d}\right]} \quad (11)$$

V_{cd} capacity of digester (m^3)

V_{fs} fresh substrate added daily (m^3/d)

$$V_R\ [m^3] = V_{fs}\left[\frac{m^3}{d}\right] * HRT\ [d] \quad (12)$$

V_R required digestion capacity (m^3)

Volume load or organic loading rate (OLR) is a measurement of how much organic material is loaded into the digester each day and is expressed as $kgVS/m^3{}_{digester}/day$. This parameter considers both the concentration and the amount of the incoming substrate and is independent of the digester size, thus representing a very good parameter for regulating the feeding of the digester and in the same time assessing the performances of the digester.

The organic loading rate is important for the plant components (esp. mixer/agitator) and for the bacteria [41]. If the organic loading rate is too high (over 4.0 kg DS/m^3 d), technical components like mixers or pumps could be damaged or you may need an earlier maintenance than calculated due to an overload [41]. The bacteria could also be stressed by too much feeding causing a biogas production and the digestion process stopping [41].

$$OLR = \frac{QC}{V} \quad (13)$$

OLR organic loading rate, (kg COD/m^3d or $kgVS/m^3{}_{digester}/day$)

Q influent flow rate m^3/d

C influent COD or VS, (kg COD/m^3 or concentration of organic matter %/100)

V volume of reactor m^3

Determination of TS and VS

Before starting digestion test, the biomass should be characterized with regard to TS and VS.

The dry matter, i.e. all inorganic and organic compounds is often expressed as TS and can be measured according to a standard protocol [39]. For a given biomass sample, it is necessary to heat the sample up to 105 °C in order to remove all water content. VS is represented by the organic compounds in the sample. After finishing the TS measurement, heating the sample up to 550 °C for 2 hours should be continued for burning up the organic matter. The weight difference between the sample after heating at 105 °C and 550 °C reflects the VS content of the biomass.

TS is calculated as the ratio between the amount of dried sample (m_{Dried}) and the initial amount of wet sample (m_{Wet}), whereas VS is calculated as the ratio between the difference in the amount of sample after drying and burning (m_{Burned}) and the initial amount of sample [39].

$$\text{TS}\ (\%) = \frac{m_{Dried}}{m_{Wet}} \qquad (14)$$

$$VS\ (\%) = \frac{m_{Dried} - m_{Burned}}{m_{Dried}} \qquad (15)$$

4.7. Digestate analysis [*Paper V*]

Digestate is the remaining liquid or solid substance which cannot be used or decomposed by the microorganisms during the anaerobic digestion; it is composed of the bacteria that died during digestion and small traces of glasses, plastic and fibre. It is can used as a fertilizer to provide soil nutrients to boost food production [42].

The aim of digestate analysis was to evaluate the agricultural use of digestate obtained from the anaerobic co-digestion laboratory scale experiments of different organic waste (glycerol, compost from landfill, fish farm sludge, and catering waste and their mixes with sewage sludge) and digestate samples from full-scale biogas plants (cattle slurry). In this scope of activities, the content of nitrogen and phosphorus, *Salmonella spp* and heavy metal concentration in digestate was monitored.

The metal content (Cd, Cr, Cu, Hg, Ni, Pb and Zn) was evaluated in digestates to examine the chemical hazard related to their use as fertilisers. The results of

The metal content (Cd, Cr, Cu, Hg, Ni, Pb and Zn) was evaluated in digestates to examine the chemical hazard related to their use as fertilisers. The results of bacterial pathogen (*Salmonella spp*) contamination were expressed as the presence/absence of pathogens.

The analysis of digestate samples (from laboratory experiments and full size biogas plants) was carried out in accredited laboratories in Estonia (Water Quality Laboratory at Tallinn University of Technology and Agricultural Research Centre at Saku, which are authorised according to EN ISO/IEC 17025).

5. RESULTS AND DISCUSSION

In planning a biogas plant, several operational parameters are required (size of biogas unit, type of digester, technology and mechanisation, mixing technology, gas processing unit, biomass feeder, monitoring and controlling [43]) but particularly data on the chemical composition along with the methane and biogas potential of different biomass that is suitable for anaerobic digestion [2].

5.1. Chemical composition of substrates

The results on the chemical composition of the substrates analysed in this study are presented in Table 5.1.
Substrates origin: Sewage sludge from Tallinn (**SS**) and Kohtla-Järve (**K-JSS**) waste water treatment plants were studied. The sewage sludge of the city of Tallinn is fermented at the waste water treatment plant in a two-stage digester. In addition to domestic waste water, a considerable amount of industrial waste water flows into the waste water treatment plant of Kohtla-Järve. The sewage sludge of Kohtla-Järve was studied in co-fermentation with cheese whey (**W**) from the dairy industry. In some cases, lagoons are used in the final stage of waste water treatment, the surface of which may be covered with duckweed (*Lemna*) (**LE**) in summer. Duckweed was studied by co-fermentation with the sewage sludge of the city of Tallinn and by fermentation without additional substrate. Samples of the raw materials to be composted were taken several times from the composting field of Tallinn landfill. Timber and stones were sorted from the samples beforehand, the sorted material was shredded (**COM**) and biogas producing potential was determined from the biomass received. The possible use of the retentate (**LEA**), which was formed after the reverse osmosis treatment stage of the leachate from Väätsa landfill for co-fermentation with sewage sludge, was studied. The purpose was to determine whether the leachate retentate is susceptible to (co-)fermentation. Glycerol is obtained as a side product in the production of biodiesel (1 tonne of glycerol from 10 tonnes of biodiesel). The raw glycerol (**GL**) was co-fermented both with sewage sludge and other substrates in order to increase the yield of biogas. We were looking for an alternative use of pool sediment formed in trout farms, by its biogas fermentation instead of discharging it into a waste water treatment plant. At the same time, alternative use for the waste from the fish industry (**FI, Fish2**) was sought by co-fermentation. Possibilities for the co-fermentation of cooking oil (**OIL**), kitchen waste (**KITC**) and catering waste (**CW**) were studied. The fermentation of yeast (**B**) from the beer industry was studied with the purpose to increasing the yield of biogas as one of the alternative solutions instead of sending it to a waste water treatment plant.

In general, the results obtained in this study are very similar to the findings of other authors and their studies.
In the sewage sludge used in biogas tests by A.E. Maragkaki et al. (2016), the content of N-tot (1.2 kg/m^3) was 4-5 times lower than in the SS used in my experiments [44].
The analyses showed that the values of chemical parameters of the cheese whey studied by us were 1 to 2 per cent lower than E. Zielewicz et al. (2012) obtained in their studies [45].
The composting resulted in higher N-tot and P-tot concentrations than the ranges presented in the "Handbook of Biogas Production and Usage" (2008) [7].
The results of chemical studies of leachate from a landfill were in the same range that were found by Peter Kjeldsen et al. (2012) and A. Montusiewicz et al. (2011) in their studies [46, 47].
The cut grass that has been dealt with in the book "Production and Usage of the Biogas" (2009) is quite similar in its chemical parameters to the duckweed studied by us [7].
Glycerol used by A.E. Maragkaki et al. (2016) in biogas tests had virtually the same chemical parameters as the glycerol used by us [44].
In the studies conducted by P. Marcet et al. (2010), the sediment (manure) from fish farms gave about a 2 times lower result than the sediment used in my studies [48].
In the studies by E. Olsen et al. (2011), the physico-chemical analyses of fish waste gave 3, 68 and 32 times higher results for N-tot, P-tot and K-tot, respectively, than the results obtained by me. This can be due to the composition of fish waste and the particular fish [49].

The chemical composition of kitchen and food waste is similar to the range presented in the "Handbook of Biogas Production and Usage" (2009) [7].
The brewery yeast (wastes) used in the studies by S. Teweldevet al. (2012) had 2 times lower N-tot and 10 times higher P-tot indicators than the brewery yeast used in my studies. The reason for this may be that their brewery waste contained spent grains, yeast biomass, etc. [50]

Table 5.1. Chemical composition of substrates [PAPERS I-IV]

Substrate	TS [%]	VS [%]	N-total [kg/m^3]	NH_4-N [kg/m^3]	P-total [kg/m^3]	K-total [kg/m^3]	Crude protein [% in TS]	Crude fat [% in TS]	pH
SS	3.2	69.5	4.1	1.4	0.09	0.04	23.6	6.1	6.89
K-JSS+SS	6.2	81.5	5.3	0.5	1.4	NT	NT	NT	6.03
W	5.0	86.6	5.8	0.09	0.9	NT	NT	NT	3.93
LE	9.8	82.1	2.0	-	0.4	NT	NT	NT	NT
COM	38.3	80.0	18.3	0.2	1.4	1.9	25.9	7.6	6.00
LEA	3.7	43.1	1.2	0.9	0.02	0.5	10.5	0.02	6.90
GL	89.4	91.4	0.2	-	0.2	2.7	NT	NT	3.50
SFP	7.1	82.9	6.1	0.1	0.04	0.02	24.0	11.8	6.80
FI	23.9	89.6	6.4	NT	0.04	0.05	39.7	46.6	NT
Fish2	89.5	91.1	NT	NT	NT	NT	NT	NT	NT
OIL	99.9	100	NT	NT	NT	NT	NT	NT	NT
KITC	28.4	91.2	4.5	0.5	0.2	NT	NT	NT	NT
CW	31.9	98.4	6.8	NF	0.16	0.31	13.7	34.2	4.10
B	12.9	91.1	9.2	0.2	2.2	0.3	NT	NT	5.65

NF – not found; NT – not tested

5.2. Biomethane potential of industrial substrates

5.2.1. Cumulative methane yield

The results of BMP experiments are presented according to the origin of the substrate. First is the result of the pure substrate and then come the results with different mixtures of substrate (e.g, with sewage sludge or some other substrate), if conducted. At the same time, the minimum, maximum and average biomethane yields for both VS and the wet mass of the substrate are presented in Table 5.2.

The test results obtained from the sewage sludge of the cities of Tallinn (**SS**) and Kohtla-Järve (**K-JSS**) coincided with the results obtained by other researchers and the actual outcome that could be obtained from biogas stations. The average biomethane yield from the sewage sludge of the city of Tallinn was 283.83 m^3 CH_4/tonne VS. At the same time, on the basis of the studies by Elena Comino et al. (2012) [51], the biogas production potential from sewage sludge is approximately 2 times higher, 451±12 m^3 CH_4/tonne VS.

Since there is no biogas station for the fermentation of sewage sludge in Kohtla-Järve, it has not been possible to compare the results with an actually operating biogas station as is possible in the case of the Tallinn waste water treatment plant. The yield of biomethane from the sewage sludge of Kohtla-Järve was 136.0 m^3 CH_4/tonne VS, 6.79 m^3CH_4/m^3 on average, which is an almost two times lower result than for the sewage sludge of Tallinn.

Menert, et al (2008) reported that Thermophilic pre-treatment increases the degree of hydrolyses of sludge; anaerobic digestion of the pretreated sludge proceeds faster than that of raw sludge [52]. In order to increase the yield of biomethane from the sewage sludge of Kohtla-Järve (**K-JSS**), it was thermally pre-treated at +70 °C for 0.5, 1 and 2 hours. The results differed very little, irrespective of the time of pre-treatment. The average yield of biomethane after thermal pre-treatment was 167.09 m^3 CH_4/tonne VS, which gave about a 2 times greater productivity for wet mass than for untreated sewage sludge of Kohtla-Järve (see Table 5.2).

Possibilities were studied for co-fermentation of the sewage sludge of Kohtla-Järve with whey (**W**) from the dairy industry (406.76 m^3 CH_4/tonne VS, 17.63 m^3 CH_4/m^3. Ghaly (1996) recorded a whey-based methane yield of about 240 l-CH_4/kg-VS [53]) and together with glycerol (**GL**) formed in biodiesel production (316.51 m^3 CH_4/tonne VS, 256.10 m^3 CH_4/m^3). In both cases, the yield of biomethane was greater than the yield from the sewage sludge of

Kohtla-Järve. In co-fermentation of sewage sludge and milk whey, the average yield of biomethane was 290.15 m^3 CH_4/tonne VS, 13.20 m^3 CH_4/m^3. Lo and Liao (1989) tested a mix of whey and cow manure with a 2:1 ratio and obtained a methane yield of 222 l-CH_4/kg-VS [53]. In the case of sewage sludge and glycerol co-fermentation, the average yield of biomethane was 295.25 m^3 CH_4/tonne VS, 48.59 m^3 CH_4/m^3.

In summer time, when there are enough nutrients in water bodies, the water bodies start to grow over. In small waste water treatment plants, where lagoons are a part of waste water treatment, the proliferation of duckweed (*Lemna minor*) may occur.

Growing aquatic plants in nutrient-rich wastewaters for phytoremediation is a promising process because of its potential for bioresource/bioenergy recovery from waste streams at low overall cost. Duckweed is a small free-floating aquatic plant that proliferates through the vegetative budding of new fronds and can double its mass within 16-24 h under ideal conditions. Duckweed has four genera: *Lemna*, *Spirodela*, *Wolffia* and *Wolffiella*. [54]

Duckweed (**LE**) was gathered from the lagoons of three small WWTP in Lääne County. Experiments were conducted with shredded and non-shredded duckweed. The purpose of shredding was to break the plant tissues, so that the biomethane producing bacterium could have better access to enchylema and shredded plant pieces. As a result of the tests, it can be said that it did not make any difference whether the duckweed was shredded or non-shredded. The average yield of biomethane was 146.33 m^3 CH_4/tonne VS. Đurđica Kovačić et al. (2014) reported the biomethane yield of duckweed (Lemna minor) is slightly higher, 176 m^3 CH_4/tonne VS [55].

In landfills, compost (**COM**) is produced from biodegradable waste. For BMP experiments, pieces of wood and stones were picked out from the material under study of Tallinn landfill. After this, the remaining biodegradable waste was shredded. The results of experiments with biomethane show that the average yield from the biodegradable waste to be composted is 228.15 m^3 CH_4/tonne VS and 67.71 m^3 CH_4/m^3. Where the material to be composted was mixed with sewage sludge in different ratios, the yield of biomethane was about two times lower, 27.68 m^3 CH_4/m^3. In published research, the biodegradability ranges from a lower biogas production of 38 L biogas/kg VS added to a higher value of 290 L biogas/kg VS. These values are within previous reported ranges. The BMP test carried by Wagland (2008) showed an average value of 300 L biogas/kg VS added for a MSW input [56]. The biogas yield of anaerobic digestion of organic fraction of municipal solid waste values reported in published research ranges between 60 and 530 L/kg VS added (Raposo et al., 2011) [57].

One big issue with landfills is leachates and their cleaning. A sample of retentate was taken after reverse osmosis cleaning in the Väätsa landfill. It was attempted to determine its potential for biogas production and to carry out its co-fermentation with sediments from Tallinn waste water treatment plant.

At first, reverse osmosis (RO) concentrate BMP test results were promising. However, the removal of inoculum productivity from total result revealed a negative outcome (Table 5.2) [58]. RO concentrate (LEA) had a negative effect on the anaerobic digestion process with or without sewage sludge [58]. Even RO concentrate dilution with distilled water did not give a positive result.

The RO discharging concentrate additions have a negative effect on the anaerobic digestion of the sewage sludge [58]. This decline in methane yield might be caused by the deterioration of methanogenic archaeal activity following treatment of RO discharging concentrate.

The production of methane is greatly dependent on the weight of nutrients in raw material, such as proteins, fats and carbohydrates. An essential element contributing to the stability of performance of the process is the ratio of carbon and nitrogen (C/N) in the substrate [59].
If the weight of carbon (C) is too high, the bacteria are not able to release the carbon to the full extent. The high C/N ratio indicates the excessive consumption of nitrogen by methane bacteria, as a result of which gas productivity decreases [7]. Otherwise, if there is too much nitrogen, harmful nitrogen compounds will form, which may increase the pH value and create a toxic environment for bacteria [7].
Ammonia (NH_3) is especially dangerous for bacteria, even in small quantities. NH_3 is balanced with ammonium ions (NH_4-). When NH_3 reacts with water, ammonium and hydroxide (OH) ions will form. If the pH value and concentration of OH ions increase, the concentration of ammonia will also grow [7].
In order to have a successfully functioning fermentation process, the ratio of carbon and nitrogen (C/N) must be 10-40 [7]. In order to have enough nutrients for bacteria, the ideal ratio of carbon, nitrogen and phosphorus is 600:15:5 [7].
In Table 5.1, it stands out that the ratio of carbon and nitrogen in the retentate of Väätsa landfill leachate is too small, at only 4.

However, there are references in scientific literature that it is possible to use the leachate from landfills as a substrate for biogas production. Although the biogas production of fresh matter is low (12.4 Nl/kg), the leachate presents high biogas production per kg of volatile solids (934.6 Nl/kg VS) due to its high humidity [60]. In the biogas yield obtained from the co-fermentation of a 20:1 sewage sludge, the intermediate leachate mixture was 1.30 m^3 per kg of removed volatile solids, while from a 10:1 mixture it was 1.24 m^3 per kg of removed VS [47].

In pursuit of renewable energies, ever more biodiesel is being produced. In the production of biodiesel, glycerol will remain at the ratio of 1:10 [PAPER I]. During the research of biomethane potential of glycerol (**GL**), it was found to be 316.51 m^3 CH_4/tonne VS and the co-fermentation with sewage sludge resulted in 256.10 m^3 CH_4/tonne VS.

Steven Nartker et al. (2014) reported that methane potential for glycerol co-digested with digestate samples was 766 ± 42 ml/gVS. The digestate alone reached a maximum methane production of 112 ± 14 ml/gVS. The average difference between the two samples was 608 ml/gVS, which is 7 times as much as the gas production of digestate alone. This indicates that glycerol adds significant methane production when co-digested with digestate, and it does not show short-term toxicity effects when loaded at 33% of the total OLR. [61]

Kiattisak Panponga et al. (2014) reported the maximum methane yield of 1% glycerol waste and 99% canned seafood wastewater were 577 ml CH_4/g VS-added [62].

A big producer of biodegradable waste is the food industry. Beginning from the production of raw materials (plants, fish, animals, etc.) to the final consumer, i.e. a human being who is not able to consume the ready-to-eat food and leaves kitchen and catering wastes behind.

The sediment in pools of trout farms (**SFP**) consists of 98-99 per cent fish excrement and 1-2 per cent of fish feed falling to the bottom. The yield of biomethane from the sediment was 334.58 m^3 CH_4/tonne VS and mixing with sewage sludge in different ratios resulted in the average yield of biomethane of 248.35 m^3 CH_4/tonne VS. The result is similar to the biogas production potential of sediment from a waste water treatment plant (283.83 m^3 CH_4/tonne VS).

Fish waste from the fish factories (**FI, Fish2**) resulted in an average yield of 553.09 m^3 CH_4/tonne VS of biomethane and co-fermentation with sewage sludge gave 321.16 m^3 CH_4/tonne VS. When studying the mix of waste from different processes and types of production, the highest yield of biomethane was received from the mixture of fish skin, fish fat and oil – 277.31 m^3 CH_4/tonne VS. The result was smaller than expected, as the average biogas production potential for rapeseed oil was determined to be 797.91 m^3 CH_4/tonne VS.

In his paper, Gopi Krishna Kafle et al. (2013) reported on the methane potential of Pacific saury fish waste 435, mackerel fish waste 526 and cuttlefish waste 543 mL/gVS, which is higher than the results obtained in our studies [63].

For studies with kitchen waste (**KITC**), everyday waste was collected separately, and all the materials (potato, meat, soup, sauces, etc.) were also studied separately. Catering companies (**CW**) provided leftover food waste from catering, which was shredded and mixed into a uniform mass. The average yield

of biomethane from kitchen and catering waste differed almost two-fold. The average yield of biomethane from kitchen waste is 227.28 m^3 CH_4/tonne VS and 502.98 m^3 CH_4/tonne VS for catering waste.
The experimental results with kitchen waste of Jingqing Ye et al. (2013) were lower than our results 95.6 m^3 CH_4/tonne VS added [64].

There was a separate study for cooking oil (**OIL**) (rapeseed oil), the biogas production potential of which was found to be 797.91 m^3 CH_4/tonne VS.
P.G. Kougias et al. (2015) reported that the rapeseed oil methane yield from batch assays was 704 ± 13 (mL/gVS added) [65]. Also, our study had same curve as P.G. Kougias et al. (2015) reported: the curve corresponding to the sample of used oil shows that the biogas produced during the experiment period did not achieve its maximum value, indicating that the process was not completed [60]. Nevertheless, it was decided to stop the test after 47 days. The biogas production during this period was found to be 970.6 m^3 CH_4/tonne VS [60].

Yeast leftovers from breweries (**B**) have a high potential for biogas production: the average yield reaches 828.27 m^3 CH_4/tonne VS, 97.69 m^3 CH_4/m^3 for wet mass; co-fermentation with sewage sludge gives 710.57 m^3 CH_4/tonne VS and 58.89 m^3 CH_4/m^3 for wet mass.
Gregor D. Zupancic et al. (2012) reported an average specific biogas production of 560 m^3 tonne-1 of volatile solids) was achieved [66]. Koplimaa et al (2009) found in there study methane production by organic matter removal in the anaerobic stage at Salutaguse Yeast Factory WWTP was 269 m^3CH_4/day (the average concentration of CH_4 measured in the biogas was 65%) [67].

Results on the BMP are grouped according to their origin, then first pure substrate and then substrate mix with sewage sludge or with some other substrate mix. Results are presented in Table 5.2 and Fig.5.2 and 5.3. On fig. 5.1 is presented average methane potential of substrates by VS and error bars indicate standard deviation which are effected by substrate samples taken in different places and season. The first 3 results columns are presented by CH_4/tonne VS and by wet weight in the last 3 columns.

Table 5.2 BMP test results m^3CH_4 [PAPERS I-IV, 58]

Substrate	min by VS	max by VS	Ave by VS	min by WW	max by WW	Ave by WW
SS	71.18	851.74	283.83	1.12	21.98	7.26
K-JSS	136.00	136.00	136.00	6.79	6.79	6.79
W	406.76	406.76	406.76	17.63	17.63	17.63
K-JSS+W	203.33	369.17	290.15	9.68	16.25	13.20
K-JSS70	162.64	170.90	167.09	8.11	8.52	8.33
K-JSS+GL	295.25	295.25	295.25	48.59	48.59	48.59
LE	116.78	174.39	146.33	11.88	14.02	12.79
COM	228.15	228.15	228.15	67.71	67.71	67.71
COM+SS	198.36	229.25	212.97	8.90	49.76	27.68
LEA	-954.12	-32.97	-430.57	-6.89	-0.24	-3.31
LEA+SS	-124.24	178.71	51.68	-1.99	4.14	1.25
LEA+DE	-413.8	532.42	-24.98	-0.76	0.49	-0.276
GL	198.89	383.49	316.51	157.34	312.37	256.10
GL+SS	86.29	338.31	242.87	5.34	33.09	17.77
GL+TV	236.30	326.09	268.14	96.52	260.87	182.49
SFP	243.31	370.19	334.58	8.44	21.66	13.25
SFP+SS	181.94	316.03	248.35	4.21	7.40	5.55
FI	553.09	553.09	553.09	118.20	118.20	118.20
FI+SS	296.03	346.29	321.16	10.27	13.59	11.93
Fish2	277.31	277.31	277.31	225.90	225.90	225.90
FI+GL+SS	299.71	299.71	299.71	11.86	11.86	11.86
OIL	797.91	797.91	797.91	797.61	797.61	797.61
OIL+GL+SS	502.55	648.14	575.345	479.11	587.92	533.515
KITC	122.78	376.62	227.28	66.71	204.62	123.43
CW	403.93	602.03	502.98	82.27	162.73	122.50
CW+SS	264.60	550.84	403.62	2.41	281.06	68.44
B	825.40	831.13	828.27	97.35	98.03	97.69
B+SS	623.97	751.72	710.57	34.30	81.38	58.89

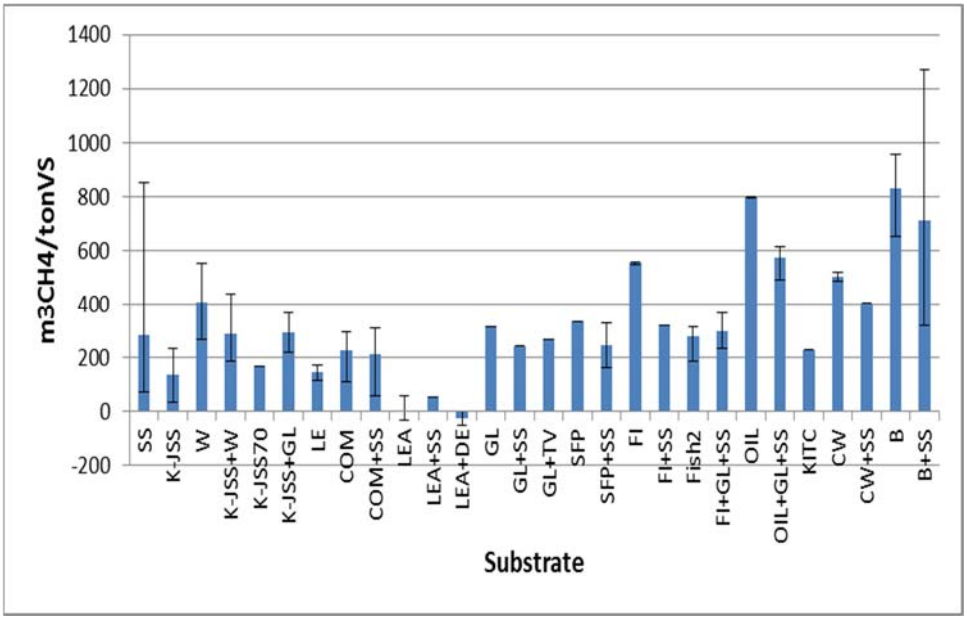

Figure 5.1 Methane potential of substrates by VS and error bars indicate standard deviation

In order to line out the course of methane fermentation for particular raw materials, Fig. 5.2 and 5.3 present the results for accumulated gas (CH_4) volumes and flow rates obtained during experiments.

It is considered that methane production curves correspond to the rapid bioconversion of readily degradable components followed by a slower bioconversion of fibrous portion of the substrates.

In addition to the results shown in Table 5.2, Fig. 5.2 and 5.3 also demonstrate that the substrates under study have significantly higher biomethane productivity than sewage sludge. The exception is the RO retentate of the leachate from Väätsa landfill, the negative result of which was addressed before.

Fig. 5.2 and 5.3 show the characteristic curve of the accumulated gas volume of glycerol and cooking oil. Glycerol is characterised by a curve emerging on days 2-4, and cooking oil by a gentle rise during days 1-15. That differs from other substrates, like sewage sludge, waste from food industry, etc.

On the basis of the study results, it can be strongly recommended to co-ferment sewage sludge with other substrates in order to increase the average yield of biomethane.

For anaerobic digestion purposes, it is important to define the optimum retention times for a defined substrate to reach its maximum potential [2]. From a technical or economical point of view, retention times can be targeted at a level when substrates have reached a certain percentage of their potential ultimate methane production [2, 68].

Anaerobic digesters are often designed to operate with a single substrate. In some cases, digesters can operate with a mixture of several substrates. In general, retention times can vary from 20 to 40 days. In this study, most of the analysed substrates had produced at least 80% of their ultimate yield within the first 7-10 days.

Generally, the main yield of biomethane is obtained from the phase during days 5 to 10; after this, methane fermentation almost ceases. The production of biomethane was only observed during days 20 to 25 in the case of cooking oil. (Fig. 5.3).

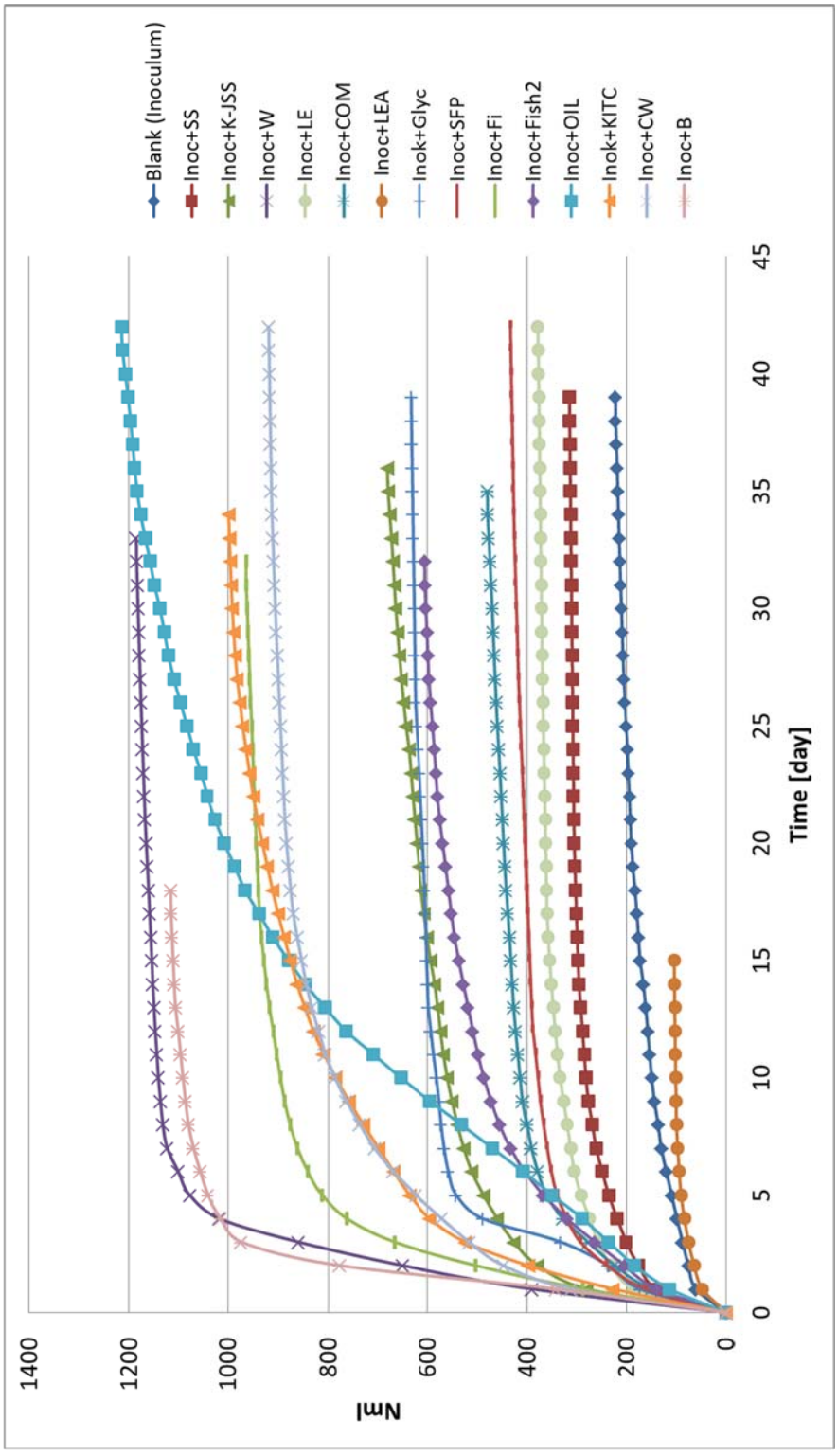

Figure 5.2 Accumulated biomethane volume Nml/day

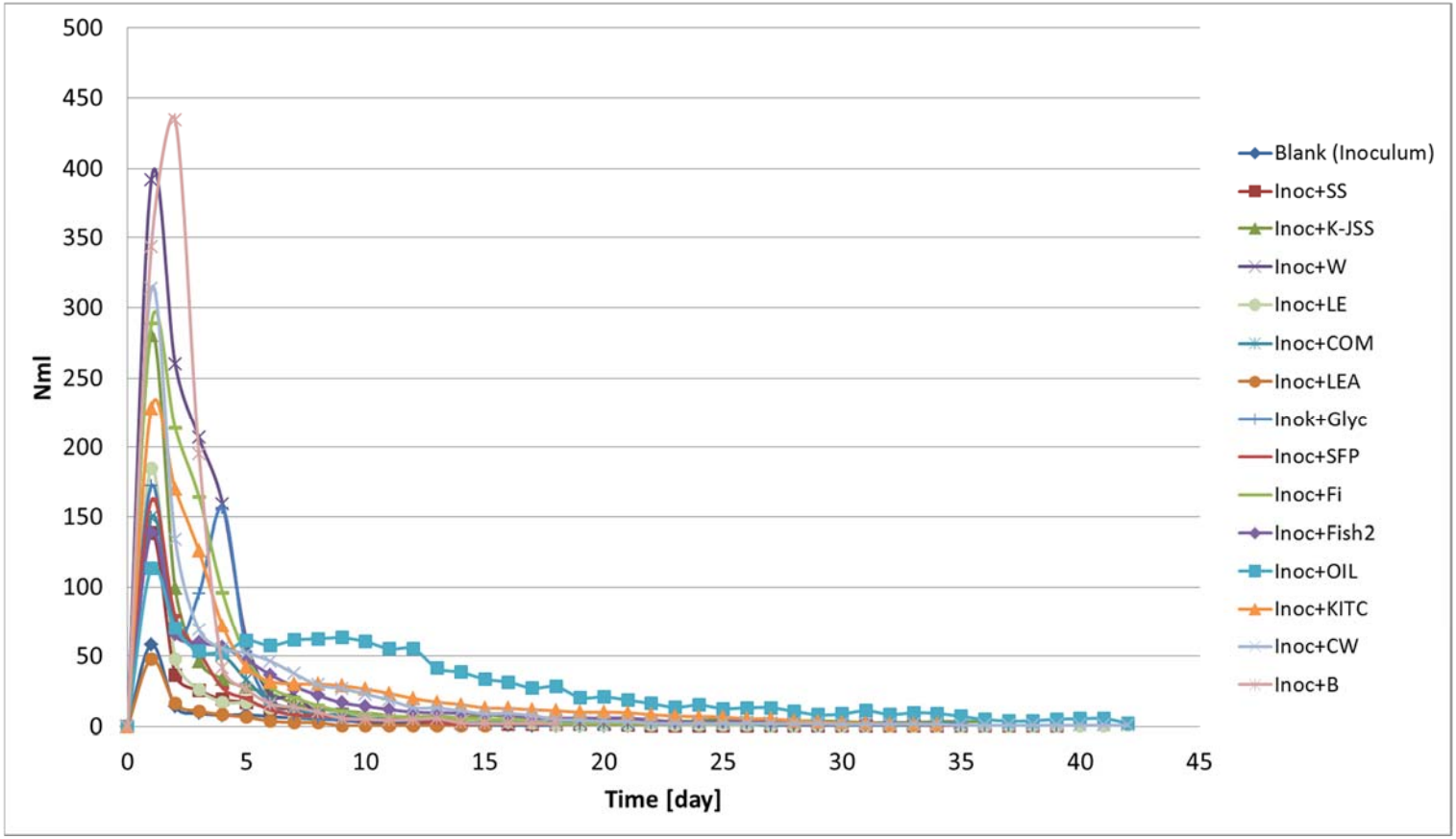

Figure 5.3 Biomethane flow rate Nml/day

Table 5.3 Time to reach corresponding percentage of ultimate methane yield

Substrate	60%	Days	70%	Days	80%	Days	100%	Days
SS	189.30	3	220.85	4	252.40	6	315.50	39
K-JSS	409.26	3	477.47	5	545.68	9	682.10	36
W	711.54	3	830.13	3	948.72	4	1,185.90	33
LE	227.58	2	265.51	4	303.44	6	379.30	42
COM	287.70	3	335.65	5	383.60	7	479.50	35
LEA	62.25	2	72.63	3	83.00	4	103.75	15
GLY	380.52	3	443.94	4	507.36	5	634.20	39
SFP	259.86	2	303.17	3	346.48	5	433.10	42
FI	578.16	2	674.52	3	770.88	4	963.60	32
Fish2	363.42	5	423.99	7	484.56	10	605.70	32
OIL	729.42	12	850.99	14	972.56	18	1,215.70	42
KITC	599.10	4	698.95	7	798.80	11	998.50	34
CW	550.92	3	642.74	6	734.56	8	918.20	42
B	670.02	2	781.69	2	893.36	3	1,116.70	18
AVERAGE	429.90	3.0	501.60	4.0	573.20	6.3	716.60	34.4

5.3. Continuous one-stage experiments

In parallel with BMP studies, experiments were carried out with some substrates in a one-stage digester (in drop and fill mode) in order to investigate the possibilities for increasing the yields of biogas. Co-digestion with sediments from Tallinn WWTP was tested. In addition to biogas yield studies, the concentrations of gases, such as CH_4, CO_2, O_2, H_2S and NH_3, were also measured. Gas analyses were carried out over 24 hours from biogas collected to a gas meter (fig. 4.1), and the results are presented in Table 5.4 and fig. 5.4.

While comparing the results of continuous experiments and the results of biomethane potential (BMP), a significantly lower yield can be shown for VS.

If the average yield of biomethane from sewage sludge (**SS**) was 284 m^3 CH_4/tonneVS, then the continuous experiment resulted in an average of 77 m^3 CH_4/tonne VS.
During experiments, the TS in fermented sludge (digestate) were in the range of 2.44 to 2.91%, and VS in the range of 53.53 to 58.42%.

The TS content of substrate influences anaerobic digestion process and biogas production efficiency. The methane and biogas production decreased with increasing the total solid contcentration. The results obtained by Liotta et al. (2014), showed that high-solids system could reach much higher volumetric methane production rate compared with low-solids system at the same solid retention time (SRT) in mesophilic anaerobic reactors treating sewage sludge [69].

For shredded compost (**COM**), co-fermented with sewage sludge, the average yield of biomethane at BMP tests was 213 m^3 CH_4/tonne VS; it was 37 m^3 CH_4/tonne VS in continuous experiments.
Wei Zheng et al. (2013) found biomethane potential for municipal biodegradable solid wastes of 253-337 m^3 CH_4/tonne VS [70].

For co-digestion of glycerol (**GL**) with sewage sludge, the yield of biomethane by BMP tests was 243 m^3 CH_4/tonne VS, but 206 to 303 m^3 CH_4/tonne VS in continuous experiments, depending on the amount of glycerol in supply. G. Silvestre et al. (2015) obtained the result of 349 to 490 m^3 CH_4/tonne VS [71]. Similarly to the results by Th. Amon et al. (2006) [72] and M.S. Fountoulakis et al. (2010) [73], our experiments also revealed that the co-digestion effect was especially high with glycerine additions of 3-6%. Glycerine was found to increase the CH_4 yield from the anaerobic digestion of protein dominated substrates. For a stable digestion process, the amount of glycerine should not exceed 6% [72, 73]. According to research data and our tests, the limiting organic loading rate is 2.6 to 3.0 kgVS(m^3*day). This is about 5% of the daily feed amount.
The yield of biomethane from BMP tests was 413 m^3 CH_4/tonne VS and 335 m^3 biogas/tonne VS in continuous experiments from the sediment from fish farm pools (**SFP**). When the fish farm pool sediment was co-digested with sewage sludge, the yield of biomethane was 248 m^3 CH_4/tonne VS, and the yield of biogas in a continuous experiments was 245 to 299 m^3 CH_4/tonne VS. For Ruth Gebauer (2004), the highest yield of biomethane from sediment of fish farm pools was 241 m^3 CH_4/tonne VS [74].
During experiments, the TS in fermented sludge were in the range of 2.39 to 2.82%, and VS in the range of 52.49 to 61.54%. Sediment from fish farm pools gave very good results in CH_4 percent having up do 70% (table 5.4, figure 5.4). All so biomethane production increased significantly at days 250 to 300 (figure 5.5) when was used fresh and more high VS content sediment after sludge centrifuge.

The yield of biomethane from BMP tests fish waste (**FI, Fish2**) was 553 m^3 CH_4/tonne VS, but only 270 to 272 m^3 CH_4/tonne VS in continuous experiments. For Carvalho, L et al. (Accessed 13.09.2016), the observed biomethane potential from fish waste was 712 m^3 CH_4/tonne VS, and the yield of biogas was 1,069 m^3 biogas/tonne VS in continuous experiments [75].

For catering waste (**CW**), the yield of biomethane from BMP tests was 503 m^3 CH_4/tonne VS, and the yield of biomethane was 249 m^3 CH_4/tonne VS in continuous experiments. For catering waste co-digested with sewage sludge, the yield of biomethane was 404 m^3 CH_4/tonne VS, and the yield of biomethane in continuous experiments was 312 m^3 CH_4/tonne VS.
Tampio E. (2016) obtained methane yields 400-430 m^3 CH_4/tonne VS in continuous food waste digestion [76].

For yeast from breweries (**B**), co-digestion with sewage sludge resulted in the yield of biomethane of 711 m^3 CH_4/tonne VS, and the yield of biomethane was 358 m^3 CH_4/tonne VS in continuous experiments.

During experiments, TS in digested sludge were in the range 2.6 to 3.2%, and VS in the range of 53 to 63%.

Adding a beer yeast into the digester feed gave 50% (total average) higher methane % and 661% higher biogas production compared with sewage sludge.

The results demonstrate that the biomethane potential from BMP tests is more than two times higher than the biomethane potential from one-stage tests. Ratios in SS and the substrate cause mixtures can be considered as the reason for the different results. On the other hand, the reactor released digestate every day, which contained not fully fermented biomass.

Blonskaja et al. (2003) recommended use of upflow anaerobic sludge blanket (UASB) reactor as the second stage of the two-stage set-up as it guarantees decreased washout of sludge and thus more stabile work of the digester [77].

From fig. 5.3 and 5.4 reveals the SS quality fluctuation depending on the place of sampling in Tallinn WWTP. SS samples were taken from primary clarifier and mud house before digester. SS from primary clarifier (days 45 – 200) gave 33% higher content and production of methane. Main differences were there in VS and TS. Samples from Primary clarifier had two times more VS and TS content then samples from mud house.

Some of the high and low peaks on fig. 5.3 and 5.4 are caused by temperature fluctuations in water heater.

Table 5.4 One-stage results [PAPERS I-IV]

Substrate	Ave by VS [m^3CH_4/ton VS]	CH_4 [%]	CO_2 [%]	O_2 [%]	H_2S [ppm]	NH_3 [ppm]	ORL [kg VS/(m^3*day)]	Note
SS	77	47.4	5.8	0.1	0	1.1	1.2	
K-JSS+SS	-	-	-	-	-	-	-	Nw
W	-	-	-	-	-	-	-	Nt
LE	-	-	-	-	-	-	-	Nw
COM+SS	37	68.7	22.6	0	0	19.4	1.6	
LEA	-	-	-	-	-	-	-	Nw
LEA+SS	-	-	-	-	-	-	-	Nw
GL+SS 2	206	59.6	14.9	3.2	0	31.8	1.7	
GL+SS 5-3	242	58.5	28.1	0.3	0	104.6	2.6	
GL+SS 3-5	303	66.3	23.9	2.1	0.1	5.7	2.1	
GL+SFP+SS	303	71.5	15.9	1.5	0.1	14.1	1.8	3M NaOH
SFP	413	69.4	23.5	0	1.1	6.9	1.2	
SFP 50% +SS	269	70.3	21.9	1.4	0	16.6	1.5	
SFP 36% +SS	245	65.5	21.8	1.6	0	19.7	2.1	
SFP 10% +SS	299	68.8	17.2	2.3	0	20.6	1.7	
FI	270	69.1	20.6	1.1	0	59.6	2.1	
Fish2	272	59.1	22.3	0.9	0.1	140.3	1.2	
OIL	-	-	-	-	-	-	-	Nt
KITC	-	-	-	-	-	-	-	Nt
CW	249	65.6	29.8	0.4	1.4	10.4	2.1	
CW+SS	312	54.6	25.5	0.4	0	12.4	2.2	
B 50% +SS	358	71.2	25.0	0	0	9.3	1.1	

Nt – not tested; Nw-not working inhibited

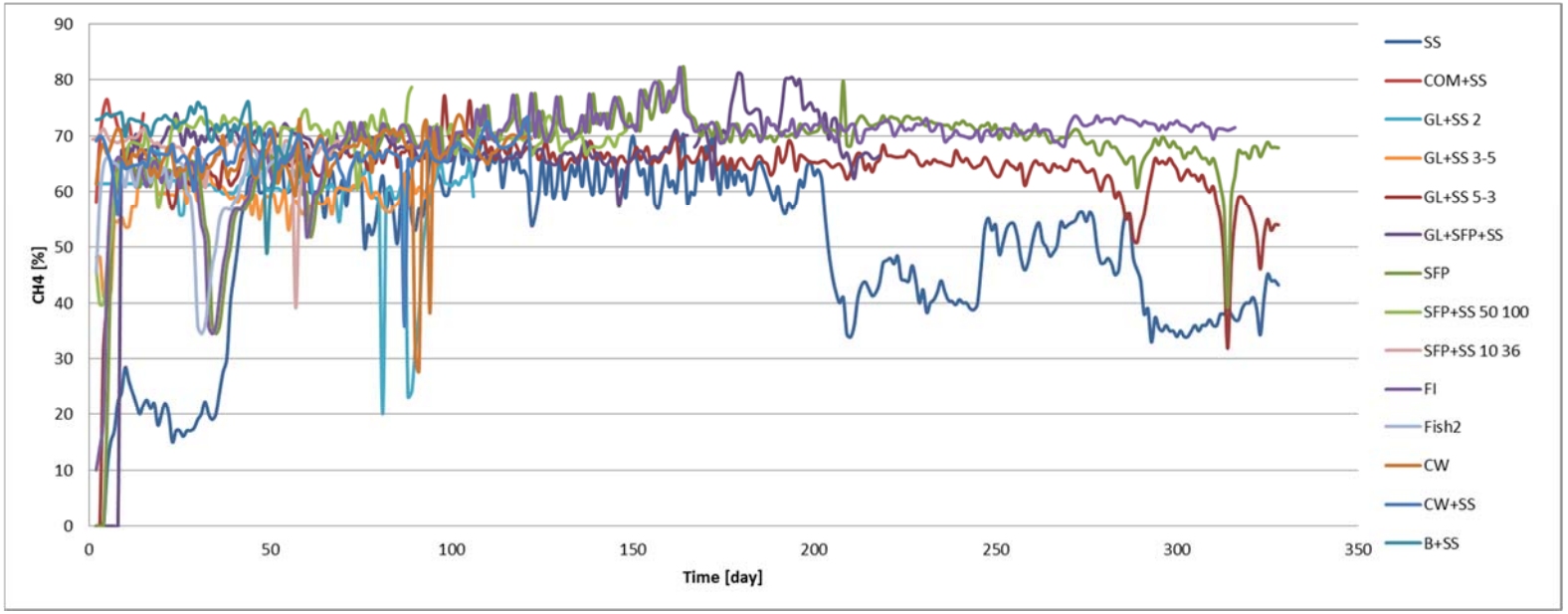

Figure 5.4 Biogas methane %

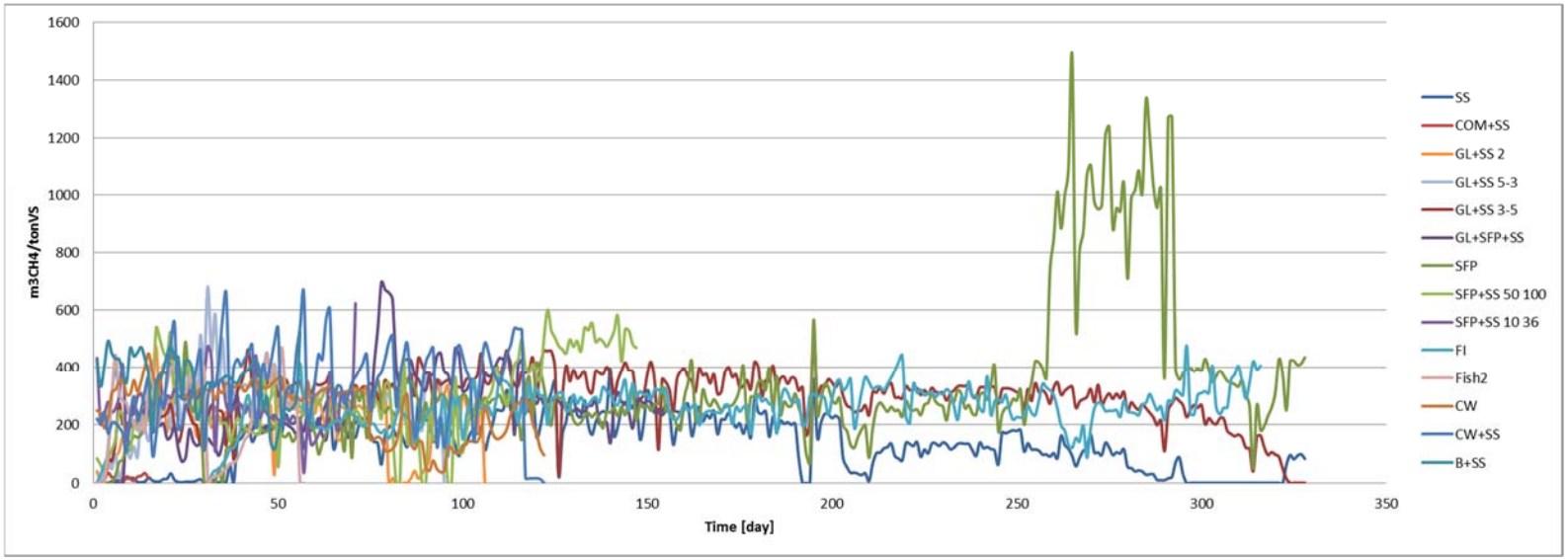

Figure 5.5 Daily methane production per added VS

5.3.1. Reduction of CO_2

Besides methane, biogas also contains CO_2 and H_2S and traces of other compounds. According to published research, one effective way of reducing CO_2 and H_2S in biogas is to drive biogas through an alkaline solution, typically 3 molar NaOH solution [78].

The biogas produced in the reactor passes through an individual flask containing an alkaline solution. Several acid gas fractions, such CO_2 and H_2S, are retained by chemical interaction with NaOH, only allowing CH_4 to pass through to the bio-methane gas clock. A pH indicator is added into the flask for controlling the acid binding capacity of the solution. [39]

The pH indicator Thymolphthalein will turn from blue to colourless when the CO_2 binding capacity of the NaOH solution decreases below optimal. At this point, replacement of the bottle with NaOH solution is recommended, to avoid the CO_2 gas from passing to the gas clock. [39]

After some discussion and literary studies, we decided to set up a one-stage test with raw sludge, glycerol and fish farming pool sludge substrate. Also, we added a CO_2 fixing unit (photo 5.1). The CO_2 fixing unit was installed after the reactor and before the gas clock. Biogas was directed on to the surface of 3M NaOH in test set I. When the pH indicator thymolphthalein turned from blue to colourless and the NaOH surface was crystallised, then replacement of the bottle with NaOH solution was done. The pH measurement from solution was before test 13 and after turning blue to colourless 9.

The test was run without CO_2 fixing unit for the first 35 days. After 35 days, we set up the CO_2 fixing unit. After the first test series, we continued tests with the CO_2 fixing unit to obtain more data, and in test set II biogas was directed in to a 3M NaOH solution (3 cm depth). In Table 5.5 are the first and second tests results.

The NaOH solution gave a 24% effect in rising the methane % and up to a 96% reduction in CO_2 (Fig. 5.6).

D. Shah and H. Nagarseth (2015) found in their study that carbon dioxide present in raw biogas can be reduced from 32.01% to 3.05% and methane content present in raw biogas can be increased from 61.22% to 94.69% [4].

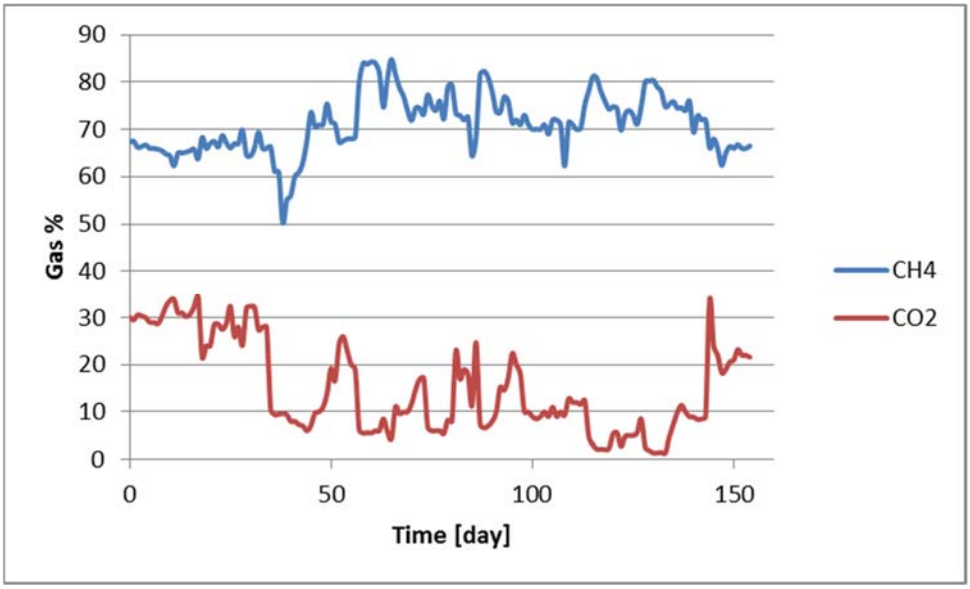

Figure 5.6 CH_4 and CO_2 content in biogas analysis

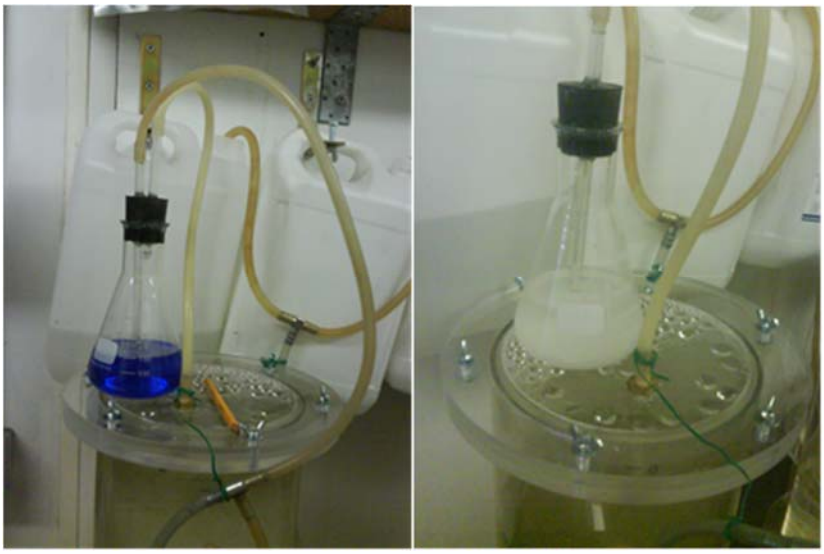

Photo 5.1. 3mol NaOH solution connected with gas clock. 3 mol NaOH solution before test (blue) and after 2 weeks testing (white).

Table 5.5 CO_2 decrease test results

Test results	CH_4 [%]	CO_2 [%]	O_2 [%]	H_2S [ppm]	NH_3 [ppm]
Before NaOH	**66.3** (62.3-69.9)	**28.2** (21.6-34.4)	**0.6** (0.1-1.5)	**0** (0-0)	**25.3** (3-53)
Test set I, after NaOH	**72.7** (64.4-84.8)	**11.7** (4.3-24.7)	**2.9** (1-4.5)	**0** (0-0)	**15.7** (0-45)
Test set II, after NaOH	**73.0** (66.3-81.2)	**9.2** (1.4-21.4)	**0.4** (0-5.9)	**0** (0-0)	**2.3** (0-26)

Naja et al (2011) found that not homogeneous and not constant quality of biogas in time must be processed before injection into the distribution network and an adequate process of purification should be optimized and monitored [79]. The purification process should allow, as a minimum requirement, to reach the technical specifications but also to control the minor elements likely to present a potential risk (heavy metals, some organochlorinated compounds, hydrogen sulphide, benzene, acetaldehyde and formaldehyde) and thus to obtain purified biogas of a quality comparable to natural gas [79]. When the biogas is purified and improved it can be utilized for various purposes which include heat and steam production, fuel oil when upgraded, used in production of chemicals, used in fuel cells as fuel, injection to national gas grid and as a source of energy for generation electricity and cooling [80].

5.4. Digestate

As previously mentioned digestate is the remaining liquid or solid substance after the anaerobic digestion. It is used as a fertilizer to provide soil nutrients to boost plant production [80].
The aim of this study was to evaluate the agricultural use of digestate obtained from the anaerobic co-digestion laboratory scale experiments of different organic waste (glycerol, compost from landfill, fish farm sludge, and catering waste and their mixes with sewage sludge) and digestate samples from full-scale biogas plants (cattle slurry). In this scope of activities, the content of nutrient, *Salmonella spp* and heavy metal concentration in digestate was monitored.

One important fertiliser value for digestates is content of nitrogen. According to our study results, the average of all investigated digestate N-total was 2.6 kg/m^3 with a minimum of 54% (1.4 kg/m^3) in ammonium form, which may be the key factor in determining the application rate to soils. Digestate N-total study results were in the range of 0.2 to 5.5 kg/m^3. The lowest result was obtained in the Tallinn WWTP sewage sludge digestate, which was 0.2 kg/m^3. Of course, this low result also depends on the time when the sewage sludge sample was taken from the Tallinn WWTP for biogas tests. In general, according to other

indicators, the Tallinn WWTP sewage sludge digestate reveals the lowest results. Higher N-total results were obtained from fish farm sludge laboratory test digestate and the Biogas Plant 3 digestate, which were 6.2 and 5.5 kg/m^3, respectively. R. Nkoa (2014) reported in his paper that typical anaerobic digestates N-total could be in the range of 3.1-14.0 (% DM) [81]. Similar results for N-total (3.0-30.0) were found in the IEA Bioenergy (Utilisation of digestate 2010) example from the UK of the approximate nutrient concentration of selected manure sources (kg/m^3 or kg/t fresh weight) and 0.4-30 (kg/m^3 or kg/t fresh weight) by Bioenergy Association of New Zealand Inc. [82, 83]. Poultry digestates have the highest N-total results (layer manure 16 and broiler/turkey litter 30 kg/m^3). The concentrations of the main nutrients P and K were also relevant (Table 5.6), which indicate that the materials can be an important source of nutrients for agricultural produce and help reduce the use of inorganic fertilisers. Biogas plants 1, 2, 3 and compost laboratory test digestate revealed higher study results in P-total and K-total than the Tallinn WWTP sewage sludge, fish farm sludge, glycerol and catering waste laboratory test digestate. R. Nkoa (2014) reported in his paper that typical anaerobic digestates P-total could be in the range of 0.2-3.5 and K-total 1.9-4.3 (% DM) [81]. By IEA Bioenergy P-total 0.5-10.9 and K-total 2.1-15 (kg/m^3) and by Bioenergy Association of New Zealand Inc. P-total 0.05-10.9 and K-total 0.2-15 (kg/m^3) [82, 83]. As mentioned above, poultry manure has the highest results.

Table 5.6 Nutrient content and *Salmonella* presence or absence in digestate [PAPER V]

Origin	TS [% ww]	VS [% TS]	N-total [kg/m^3]	NH_4-N [kg/m^3]	P-total [kg/m^3]	K-total [kg/m^3]	pH	*Salmonella*
Biogas Plant 1	6.2	81.5	3.7	1.6	0.7	2.7	7.9	Present
Biogas Plant 2	7.1	81.3	3.8	1.8	0.6	3.3	8.3	Absent
Biogas Plant 3	4.8	63.9	5.2	3.2	1.5	2.1	8.4	Absent
SS	2.4	60.1	0.2	0.1	0.03	0.04	7.0	Absent
SFP	2.7	56.4	6.2	3.5	0.06	0.02	7.1	Absent
GL	2.3	36.1	1.2	0.9	0.2	0.1	6.9	Absent
CW	3.1	68.6	3.5	1.5	0.06	1.1	7.3	Absent
COM	3.7	77.6	3.5	2.6	0.4	0.5	7.5	Present

From research publications, references can be found about the presence of *Salmonella* spp in different digestates [84, 85, 86, 87]. In our research, the presence of *Salmonella* spp. was reported in some digestates collected from the laboratory reactors and in some samples collected from the full-scale biogas plants. *Salmonella* mostly occurred in WWTP sewage sludge and in manure. In some cases, the presence of *Salmonella* was not observed after anaerobic digestion. *Salmonella* spp was absent in food industry substrates, but the

presence of *Salmonella* spp was noticed in some cases after digestion. It might be caused by inoculum that came from the WWTP digester or sewage sludge that already contained *Salmonella* spp [PAPER V].
Pathogen inactivation/destruction is mainly the result of the combined effect of process temperatures (thermophilic or mesophilic) and the retention times of feedstock inside the digester. In countries like Denmark and Germany, methods to measure the sanitation efficiency of AD based on “indicator organisms” were developed. A commonly used indicator organism is Streptococcus faecalis, and this was chosen because it takes longer to be destroyed during the AD process compared with other pathogenic bacteria, viruses and parasite eggs.[86]
If anaerobic digestion is used as a biological treatment, the recommended treatment process in Sweden is preheating at 70 ºC for 1 h, which is sufficient to kill vegetative bacteria, such as faecal streptococci, Salmonella and Listeria, different viruses and non-cystic Parasites [87].

According to the regulations valid in Estonia, sewage sludge digestate has to be monitored separately from other digestates. The allowable concentrations of heavy metals in sewage sludge to be applied in farming in Estonia are regulated by Minister of the Environment Regulation No. 78, "Sewage sludge in agriculture, landscaping and recultivation requirements for use"(EST limit) and allowable concentrations of heavy metals in digestate in Estonia are regulated by Minister of the Environment Regulation No. 12 "Requirements for digestate from biodegradable waste from biogas production" Annex 2 Digestate safety and quality indicators (EST limit I). European Directive No. 278 of 12 June 1986 "Environment and in particular protection of the soil, when sewage sludge is used in agriculture" is currently valid together with a number of amendments. The most recent document on sludge and biowaste was published by the Estonian Environmental Research Centre in March 2012 [Paper V].
Heavy metal concentration that was measured during our research was well below the maximum admissible concentration according to the Estonian Fertilisers Act (Table 5.7).

Table 5.7 Heavy metal content (mg/kg TS) in digestates [PAPER V]

Origin	Zn	Cu	Hg	Cd	Cr	Ni	Pb
Biogas plant 1	15.1-19.5	3.7-8.21	NF	<0.01-0.03	0.19-0.23	NF-<0.3	0.09-0.33
Biogas plant 2	13.6-15.0	2.7-3.5	NF	<0.01-0.018	0.1-0.2	NF-<0.3	0.037-0.2
Biogas plant 3	35.8-80.2	6.48-13.9	NF	<0.01-0.03	0.32-0.82	0.39-0.54	NF-0.32
SS	5.8	5.02	NF	0.03	0.35	<0.3	0.18
SFP	10.2-15.8	5.23-7.49	NF	0.05-0.093	0.52-0.81	<0.3	0.299-0.383
GL	985	362	<0.0005	2.8	39.3	21.2	41.0
CW	323-462	108-197	0.13-0.37	1.1-1.61	12.8-30	15-50.6	10.5-25.4
COM	15.6	7.91	NF	<0.6	0.708	1.35	1.68
EST limit	2500	1000	16	20	1000	300	750
EST limit I	600	200	0.45	1.3	60	40	130

NF – not found

EST limit – Sewage sludge in agriculture, landscaping and recultivation requirements for use

EST limit I – Digestate safety and quality indicators

The Cd concentration showed values lower than 0.1 mg/kg TS, while the legally permissible limit value for digestate is 1.3 mg/kg TS. Only glycerol and catering waste digestate showed slightly higher results than the limit rate for Cd in digestate. According to the sewage sludge use rate, all Cd values were lower than 20 mg/kg TS. IEA Bioenergy (2010) report that approximate Cd concentrations in animal slurry can be 0.2-30 mg/kg TS, in crops 0.2 mg/kg TS and in agri-food products <0.25 [82].

The Cr limit value according to digestate safety and quality indicators is 60 mg/kg TS; our digestate test results were much lower than the limit. The Cr limit value according to sewage sludge Environment Regulation is 1,000 mg/kg TS, but the digestate study results were in the range of only 0.1 to 0.82. Only glycerol and catering waste digestate analyses showed higher results of 39.3 and 12.8 to 30 mg/kg TS, which at the same time is 40 times higher than in other digestates but 25 times lower than the permissible limit for sewage sludge limit in agriculture. IEA Bioenergy (2010) report that approximate Cr concentrations in animal slurry can be 2.4-5.1 mg/kg TS, in crops 0.0-0.5 mg/kg TS and in agri-food products <0.15-<1.0 [82].

On the other hand, the Hg was present only in catering waste digestate and was in the range of 0.13 to 0.37, which is lower than the digestate safety limit 0.45 mg/kg TS, and the result were 40 times lower than the permissible sewage

approximate Hg concentrations in animal slurry and in crops were not found, but in agri-food products they can be <0.01 mg/kg TS [82].
Pb concentrations were in the range of NF to 41 mg/kg TS, which is 3 times lower result then digestate safety limit of 130 mg/kg TS and 18 times lower than the sewage sludge use limit of 750 mg/kg TS. IEA Bioenergy (2010) report that approximate Pb concentrations in animal slurry can be <1.0-9.8 mg/kg TS, in crops 2.0-3.0 mg/kg TS and in agri-food products <1.0-0.25 [82].
Digestates Zn test results were 2.5 times below the sewage sludge use limit of 2,500 mg/kg TS, but at the same time glycerol digestate were 1.6 times higher than digestate safety limit of 600 mg/kg TS resulting in 985 mg/kg TS. IEA Bioenergy (2010) report that approximate Zn concentrations in animal slurry can be 176-423 mg/kg TS, in crops 35-56 mg/kg TS and in agri-food products 3.7-6.1 [53].
Digestates Cu test results were 2.8 times lower than the sewage sludge use limit of 1,000 mg/kg TS, but at the same time the glycerol digestate Cu test results were 1.8 times higher than digestate safety limit of 200 mg/kg TS, resulting in 362 mg/kg TS, and catering waste digestate were quite near the digestate safety limit with a result of 197 mg/kg TS. IEA Bioenergy (2010) report that approximate Cu concentrations in animal slurry can be 51-364 mg/kg TS, in crops 4.5-9.5 mg/kg TS and in agri-food products 1.2-3.7 [82].
Digestates Ni test results were 5.9 times lower than the sewage sludge use limit of 300 mg/kg TS, but at the same time catering waste digestate were 1.3 times higher than the digestate safety limit of 40 mg/kg TS, resulting in 51 mg/kg TS. IEA Bioenergy (2010) report that approximate Ni concentrations in animal slurry can be 5.5-7.8 mg/kg TS, in crops 2.1-5.0 mg/kg TS and in agri-food products <1.0 [82].

In general catering, waste digestate and glycerol digestate heavy metal tests show much higher heavy metal (Zn, Cu) content in digestate than in other digestates. On the one hand, this might be caused by fish (salmon, pike perch, Baltic herring, etc.) and fish waste (heads, tails, backbone), which typically contains more heavy metals. Glycerol digestate high heavy metal content might be caused by glycerol quality and on the other hand the quality of Tallinn WWTP sewage sludge that was used as a co-substrate in biogas fermentation experiments.
Comparing our results with published research data reveals that there was a much lower content of heavy metals in our digestate.
The biodegradable waste must, in particular, be used in the production of biogas and the residues resulting from the fermenting process should be regarded as a potential reproducible product, particularly in agriculture as a fertiliser and soil conditioners.

6. CONCLUSIONS

AD is an environmentally sustainable technology to manage organic waste (e.g., food, garden, household, agricultural, food processing industrial wastes).

The results of the doctoral thesis showed that biodegradable waste, sediments and their mixes in Estonia have a high methane producing potential. The biomethane yields obtained varied in the range of 136 m^3 CH4/tonne VS (K-JSS) to 828 m^3 CH4/tonne VS (B) [PAPERS I-IV]. The quickest bioconversion of the substrate into biomethane occurred in experiments with yeast from breweries (B), for which achieving the cumulative yield of 80% took three days. Cooking oil (OIL) required 18 days for the same process.

By adding waste glycerol 2-5% by weight, the methane productivity per volume of reactor increased around 250-400%. When adding fish residue 2% by weight, the methane productivity per volume of reactor increased up to 290% [PAPER II, IV].

The sufficient testing period with AMPTS II is 20 days [PAPER II, III].
Although the results demonstrated a great potential for producing biogas from substrates, the analysis of different aspects of the fermentation process must be accompanied by investigations with pilot devices in order to avoid the excessive feeding of the reactor, resulting in process disturbances, and the co-influence of different substrates in the co-fermentation process. In addition to maintaining the anaerobic environment, the main parameters (pH, alkalinity, temperature, residence time, etc.) must be monitored, as they may affect the process of biogas production in different stages. Certainly, preliminary studies should be conducted with raw materials before using them as co-substrates in a biogas station, as the literature references show that the biomethane production potentials for substrates with similar parameters may vary widely.

A remarkable effect was achieved by using 3M NaOH for cleaning the biogas from CO_2 and H_2S. A question arose as to whether the yield of biomethane studied with AMPS II device can be contributed to pure biomethane, seeing as the biogas is not in immediate contact with 3M NaOH solution but instead only touches NaOH superficially while passing through the CO_2 purification unit. The results of biogas analyses obtained in flow-through experiments showed differences in the purity of biomethane in cases when the biogas was passed through a 3M NaOH solution or when it passed over the surface of the 3M NaOH solution. When the biogas was passed through the 3M NaOH solution, the percentage of biomethane was higher.

The use of the digestate formed in the biogas production as a fertiliser in agriculture has a tempting potential.

The microbiological analysis of digestate performed in this study revealed the presence of *Salmonella* during the digestion process, in both the laboratory reactors and full size biogas plants [PAPER V].
Certainly, the microbial pathogens should be continuously monitored both in the substrate and the digestate, as the studies have demonstrated that instead of being reduced or destroyed in the reactor, the pathogens may even increase their number in the digestate. Therefore, the main concern is the entrance of pathogens into the food chains of humans and animals, which depends on the survival of pathogens in soil and plants.

The digestate may become a very essential source of macro- and micro-nutrients in agriculture, primarily of nitrogen, phosphorus and potassium. At the same time, the excessive amounts of nitrogen or phosphorus introduced into the soil may act as sources of pollution of ground and surface waters and cause eutrophication.

If the digestate is used as a fertiliser, it is possible to diminish the deficit of trace elements in plants. For example, there is often a lack of Zn in soil, resulting in inhibition of the growth of cereals. The remaining heavy metals or indicator elements are toxic to animals, humans and plants [8]. Introducing these elements to the soil certainly should be monitored and decreased in order to avoid the pollution of soil with heavy metals. The observed accumulation of heavy metals in the digestate was not big enough to cause legal obstacles in Estonia and Europa on spreading the digestate onto fields as a fertiliser.

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ABSTRACT

Anaerobic fermentation is globally becoming a more and more essential part of the technologies for handling biodegradable waste, in addition to technologies for producing electric, thermal and fuel energy.
The production of biogas is mainly based on the process of mesophilic or thermophilic anaerobic fermentation, where the organic substances (proteins, fats and carbohydrates) in the raw material (substrate) are decomposed, producing biogas and the leftover of fermentation, or digestate. Primarily, the most easily degradable fats, proteins and carbohydrates are converted into biogas. On average, 3,5-50 per cent of hydrocarbons in the biomass of the raw material are transformed into biogas, while the remaining part will stay in the digestate. In addition to the aforementioned factors, during the production of biogas the concentrations of pathogens will decrease, the seeds of plants are destroyed, the share of ammonium ion (NH4-) in N-tot will increase, and, during co-fermentation, the concentrations of micro- and macro nutrients in the digestate will increase.
One of the ways for processing different biowaste is their co-fermentation with sewage sludge, which allows for increasing the production of biogas.
The aim of the present work was to determine the potential of production of biogas from biodegradable waste and to carry out one-stage experiments in flow-through reactors. The purpose of the experiments was to quantitate the production of biogas in the process of co-fermentation.
Sewage sludge, the manure produced in animal farming and the biodegradable waste formed in the agriculture (cut grass, waste from food industry, waste and sediment from fish farming, etc.), the adding of which allows to increase the production of biogas, should be treated as the main sources for biogas stations. Yeast from breweries, cooking oils and the glycerol produced in the production process of biodiesel fuel might serve as raw materials.
The composition of 14 substrates of Estonian origin was analysed, their yield of methane from co-fermentation with sewage sludge was quantified and the chemical composition of the digestate obtained in experiments was analysed. In parallel with laboratory experiments, the substrates and digestates from three biogas stations operating in Estonia were monitored.
Inoculate for experiments was obtained from the anaerobic reactor of Tallinn waste water treatment plant. The experiments were carried out in the anaerobic environment, at temperatures of 38 °C and 55 °C.
The cumulative yield of methane from the substrate was calculated, subtracting the yield obtained in the control experiment with inoculum from the total amount of the methane produced during incubation. The production of gas was expressed as cubic metres of methane or biogas in standard conditions (0 °C and 1 atm) per volatile substances or wet mass of the substrate added during the experiment.

The data on the chemical composition of the substrate and the digestate were in mainly similar to the results published by other authors.
The yields of methane from sewage sludge were in the range of 71-851 m^3 CH_4/tonne VS. The yields of methane from landfill leachate and compost were in the range of 0-228 m^3 CH_4/tonne VS. The methane yields from glycerol formed in biodiesel production were in the range of 199-383 m^3 CH_4/tonne VS. The yields of methane from food industry waste were in the range of 122-831 m^3 CH_4/tonne VS.
The compost from landfill had the highest yield of methane (228 m^3 CH_4 /tonne VS), while the leachate from landfill inhibited the process of methane fermentation.
Among samples from food industry the beer yeast had the highest methane yield (831 m^3 CH_4/tonne VS) and the kitchen waste the lowest (122 m^3 CH_4/tonne VS).
According to research sewage sludge and waste from industries are suitable substrates for co-fermentation. The analyses of digestates carried out during studies showed that the digestate is also suitable for use as a fertiliser in agriculture and as a potential material after increasing the content of dry matter. In comparison with the world's practice the results of this research are quite similar. However before use of substrate and digestate studies and tests should be carried out to ensure the increase in biogas yield and digestate safety. The study results reveal differences in biogas yield and digestate analysis for some similar substrates used compared to the world practice. Collaboration among the producers of waste and biogas stations is the key question in order to promote co-fermentation and the adaption of legislative acts to widen co-fermentation and the uses of digestate.

KOKKUVÕTE

Anaeroobne kääritamine ning saadava biogaasi ja digestaadi kasutamine on maailmas muutumas järjest olulisemaks biolagunevate jäätmete käitlemise tehnoloogiaks lisaks elektri- ja soojusenergia tootmisele jäätmete põletamise teel.

Biogaasi tootmine põhineb peamiselt mesofiilsel või termofiilsel anaeroobse kääritamise protsessil, mille käigus lagundatakse tooraines (substraadis) sisalduv orgaaniline aine (proteiinid, rasvad ja süsivesikud) ja saadakse biogaas ja kääritusjääk ehk digestaat. Keskmiselt muundatakse 35-50% tooraine biomassis sisalduvatest süsivesinikest (eelkõige kergemini lagundatavad rasvad, proteiinid ja süsivesikud) anaeroobse kääritamise käigus biogaasiks ning ülejäänud osa jääb alles kääritusjääki. Lisaks väheneb biogaasi tootmise käigus toormes patogeenide sisaldus, hävinevad taimede seemned, suureneb ammooniumi (NH_4-N) osakaal üldlämmastikust ning kooskääritamise puhul summeerub toorainete mikro- ja makrotoitainete sisaldus kääritusjäägis.

Erinevate biojäätmete üheks võimalikuks käitlemisviisiks on kooskääritamine reoveesettega, mis võimaldab suurendada biogaasi saagist.

Käesoleva uurimustöö eesmärgiks oli teostada ühe-astmelised katsed läbivoolureaktorites, et määrata biolagunevate jäätmete biogaasi saagis ja hinnata biogaasi tootmise väljavaateid. Katsed teostati kooskääritamise protsessis.

Biogaasijaamade peamise toorainena tuleb käsitleda reoveesetet, loomakasvatuses tekkivat sõnnikut ning erinevaid põllumajanduses tekkivaid biolagunevaid jäätmeid (niidetud rohi, toiduainetööstuse jäätmed, kalakasvatuse jäätmed ja setted jne), mille lisamisel saab suurendada biogaasi tootlikkust ja selle läbi väärindada suuremat hulka biolagunevaid jäätmeid. Tooraineteks saab kasutada ka õlletööstuse pärmi, toiduõlisid ja biodiisli tootmises tekkiv glütserooli.

Analüüsiti 14 Eesti päritolu substraadi keemilist koostist, määrati nende metaanisaagis kooskääritamisel reoveesettega ja analüüsiti katsetest saadud digestaadi keemilist koostist. Paralleelselt laboratoorsete katsetega jälgiti kolmes Eestis töötavas biogaasijaamas kasutatavaid substraate ja saadavat digestaati.

Inokulum katsete tarbeks saadi Tallinna reoveepuhastusjaama anaeroobsest kääritist. Katsed teostati mesofiilses anaeroobses keskkonnas temperatuuridel 38 ja 55 °C.

Metaani kumulatiivne saagis substraadis arvutati järgnevalt: inkubatsiooni aja jooksul tekkinud metaani summast lahutati inokulumi võrdluskatses tekkinud metaani saagis. Gaasi saagis väljendati kuupmeetrites metaani või biogaasi, arvutatuna 0 °C ja 1 atm (ehk standardtingimustes) katses lisatud substraadi lenduvaine või märgmassi kohta.

Uuritud ja katsetatud substraatide keemiline koostis ja digestaadi ainesisaldus sarnanesid teiste autorite poolt avaldatud tulemustega.

Reoveesetete metaanisaagised olid vahemikus 71-851 m^3CH_4/ton VS. Prügila nõrgvee ja komposti metaanisaagised olid vahemikus 0-228 m^3CH_4/ton VS. Biodiisli tootmises tekkiva glütserooli metaanisaagised olid vahemikus 199-383 m^3CH_4/ton VS. Toiduainetööstuse jäätmete metaanisaagised olid vahemikus 122-831 m^3CH_4/ton VS.

Kõrgeim metaanisaagis oli prügila kompostil 228 (m^3CH_4/ton VS), kui samal ajal prügila nõrgveel oli inhibeeriv toime metaankääritusprotsessile.

Toiduainetööstuse proovides oli õllepärmil kõrgem metaanisaagis (831 m^3CH_4/ton VS), kui köögijäätmetel (122 m^3CH_4/ton VS).

Käesoleva doktoritöö tulemusena on reoveesete ja tööstusettevõtete jäätmed sobivateks substraatideks kooskääritamisel. Uuringute käigus tehtud digestaadi analüüsid näitasid selle sobivust kasutamiseks põllumajanduses eeskätt väetisena ja mulla omaduste (huumusesisalduse) parandajana ning peale kuivaine sisalduse tõstmist ka potentsiaalse haljastusmaterjalina. Võrdluses maailma praktikaga on uurimustöö tulemused küllaltki sarnased, ent enne substraadi ja digestaadi kasutusele võttu tuleks kindlasti läbi viia uuringud ja katsetused veendumaks biogaasi saagise suurenemise ning digestaadi ohutuses, sest nagu uurimustööst välja tuli ei pruugi sarnastel toormetel tulemused ühtida maailma praktikaga.

Võtmeküsimusteks on veel koostöö biolagunevate jäätmete tekitajate ja biogaasijaamade omanike vahel, edendamaks kooskääritamist ning seadusandluse kohandamine soodustamaks kooskääritamist ja digestaadi kasutamise valdkondade laiendamist.

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APPENDIX I ORIGINAL PUBLICATIONS

PAPER I

Kuusik, Argo, Loigu, E., Kuusik, A., Sokk, O. (2013). Possibility of Enhancing Methane Productivity in Anaerobic Reactors in the Treatment of Excess Sludge from Wastewater Treatment Plants. International Journal of Science and Engineering Investigations, 2 (12), 33-36.

PAPER II

Kuusik, Argo, Kuusik, A., Loigu, E., Sokk, O., Pachel, K. (2013). Selection of Most Promising Substrates for Biogas Production. International Journal of Energy and Environment, 7 (3), 115-124.

PAPER III

Kuusik, Argo, Kuusik, A., Pachel, K., Loigu, E., Sokk, O. (2013). Generalised Intergation of Solid Waste Treatment Practices to Enhance Methane Productivity, Generate Suspension Fertiliser and Upgrade Biogas. European Scientific Journal, 9 (36), 14-30.

PAPER IV

Kuusik, Argo, Pachel, K., Kuusik, A., Loigu, E. (2014). Anaerobic co-digestion of sewage sludge with fish farming waste. In: 9th International Conference on Environmental Engineering: Water Engineering (1-8). Vilnius, Lithuania: VGTU Press “Technika”.

PAPER V

Kuusik, Argo, Pachel, K., Kuusik, A., Loigu, E. (2017). The possible agricultural use of digestate. Proceeding of the Estonian Academy of Security Sciences, 66(1), 64-74.

PAPER I

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Possibility of Enhancing Methane Productivity in Anaerobic Reactors in the Treatment of Excess Sludge from Wastewater Treatment Plants

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Abstract- This paper describes technological possibilities to enhance methane production in the anaerobic stabilisation of wastewater treatment plant excess sludge. 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 percentage increase of total solids 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- *Enhancement of methane production; fishery residues; waste glycerol.*

I. INTRODUCTION

Nowadays, the investigation and use of alternative energy sources have become progressively more topical [1]. Among these sources, biodiesel as a liquid fuel from rapeseed and biogas from the anaerobic digestion of different organic waste are comparatively well known. In general, one of the sources of biogas is the anaerobic digestion of wastewater treatment plant excess sludge. The liquid fuel production also creates waste by-products. One of these is glycerol. Its need for industrial use is limited. The production of 100 kg of biodiesel creates 10–11 kg of waste glycerol [2]. The aim of the investigation was to ascertain how to incorporate ordinary waste sludge and glycerol anaerobic digestion in the best way. Also of interest was whether it is reasonable to use fishery residues in the same manner. Reference [3] claims that concentrated glycerol, as a single raw material, is not treatable by anaerobic digestion technology. Due to the co-substrate effect, glycerol is more easily digested in a mixture of different organic materials where it is in the role of admixture [4].

II. EXPERIMENTAL PROCEDURE

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 performed with raw sludge obtained from Tallinn wastewater treatment plant (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. 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. 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 pH was measured by a pH meter (Denver Instrument, UP-5). 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.

III. EXPERIMENTS AND RESULTS

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 I below gives 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 II and III) 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 II, and III).

TABLE I 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 II 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 III CONTINUE OF THE TABLE II

Retention time, days	*Tempe-rature, ^oC*	*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 II and III, 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 IV and V. The bracketed values are minimums and maximums considering the weighted average.

TABLE IV THE SUMMARISED DATA OF THE EXPERIMENTS ON THE LEVEL OF WEIGHTED MEANS

Substrate	*Days considered*	*Retention 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 II and III 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.

Summarizing the results of tables IV and V 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 V CONTINUE TABLE IV

Substrate	*Methane yield*		*Methane contents in biogas, %*	*Solid removal, %*	
	Per volume, L/m³	*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 VI was derived on the basis of tables IV and V. 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 VI 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)

IV. CONCLUSION

1. The yield of methane production of existing anaerobic reactors can be efficiently enhanced by adding glycerol or fishery residues. Methane concentration in the biogas is also higher.

2. Both additives are industrial waste. Their utilization 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%.

3. 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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PAPER II

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.

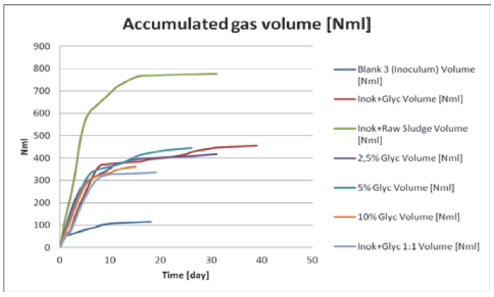

Fig.1, Cumulative methane generation (test set 1)

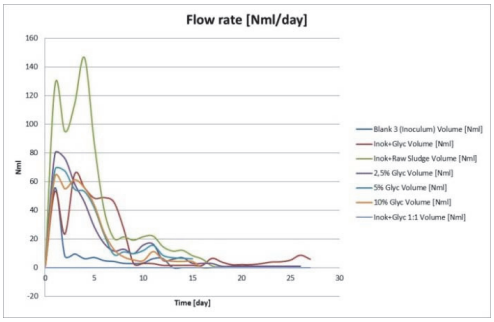

Fig.2, Daily methane generation intensity (test set 1)

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.

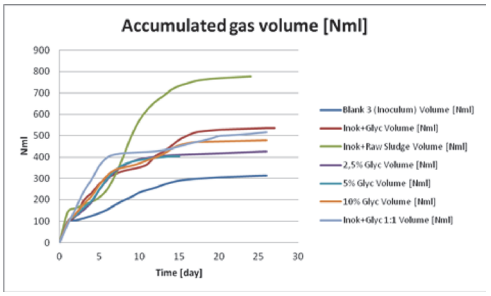

Fig. 3, Cumulative methane generation (test set 2)

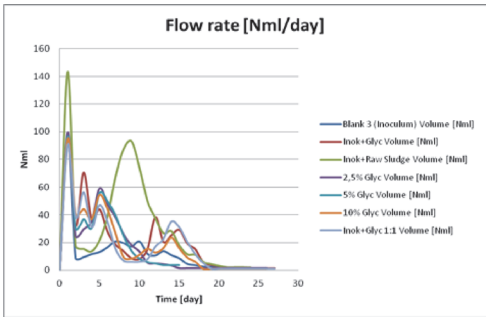

Fig. 4, Daily methane generation intensity (test set 2)

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.

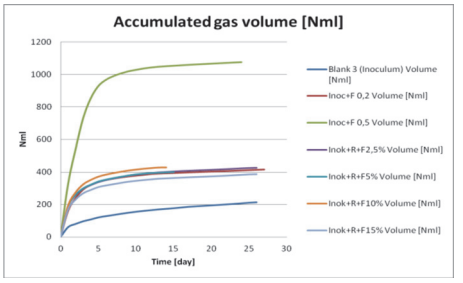

Fig. 5, Cumulative methane generation (test set 4)

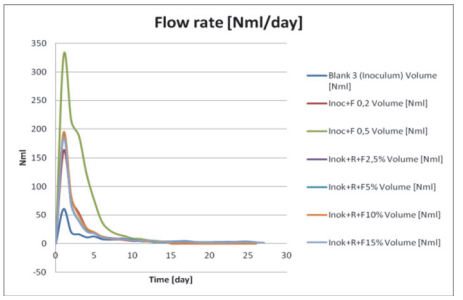

Fig. 6, Daily methane generation intensity(test set 4)

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 ^{o}C)

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	Retention 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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PAPER III

GENERALISED INTEGRATION OF SOLID WASTE TREATMENT PRACTICES TO ENHANCE METHANE PRODUCTIVITY, GENERATE SUSPENSION FERTILISER AND UPGRADE BIOGAS

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Abstract

This paper presents a general solution of how to link together the treatment of different solid waste: excess sludge, wastes glycerol or fishery residues and waste ash. The aim of the solution is to enhance biogas production and to produce an organic–mineral suspension fertiliser. The enhancement of biogas productivity is achieved by adding waste glycerol from biofuel plants or fish residues from fish farming and fishing industries into anaerobic reactors of wastewater treatment plants. The enhancement of biogas productivity lies in the range of 200–400%. The fertiliser is produced as a mixture suspension on the basis of waste sludge, waste ash and mineral fertilisers. The mixture is treated by mechanical disintegration, which is responsible for homogenisation and dehelminthing. If the pH of the suspension fertiliser must be reduced, the bubbling of biogas through the suspension can be used. The carbon dioxide content is diminished and the calorific value of the biogas is elevated.

Keywords: Excess sludge, ash, waste glycerol, disintegration, suspension fertiliser, biogas enhancement and upgrading

Introduction

This article gives an overview on how to implement the integrated treatment of different solid wastes: wastewater treatment plant (WWTP)

excess sludge, waste glycerol from biodiesel production, fishery residues, and oil shale fly ash from electric power stations. The objective was to find the best way to deal with the particular waste management problem and to generate usable products.

The waste glycerol used in anaerobic degradation is today one of the sources of alternative energy (Mousdale, 2008; Kuusik *et al*., 2012). Biodiesel production creates 10–11kg waste glycerol per 100 kg biodiesel (Miele *et al.,* 2008). The aim of the investigation was to ascertain best way to incorporate ordinary waste sludge and glycerol into anaerobic digestion. It was also of interest whether fishery residues could be used in the same manner. Hutňan (2009) claims that concentrated glycerol, as a single raw material, is not treatable by anaerobic digestion technology. Due to the co-substrate effect, glycerol is more easily digested in a mixture of different organic materials where it is in the role of an admixture (Fountoulakis, 2010; Kaosal *et al.,* 2012).

The solution to this problem causes another problem of how to use the remaining sludge. The proper method is to produce suspended fertilisers on the bases of stabilised waste sludge and waste fly ash, which can be linked together with mechanical disintegration.

Suspension fertilisers are mixtures of liquid, stabilisation matter, and dissolved and non-dissolved mineral nutrients. Stabilisation material commonly has a clayish nature and its purpose is to hold the non-solute fertiliser particles homogeneously in suspension. Clay or similar matter is generally substitutable by non-settle able excess sludge that originates from activated sludge treatment (Loit H., 1989). The sludge content of dry solids has to be around 4% ($\geq$40g L^{-1}) and may reach (6–8%). When excess sludge and shale ash are used together in the mass, the concentration of sludge may be less (Sokk *et al.,* 2007). Even 20 g L^{-1} may be sufficient.

The waste sludge must not contain viable helminth eggs. If required, the dehelminting process can be carried out by mechanical disintegration.

The disintegrator (Hint, 1981) is a mill where opposing discs are equipped with milling elements (Figure 1) positioned in intermeshing circles. The material to be ground is directed to the centre. A centrifugal force carries the material outward through the counter-rotating milling elements. The collision velocity between material particles and the milling elements depends on the rotating speed and element placement radius and may reach 300 m s^{-1}. It was expected that helminth eggs would be damaged and lose germinating ability in such a highly energetic mechanically agitating environment. Such treatment is not sufficient to decrease the viability of infectious bacteria; therefore, separate treatment to degrade bacterial germinating is needed. Utilisation of shale ash in the mixture of suspension

fertilisers raises the pH level and that in turn suppresses the viability of the micro flora.

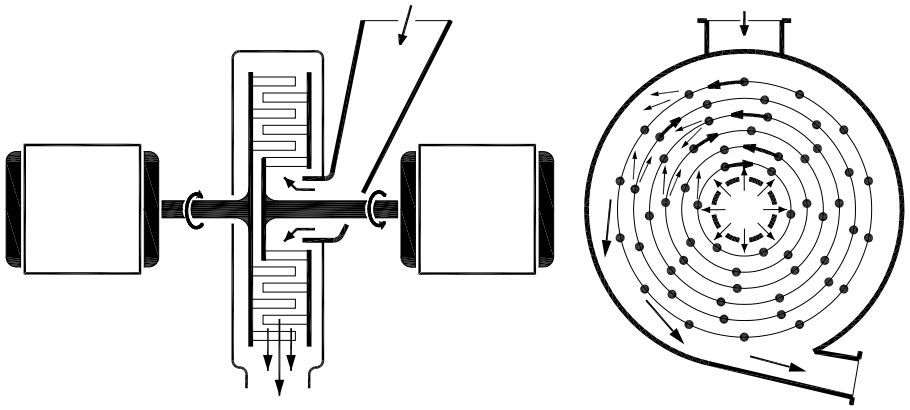

Figure 1 The schematic construction of disintegrator: side view on the left; treatment process on the right (according to Hint, 1981).

I. Experimental procedure

1. Anaerobic degradation

A series of continuous experiments were carried out in order to investigate the influence of glycerol and fish residue concentrations on the process. One experiment was performed with raw sludge obtained from Tallinn WWTP). Other experiments were performed 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 biodiesel pilot plant in Estonia (Viljandi). Fishery residues were obtained from the salmon treatment department of Kakumäe fishery near Tallinn. 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 transmission. The temperature in the reactors of inner reactive mass of 1.6 and 4.5 was maintained by using water jackets surrounding them. The reactor with the inner reactive mass of 5 kg was surrounded by an electric heating pad. The temperature of the digesters was kept mesophilic (between 35 and 40 ^{o}C), mainly around 36–38 ^{o}C. Mixing was effected with magnetic spinners. That was done every morning before and after feeding. Biogas was collected into a gas clock, which was filled by 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 20 to 40 days. Initially, the reactors were inoculated with anaerobic sludge originating from Tallinn WWTP. Sewage

sludge and its mixtures with glycerol were inserted by syringe. A mixture of sludge and fish residue was added through a tube on the top of the reactor. The sludge and fish residue was stored in a refrigerator at +4 to +6 ^{o}C. The pH was measured by a pH meter (Denver Instrument, UP-5). Each day, sludge removal from the digester took place before feeding the reactor. A gas sample was taken and measured every morning. 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 determined with biogas analyser (Gas Data GFM416 Biogas Analyser). The methane yield of an anaerobic process depends on the amount of organics (represented by VS content) and the biochemical characteristics of the organics (Zheng *et al*., 2013). 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.

2. Preparing suspension fertilisers

Pilot plant dehelminting experiments were carried out in Uzbekistan on a disintegrator of 1.2 m rotor diameter and a rotation speed 1500/1500 min^{-1} (impact velocities $\leq$ 185 m s^{-1}). Local specialists in the laboratory of the Tchirchik WWTP counted the helminth eggs in one litre samples. The method was known and accepted at that time in the former USSR. In a highly concentrated salt solution, the eggs float to the liquid surface from where they are gathered and countered under a microscope. Infection tests on guinea pigs were accomplished in the Hygienic Institute in Samarkand.

Experiments to reduce the concentration of viable intestine micro-flora were carried out in Tallinn University of Technology on a 35 cm rotor diameter disintegrator at a rotation speed of 3000/3000 min^{-1}, which gave an impact velocity 110 m s^{-1}.). The concentration of viable intestine micro flora was determined as the number of CFU (colony formed units) per one gram of suspension. This number was determined for *Escherichia coli* as the representative of intestine micro flora and indicator of contagiousness. CFU was measured by the most probable number method in the microbiology laboratory of the Estonian Environmental Research Centre. The value of the pH was determined by measuring the sludge water solutions (1:5).

A number of tests of interacting between suspension fertilisers and biogas were carried out in a hermetically closed plastic bottle; biogas and suspension fertilisers were introduced into the bottle. These components were shaken for 3–5 minutes. The bottle had a hose connection with a vessel containing liquid or suspension. It was necessary for the elimination of the vacuum created by absorbed CO_2 in the bottle. Biogas was obtained from an anaerobic reactor treating the liquid wastes of a yeast factory.

II. Experiments and results

1. Anaerobic degradation with mixtures

All the tests started with a 40 day retention time. The goal was to reduce the time to 20 days. At the same time, the amount of methane production from digestion matter and the percentage of methane in biogas were measured. Among these experiments, the raw sludge digestion without an additive (Table 1 and 2) was specified as the standard process. The result obtained in the presence of additives were evaluated and compared with the 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 experiments with 100% sludge and its mixture with glycerol were started on the same calendar day and finished in 82 days. The experiment with the fish residue started later and was observed for the duration of 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 1, and 2).

Table 1 data from single raw waste sludge digestion by reactor volume 1.7 litres

Days considered	*Retention time, days*	*Volume load TS kg/m^3/d*	*Input, g/L*		*Output, g/L*		*Organic removal input-output, g/L*	
			TS	*VS*	*TS*	*VS*	*ΔTS*	*ΔVS*
9–21	40	0.885	35.4	26.625	22.375	14.05	13.025	12.625
22–30	35	1.011	35.394	26.62	22.159	13.234	13.234	13.388
31–41	30	1.088	32.644	24.169	22.331	13.819	10.313	10.35
42–55	25	1.048	26.203	16.25				
56–82	20	1.601	32.025	22.375	21.863	13.713	10.163	8.663
Average		1.227	31.972	22.693	22.1	13.728	11.242	10.504

Table 2 data from single waste sludge digestion reactor volume 1.7 litres (continuation of Table 1)

Retention time days	*Temperature ^oC*	*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 shown.

Similarly as tables I and II, 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).

These tables about the mixtures are not presented and only the last rows presenting weighted averages are shown in tables 3 and 4. The bracketed values are minimums and maximums regarding weighted average.

Table 3 summarised data according to weighted means

Substrate	***Days considered***	***Retention 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)

Summarising the results of tables 3 and 4 against the data of Table 1 points towards the following conclusions:

1. Admixed sludge has a higher volume load and higher concentration.
2. The difference between the input output concentrations are more directly related to 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; the yield per removed organics varies around a mean value.
5. Methane production is increased by additives more than the production of 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 4 summarised data according to weighted means (continuation of Table 3)

Substrate	*Methane yield*		*Methane contents in biogas %*	*Solid removal %*	
	Per volume ,L/m³	*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 98% +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 95% + 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 98% +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 5 was derived on the basis of tables 3 and 4. It compares the influence of additives on methane productivity. Methane production increased up to about 400% without a remarkable increase of residue solids in output sludge. This shows how to use the existing anaerobic facilities of WWTP for the production of alternative and green energy.

Table 5 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 (1,227)	100 (22.1)	100 (128)
Sludge+2% glycerol	40–20	164 (2.016)	111.3(24.588)	252 (323)
Sludge+5% glycerol	40–20	173.1(2.124)	122.1(26.994)	382 (488.6)
Sludge+2%fish residues	40–30	99 (1.215)	107.9(23.836)	288.6(369.4)

2. Suspension fertilisers

Technology for the production of suspension fertilisers with excess sludge as the stabilising matter was developed in the years 1986–1990 and put into pilot scale use in Central Asia. However, the sludge had a very high concentration of helminth eggs (hundreds per litre). Mechanical disintegration was investigated for the dehelminthing of sludge.

Experiments without mineral nutrients were carried out in excess sludge solid concentrations of 2–4% and with minerals in concentrations of 6–10%.

The results of sludge dehelminting experiments for a throughput of 5 m^3 h^{-1} are summarised in Table 6 (Loopere *et al.,* 1987). A detailed description of their technical specifications is not the goal of this article. We see that complete dehelminting is available when disintegration is carried out in a blend of sludge and mineral fertilisers.

Table 6 Characteristics of experiments.

Variant	*Type of rotor*	*Material treated and dry solid content in sludge*	*Efficiency of dehelminthing, %*	*Specific energy consumption, kJ kg^{-1}*
1	Blade	Sludge 2–4%	88	72
2	Blade	Sludge 6–8% and minerals	100	72
3	Blade densified	Sludge 2–4%	96	60
4	Blade densified	Sludge 6–8% and minerals	100	60

Parallel fertilising trials with the same quantity of mineral nutrients, in one case as dry solid and in the other case in suspension, were accomplished. In Uzbekistan, on-field productivity increase was in the range of 3–9% in the case of onion, tomato and maize cultivation. A few samples from sludge containing 15–25 % helminth eggs after treatment were sent to the laboratory to investigate the viability of the remained eggs. Infection tests on guinea pigs showed that the eggs of untreated sludge had infectiousness of over 90%. Untreated sludge had lost this capability (Loit *et al.,* 1989).

New experiments to create suspension fertilisers, based on non-stabilised wastewater treatment sludge, were launched in the autumn of 2006 in Tallinn (Sokk *et al*., 2007). Oil shale ash, obtained from a thermal power station, was used for the stabilisation of the waste sludge and the reduction of intestine micro flora. At the present time, there are no emerging problems with helminth eggs in high income countries (Jimenez, 2011); moreover, the complete neutralisation viability of helminth eggs is achieved in a lime environment (Jimenez-Cisneros, 2007). In Tallinn wastewater sludge, only single eggs in a few sludge samples have been discovered and the regulation allowing an average permissible number of one helminth eggs per litre is being met. The concentrations of heavy metals in sludge and ash were considered. In principle, it was revealed that their mixture could be used as fertiliser because the concentration of heavy metals is not significant (Table 7). Therefore, the objective of the experiments was how to reduce the number of *Escherichia coli* (Table 8). The permissible number of CFU for *Escherichia coli* is no more than 1,000 per 100 ml sludge suspension. This enables to indicate the sludge to be innocuous.

Table 7 Concentrations of heavy metals in dry solids, mg/kg.

Metal	*In sludge*	*In ash*[1]	*In mixture*[2]	*Permissible in sludge****
Cd	0.73–6.0	0.19–3.5	≤ 4	20
Cu	41–700	5.6–17.9	≤ 132	1,000
Ni	6.0–200	19	≤ 50	300
Pb	5.0–98	13.4–383	≤ 340	750
Zn	181–1120 724–3933[3]	284	≤ 425	2,500
Hg	0.1–1.7	1	≤ 1.2	16
Cr	4.9–180 126–3995[3]	15.5–58.6	≤ 80	1,000

[1]Häsänen, et al (1997)
[2]Calculated as maximum for dry mixture that is derived from raw mixture with 40% dry ash and 60% raw sludge containing 8% dry solids
[3]These extreme concentrations are measured only by Keila WWTP (Estonia).

Table 8 CFU g^{-1} of *Esherichia coli* in suspensions.

Mixture	*CFU measured*		*pH*	*Dry solids %*	*Experiment*
	Day of disintegration	*After 3 days*			
Natural sludge	3,155,354		7.11	6.63	First
Natural sludge disintegrated	13,220,556		7.25	4.67	
Mixture (sludge 60%, mineral fertiliser 40%) disintegrated	18,071	11,556	5.65	41.5	
Mixture (sludge 60%, fertiliser 32%, ash 8%) disintegrated	54,361	11,556	6.86	41.5	
Natural sludge disintegrated	19,259,046		6.91	6.5	Second
Mixture (sludge 60%, fertiliser 20%, ash 20%) disintegrated	15,196		8.36	37.7	
Mixture (sludge 60%, fertiliser 10%, ash 30%) disintegrated	198		9.19	41.9	
Mixture (sludge 60%, ash 40%) disintegrated	<12.3		12.26	45.5	

The calculated CFU in raw sludge of the two last versions in Table 8 gives 8,300 and <560 per 100 ml respectively.

On the basis of Table 8, the following conclusions can be drawn:

1. Disintegration is not a diminishing factor for CFU number in waste sludge.
2. Prolonging the contact time between the sludge mixture components diminishes CFU.

3. The main-diminishing factor of the CFU in the sludge mixture is a pH of over 12. With that, the required CFU number is achieved.

It was concluded that disintegration of the sludge with mineral fertilisers has a great impact on dehelminthing but not for the sanitation in regard to intestine bacteria. Sanitation is achieved by increasing the sludge mixture pH. Preparing the fertiliser mixture and its disintegration was accomplished simultaneously. The process was completed in about half an hour. Table 8 indicates both that the presence of mineral fertilisers decreases the mixture pH and that only comparatively high ash concentrations can increase it. Tests to reveal the influence of the ash concentration and its contact time to pH value without fertilisers are presented in Table 9.

Table 9 CFU g^{-1} of *Escherichia coli* in the mixture of waste sludge and shale ash.

Ash %	***CFU measured***		***pH***	***Dry solids %***
	After 1 day of contact time	***After 4 days of contact time***		
Natural sludge	214,720	50,384	6.81	2.17
2.5	240,800	4582	8.99	5.29
5	621	925	10.58	6.72
10	23	<5.6	11.58	12.4

Different mineral fertilisers were added to sludge, which contained 10% ash and had been in contact for 4 days. A fertiliser concentration of 20% was maintained in the suspension. From these mixtures, the CFU of *Escherichia coli* was measured. The results are presented in Table 10.

Table 10 CFU by different fertiliser suspensions.

Fertiliser	***Dry solids %***	***pH***	***CFU g^{-1}***
Ammonium nitrate	29.8	7.43	<5.6
Sodium nitrate	30.8	10.59	<5.6
Superphosphate	28.5	6.63	<5.6

Table 5 indicates that chemical processes took place in the mixture of sludge, as every mineral fertiliser caused a different pH. This phenomenon would have no influence on CFU if the contact time between ash and sludge had been sufficient before the fertilisers were added. Here, all CFU stayed under the determination threshold.

Primarily, the decrease of the pH takes place in the mixture containing NH_4^+ ions. When they are absent (for instance sodium nitrate in our case), the falling of pH is insignificant. Therefore, the neutralisation of the fertiliser suspension is recommended.

When the pH of the suspension is too high, it is possible to decrease it by bubbling biogas through the suspension.

In the contacting tests, the initial volume ratio of the biogas and suspension fertiliser was 4:1. The nutrition component in the suspension was sodium nitrate ($NaNO_3$) in a mass concentration of 20% and ash concentration of 10%. The average values of the three repeated tests were:

1. The concentration of CH_4 increased from 57% to 93.5% with a variation of <3%.

2. The pH of the suspension fertiliser dropped from 12.23 to 10.05.

3. By smelling, the concentration of hydrogen sulphite (H_2S) and other malodorous components was obviously decreased. Instrumental analysis didn't show the presence of H_2S.

4. When treated suspension fertilisers with a pH of 10–11 were bubbled again (under previous conditions), the pH continued to drop and the new value was 7.2–7.5.

Discussion

Electric power production based on the combustion of oil shale results in large-scale formation of lime-containing ash and a high CO_2 emission in flue gases (carbon emission is as high as 29 tons per TJ of produced energy) (Kuusik *et al*., 2005; Kuusik *et al*., 2005). The possibility of using ash in the process of oil shale combustion to capture the carbon dioxide contained in the flue gases was investigated (Uibu *et al*., 2007). Waste ash suspension in water was prepared and flue gases were bubbled through it. Satisfying results for the absorption of CO_2 in ash suspension were obtained (Uibu *et al*., 2009). This phenomenon is closely related to the suspension pH, and the pH drops in the process of CO_2 absorption (Uibu *et al*., 2010). This knowledge encouraged us to examine this principle in regard to suspension fertilisers, where the source of CO_2 is biogas (Sokk *et al*., 2008).

In view of this, it is clear that lowering the pH by means of biogas will cause its purification and increase its calorific value (Mostbauer, 2008; Lombardi *et al., 2008*). This linked together with suspended fertiliser production can be regarded as a method for biogas upgrading for use as a consumable energy carrier. In this case, biogas productivity becomes important and it is reasonable to treat liquid wastes of high organic concentration anaerobically. Wastewaters from different food production industries have high BOD and COD concentrations. We have anaerobically tested wastewaters originating from cheese and vegetable oil production and alcohol distilleries (Blonskaja *et al.,* 1999; Blonskaja *et al*., 2006).

A short review of these experiments is presented in table 11.

Table 11 The main investigated parameters of the anaerobic treatment processes.

Reactor type	*Origin of wastewater*	*HRT days*	*Load, kg COD $m^{-3}d^{-1}$*	*COD input*	*COD removal %*	*Energy produced kJ/ $m^{-3}d^{-1}$*
Contact process[0]	Cheese whey	5–10	4.32–18.28	60 300–66 700	40–83	78.2
UASB[0]	Cheese whey	2.5–12	0.5–16		58–98	72.4
Fixed bed[0]	Distillery	10–19	2.5–5.1	49 000–53 000	≤54	≤23.1
UASB[0]	Distillery	20–39	0.6–2.5		≤93	≤16.2
Fixed bed[0]	Vegetable oil	7–90	0.1–2.2	6 700–11 000	≤85	≤11
Fixed bed[1]	Vegetable oil	1–1.5	6–9		≤85	≤71.7
Fixed bed[2]	Vegetable oil	3–4	1.6–2		≤85	≤17.2

[0]Single stage reactor
[1]First stage anaerobic reactor
[2]Second stage anaerobic reactor

Considering the average values of the 4th and 6th columns in Table 11, the 7th column for potential energy production is created. In this, the facts that one kg CH_4 corresponds to four kg COD and combustion (oxidising) of one kg CH_4 produces 50 kJ energy were taken into account (Mitzlaff, 1988). In these calculations, it was assumed that 10% of COD removal was caused by anaerobic biomass synthesis (Olvera *et al.,* 2012). COD removal in the case of cheese whey was calculated on the basis of median values of COD.

The stored potential energy of refined biogas (97% methane) is 9.67 kWh/m^3 and of natural gas is 11.0 kWh/m^3. They are equivalent to about 1.1 and 1.2 litres of petrol accordingly (Swedish Gas Technology Centre, 2007). The production cost of biogas energy can be about 2.5–6 times cheaper than the retail cost of fossil energy (Technical Note No. 1, October 2007).

In principle, it is possible to use excess sludge with waste admixtures; with waste ash, biogas production is enhanced, mineral organic suspension fertiliser is obtained and biogas as an alternative energy carrier is upgraded. An advantage of this is also that by bubbling with biogas a part of the CO_2 that is released earlier is captured as carbonates and the "greenhouse effect" is retarded. In the above-mentioned waste treatment technologies, the only marketable material used is real mineral fertiliser.

The used tests demonstrate the possibilities and ways for resolving problems related to the reduction of environmental pollution. On the basis of the above presented experiments and cited literature sources, some general

methods to direct excess sludge from WWTP into soil as fertiliser can be devised:

A. The use of stabilised sludge for horticulture and/or agriculture when heavy metal containment is in the permissible range and helminths or/and microbial infection danger is absent or is not a problem.

Input: excess sludge and glycerol or fishery waste.

Process: anaerobic degradation of sludge with additives.

Output: increased amount of biogas produced and stabilised sludge as raw material for bio solids.

B. The use of stabilised sludge for horticulture or/and agriculture when heavy metal concentration is in the permissible range and dehelminthing or/and sanitation is needed.

Input: excess sludge, glycerol or fishery waste, mineral fertilisers.

Process: 1. Anaerobic degradation of sludge with additives.

2. Making a mixture of stabilised sludge and ash and holding it ≥ 4days.

3. Neutralising of alkali mixture of sludge and ash via bubbling of biogas through the mixture.

Output: Increased amount of refined biogas where methane contents may be over 90%. Dehelminthed and stabilised sludge may be regarded as biosolid.

C. The use of stabilised sludge in the composition of suspended fertilisers for horticulture or/and agriculture when heavy metal concentrations are in a permissible range, dehelminthing is needed and sanitation is not needed or is not a problem.

Input: excess sludge, glycerol or fishery waste, mineral fertilisers.

Process: 1. Anaerobic degradation of sludge with additives.

2. Preparing a mixture of anaerobically treated sludge and mineral fertilisers.

3. Disintegration the mixture of sludge and mineral fertilisers.

Output: increased amount of biogas produced and dehelminthed-stabilised suspension fertiliser.

D. Production of suspension fertilisers using stabilised excess sludge. Microbial sanitation is needed

Input: Excess sludge, glycerol or fishery waste, ash which creates pH ≥ 12 in water

Process: 1. Anaerobic degradation of sludge with additives.

2. Mixture of stabilised sludge, and ash that is held ≥ 4 days before disintegration.

3. Disintegration of mixture with mineral fertilisers.

4. Neutralising of alkali mixture of sludge, ash and mineral fertiliser via bubbling of biogas through the mixture

Output: refined biogas, where methane content may be over 90%, dehelminthed and sanitised sludge as bio solid.

E. The use of suspension fertilisers or raw sludge for quick or immediate use in plantations where dehelminthing and sanitation is needed and heavy metal concentrations are in the permissible range.

Input: mixture of raw and surplus sludge of WWTP, mineral fertilisers, ash creating pH ≥ 12.

Process: 1. Making a mixture of ash and sludge and holding it for ≥ 4 days

2. Disintegrating the mixture of sludge, ash and mineral fertiliser

3. Neutralising the mixture via biogas bubbling gained from another process.

4. Bringing the mixture as suspension fertiliser into the soil not later than 3 days.

Output: Refined biogas where methane concentration may be over 90% and dehelminthed and sanitised suspension fertiliser for instant use.

Conclusion

1. The yield of methane production from anaerobic excess sludge reactors can be efficiently enhanced by adding glycerol or fishery residues. Methane concentration in the biogas is also higher.

2. Both additives are industrial waste. Their utilisation is environmentally desirable. Adding waste glycerol 2–5% by weight, the methane productivity per volume of reactor increased around 250–400%. Adding fish residue 2% by weight, the methane productivity per volume of reactor increased up to 290%.

3. The per cent increase of methane production by additives is more than ten times higher than the increase of solid residues in the outgoing sludge.

4. A special rotor construction is required and a simultaneous disintegration of sludge and mineral nutrients is needed for complete dehelminthing of excess sludge.

5. By combining waste sludge, ash and mineral nutrients and experimenting with different contacting times and mechanical disintegrations with different rotors, environmentally hygienic and safety suspension is attainable.

6. The disintegrated mixture is extremely fine and homogeneous; it also may be considered a bio solid, but containment of mineral nutrients makes it more valuable and it is regarded as a suspension fertiliser**.**

7. If the pH of fertiliser suspension is too high after disintegration, the treatment of the suspension with biogas decreases the pH and the quality of the biogas as an energy source improves.

8. Several technologies can be created to direct excess sludge into the soil as fertiliser. Permissible concentrations of heavy metals must be considered and adequate legislation followed.

9. Aside from industrially produced mineral fertilisers, all other components, such as sludge, ash, glycerol and fishery residues, are wastes that are directed into the soil as fertiliser. The complex processing produces an enhanced quantity of gas energy.

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PAPER IV

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Section: Water Engineering

Anaerobic co-digestion of sewage sludge with fish farming waste

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Abstract

Costly management of wastes from Estonia fish processing plants usually sends their sludge from the sludge filter press process to wastewater treatment plant or composting. To reduce the cost, the potential use of this waste for the production of biogas through the anaerobic process was investigated. Anaerobic digestion has long proven to be an efficient way for the production of a renewable fuel, biogas, which can be used as a source of energy to produce electricity or heat. This renewable energy resource can be used to reduce processing costs of plants. As a result, fish waste becomes a valuable resource instead of a waste which has tipping fee. In this study, both batch and continuous flow anaerobic digestion experiments were performed at mesophilic (38 ± 1°C) condition. The inoculum used was from an anaerobic mesophilic digester from municipal wastewater treatment plant (WWTP). Primary substrate was sewage sludge from WWTP and secondary substrate was sludge from Saaremaa salmon breeding farm. The mesophilic anaerobic treatment of concentrated sludge from an Saaremaa salmon breeding farm pools with total solids (TS) from 3.2 to 7.0% was investigated in a one-stage periodically stirred tank reactor at 38 °C and 20–25 days hydraulic retention time (HRT). Organic loading rate (OLR) ranged from 1.08 up to 1.22 kg volatile solids (VS)/(m^3*d). Methane yields between 223.13–370.19 m^3 CH_4/ton VS and 4.34–8.65 m^3 CH_4/ton were achieved. The pH-value was hold at 6.92–7.45 during the whole operation. The fertilizing value of the treated sludge was estimated to be 0.87–1.12 kg N and 0.42–0.99 kg P per ton. The biogas analysis shows that CH_4 content varied from 63.3 to 74.6% and CO_2 content ranged from 11.2 to 29.0%.

Keywords: Anaerobic treatment; fish farming sludge; energy production; biogas; sludge treatment.

1. Introduction

Typical sewage sludge consists of primary sludge separated from wastewater during pre-settling, and biological excess sludge from the activated sludge system. Anaerobic digestion is an appropriate technique for the degradation and stabilisation of sludge before their final disposal. In recent years, much attention has been focused on the improvement of digester biogas production, in order to upgrade their role in stabilizing the sludge and also to produce a feasible bio-energy power plant. One option for improving methane yields is co-digestion. This process is well known and resulting in much higher methane yields when glycerol, food waste and similar types of organic waste were combined with sewage sludge, cow and pig slurries at biogas plants [1], [2]. In recent years there have been many successful efforts in the co-digestion of sewage sludge with several other substrates, such as the source-sorted organic fraction of municipal solid waste [3–5], glycerol from the biodiesel industry [6], cattle manure [7], pig manure [8] and solid slaughterhouse wastes [9].

Literature on biogas plants indicates that high biogas production is positively correlated with the addition of high concentrate organic by-products. Fish farms produce large quantities of organic waste. This material can accumulate on the pool, as well as be suspended in the water column. Its composition is determined according to several parameters, such as the non-consumed scraps of feeding stuffs and excrements, or other organic droppings from fish. [10]. Sludge from fish farms has three origins: fish faeces, drum filters and biofilters. In recirculated fish farms, significant sludge is produced and has a high content of fat and volatile suspended solids. Dewatering and managing the sludge is a challenging task, as it is very unstable. Fish farming sludge is an organic, readily digestible substance which cannot be easily stored over a long period. Requirements for the storage and disposal of wastes in an environmentally safe manner have to be considered in waste management.

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An average to large land-based fish farm (1000 tons feed/year) can produce up to 15 tons of sludge (dry matter) each month equivalent to 150 m^3 wet sludge (10% TS in wet sludge) with approximately 200 g of suspended solids (SS) per kilogram of fish feed [11]. This sludge needs to be managed and discarded properly. Besides suspended solids, however, the sludge also contains high amounts of chemical oxygen demand (COD) and nutrients. Therefore, instead of considering sludge as a pure waste, it can also be used as a source of carbon needed for denitrification. Nitrate commonly accumulates in the production water due to the intense nitrification that has to occur in the biofilters by changing ammonia into nitrate. The micro-organisms reducing the nitrate (denitrifiers) require carbon from the sludge as an energy source to carry out the reaction.

These advantages make fish farming sludge an ideal co-substrate for the anaerobic digestion process. Recent experiments with co-digestion, applying fish farming sludge, glycerol, brewery yeast, whey, municipal solid waste, pig manure and kitchen waste to mixtures of sewage sludge, have shown a significant increase in the methane yield. The main objective of this work was to evaluate the use of fish farming sludge as a co-substrate, in order to boost biogas production during the anaerobic treatment of sewage sludge. The effect of fish farming sludge supplementation on methane yield was examined in continuous experiments, and the fish farming sludge limiting concentration in the feed for a stable digestion process was estimated (the risk of organic overloading).

2. Materials and methods

2.1. Feedstock

Sewage sludge was sludge originating from the municipal sewage treatment plant of the city of Tallinn (population 420,000), Estonia. The sludge was stored fridge at +4ºC until use. The characteristics of the sludge are summarized in Table 1. The inoculum (Inoc) was taken from the city of Tallinn WWTP biogas plant anaerobic digester what is operating at +38ºC with sewage sludge.

Table 1 Main characteristics of sewage sludge used in the experiments

	TS %	VS %	COD gO_2/l	P_{tot} gP/l	N_{tot}, gN/l	NH_4-N gN/l	pH
Inoc	2.30	55.85	29.2	0.77	480	0.87	7.13
SS	2.67	69.27	36.2	0.63	400	0.42	6.49
Inoc	2.44	52.95	32.2	0.81	496	0.96	7.12
SS	3.36	64.97	38.9	0.58	422	0.54	6.03

Fish farming sludge (FS) was obtained from a fish farming pool WWTP in Saaremaa Estonia (Table 5). These sediments that were formed by fish stool and settled fish feed. Under the study of Mizanur *et al*. tank sediment is enriched with organic matter, nitrogen, phosphorus and macro and micro nutrients as well, and hence it can be a potential fertilizer [12]. This description shows that fish tank residue is appropriate for to using it as substrate for biogas producing. In the fish pond residue there are two main nutrient sources, fish feed and fertilizers. Addition of manure and feed provides organic N and P, while inorganic form comes from chemical fertilizers. The organic form of the sediment constitutes about 35–40% of the total P [12]. Fish farming takes place inside the premises. Pools are made of concrete and plastic. Fish breeding capacity is 100 tons per year. Water temperature is 15 °C and aeration air is hold on 16.0–16.5 °C for to avoiding from steaming. Farming fish was Trout while sampling of sludge in the pond. Breeding period is 12–14 months and approximately g weight up to 1.5 Kg per fish. 1–2% of feeding material falls in sediment sludge. Contaminated water treated by Drum filter system and effluent compensated with well fresh water and some organic effluent goes back to the pools. The farm produces 50 tons sludge in a year.

Table 2 Main characteristics of fish farming sludge

	TS %	VS %	COD gO_2/l	P_{tot} gP/l	N_{tot}, gN/l	NH_4-N gN/l	pH
FS	7.06	82.90	82.4	1.83	616	O.10	5.70
FS	3.27	72.23	88.2	2.43	648	0.23	5.15

2.2. Experimental procedure

2.2.1. Continuous experiments

Two series of continuous experiments were carried out in order to investigate: (a) the limiting concentration of fish farming sludge in the feed, (b) the methane production of the fish farming sludge-supplemented sludge during anaerobic digestion and (c) heavy metals content and sludge suitability for agriculture. First, three digesters with a working volume of 4.5 l were constructed using fiberglass. The digesters supply pipes on the top of digesters were sealed with rubber stoppers containing an influent to allow injection of wastes. Effluent port on the bottom was sealed with hose clamp to allow sludge outlet. A

water heater was used to maintain the temperature of the digesters at +38oC. The digesters were connected to gas clocks. Biogas was collected by displacement of water. The reactors were operated in a draw-and-fill mode (on a daily basis) with a hydraulic retention time (HRT) of 20 days. Initially, the reactors were inoculated with anaerobic sludge originating from the municipal biogas plant of the city of Tallinn. The feed in the reactors was sewage sludge: as sole substrate (R1), supplemented with 50% (w/w) fish farming sludge (R2), and supplemented with 100% (w/w) fish farming sludge (R3). The digesters were operated using this feed for 147 days. The reactor was fed once a day (every 24 h) with a total feeding volume of 225 ml/d, resulting in a hydraulic retention time of 20–23 d. Organic loading rate was in range 1.08 up to 1.22 kg VS/(m3*d). The mixed liquid from the reactor was stirred periodically for 15 min, once an hour. The temperature was maintained at 38oC via water heater through water jackets surrounding the reactors. The initial feed was sewage sludge and the bioreactor was operated using this feed for 20 days. Fish farming sludge was then added to the feed so that the reactor was fed continuously with sewage sludge containing 50% fish farming sludge.

2.2.2. Batch experiments

Methane production potential (MPP) tests were done with Automatic Methane Potential Test System II (AMPTS). The AMPTS II follows the same measuring principles as conventional methane potential tests which make the analysis results fully comparable with standard methods. Sample material was mixed in to 500 ml serum bottle reactors, in 400 ml amounts. Each reactor contained the individual materials, nutrient medium, and inoculum. Zheng et al. (2013) suggested that an inoculum-to-substrate ratio (ISR) of ≥2 has never been reporter as inhibitory [13]. In these experiments we used substrate-to-inoculum ratio of 0.2 and 0.5. The serum bottles were sealed with tube clamps immediately after blow out with nitrogen (2 min). Bottles was put into incubation unit (+38 ± 0.2°C) and mixed by a slow rotating agitator. Produced biogas in each reactor goes through an individual vial containing 3 M alkali solution (NaOH). Gases such as CO_2 and H_2S are removed by chemical reactions and CH_4 is the only gas that passes through unchanged. All the tests were run in duplicate. With the AMPTS II both the gas volume measurements and data logging are fully automatic during the long incubation period and experimental data is calculated and generated into a standard data sheet.

2.3. Analytical methods

The pH was measured by an electrode (Denver Instrument, UP-5), while total (TS) and volatile (VS) solids, total and soluble chemical oxygen demand (COD), total nitrogen (TN), ammonium (NH_4-N) and total phosphorus (TP) were determined according to standard methods [14]. Gas samples from continuous experiments were took by biogas analyser (Gas Data GFM416 Biogas Analyser).

3. Results and discussion

3.1. Continuous experiments

The methane yield of an anaerobic process depends on the amount of organics (represented by VS content) and the biochemical characteristics of the organics [13]. Therefore, it is necessary to distinguish the biochemical characteristics of the organics. Table 3 and 4 shows overviews of the VS values. As the Fig.1 shows the maximum methane production in terms of VS added took place at 100% fish farming sludge. Although due to the risk of crust formation it is not the most recommended concentration. The crust formation forms as a result of decrease of the pH value. To avoid crust formation the feeding took place every second day. Concentrations 50% and 100% are mostly influenced by the fish farming sludge. Concentrations 10% and 35.6% are mostly influenced by the raw sludge and therefore less stable. Methane production of CH_4 produce about 70% (fluctuates between 65 and 75).

Table 3. Experimental Results

Mix	Retention time, Day	VS in,	Per added VS, m^3/tonVS	ORL.RT kg VS/(m3*day)	VS out, %	TS out, %
10% FS + 90%SS	35	4.40-5.06 (4.59)	108.52-561.32 (298.01)	0.98-1.13 (1.02)	50.31-52.12 (51.21)	1.19-1.55 (1.37)
35,6% FS + 64,4%SS	36	6.46-6.89 (6.71)	34.57-623.80 (252.46)	1.44-1.53 (1.49)	45.57-47.62 (46.59)	2.36-2.37 (2.37)
50 % FS +50%SS	72	4.82-8.07 (5.94)	56.41-537.32 (269.18)	1.07-1.79 (1.32)	50.00-49.39 (49.69)	2.29-2.38 (2.34)
100 % FS	49	4.92-5.48 (5.22)	105.03-601.34 (413.11)	1.09-1.22 (1.16)	43.38-69.01 (51.03)	1.59-2.31 (2.02)

Table 4. Biogas Production

Mix	Retention time, Day	VS in,	Per added VS, m^3/tonVS	ORL.RT kg VS/(m3*day)	VS out, %	TS out, %
10% FS + 90%SS	35	4.40-5.06 (4.59)	108.52-561.32 (298.01)	0.98-1.13 (1.02)	50.31-52.12 (51.21)	1.19-1.55 (1.37)
35,6% FS + 64,4%SS	36	6.46-6.89 (6.71)	34.57-623.80 (252.46)	1.44-1.53 (1.49)	45.57-47.62 (46.59)	2.36-2.37 (2.37)
50 % FS +50%SS	72	4.82-8.07 (5.94)	56.41-537.32 (269.18)	1.07-1.79 (1.32)	50.00-49.39 (49.69)	2.29-2.38 (2.34)
100 % FS	49	4.92-5.48 (5.22)	105.03-601.34 (413.11)	1.09-1.22 (1.16)	43.38-69.01 (51.03)	1.59-2.31 (2.02)

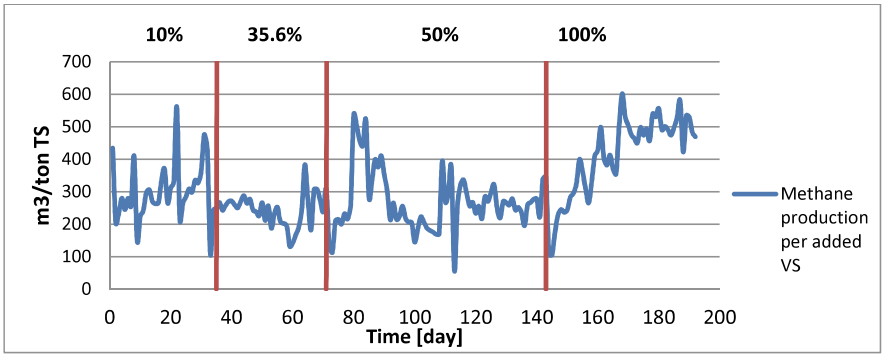

Fig. 1. Methane production per added VS

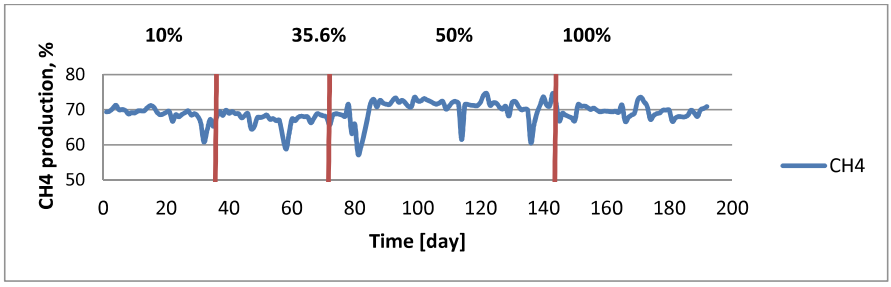

Fig. 2. Production of CH_4, in percentage

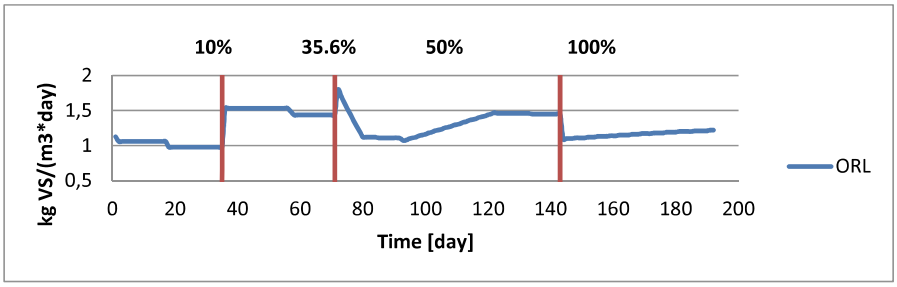

Fig 3. ORL during the experiments, kg VS/(m3*day)

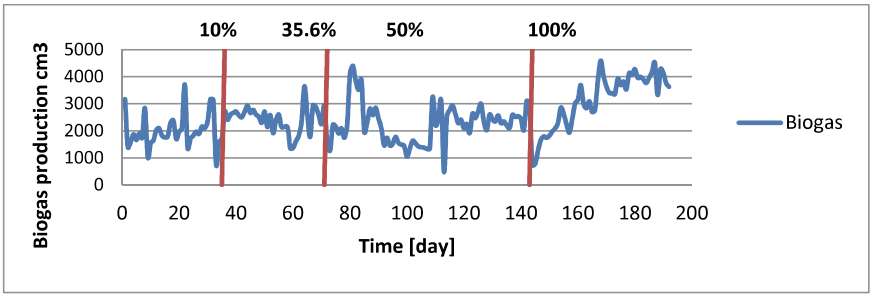

Fig. 4. Biogas production, cm^3/day

Table 5. Nutrients and Minerals in Tests

	Test no. 1 Inoculum	Test no. 2 Inoc+F0.2	Test no. 3 Inoc+F0.5	Test no 4 Inoc+F0.2F35%	Test no. 8 Inoc+F0.2F50%	Test no. 11 Inoc+F0.2F75%	Test no. 12 Inoc+F0.2F90%	Fish farm pool sludge	Sewage sludge	Inoculum	Limit values in Estonia [16]
Dry solids %	2.39	2.31	2.22	2.35	2.36	2.44	2.31	2.92	3.2	2.41	–
Phosphorus – P %	0.044	0.067	0.057	0.045	0.055	0.052	0.053	0.042	0.099	0.027	–
Potassium –K %	0.039	0.037	0.023	0.034	0.04	0.039	0.036	0.024	0.043	0.036	–
Sulfur – S %	–	–	–	–	–	–	–	0.008	0.048	0.011	–
Zink – Zn mg/kg	11.3	15.8	14	10.2	13.6	13	13.1	7.25	25.9	5.8	2500.0
Copper – Cu mg/kg	6.57	7.04	5.35	5.23	7.49	6.62	6.45	1.73	12.3	5.02	1000.0
Mercury – Hg mg/kg	<0.01	<0.01	Non found	Non found	<0.01	0.01	Non found	Non found	<0.01	Non found	16.0
Cadmium – Cd mg/kg	0.066	0.05	0.057	0.061	0.093	0.08	0.074	0.016	0.176	0.027	20.0
Chromium – Cr mg/kg	0.671	0.812	0.617	0.522	0.741	0.694	0.747	0.064	1.6	0.353	1000.0
Nickel – Ni mg/kg	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	300.0
Lead – Pb mg/kg	0.359	0.341	0.297	0.298	0.383	0.299	0.345	0.03	0.516	0.175	750.0
N %	2.33	3.02	3.02	2.98	3.08	3.08	3.10	3.84	3.77	2.59	–
C %	24.69	–	–	–	–	–	–	40.17	32.09	26.29	–
H %	3.49	–	–	–	–	–	–	5.59	4.50	3.66	–
Crude protein %	–	–	–	–	–	–	–	24.00	23.56	16.19	–
Crude fat %	–	–	–	–	–	–	–	11.78	6.09	2.38	–

High concentration of light metals such as calcium, sodium, potassium and magnesium are known to be inhibitory to methanogens [17]. The heavy-metal content of the processed sludge meets requirements set by the Estonian law [16]. The contents of N, P and K are in line with, or higher than those of e.g. swine or cattle manure, which should make this sludge attractive to use as a bio-fertilizer, similar results were found in study conducted in Department of Biotechnology, Lund University [17].

3.2.1 Batch experiment results

Two identical batch experiments were conducted. In the first experiment the AMPTS II was operated for 42 days and in the second 21 days. The experiments operation time was reduced because the main process occurs during the first 7 days. Main characteristics of sewage sludge used in the experiments are presented in Table 1 and main characteristics of fish farming sludge in Table 2. The theoretical value for the production of methane by VS was calculated to be 393.91 m3CH4/tVS and the production from wet weight 9.29 m3/m3. The calculations were made using 100% TS, crude protein, crude fat and carbohydrates [18]. Tests results are expected to be lower than the theoretical calculations. It is due to the instability of the anaerobic digestion process and the degradability of the organic matter. All organic matter is not easily decomposable and may need thermal pre-treatment.

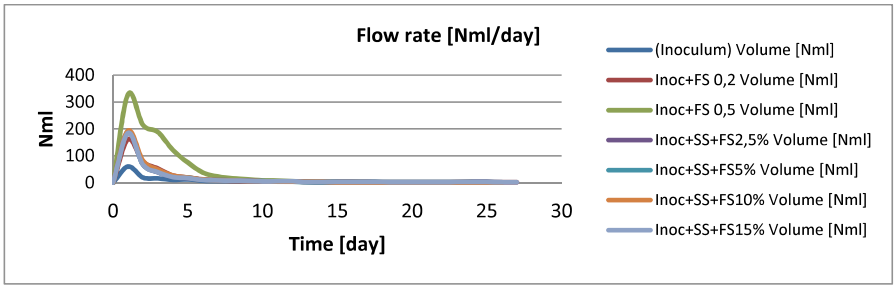

Fig.5. Flow rate [Nml/day], Tests set no. 1

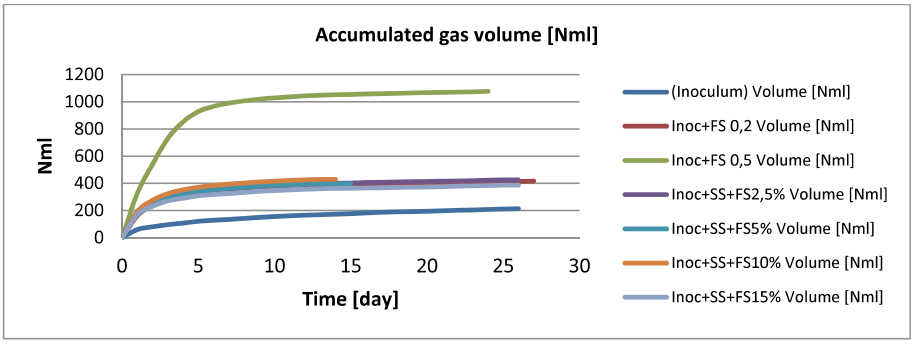

Fig.6. Accumulated gas volume [Nml], Tests set no. 1

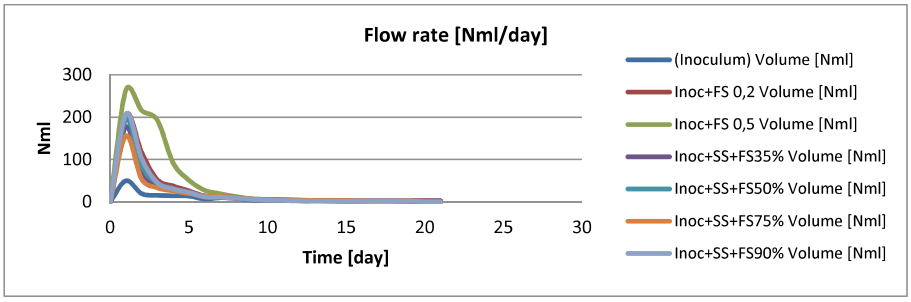

Fig.7. Flow rate [Nml/day], Tests set no. 2

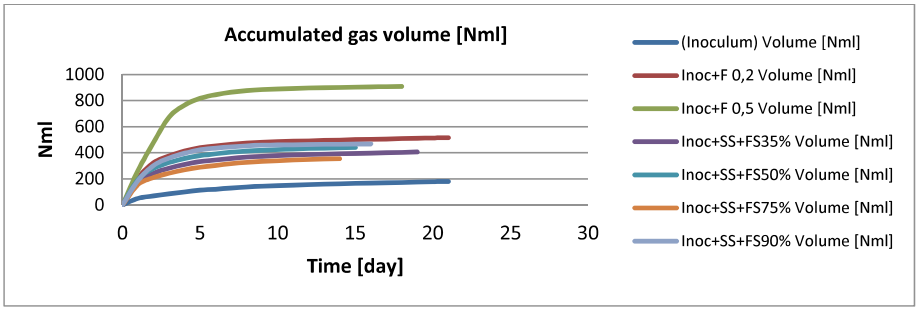

Fig.8. Accumulated gas volume [Nml], Tests set no. 2

At the first day the gas flow rate was the highest. After 10 days the average gas flow rate was 5.50 [Nml/day] and after 15 days the average gas flow rate went down to 2.29 [Nml/day]. (Using only inoculum was not taken into account in these calculations). From the figures 7 and 8 it can be seen that the main volume of accumulated gas is formed after 7 days. After

7 days the change in accumulated gas volume was minimal. This result is similar to a study conducted at Technical University of Lisbon where after 10 days, 81% of the total biogas was formed [19].

Table 6. The minimal and maximal gas flow and for each test mix combination used

Mix	Max [Nml/day]	Min [Nml/day]
(Inoculum) Flow [Nml/day]	61.07	1.40
Inoc+FS 0.2 Flow [Nml/day]	205.25	0.90
Inoc+FS 0.5 Flow [Nml/day]	310.85	1.40
Inoc+SS+FS 2,5% Flow [Nml/day]	188.20	0.90
Inoc+SS+FS 5% Flow [Nml/day]	193.05	0.50
Inoc+SS+FS 10% Flow [Nml/day]	190.65	1.00
Inoc+SS+FS 15% Flow [Nml/day]	177.60	0.95
Inoc+SS+FS 35% Flow [Nml/day]	174.55	2.60
Inoc+SS+FS 50% Flow [Nml/day]	191.55	2.80
Inoc+SS+FS 75% Flow [Nml/day]	156.80	3.00
Inoc+SS+FS 90% Flow [Nml/day]	197.55	1.70

The most effective gas production was when the substrate/inoculum rate was 0.5. The least effective was using only inoculum. The proportion of 0.2 was tested more thoroughly to find out what results if using fish and raw sludge give. The best gas flow came from Inoc+SS+FS 90% (197.55 Nml/day) and the lowest was Inoc+SS+FS 35% (147.55 Nml/day). The first and the second test sludge may be slight different due to the difference of the fish feed used. Also the TS and VS were different during the tests.

Table 7. Characteristics and outcomes from tests set no. 1

Tests	Dry components in pulps [g]					Production of methane	
	Inoculum	FS	Sewage sludge	Substrate (VS)	Inoculum (VS)	Production by wet weight m^3/m^3	Production by VS m^3CH_4/tVS
Inoc	400			0.000	5.143		
Inoc+FS 0.2	383.16	16.84		0.985	4.926	14.23	243.31
Inoc+FS 0.5	360.39	39.61		2.317	4.634	21.66	370.19
Inoc+SS+FS2,5%	353.32	1.17	45.51	0.908	4.543	4.34	223.13
Inoc+SS+FS5%	355.34	2.23	355.34	0.914	4.569	5.05	246.91
Inoc+SS+FS10%	358.91	4.12	36.98	0.923	4.615	5.45	242.72
Inoc+SS+FS15%	361.95	5.71	32.34	0.931	4.654	5.69	232.45

Table 8. Characteristics and outcomes from tests set no. 2

Tests	Dry components in pulps [g]					Production of methane	
	Inoculum	FS	Sewage sludge	Total solids (TS)	Volatile solids (VS)	Production by wet weigh m^3/m^3	Production by VS m^3CH_4/tVS
Inoc	400			0.000	5.166		
Inoc+FS0.2	360.52	39.48		0.931	4.656	8.44	357.94
Inoc+FS0.5	314.02	85.98		2.028	4.056	8.65	366.88
Inoc+SS+FS35%	358.70	14.46	26.85	0.927	4.633	5.57	248.14
Inoc+SS+FS50%	359.14	20.43	20.43	0.928	4.638	6.71	295.47
Inoc+SS+FS75%	359.84	30.12	10.04	0.929	4.648	4.21	181.94
Inoc+SS+FS90%	360.25	35.78	3.98	0.930	4.653	7.40	316.03

4. Conclusions

The study showed that the potential use of this substrate for the production of biogas through the anaerobic process technology is promising. The co-digestion increased the methane yields, biogas production and also stabilized the process. The sludge would be attractive to use as a bio-fertilize in agriculture. Due to the risk foaming of crust, further tests are needed for using 100% fish farming sludge with residence time of 20 days. It should be investigated what is the reason for

the decrease of the pH value because under these conditions was noticed drop of pH and crust formation. The fish pool sludge should also be tested using different fish feeds, since the sludge properties are conditioned by the feed properties.

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PAPER V

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ENVIRONMENTAL
ENGINEERING

Possible agricultural use of digestate

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Abstract. The aim of this study was to evaluate the agricultural use of digestates obtained from laboratory-scale experiments of anaerobic co-digestion of different organic wastes (glycerol, compost from landfill, fish farm sludge, and catering waste and their mixes with sewage sludge) and from full-scale biogas plants (cattle slurry). The concentration of nitrogen, phosphorus, and heavy metals and presence of *Salmonella* spp. in digestates were monitored.

The co-digestion trials were performed using laboratory-scale reactors. The microbiological analyses of digestate showed the presence of *Salmonella* spp. in both the laboratory-scale reactors and samples taken from full-scale biogas plants. Some digestate samples highlighted the importance of the microbiological quality evaluation of the digestate in studying the possible health risks for consumers. The heavy metals concentrations did not exceed the maximum levels permitted by the Estonian Minister of the Environment Regulation No. 78 of 01.02.2003 'Requirements for the application of sewage sludge in agriculture, landscaping, and recultivation'. Although Cd concentration showed values lower than 3 mg/kg TS and Hg was only found in catering digestate, environmental contamination would be possible if digestates were used for agricultural purposes.

This work can be considered as a preliminary study in evaluating the possible agricultural use of the digestate obtained from the co-digestion of different organic wastes.

Key words: anaerobic co-digestion, agricultural use, fertilizer, heavy metals, nitrogen, phosphorus, *Salmonella* spp.

1. INTRODUCTION

Millions of tonnes of wet and solid waste are produced from municipal, industrial, and agricultural sources. The decomposition of these organic wastes results in the contamination of land, water, and air [1]. The European Commission has set the ambitious goal of increasing the target of energy generated from renewable sources to 20% in 2020 compared to 8.5% in 2005 [2]. To reach this goal, the use of all existing renewable energy sources must increase [3]. Anaerobic digestion is a suitable option for the production of renewable energy in the form of biogas; this process can be used for treating organic wastes such as manure, slurry, food processing waste, as well as sewage sludge and other organic fractions of municipal solid waste [3].

Methane fermentation is a complex process. It can be divided into four phases of degradation: hydrolysis, acidogenesis, acetogenesis, and methanation, according to the main process of decomposition in this phase (Fig. 1) [4]. The individual phases are carried out by different groups of microorganisms, which partly stand in syntrophic interrelation (some species of micro-organisms acting together degrade certain compounds of substrates that they cannot degrade on their own, e.g. *Nitrosomonas* and *Nitrobacter*) and present different requirements for the environment [4].

The temperature for acidifying bacteria has two optimum levels: a smooth level at about 32–42 °C for mesophilic microorganisms and a sharp level at 48–55 °C for thermophilic microorganisms (Fig. 2). Most of the methanogenic microorganisms are mesophilic.

Under mesophilic operating conditions, the inhibition of ammonium is reduced due to the lower content of

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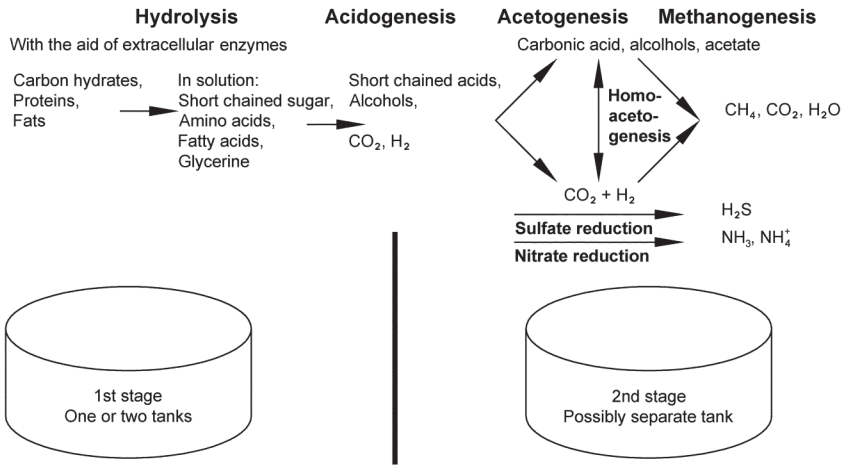

Fig. 1. Biochemistry of methane gas production [4].

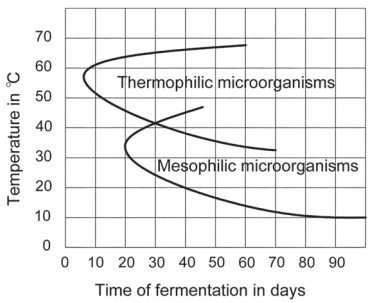

Fig. 2. Influence of the temperature on fermentation time [4].

inhibiting free ammonia. It should be mentioned that generally the energy balance is better in the mesophilic range than in the thermophilic range. The thermophilic mode of operation results in an about 50% higher rate of degradation and, notably with fat-containing materials, a better microbial availability of the substrates and thus a higher biogas yield.

Epidemic and plant pathogenic bacteria are inactivated by higher process temperatures. Therefore no special hygienic procedures are necessary at temperatures higher than 55 °C and longer than 23 h material retention time.

In some two-stage plants, different temperatures are used at the two stages. There are good reasons to drive the methanation reaction thermophilically and the hydrolysis mesophilically. However, depending on the substrate, it may also be favourable to operate hydrolysis at higher temperatures than methanation [4].

The anaerobic co-digestion technology has two main advantages: the co-digestion of combined wastes results in a higher biogas yield compared to single waste digestion and the methane concentration in the biogas is also higher than in single waste digestion [1,5]. There are several studies in published research that refer to the utilization of co-digestion, such as co-digestion of the organic fraction of municipal solid waste [6], cattle manure [7], pig slurry [8] and agricultural residues [9,10], organic solid waste and sewage sludge [11] or more specific waste (fish farm waste, slaughterhouse waste, glycerol, kitchen waste) [12–15].

In addition to biogas, anaerobic digestion generates a digestate – a product that can be used as an agricultural fertilizer because the nutrients present in the raw input material remain in it and are accessible for crops after the digestion process [1]. The diverse origins of the input material used for biogas production indicate that biogas plants produce fertilizers that vary in nutrient content [1].

Two types of digestate are distinguished on the basis of their dry matter content. The liquid digestate contains less than 15% dry matter, while the solid digestate contains more than 15% dry matter. Solid digestate can be used in a similar fashion as compost or composted with other organic residues; it can be transported more economically over long distances than liquid material [16].

According to published research, the physical–chemical properties of digestates have been widely investigated, while fertilization studies are still scarce [3,16]. However, digestates are not harmless products, as they contain heavy metals and may contain organic pollutants, pesticides, and pathogenic bacteria that are introduced to the soil ecosystem by their application [3]. Heavy metals can be present in wastewater treatment plant (WWTP) sewage sludge substrate used for biogas production and are not altered in the anaerobic digestion process; therefore, they may be concentrated due to the mass reduction during the process [3]. Pathogenic bacteria can be found in the substrate or in the digester. There is a risk that the digestate could be polluted with pathogenic bacteria after the digestion process, even if no pathogenic bacteria were found in the substrate.

The soil application of digestates requires a quality evaluation in terms of microbial stability, hygiene, the presence of organic and inorganic toxic compounds, and the concentration of organic matter and nutrients [17]. The application of digestate on fields can potentially spread pathogens from one farm to another, resulting in crop contamination. The potential health risk of digested residues from biogas production is partly caused by the substrates that are treated in the biogas plants; for instance, organic wastes may contain pathogenic bacteria, depending on the source and type of waste. In particular, waste of animal and human origin can contain various pathogenic bacteria, parasites, viruses, fungi, and moulds [3,18]. Some studies have posited that pathogenic weed seeds can survive after anaerobic digestion, and the growth of the remaining viable bacteria after the application of digestate to soil has been demonstrated for some bacterial species [3,19,20].

Although the combination of process temperatures and retention time is the most important sanitation/pathogen inactivation factor, the research results indicate that pathogen inactivation is more complex and occurs in the combined effect of these with other process parameters such as pH and NH_3 concentration inside the digester [21]. For this reason, it is important to optimize and closely monitor the anaerobic digestion process and the process parameters [22].

The aim of this study was to investigate the content of faecal pathogens as well as the heavy metal concentration of digestates obtained from the anaerobic co-digestion of organic waste.

2. MATERIALS AND METHODS

2.1. Anaerobic digestion reactors

The study of anaerobic co-digestion based on biodegradable waste was carried out using three different experimental devices: six laboratory-scale reactors, one Armfield W8 anaerobic digester, and one Automatic Methane Potential Test System (AMPTS) II.

The laboratory-scale reactors with working volumes of 5 litres were constructed using fibreglass. The digesters were sealed with rubber stoppers and tube clamps containing an influent/effluent port to allow the injection of wastes. A water jacket and an electric heating pad around the digester were used to maintain the temperature of the digesters, while magnetic spinners were used for mixing. Mixing was performed every morning before and after feeding and, by using a timer, once every hour for 15 min. Biogas was collected through the displacement of water in gas clocks. The reactors were operated in draw and fill mode (on a daily basis) and were fed daily with 250 g of organic waste substrates with a hydraulic retention time of 20 to 30 days. The organic loading rate was up to 2 kgVS/(m^3 · day). The digestate collection for chemical analyses was performed in the middle and at the end of the test.

The Armfield W8 anaerobic digester comprises two 5 litre upward-flow packed bed reactors with feed rate and temperature control facilities to allow for steady, continuous operation at up to 7 L per day over periods of months. The reactors may be operated in series or in parallel. A buffer vessel between the reactors permits the discharge of excess flow from the first reactor when the second reactor is operated in series but at a lower flow rate. The flow rates to the vessels are set and controlled by calibrated peristaltic pumps.

The temperature of each reactor is controlled by an electric heating mat wrapped around the reactor's external wall. The temperature distribution within each reactor is maintained at ±0.5 °C. Reactor temperatures may be separately set at any desired value in the range from ambient to 55 °C.

The gas off-take from each reactor is taken to a volumetrically calibrated collector vessel operating by water displacement. A constant head, a liquid sealing device, ensures that the gas pressure in the reactor is maintained at a constant value throughout the test run. The collected gas can be exhausted from the vessel and the volume re-filled with water during a run without breaking the liquid seal.

Liquid and gas sampling points are located at all strategic points around the reactors. Non-return valves and liquid seal siphon breaks are included in the process pipework to ensure each reactor operates at a constant volume without the ingress of air or the danger of accidental siphonic action.

Methane production potential tests were conducted with an AMPTS II. The AMPTS II follows the same measuring principles as conventional methane potential tests, which makes the analysis results fully comparable

with those of standard methods. Sample material was mixed in 400 mL amounts in 500 mL serum bottle reactors. Each reactor contained the individual materials, nutrient medium, and inoculum. In these experiments, substrate-to-inoculum ratios of 0.2 and 0.5 were used. The serum bottles were sealed with tube clamps immediately after the blow out with nitrogen (2 min). The bottles were put into the incubation unit (+38 ± 0.2 °C) and mixed for 60 s with a 2 min pause at 24 h over 42 days by a slow rotating agitator. The produced biogas in each reactor was directed through an individual vial containing 3 M alkali solution (NaOH). Gases such as CO_2 and H_2S were removed by chemical reactions and CH_4 was the only gas that passed through unchanged. From the carbon dioxide absorption unit, the gas was directed to a flow cell array. All experiments were carried out twice. With the AMPTS II, both the gas volume measurements and data logging are fully automatic during the long incubation period. Experimental data was calculated and generated into a standard data sheet. The digestate products collection was performed at the end of the test.

Initially, the laboratory-scale reactors were inoculated with anaerobic sludge (+38 °C) obtained from the WWTP biogas station of the city of Tallinn. Other substrates for laboratory tests and their origin are outlined in Table 1.

During the research, three full-scale biogas plants were under examination. Biogas Plant 1 processes a mixture of cattle manure and pig slurry (90% + 10%), Biogas Plant 2 cattle manure, and Biogas Plant 3 pig slurry. They operated at mesophilic temperatures (+41 ± 2 °C). The digestate products for analyses were collected from the manure storage, before the digester and digestate takeout.

At the end of each digestion trial, representative samples of digestate (~1.5 L) were collected from the reactors. The input substrate samples were collected before commencing the digestion process according to CEN/RT 15310-2 and ISO 5667-13. Samples were treated according to CEN/TR 15310-4 and ISO 5667-15.

Table 1. Substrates and their origin for laboratory tests

Substrate	Origin
Inoculum	Tallinn WWTP
Sewage sludge	Tallinn WWTP
Fish farm sludge	Saaremaa fish farm
Glycerol	Biodiesel production plant
Catering & kitchen waste	Catering industry
Compost	Tallinn Landfill

2.2. Analyses

The pH was measured by an electrode (Denver Instrument, UP-5), while total solids (TS) and volatile solids (VS), total and soluble chemical oxygen demand (COD), total nitrogen (N-tot), ammonium nitrogen (NH_4-N), total potassium (K-tot), and total phosphorus (P-tot) were determined according to standard methods. Gas samples from continuous experiments were taken by a biogas analyser (Gas Data GFM416 Biogas Analyser).

The content of metals (Cd, Cr, Cu, Hg, Ni, Pb, and Zn) was evaluated in digestates to examine the chemical hazard related to their use as fertilizers. The results of bacterial pathogen (*Salmonella* spp.) contamination were expressed as the presence/absence of pathogens.

The analyses of substrate and digestate samples (from laboratory experiments and full-scale biogas plants) were carried out in accredited laboratories in Estonia (Water Quality Laboratory at Tallinn University of Technology and Agricultural Research Centre at Saku, which are authorized according to EN ISO/IEC 17025).

3. RESULTS AND DISCUSSION

3.1. Storage of digestate

Digestate is usually produced throughout the year and it will therefore need to be stored until the appropriate time for its application as a fertilizer during the growing season. The length of the storage period depends on the geographical area, soil type, winter rainfall, crop rotation, and national regulations governing manure applications. In many cases, a 6–9 month storage capacity is recommended and in some countries it is obligatory [23]. For example, the Estonian Water Act does not permit fertilizing from the beginning of December until the end of March and an 8-month storage capacity is required [24].

During the storage, the digestate, unlike whole slurry from cows in particular, does not usually form a crust because the solid material that would have formed the crust is broken during the digestion process. When the digestate is actually stored in open tanks in the same way as manure, ammonia and methane gases will volatilize. Natural crusts (provided that they are 10–20 cm thick) and a floating layer of plastic pieces, clay pebbles or chopped straw, etc. minimize ammonia losses. Another approach that minimizes both methane and ammonia losses is to cover the storage tanks with airtight membranes or to use flexible storage bags. After digestion with an energy crop, up to 100 days of (covered) storage is necessary to reduce the emission of methane to less than 1% [23].

In some European countries with a developed biogas sector (e.g. Germany, Denmark, and Austria), there are financial incentives to cover digestate stores, with the main objective being to reduce methane emissions [23]. Simultaneously ammonia losses are also avoided.

3.2. Nitrogen and phosphorus

The agronomic value of applying treated waste is mainly related to its chemical composition and to its soil physical conditioning value. The three major plant nutrients are nitrogen, phosphorus, and potassium. Evaluating the agronomic value of waste on soil relies largely on the evaluation of the ability of the waste to supply N, P, and K to crops in terms of commercial fertilizer equivalence [25].

The composition of fermented biomass (digestate) mainly depends on the basic material of organic matter and its nitrogen content and the form of nitrogen. The nutrient content of digestate is also influenced by the length of the fermentation process, its parameters (such as temperature, pressure, etc.), and the origin and composition of the raw material. The fermentation process reduces the organic dry matter content of original material to 24–80% [26]. The higher N content of a digestate compared to composts is a consequence of the N concentration effect because the carbon sources are degraded to CO_2 and CH_4, and N is preserved during anaerobic digestion [16]. Nitrogen is a major plant nutrient in the form of NH_4 and NO_3, and it is the most common plant growth-limiting factor of agricultural crops. The fertilizing effect of added N is decreased by the inadequate synchrony of crop N demand and the soil N supply [16]. The advantage of digestate application is the possibility of reallocation of the nutrients within the crop rotation from autumn to spring, when the crop nutrient demand arises [16].

During organic matter degradation, part of the organically bound nitrogen is reduced to the NH_4 form, mainly ammonium carbonate. The NH_4 content of the digestate is about 60–80% of total N content. Generally, the NH_4-N concentration is increased by protein-rich feedstock such as dry by-products and slaughterhouse waste [16]. The conversion of organic N to NH_4-N allows for its immediate utilization in crops. The higher amount of NH_4-N and the higher pH predominate over factors (lower viscosity, lower dry matter content) that could reduce the ammonia volatilization from the digestate. The emission of ammonia could be decreased by various injection techniques that lower the air velocity above the digestate [16]. The application depth has a significant effect on NH_3 volatilization. The surface application of a liquid biofertilizer causes the loss of 20–35% of the applied total ammoniacal N, while disc coulter injection at a 5–7 cm depth reduces the ammoniacal loss to 2–3% [27]. This method should also be used in digestate application to reduce ammonia volatilization [16].

Other important nutrients (P, K, Ca, and Mg) in the digestate do not change. As with nitrogen, some phosphorus is turned into an inorganic form that is easily assimilable to plants. In the farm manure Mg and K are mainly in dissolved form, and are easily available to plants. These elements do not have any particular effect on the fermentation process. The sulphur content of the substrate can be reduced during the fermentation process, because the sulphur from hydrogen sulphide is converted into a gaseous state and comes out of the process with the other gases [26].

Digestate has a higher P and K concentration than composts. For this reason, it is more suitable for supplementation of these missing macronutrients in soils. Furthermore, it has been assumed that all phosphorus in the digestate is in available forms; therefore, digestate seems to be a useful material for adding the missing nutrients to soil, especially P and K [16]. The accumulation of P and K in soil could be avoided through a reduction of the applied digestate dose, but an artificial fertilizer has to be used for filling the nitrogen gap in this case [16].

Research data reveal a reduction of dry matter content in substrate as a result of anaerobic digestion with an overall difference of up to 30% between the input and output of dry matter content in substrate. This reflects the breakdown of organic matter and the loss of carbon from the substrate, with the generation of CH_4 and CO_2. Increases in the effluent NH_4-N content and pH are also expected to be a result of the generation of NH_4-N (resulting from the degradation of proteins) and the production of CO_2 [28]. Such changes were recorded in most of researches.

An important indicator of fertilizer value for digestates is their N-tot content. According to our study results, the average N-tot of all investigated digestates was 2.6 kg/m^3 with a minimum of 54% (1.4 kg/m^3) in ammonium form, which may be the key factor in determining the application rate to soils. Digestate N-tot ranged from 0.2 to 5.5 kg/m^3. The lowest N-tot content was recorded in the Tallinn WWTP sewage sludge digestate, which was 0.2 kg/m^3. Of course, this low result also depends on the time when the sewage sludge sample was taken from the Tallinn WWTP for biogas tests. In general, according also to other indicators, the Tallinn WWTP sewage sludge digestate revealed the lowest results. Fish farm sludge laboratory test digestate and the digestate from Biogas Plant 3 had higher N-tot values, respectively 6.2 and 5.5 kg/m^3.

The concentrations of the main nutrients P and K were also relevant (Table 2). These indicate that the

Table 2. Nutrient content in digestate

Origin	TS, % ww	VS, % of TS	N-total, kg/m^3	NH_4-N, kg/m^3	Total P, kg/m^3	Total K, kg/m^3	pH
Biogas Plant 1	6.2	81.5	3.7	1.6	0.7	2.7	7.9
Biogas Plant 2	7.1	81.3	3.8	1.8	0.6	3.3	8.3
Biogas Plant 3	4.8	63.9	5.2	3.2	1.5	2.1	8.4
Tallinn WWTP	2.4	60.1	0.2	0.1	0.03	0.04	7.0
Fish farm sludge	2.7	56.4	6.2	3.5	0.06	0.02	7.1
Glycerol	2.3	36.1	1.2	0.9	0.2	0.1	6.9
Catering waste	3.1	68.6	3.5	1.5	0.06	1.1	7.3
Compost	3.7	77.6	3.5	2.6	0.4	0.5	7.5

TS – total solids; VS – volatile solids.

materials can be an important source of nutrients for agricultural production and help reduce the use of inorganic fertilizers. The content of P-tot and K-tot revealed by biogas plants 1–3 and compost laboratory test digestate were higher than in the Tallinn WWTP sewage sludge, fish farm sludge, glycerol, and catering waste laboratory test digestates.

However, the great variability of their composition, which depends on original materials used for anaerobic co-digestion, makes it necessary to analyse digestates chemical characteristics prior to soil use in order to avoid over-application [17]. The fertilizing value of these materials should be evaluated according to the total concentration of nutrients and the availability of nutrients to plants, which should take into consideration the transformation processes in the soil, such as mineralization, nitrification, or soil fixation [17].

3.3. Microbiological analyses

The use of digestate as a fertilizer is usually governed by regulations and standards that protect animal and human health as well as the quality of crops. Each country has its own standards while Regulation No. 1069/2009 of the European Parliament and of the Council applies to all EU Member States when the digestate contains industrial residues and animal by-products [29]. According to EU requirements, substrates of animal and human source have to be processed for the purpose of reducing and eliminating infectious agents. The substrates must be thermally treated at a temperature of 70 °C, or even sterilized at 133 °C [26].

The disposal of infectious agents in the substrate takes place in the fermentation process. The result depends on the length of the fermentation process, the temperature, and the physical and chemical conditions in digesters. At an intensive mixing of the substrate in the digesters, a risk arises that some added part of the substrate is carried off immediately. In this case, there is a possibility that some pathogens are in a digester for a short time and are not destroyed. These will be in digestate and can cause plant disease, enter domestic and wild animals, and reach people via the food chain [26]. The temperature at which the fermentation takes place has the most significant impact on the destruction of pathogens [26].

In our research, the presence of *Salmonella* spp. was reported in some digestates collected from the laboratory reactors and in some samples collected from the full-scale biogas plants. *Salmonella* mostly occurred in WWTP sewage sludge and in manure. In some cases, the presence of *Salmonella* was not observed after anaerobic digestion. No *Salmonella* was found in food industry substrates, but the presence of *Salmonella* was noticed in some cases after digestion. It might be caused by the inoculum that came from the WWTP digester and already contained *Salmonella* (Table 3).

As a rule, up to 90% of the bacteria causing diseases (such as *Salmonella*) will be destroyed in mesophilic conditions within a few days. In thermophilic conditions, a similar effect is achieved, though within a few hours. However, about 10% of the bacteria can survive in mesophilic (35 °C) conditions after 20 days. With the use of two-stage or two consecutive digesters in mesophilic conditions, 99% of bacteria will be destroyed [26].

Table 3. *Salmonella* presence/absence in substrate and digestate

Origin	In substrate	In digestate
Biogas Plant 1	present	present
Biogas Plant 2	present	absent
Biogas Plant 3	absent	absent
Tallinn WWTP	present	absent
Fish farm sludge	absent	absent
Glycerol	absent	absent
Catering waste	absent	absent
Compost	present	present

According to the EU standard, pasteurization of substrates where animal by-products (slaughterhouse waste) are present in the feedstock is required, at 70 °C for 1 hour or its equivalent with thermophilic digestion with a guaranteed retention of 5 hours at 53 °C (in Germany: 24 hours at 55 °C). These treatments result in the minimal risk (if any) of transferring pathogens via digestate [23].

Incomplete destruction of pathogens is often due to an insufficient duration and mixing of the fermentation substrate. It is suggested that vegetable substrates from different sources (household, garden, farm, etc.) may contain a large concentration of weed seeds. Their insufficient treatment may result in an increase of weed growth in the cultivated landscape. It is possible that fermentation digestate is contaminated by pathogens and various seeds after the fermentation process, such as during storage and/or in the field [26].

The elimination of pathogens depends on a number of factors, including pH, temperature, and retention time of the biological treatment. Figure 3 and Table 4 illustrate how various combinations of temperature and retention time may be used to safely kill off all relevant pathogens, e.g. 70 °C for <1 h, 55 °C for >1 day, or 45 °C for >1 month [25].

The eggs of common gastrointestinal worms and larvae of lungworm are inactivated within 4 hours at 53 °C and after 8 days at 35 °C. Mesophilic digesters are the most common on-farm type in Europe and are very effective at lowering pathogen numbers (Table 4).

Many common viruses are also killed during mesophilic and thermophilic digestion; for example, *bovine viral diarrhoea* (5 min at 55 °C; 3 h at 35 °C) and those causing Aujeszky's disease in pigs (10 min at 55 °C; 5 h at 35 °C) and Johne's disease (0.7 hours at 55 °C, 6 days at 35 °C) [23]. Hygienization may also be achieved by increasing the pH to 12, for example, by liming or by using other alkaline agents [25].

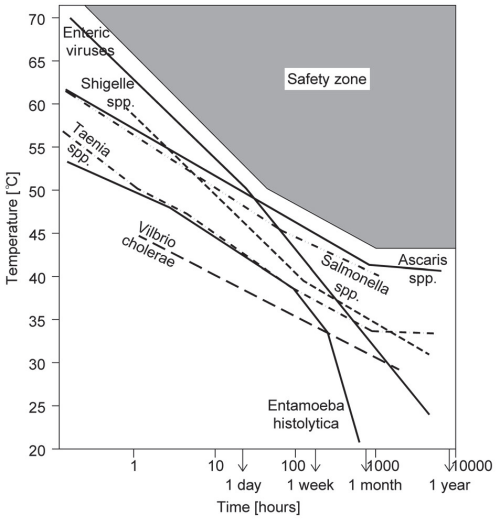

Fig. 3. Time–temperature relation for the safe killing off of various pathogens in sewage sludge [25].

In summary, anaerobic digestion in biogas plants (particularly thermophilic digestion at 52–55 °C) can offer a useful means of lowering the numbers of pathogens in waste (substrate). Once a digestate is applied to soils, a relatively quick die-off of most pathogens occurs due to the competitive advantage of native organisms present in agricultural and forest soils. The survival time for most waste-borne microorganisms following soil application is usually very short (from hours to days), but a few species, such as the persistent *Escherichia coli* O157:H7, were shown to be able to survive somewhat longer (several months) [25].

Table 4. Pathogen and nematode survival times in digestate and raw slurry [9,23,30]

Pathogen	Biogas system			Raw slurry	
	70 °C (seconds)	53 °C (hours)	35 °C (days)	18–21 °C (weeks)	6–16 °C (weeks)
Salmonella typhimurioum	6	0.7	2.4	2.0	5.9
Salmonella dublin	6	0.6	2.1	–	–
Coliform bacteria	20	0.6	3.1	2.1	9.3
Staphilococcus auraeus	8	0.5	0.9	0.9	7.1
Mycobacterium paratuberculosis	8	0.7	6.0	–	–
Streptococcus faecalis	3.9 min	1.0	2.0	–	–
Group D streptococci	20	–	7.1	5.7	21.4
Larvae of nematodes	<0.6	<0.7	<2.4	<2.0	<5.9
Escherichia coli	–	0.4	1.8	2.0	8.8

– Not determined or no result obtained.

Generally, survival of pathogens depends on a variety of climatic and soil conditions, including temperature, moisture content, and pH. Low temperatures and high soil moisture result in the longest survival of pathogens [25].

3.4. Metal analyses

Plants, animals, and humans require trace amounts of some heavy metals such as copper and zinc, while others like cadmium, chromium, mercury, and lead are toxic to them. The heavy metals in the feedstock usually come from an anthropogenic source and are not degraded during anaerobic digestion. The main origins of heavy metals are animal feed additives, the food processing industry, flotation sludge, fat residues, and domestic sewage.

According to the regulations valid in Estonia presently sewage sludge digestate has to be monitored separately from other digestates. The allowable concentrations of heavy metals in sewage sludge to be applied in farming in Estonia are regulated by Minister of the Environment Regulation No. 78 [31] and the allowable concentrations of heavy metals in digestate in Estonia are regulated by Minister of the Environment Regulation No. 12 [32]. European Directive No. 278 of 12 June 1986 'Environment and in particular protection of the soil, when sewage sludge is used in agriculture' is currently valid together with a number of amendments. The most recent document on sludge and biowaste was published by the Estonian Environmental Research Centre in March 2012 [33].

It is important to note that the composition of organic substances during anaerobic digestion results in an increase of heavy metal concentrations in the dry matter of sludge [3,26]. This may cause problems with existing legislation in which the heavy metals are shown in the dry matter (mg/kg DM or mg/kg TS): 50% decomposition of the organic matter may double the heavy metal content without any change in the total quantity of sludge/digestate [26].

The presence of significant amounts of Cu, Ni, and Zn in digestates suggests that there is a possibility of environmental contamination if the digestates are used for agricultural purposes. In addition to environmental concerns, the release of heavy metals (e.g. Cu, Zn, Pb, and Cd) into soils, water, and plants through the use of digestates as fertilizers could also pose public health risks throughout the food chain [3].

Heavy metal concentrations measured during our research were well below the maximum admissible concentrations according to the Estonian Minister of the Environment Regulation No. 78 [3] (Table 5).

Digestates Zn test results were in most cases 2.5 times below the sewage sludge use limit 2500 mg/kg TS. However, in the glycerol digestate its content was 985 mg/kg TS, which is 1.6 times higher than the digestate safety limit 600 mg/kg TS.

Compared to its sewage sludge use limit 1000 mg/kg TS, digestates Cu test results were mostly 2.8 times lower. However, the glycerol digestate Cu test result 362 mg/kg TS was 1.8 times higher than the digestate safety limit 200 mg/kg TS and the Cu content in catering waste digestate, 197 mg/kg TS, is quite close to its safety limit.

On the other hand, Hg was present only in the catering waste digestate. There its content was in the range from 0.13 to 0.37 mg/kg TS, which is lower than the digestate safety limit 0.45 mg/kg TS and 40 times lower than the permissible sewage sludge limit 16 mg/kg TS. The glycerol Hg value was below the determination limit, i.e. < 0.0005 mg/kg TS.

Table 5. Heavy metal content (mg/kg TS) in digestates

Source	Zn	Cu	Hg	Cd	Cr	Ni	Pb
Biogas Plant 1	15.1–19.5	3.7–8.21	NF	<0.01–0.03	0.19–0.23	NF–<0.3	0.09–0.33
Biogas Plant 2	13.6–15.0	2.7–3.5	NF	<0.01–0.018	0.1–0.2	NF–<0.3	0.037–0.2
Biogas Plant 3	35.8–80.2	6.48–13.9	NF	<0.01–0.03	0.32–0.82	0.39–0.54	NF–0.32
Tallinn WWTP	5.8	5.02	NF	0.03	0.35	<0.3	0.18
Fish farm slugde	10.2–15.8	5.23–7.49	NF	0.05–0.093	0.52–0.81	<0.3	0.299–0.383
Glycerol	985	362	<0.0005	2.8	39.3	21.2	41.0
Catering waste	323–462	108–197	0.13–0.37	1.1–1.61	12.8–30	15–50.6	10.5–25.4
Compost	15.6	7.91	NF	<0.6	0.708	1.35	1.68
EST limit	2500	1000	16	20	1000	300	750
EST limit I	600	200	0.45	1.3	60	40	130

NF – not found.
EST limit – Minister of the Environment Regulation No. 78 [31].
EST limit I – Minister of the Environment Regulation No. 12 [32].

The Cd concentrations were in most cases lower than 0.1 mg/kg TS, while the legally permissible limit value for digestate is 1.3 mg/kg TS. Only glycerol and catering waste digestate showed slightly higher results than is the limit for Cd in digestate. By the sewage sludge use rate all Cd values were lower than 20 mg/kg TS.

The Cr limit value according to digestate safety and quality indicators is 60 mg/kg TS. Our digestate test results were much lower than the limit. The Cr limit value according to the relevant environment regulation is 1000 mg/kg TS [31], but the digestate study results were in the range from only 0.1 to 0.82 mg/kg TS. Only glycerol and catering waste digestate analyses showed higher values (39.3 and 12.8–30 mg/kg TS, respectively). Although 40 times higher than in other digestates, these levels are 25 times lower than the permissible limit for sewage sludge in agriculture.

Digestates Ni levels were 5.9 times lower than the sewage sludge use limit 300 mg/kg TS yet the catering waste digestate Ni level, 51 mg/kg TS, is 1.3 times higher than the digestate safety limit 40 mg/kg TS.

The Pb concentrations were in the range from not found to 41 mg/kg TS. This highest content, determined in glycerol, is 3 times lower than the digestate safety limit 130 mg/kg TS and 18 times lower than the sewage sludge use limit 750 mg/kg TS.

In general, the heavy metal tests of catering waste digestate and glycerol digestate showed much higher heavy metal concentrations than the other digestates. The high levels in catering waste might be caused by fish (salmon, pikeperch, Baltic herring, etc.) and fish waste (heads, tails, backbone), which typically contain more heavy metals. As to glycerol digestate, the reason of the high heavy metals content might be the quality of both glycerol and Tallinn WWTP sewage sludge, which was used as a co-substrate in biogas fermentation experiments.

4. CONCLUSIONS

Agricultural use of digestates produced by the anaerobic co-digestion of Tallinn WWTP sludge, glycerol, fish farm sludge, catering waste, and compost in laboratory experiments and cattle slurry from full-scale biogas plants was examined. The microbiological analyses of digestates performed in this study revealed the presence of *Salmonella* during the digestion process, in both the laboratory reactors and full-scale biogas plants. The presence of pathogens in some digestate samples highlights the importance of the microbiological quality evaluation of the digestates to study their suitability as an agricultural fertilizer.

As the metals content of the analysed digestates was low, it should not cause environmental contamination. Nevertheless, heavy metal pollution ought to be a concern when applying digestate to soil, particularly in relation to the possible health risks for humans caused by some heavy metals (e.g. Cd, Cr and Pb). Therefore, random monitoring for heavy metals is highly recommended.

In conclusion, this work can be considered as a preliminary study in evaluating the possible agricultural use of digestates obtained from different organic wastes. Further research on the fertilizing performance on different plants by means of field trials is required.

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Metaankääritamise käigus tekkinud digestaadi võimalik kasutamine põllumajanduses

Argo Kuusik, Karin Pachel, Aare Kuusik ja Enn Loigu

Biogaasitootmine tööstuslikest biolagunevatest ja põllumajanduslikest jäätmetest on Eestis rohkemal või vähemal määral olnud aktuaalne juba viimased 15 aastat.

Biogaasitootmine põhineb anaeroobse kääritamise protsessil, mille käigus lagundatakse tooraines sisalduvaid eelkõige kergemini lagundatavaid orgaanilisi aineid – proteiine, rasvu ja süsivesikuid –, mille saadustena tekivad biogaas ning kääritusjääk ehk digestaat.

Põhiliste toorainetena kasutatakse biogaasijaamades eelkõige reoveesetet, loomakasvatuses tekkivat sõnnikut ja põllumajanduses tekkivaid biolagunevaid jäätmeid, samuti toiduainetööstuse jäätmeid, biodiislitööstuses tekkivat glütserooli, aiandusjäätmeid, kalakasvatusbasseinide setet jne, mille lisamisel saab suurendada biogaasi tootlikkust, väärindades muid kasutuseta biolagunevaid jäätmeid. Mõningate toormete puhul, näiteks loomsed kõrvalsaadused, mis on suure biogaasi potentsiaaliga, on kindlasti vajalik rakendada hügieniseerimise tehnoloogiat, millest tulenevalt rakenduvad biogaasijaamale rangemad veterinaarohutuse kontrolli meetmed.

Keskmiselt muundatakse 35–50% biomassis sisalduvatest süsivesinikest anaeroobse kääritamise käigus biogaasiks, ülejäänud osa jääb alles kääritusjääki.

Lisaks vähenevad biogaasitootmisel lõhna intensiivsus ja patogeenide sisaldus ning suureneb ammooniumi (NH_4-N) osakaal üldlämmastikust (Nüld), kooskääritamise puhul summeerub mikro- ja makrotoitainete sisaldus kääritusjäägis ning seda on lihtsam põllule laotada, sest see on homogeenne.

Erinevate toormete nii eraldi kui ka kooskääritamisel tekkinud kääritusjäägi tarbimine on oluline uurimissuund, mille eesmärgiks on kasutada kääritusjääki põllumajanduses ohutu väetisena.

Käesoleva uurimuse eesmärgiks on kaardistada TTÜ keskkonnatehnika instituudis tehtud kooskääritamise protsessis biogaasi potentsiaali uuringute käigus tekkinud kääritusjäägi keemilised analüüsid ja kõrvutada saadud tulemusi kehtiva Eesti Vabariigi seadusandlusega, mis käsitleb kääritusjäägi laotamist põllule väetisena.

APPENDIX II CURRICULUM VITAE

1. Personal data

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3. Education

Educational institution	Graduation year	Education (field of study/degree)
Tallinn University of Technology	2010	Master degree (Diploma MB 001796), speciality: Civil Engineer (Water Engineering)
Kolga High School	2004	Secondary education

Official title Civil Engineer, M.Sc (Eng.)

4. Language competence/skills

Language	Level
Estonian	Native language
Russian	Basic
English	Intermediate

5. Professional experience

Period	Organisation	Position
01.01.2017-...	Tallinn University of Technology, School of Engineering, Department of Civil Engineering and Architecture	Assistant
01.09.2012-31.12.2016	Tallinn University of Technology, Faculty of Civil Engineering, Department of Environmental Engineering, Chair of Water Engineering	Assistant
2010-31.08.2012	Tallinn University of Technology, Faculty of Civil Engineering, Department of Environmental Engineering, Chair of Water Engineering	Other staff

6. Defended theses

Master theses: Landfill water treatment with filtration and reverse osmosis equipment. Supervisor Valdu Suurkask.

7. Main areas of scientific work / current research topics
Water management, water quality, wastewater treatment, water supply, biogas.

8. Scientific work:
Publications and presentations:
Kuusik, A.; Pachel, K.; Kuusik, A.; Loigu, E. (2017). The possible agricultural use of digestate. Proceedings of the Estonian Academy of Sciences, 66(1), 64-74.
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Kuusik, A.; Kuusik, A.; Pachel, K.; Loigu, E.; Sokk, O. (2013). Generalised Integration of Solid Waste Treatment Practices to Enhance Methane Productivity, Generate Suspension Fertiliser and Upgrade Biogas. European Scientific Journal, 9 (36), 14−30.

Kuusik, A.; Kuusik, A.; Loigu, E.; Sokk, O.; Pachel, K. (2013). Selection of Most Promising Substrates for Biogas Production. International Journal of Energy and Environment, 7 (3), 115−124.
Kuusik, A.; Loigu, E.; Kuusik, A.; Sokk, O. (2013). Possibility of Enhancing Methane Productivity in Anaerobic Reactors in the Treatment of Excess Sludge from Wastewater Treatment Plants. International Journal of Science and Engineering Investigations, 2 (12), 33−36.
Kuusik, A.; Kuusik, A.; Loigu, E.; Sokk, O. (2013). Predicting Preferable Substrate Blends for the Production of Biogas. World Scientific and Engineering Academy and Society: Recent Advances in Environmental Science, Lemesis, Cyprus, March 21-23, 2013. WSEAS, 192−197.
Kuusik, A.; Loigu, E.; Sokk, O.; Kuusik, A. (2012). Enhancement of Methane Productivity of Anaerobic Reactors of Wastewater Treatment Plants. World Academy of Science, Engineering and Technology (Issue 65): WASET 2012 Tokyo, Japan International Conference, May 29-30, 2012. WASET, 1191−1193.

9. Excerpt from published books, projects and expertise:

Projects in progress:
VIR16015 "Water emissions and their reduction in village communities - villages in Baltic Sea Region as pilots (Village Waters) (1.03.2016−28.02.2019)". Tallinn University of Technology, Faculty of Chemical and Materials Technology, Department of Chemical Engineering, Chair of Environmental and Chemical Technology; Faculty of Civil Engineering, Department of Environmental Engineering, Chair of Water Engineering.

Completed projects:
VFP616 "Monitoring and management of flowing rain water in Baltic Sea catchment areas Grant agreement No 319923 (Baltic Flows) (1.10.2013−30.09.2016)". Tallinn University of Technology, Faculty of Civil Engineering, Department of Environmental Engineering, Chair of Water Engineering.
KIK14150 "Monitoring water quality, pollution load and storm water in the Mustoja catchment area (1.11.2014−31.03.2016)". Tallinn University of Technology, Faculty of Civil Engineering, Department of Environmental Engineering, Chair of Water Engineering.
Lep12034 "Monitoring storm water in Tallinn (20.02.2012−15.12.2014)". Tallinn University of Technology, Faculty of Civil Engineering, Department of Environmental Engineering, Chair of Water Engineering.
Lep12035 "Monitoring water quality in Lake Harku (20.02.2012−15.12.2014)". Tallinn University of Technology, Faculty of Civil Engineering, Department of Environmental Engineering, Chair of Water Engineering.

VIR459 "Sustainable utilization of waste and industrial non-core materials (SUSBIO) (1.05.2010−30.04.2013)". Tallinn University of Technology, Faculty of Civil Engineering, Department of Environmental Engineering, Chair of Environmental Protection.
KIK10090 "Draft of technological and technical recommendations and manuals of small wastewater treatment units for local municipalities. (15.06.2010−31.12.2011)". Tallinn University of Technology, Faculty of Civil Engineering, Department of Environmental Engineering, Chair of Water Engineering.

APPENDIX III ELULOOKIRJELDUS

1. Isikuandmed

Eesnimi	**ARGO**
Perekonnanimi	**KUUSIK**
Sünniaeg ja -koht	11/07/1985, Tallinn, Eesti
Isikukood	38507110212
Kodakondsus	Eesti

2. Kontaktandmed

Aadress	74626,Vainu, Pudisoo, Harjumaa, Eesti
Telefon	+372 53909716
E-post	argo.kuusik@ttu.ee

3. Hariduskäik

Õppeasutus	Lõpetamise aeg	Haridus (eriala/kraad)
Tallinna tehnikaülikool	2010	Tehnikateaduste magister, ehitusinsener (Veetehnika) (Diplom MB 001796)
Kolga keskkool	2004	Keskharidus

Ametlik nimetus Ehitusinsener, M.Sc (Eng)

4. Keeleoskus

Keel	Tase
eesti keel	emakeel
vene keel	algtase
inglise keel	kesktase

5. Erialane töö

Periood	Organisation	Position
01.01.2017-...	Tallinna Tehnikaülikool, Inseneriteaduskond, Ehituse ja arhitektuuri instituut	Assistent
01.09.2012-31.12.2016	Tallinna Tehnikaülikool, Ehitusteaduskond, Keskkonnatehnika instituut, Veetehnika õppetool	Assistent
2010-31.08.2012	Tallinna Tehnikaülikool, Ehitusteaduskond, Keskkonnatehnika instituut, Veetehnika õppetool	Insener

6. Kaitstud lõputööd

Magistritöö: Prügilavee puhastamine filtratsiooni- ja pöördosmoosi seadmetega. Juhendaja Valdu Suurkask

7. Teadustöö põhisuunad
Veemajandus, vee kvaliteet, reoveepuhastus, veevarustus, biogaas.

8. Teadusartiklid
Kuusik, A.; Pachel, K.; Kuusik, A.; Loigu, E. (2017). The possible agricultural use of digestate. Proceedings of the Estonian Academy of Sciences, 66(1), 64-74.

Kuusik, A.; Pachel, K.; Kuusik, A.; Loigu, E. (2016). Assessment of landfill wastewater pollutants and efficiency of different treatment methods. Proceedings of the Estonian Academy of Sciences, 65 (4), 452−471, 10.3176/proc.2016.4.10.

Kuusik, A.; Pachel, K.; Kuusik, A.; Loigu, E.; Tang, W. Z (2014). Reverse osmosis and nanofiltration of biologically treated leachate. Environmental Technology, 35 (19), 2416−2426, 10.1080/09593330.2014.908241.

Kuusik, A.; Pachel, K; Kuusik, A.; Loigu, E. (2014). Anaerobic co-digestion of sewage sludge with fish farming waste. In: 9th International Conference on Environmental Engineering: Water Engineering (1−8). Vilnius, Lithuania: VGTU Press "Technika".

Kuusik, A.; Pachel, K; Kuusik, A.; Loigu, E. (2014). Landfill runoff water and landfill leachate discharge and treatment. In: 9th International Conference Environmental Engineering: Water Engineering: Selected Papers (1−6). Vilnius, Lithuania: VGTU Press "Technika".

Kuusik, A.; Kuusik, A.; Pachel, K.; Loigu, E.; Sokk, O. (2013). Generalised Integration of Solid Waste Treatment Practices to Enhance Methane Productivity, Generate Suspension Fertiliser and Upgrade Biogas. European Scientific Journal, 9 (36), 14−30.

Kuusik, A.; Kuusik, A.; Loigu, E.; Sokk, O.; Pachel, K. (2013). Selection of Most Promising Substrates for Biogas Production. International Journal of Energy and Environment, 7 (3), 115−124.

Kuusik, A.; Loigu, E.; Kuusik, A.; Sokk, O. (2013). Possibility of Enhancing Methane Productivity in Anaerobic Reactors in the Treatment of Excess Sludge from Wastewater Treatment Plants. International Journal of Science and Engineering Investigations, 2 (12), 33−36.

Kuusik, A.; Kuusik, A.; Loigu, E.; Sokk, O. (2013). Predicting Preferable Substrate Blends for the Production of Biogas. World Scientific and Engineering Academy and Society: Recent Advances in Environmental Science, Lemesis, Cyprus, March 21-23, 2013. WSEAS, 192−197.

Kuusik, A.; Loigu, E.; Sokk, O.; Kuusik, A. (2012). Enhancement of Methane Productivity of Anaerobic Reactors of Wastewater Treatment Plants. World Academy of Science, Engineering and Technology (Issue 65): WASET 2012 Tokyo, Japan International Conference, May 29-30, 2012. WASET, 1191−1193.

9. Väljavõte publikatsioonidest, ekspertiisidest ja projektidest

Jooksvad projektid:
VIR16015 "Hajaasustusalade reostuskoormuse vähendamine - Läänemere piirkonna külad pilootobjektidena (1.03.2016−28.02.2019)". Tallinna Tehnikaülikool. Keemia ja materjalitehnoloogia teaduskond, Keemiatehnika instituut, Keskkonnakaitse ja keemiatehnoloogia õppetool; Ehitusteaduskond, Keskkonnatehnika instituut, Veetehnika õppetool.

Lõppenud projektid:
VFP616 "Monitoring and management of flowing rain water in Baltic Sea catchment areas Grant agreement No 319923 (BalticFlows) (1.10.2013−30.09.2016)". Tallinna Tehnikaülikool, Ehitusteaduskond, Keskkonnatehnika instituut, Veetehnika õppetool.
KIK14150 "Mustoja valgala veekvaliteedi, reostuskoormuse ja sademevee äravoolu uuring (1.11.2014−31.03.2016)". Tallinna Tehnikaülikool, Ehitusteaduskond, Keskkonnatehnika instituut, Veetehnika õppetool.
Lep12034 "Tallinna sademevee seire (20.02.2012−15.12.2014. Tallinna Tehnikaülikool, Ehitusteaduskond, Keskkonnatehnika instituut, Veetehnika õppetool.

Lep12035 "Harku järve vee kvaliteet (20.02.2012−15.12.2014)". Tallinna Tehnikaülikool, Ehitusteaduskond, Keskkonnatehnika instituut, Veetehnika õppetool.
VIR459 "Sustainable utilization of waste and industrial non-core materials (SUSBIO) (1.05.2010−30.04.2013)". Tallinna Tehnikaülikool, Ehitusteaduskond, Keskkonnatehnika instituut, Keskkonnakaitse aluste õppetool.
KIK10090 "Reovee väikepuhastite tehnoloogiliste ja tehniliste lahenduste soovituste ja juhendmaterjalide koostamine kohalike omavalitsuste tarbeks (15.06.2010−31.12.2011)". Tallinna Tehnikaülikool, Ehitusteaduskond, Keskkonnatehnika instituut, Veetehnika õppetool.

DISSERTATIONS DEFENDED AT TALLINN UNIVERSITY OF TECHNOLOGY ON *CIVIL ENGINEERING*

1. **Heino Mölder**. Cycle of Investigations to Improve the Efficiency and Reliability of Activated Sludge Process in Sewage Treatment Plants. 1992.

2. **Stellian Grabko**. Structure and Properties of Oil-Shale Portland Cement Concrete. 1993.

3. **Kent Arvidsson**. Analysis of Interacting Systems of Shear Walls, Coupled Shear Walls and Frames in Multi-Storey Buildings. 1996.

4. **Andrus Aavik**. Methodical Basis for the Evaluation of Pavement Structural Strength in Estonian Pavement Management System (EPMS). 2003.

5. **Priit Vilba**. Unstiffened Welded Thin-Walled Metal Girder under Uniform Loading. 2003.

6. **Irene Lill.** Evaluation of Labour Management Strategies in Construction. 2004.

7. **Juhan Idnurm**. Discrete Analysis of Cable-Supported Bridges. 2004.

8. **Arvo Iital**. Monitoring of Surface Water Quality in Small Agricultural Watersheds. Methodology and Optimization of monitoring Network. 2005.

9. **Liis Sipelgas**. Application of Satellite Data for Monitoring the Marine Environment. 2006.

10. **Ott Koppel**. Infrastruktuuri arvestus vertikaalselt integreeritud raudtee-ettevõtja korral: hinnakujunduse aspekt (Eesti peamise raudtee-ettevõtja näitel). 2006.

11. **Targo Kalamees**. Hygrothermal Criteria for Design and Simulation of Buildings. 2006.

12. **Raido Puust**. Probabilistic Leak Detection in Pipe Networks Using the SCEM-UA Algorithm. 2007.

13. **Sergei Zub**. Combined Treatment of Sulfate-Rich Molasses Wastewater from Yeast Industry. Technology Optimization. 2007.

14. **Alvina Reihan**. Analysis of Long-Term River Runoff Trends and Climate Change Impact on Water Resources in Estonia. 2008.

15. **Ain Valdmann**. On the Coastal Zone Management of the City of Tallinn under Natural and Anthropogenic Pressure. 2008.

16. **Ira Didenkulova**. Long Wave Dynamics in the Coastal Zone. 2008.

17. **Alvar Toode**. DHW Consumption, Consumption Profiles and Their Influence on Dimensioning of a District Heating Network. 2008.

18. **Annely Kuu**. Biological Diversity of Agricultural Soils in Estonia. 2008.

19. **Andres Tolli**. Hiina konteinerveod läbi Eesti Venemaale ja Hiinasse tagasisaadetavate tühjade konteinerite arvu vähendamise võimalused. 2008.

20. **Heiki Onton**. Investigation of the Causes of Deterioration of Old Reinforced Concrete Constructions and Possibilities of Their Restoration. 2008.

21. **Harri Moora**. Life Cycle Assessment as a Decision Support Tool for System optimisation – the Case of Waste Management in Estonia. 2009.

22. **Andres Kask**. Lithohydrodynamic Processes in the Tallinn Bay Area. 2009.

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24. **Dmitry Kurennoy.** Analysis of the Properties of Fast Ferry Wakes in the Context of Coastal Management. 2009.

25. **Egon Kivi**. Structural Behavior of Cable-Stayed Suspension Bridge Structure. 2009.

26. **Madis Ratassepp**. Wave Scattering at Discontinuities in Plates and Pipes. 2010.

27. **Tiia Pedusaar**. Management of Lake Ülemiste, a Drinking Water Reservoir. 2010.

28. **Karin Pachel**. Water Resources, Sustainable Use and Integrated Management in Estonia. 2010.

29. **Andrus Räämet**. Spatio-Temporal Variability of the Baltic Sea Wave Fields. 2010.

30. **Alar Just**. Structural Fire Design of Timber Frame Assemblies Insulated by Glass Wool and Covered by Gypsum Plasterboards. 2010.

31. **Toomas Liiv**. Experimental Analysis of Boundary Layer Dynamics in Plunging Breaking Wave. 2011.

32. **Martti Kiisa**. Discrete Analysis of Single-Pylon Suspension Bridges. 2011.

33. **Ivar Annus**. Development of Accelerating Pipe Flow Starting from Rest. 2011.

34. **Emlyn D. Q. Witt**. Risk Transfer and Construction Project Delivery Efficiency – Implications for Public Private Partnerships. 2012.

35. **Oxana Kurkina**. Nonlinear Dynamics of Internal Gravity Waves in Shallow Seas. 2012.

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37. **Tiina Hain**. Characteristics of Portland Cements for Sulfate and Weather Resistant Concrete. 2012.

38. **Dmitri Loginov**. Autonomous Design Systems (ADS) in HVAC Field. Synergetics-Based Approach. 2012.

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40. **Viktoria Voronova**. Assessment of Environmental Impacts of Landfilling and Alternatives for Management of Municipal Solid Waste. 2013.

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42. **Inga Zaitseva-Pärnaste**. Wave Climate and its Decadal Changes in the Baltic Sea Derived from Visual Observations. 2013.

43. **Bert Viikmäe**. Optimising Fairways in the Gulf of Finland Using Patterns of Surface Currents. 2014.

44. **Raili Niine**. Population Equivalence Based Discharge Criteria of Wastewater Treatment Plants in Estonia. 2014.

45. **Marika Eik**. Orientation of Short Steel Fibers in Cocrete. Measuring and Modelling. 2014.

46. **Maija Viška**. Sediment Transport Patterns Along the Eastern Coasts of the Baltic Sea. 2014.

47. **Jana Põldnurk**. Integrated Economic and Environmental Impact Assessment and Optimisation of the Municipal Waste Management Model in Rural Area by Case of Harju County Municipalities in Estonia. 2014.

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49. **Andrea Giudici**. Quantification of Spontaneous Current-Induced Patch Formation in the Marine Surface Layer. 2015.

50. **Tiina Nuuter**. Comparison of Housing Market Sustainability in European Countries Based on Multiple Criteria Assessment. 2015.

51. **Erkki Seinre**. Quantification of Environmental and Economic Impacts in Building Sustainability Assessment. 2015.

52. **Artem Rodin**. Propagation and Run-up of Nonlinear Solitary Surface Waves in Shallow Seas and Coastal Areas. 2015.

53. **Kaspar Lasn**. Evaluation of Stiffness and Damage of Laminar Composites. 2015.

54. **Margus Koor**. Water Distribution System Modelling and Pumping Optimization Based on Real Network of Tallinn. 2015.

55. **Mikk Maivel**. Heating System Efficiency Aspects in Low-Energy Residential Buildings. 2015.

56. **Kalle Kuusk**. Integrated Cost-Optimal Renovation of Apartment Buildings toward Nearly Zero-Energy Buildings. 2015.

57. **Endrik Arumägi**. Renovation of Historic Wooden Apartment Buildings. 2015.

58. **Tarvo Niine**. New Approach to Logistics Education with Emphasis to Engineering Competences. 2015.

59. **Martin Thalfeldt**. Total Economy of Energy-Efficient Office Building Facades in a Cold Climate. 2016.

60. **Aare Kuusik**. Intensifying Landfill Wastewater and Biodegradable Waste Treatment in Estonia. 2016.

61. **Mart Hiob**. The Shifting Paradigm of Spatial Planning in Estonia: The Rise of Neighbourhood Participation and Conservation of Built-up Areas through the Detailed Case Study of Supilinn, a Historic Suburb of Tartu City, Estonia. 2016.

62. **Martin Heinvee**. The Rapid Prediction of Grounding Behavior of Double Bottom Tankers. 2016.

63. **Bharat Maharjan**. Stormwater Quantity and Quality of Large Urban Catchment in Tallinn. 2016.

64. **Nele Nutt**. The Restoration of Nationally Protected Estonian Manor Parks in the Light of the Florence Charter. 2017.