Proceedings of the Estonian Academy of Sciences,
2017, **66**, 1, 64–74
https://doi.org/10.3176/proc.2017.1.10
Available online at www.eap.ee/proceedings

ENVIRONMENTAL ENGINEERING

# Possible agricultural use of digestate

Argo Kuusik*, Karin Pachel, Aare Kuusik, and Enn Loigu

Department of Environmental Engineering, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia

Received 15 August 2016, revised 28 September 2016, accepted 29 September 2016, available online 2 March 2017



**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

* Corresponding author, argo.kuusik@ttu.ee

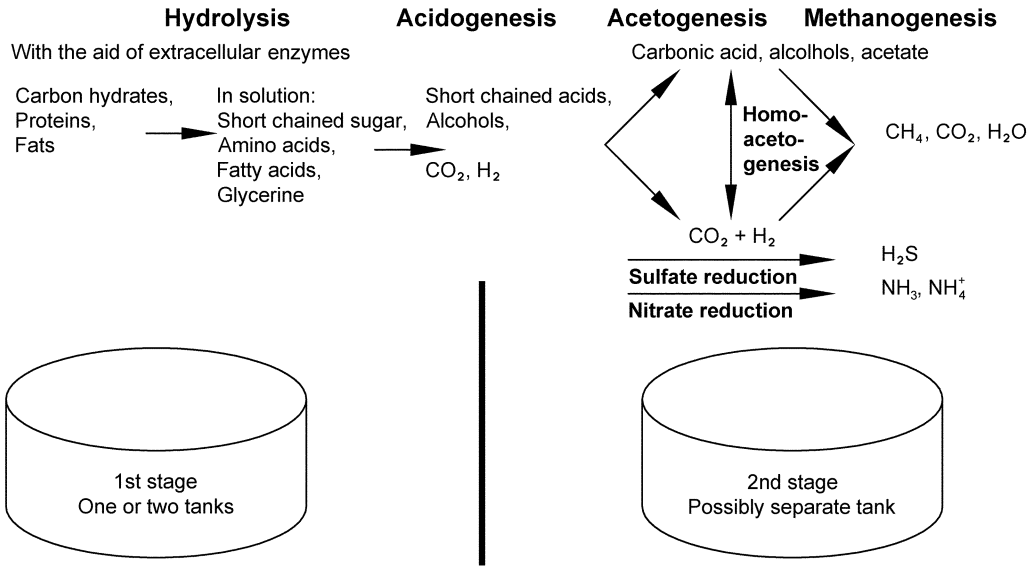


**Fig. 1.** Biochemistry of methane gas production [4].

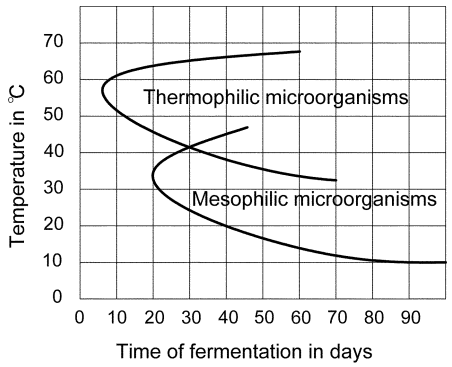


**Fig. 2.** Influence of the temperature on fermentation time [4].

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].

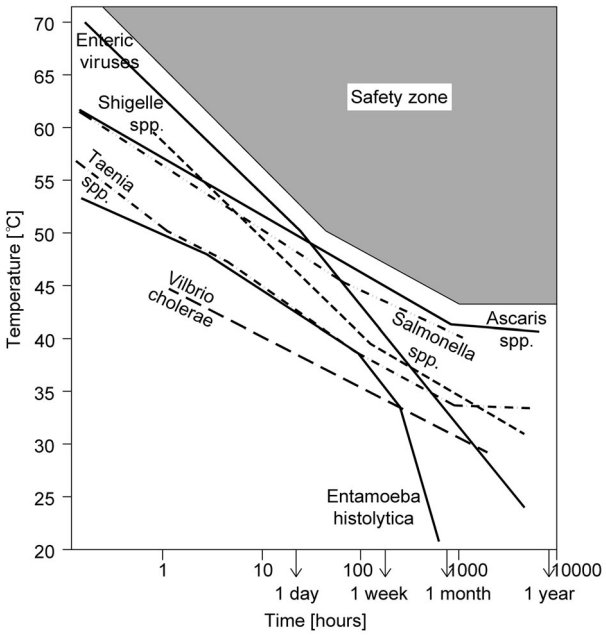


**Fig. 3.** Time–temperature relation for the safe killing off of various pathogens in sewage sludge [25].

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.

## ACKNOWLEDGEMENTS

Estonian Ministry of Education and Research is greatly acknowledged for funding and supporting this study. European Social Foundation financing task 1.2.4 ‘Cooperation of Universities and Innovation Development’ Doctoral School project ‘Civil Engineering and Environmental Engineering’ (code 1.20401.09-0080) has made the publishing of this article possible. The authors thank the Central Baltic Interreg IV A programme 2007–2013, Project No. SFE25 ‘Sustainable utilization of waste and industrial non-core materials’.

The publication costs of this article were covered by the Estonian Academy of Sciences.

## REFERENCES

1. Nasir, M. I., Mohd Ghazi, T. I., and Omar, R. Production of biogas from solid organic wastes through anaerobic digestion: a review. *Appl. Microbiol. Biotechnol*., 2012, **95**, 321–329.
2. EREC. *Renewable Energy Technology Roadmap: 20% by 2020*. European Renewable Energy Council, 2008.
3. Bonetta, S., Bonetta, S., Ferretti, E., Fezia, G., Gilli, G., and Carraro, E. Agricultural reuse of the digestate from anaerobic co-digestion of organic waste: microbiological contamination, metal hazards and fertilizing performance. *Water Air Soil Pollut*., 2014, **225**, 2046.
4. Deublein, D. and Steinhauser, A. *Biogas from Waste and Renewable Resources. An Introduction.* WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2008.
5. Kuusik, A., Loigu, E., Sokk, O., and Kuusik, A. Enhancement of methane productivity of anaerobic reactors of wastewater treatment plants. In *World Academy of Science, Engineering and Technology*, No. 65: *WASET 2012 Tokyo, Japan, International Conference, May 29–30, 2012*. WASET, 2012, 1191–1193.
6. Kuusik, A., Pachel, K., Kuusik, A., and Loigu, E. Assessment of landfill wastewater pollutants and efficiency of different treatment methods. *Proc. Estonian Acad. Sci*., 2016, **65**, 452–471.
7. Yufang Wei, Xiujin Li, Liang Yu, Dexun Zou, and Hairong Yuan. Mesophilic anaerobic co-digestion of cattle manure and corn stover with biological and

chemical pretreatment. *Biores. Technol*., 2015, **198**, 431–436.
8. Bonmatí, A. and Flotats, X. Pig slurry treatment strategy in a high livestock concentration area: anaerobic digestion as the key process. http://www.bvsde.paho.org/bvsacd/unam7/pigslurry.pdf (accessed 2016-09-15).
9. Alkaya, E., Hande Erguder, T., and Demirer, G. N. Effect of operational parameters on anaerobic co-digestion of dairy cattle manure and agricultural residues: a case study for the Kahramanmaras, region in Turkey. *Eng. Life Sci*., 2010, **10**, 552–559.
10. Søndergaard, M. M., Fotidis, I. A., Kovalovszki, A., and Angelidaki, I. Anaerobic co-digestion of agricultural byproducts with manure for enhanced biogas production. *Energy Fuels*, 2015, **29**, 8088−8094.
11. Murto, M., Bjornsson, L., and Mattiasson, B. Impact of food industrial waste on anaerobic co-digestion of sewage sludge and pig manure. *J. Environ. Manage*., 2004, **70**, 101–107.
12. Kuusik, A., Pachel, K., Kuusik, A., and Loigu, E. Anaerobic co-digestion of sewage sludge with fish farming waste. In *9th International Conference "Environmental Engineering": Water Engineering. Vilnius, Lithuania*. VGTU Press "Technika", 2014, 1–8.
13. Pitk, P., Kaparaju, P., and Vilu, R. Methane potential of sterilized solid slaughterhouse wastes. *Biores. Technol*., 2012, **116**, 42–46.
14. Kuusik, A., Loigu, E., Kuusik, A., and Sokk, O. Possibility of enhancing methane productivity in anaerobic reactors in the treatment of excess sludge from wastewater treatment plants. *IJSEI*, 2013, **2**(12), 33–36.
15. Iqbal, S. A., Rahaman, S., Rahman, M., and Yousuf, A. Anaerobic digestion of kitchen waste to produce biogas. *Procedia Engineering*, 2014, **90**, 657–662.
16. Makádi, M., Tomócsik, A., and Orosz, V. Digestate: a new nutrient source – review. In *Biogas* (Kumar, S., ed.), InTech, 2012, 295–310, http://www.intechopen.com/books/biogas/digestate-a-new-nutrient-source-review (accessed 2016-09-15).
17. Bernal, M. P., Alburquerque, J. A., de la Fuente, C., Clemente, R., Caravaca, F., Díaz-Pereira, E., et al. The probiogas project: an integrated approach of the anaerobic co-digestion of agricultural wastes for production of biogas and fertilisers. In *Pre-Processing of Manure and Organic Waste for Energy Production*, 2010.
18. Venglovsky, J., Martinez, J., and Placha, I. Hygienic and ecological risks connected with utilization of animal manures and biosolids in agriculture. *Livestock Science*, 2006, **102**, 197–203.
19. Engeli, H., Edelmann, W., and Fuchs, J. K. Survival of plant pathogens and weed seeds during anaerobic digestion. *Water Sci. Technol*., 1993, **27**(2), 69–76.
20. Martens, W., Fink, A., Philipp, W., Weber, A., Winter, D., and Boehm, R. Inactivation of viral and bacterial pathogens in large scale slurry treatment plants. In *Ramiran 98: Proceedings; International Conference on Management Strategies for Organic Waste Use in Agriculture, Sess. 8, Rennes (France), 26–29 May 1998* (Martinez, J. and Maudet, M.-N., eds). FAO, Rome (Italy). Agricultural Services Div., 1999, 529–539.
21. Carrington, E. G. Evaluation of sludge treatment for pathogen reduction – Final Report, WRc Ref: CO 5026/1, 2001. http://ec.europa.eu/environment/waste/sludge/pdf/sludge_eval.pdf (accessed 2016-09-15).
22. Al Seadi, T. and Lukehurst, C. *Quality Management of Digestate from Biogas Plants Used as Fertiliser*. IEA Bioenergy, 2012.
23. Lukehurst, C. T., Frost, P., and Al Seadi, T. *Utilisation of Digestate from Biogas Plants as Biofertiliser*. IEA Bioenergy, 2010.
24. Riigikogu. Veeseadus [Water Act]. *Riigi Teataja I*, 27.12.2016, 6.
25. Holm, P. E., Jensen, L. S., and McLaughlin, M. J. *Utilization of Biologically Treated Organic Waste on Land. Solid Waste Technology and Management* (Christensen, T., ed.). Blackwell Publishing Ltd, 2010.
26. *Handreichung. Biogasgewinnung und -nutzung*. 3., revised edition. Fachagentur Nachwachsende Rohstoffe e. V., Gülzow, 2006.
27. Nyord, T., Søgaard, H. T., Hansen, M. N., and Jensen, L. S. Injection methods to reduce ammonia emission from volatile liquid fertiliser applied to growing crops. *Biosystem Engineering*, 2008, **100**(2), 235–244.
28. Nutrient Value of Digestate from Farm-Based Biogas Plants in Scotland. Report for Scottish Executive Environment and Rural Affairs Department – ADA/009/06. July 2007.
29. Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009. *Official Journal of the European Union*, 14.11.2009, L 300/1. http://data.europa.eu/eli/reg/2009/1069/oj (accessed 2016-09-15).
30. Al Seadi, T. Good practice in quality management of AD residues from biogas production. Report. University of Southern Denmark (SDU), Bioenergy Department Niels Bohrs Vej 9, DK-6700 Esbjerg, Denmark, IEA Bioenergy, 2001.
31. Keskkonnaministri 01.02.2003 määrus nr 78 " Reoveesette põllumajanduses, haljastuses ja rekultiveerimisel kasutamise nõuded" [Minister of the Environment. Regulation No. 78 of 01.02.2003 'Requirements for the application of sewage sludge in agriculture, landscaping, and recultivation]. *Riigi Teataja L*, 2003, 5, 48 (in Estonian).
32. Keskkonnaministri 10.05.2016 määruse nr 12 "Nõuded biolagunevatest jäätmetest biogaasi tootmisel tekkivale kääritusjäägile" lisa 2 Kääritusjäägi ohutus- ja kvaliteedinäitajad [Minister of the Environment. Regulation No. 12 of 10.05.2016 'Requirements for the digestate from biogas production from biodegradable waste', Annex 2: Digestate safety and quality indicators]. *Riigi Teataja I*, 19.05.2016, 9 (in Estonian).
33. Reoveesette töötlemise strateegia väljatöötamine, sh ohutu taaskasutamise tagamine järelevalve tõhustamise, keemiliste- ja bioloogiliste indikaatornäitajate rakendamise ning kvaliteedisüsteemide juurutamise abil. III ETAPP [Development of the strategy for the treatment of sewage sludge, including raising the effectiveness of monitoring safe recycling, application of chemical and biological indicators, and introduction of quality assessment systems. Stage III]. OÜ Eesti Keskkonnauuringute Keskus, 2012 (in Estonian).

# 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.