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Preliminary study of acrylamide monomer decomposition during methane fermentation of dairy waste sludge

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ABSTRACT

Polyacrylamide (PAM) used in sludge dewatering exists widely in high-solid anaerobic digestion. Acrylamide is registered in the list of chemicals demonstrating toxic, carcinogenic and mutagenic properties. Therefore, it is reasonable to ask about the mobility of such residual substances in the environment. The study was carried out to assess the impact of the mesophilic (39 ± 1°C) and thermophilic (54 ± 1°C) fermentation process on the level of acrylamide monomer (AMD) content in the dairy sludge. The material was analysed using high-performance liquid chromatography (HPLC) for quantification of AMD. The results indicate that the process of methane fermentation continues regardless of the temperature effects on the degradation of AMD in dairy sludge. The degree of reduction of acrylamide monomer for thermophilic fermentation is 100%, while for mesophilic fermentation it is 91%. In practice, this means that biogas technology eliminates the risk of AMD migration to plant tissue. Moreover, it should be stressed that 90% of cumulative biogas and methane production was reached one week earlier under thermophilic conditions — the dynamics of the methanisation process were over 20% faster.

Introduction

Polyacrylamide (PAM) based flocculants have been accepted and implemented for decades in many applications including pulp, paper, cosmetic and textile industries. Acrylamide flocculants are also used in water treatment, chemical treatment of sludge and in the case of food processing it is recommended for organic fertilisation. The food industry typically generates solids with beneficial fertiliser properties, suitable after appropriate treatment, both to improve the properties of the soils used for agricultural purposes and for effective soil reclamation. A high content of organic matter and a significant amount of beneficial macro- and micro-nutrients have a positive influence on the physicochemical properties of the soil. In many European countries, national authorities have implemented policies to support the use of sewage from the food industry in agriculture, as it is often considered the best economic and environmental solution (European Commission, 2001). Currently, due to their importance accompanied by many technological benefits, PAM polymers associated with trivalent metal ions, such as iron and aluminium in the form of commercially available coagulants are among the chemicals used in wastewater and sludge management, including the food industry in a large volume (Vanerkar et al., 2013).

According to European Union regulations (EEC, 1774/2002), sludge like other organic residues must be adequately treated

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and proven hygienically safe (Arthurson, 2008). More specifically, the purpose of sludge stabilisation is to reduce pathogens, eliminate offensive odours and inhibit, reduce or eliminate the potential for putrefaction, which is done by biological reduction of the volatile organic fraction or the addition of chemicals. Among various biological, chemical or thermal methods of sewage sludge treatment taken into consideration, before further utilisation in the agro-food sector, anaerobic digestion by converting a part of its organic matter into biogas is currently recommended as the most effective biomass valorisation technology for smaller reactor volumes, lower energy requirements for heating and lower levels of material handling (Duan et al., 2012; Fernandez Rodriguez et al., 2012). Anaerobic digestion of sewage sludge or co-digestion of sludge with the use of several food wastes has been studied recently by several authors (Lee and Shoda, 2008; Wan et al., 2011; Dai et al., 2013; Uma Rani et al., 2013; Di Maria et al., 2014; Buranasilp and Charoenpanich, 2011).

With the identification of environmental and health risks of acrylamide monomer (AMD) used in the production of a major class of water soluble PAM based polymers, concerns related to these topics have been increasingly raised. It is known that AMD adversely affects the peripheral nervous system of organisms as a highly toxic and potent carcinogenic compound (Tareke et al., 2002;). Therefore, guidelines for PAM use in variety of applications typically recommend products with <0.05% AMD content only (Sojka and Surapaneni, 2001; Lu et al., 2014).

The twenty year plus period until now indicates that several problems related to the fate of AMD, following application of PAM in cropland, were studied and have been summarised in various articles including the most important topics like PAM degradation mechanism, AMD chemical reactivity and its mobility and accumulation risk in plants (Friedman, 2003). Among others our own study with hydroponically grown head lettuce confirmed that the problem of AMD mobility and its uptake by plants should be an important topic with respect to safe polyacrylamide handling in the agro-food area (Mroczek et al., 2014).

Since degradation of polyacrylamide accompanied with accumulation of its toxic monomer is important to the disposition of biogas residues generically termed as "digestate" (Chu et al., 2003; Zhang et al., 2009; Duan et al., 2012,) biodegradation of polyacrylamide by anaerobic digestion under mesophilic conditions in a dewatered sludge system was studied by Dai et al. (2014). The authors concluded that PAM can be consumed as the carbon source by the breakdown of the carbon chain backbone in different groups. These results seem contradictory to those reported by other researchers, which indicated that PAM could be utilised as a nitrogen source rather via microbial amidase activity and transformation in polyacrylates (Kay-Shoemake et al., 1998). It has been reported that aerobic and anaerobic treatment seems to be a perfect way for biodegradation of many substances (Costa et al., 2014; Lina et al., 2013; Brovelli et al., 2011). On the other hand, Hamelin et al. (2010) reported that the solid fraction from PAM-separated slurry is not suitable to be degraded during anaerobic digestion.

In the present study we focused on the examination of how the selected PAM flocculants, used in commercial thickening operation, affected AMD content during the digestion of sewage sludge collected from a dairy processing plant. Experiments were conducted under the same analytical conditions, in lab bioreactors, to determine and to compare changes in free residual acrylamide monomer content in digestates following methane fermentation of the raw material under mesophilic and thermophilic conditions. It should be underlined that biogas production based on biomass seems to be one of the most promising directions of renewable energy development in Poland (Cerbin et al., 2012).

1. Materials and methods

1.1. Materials

The material tested was PAM treated sludge collected from a local dairy processing plant generating typical milk waste and cleaning wastewaters from the facilities of the factory. The sludge samples were obtained from dairy effluent treatment plant characterized by flow about 600 m^3/day and Population Equivalent about 23,500. The characteristics of the influent wastewater of the plant making drinking milk, yoghurt, kefir and quark and working without whey protein recovery system were as follows: pH was 5.5, total COD was 5000 mg/L, total nitrogen was 30 mg/L and total phosphorus was 7.1 mg/L. Chemicals used during wastewater and sludge treatment included alum, iron chloride, iron sulphate and selected PAM flocculants. Following technological procedure, the highly charged, very high molecular weight cationic product in the form of emulsion with residual acrylamide monomer content below 1000 mg/L was employed for sludge thickening in dosage of 3.5 kg/Mg dry matter (DM). As inoculum — the liquid fraction of digested pulp from a local agricultural biogas plant was used, which is common practice in laboratory scale experiments (Djelal and Amraneb, 2013; Chen et al., 2015).

The study was conducted in a biogas Laboratory of Ecotechnologies working within the Institute of Biosystems Engineering at Poznan University of Life Sciences. The research was based on modified German standard DIN 38414/S8, while chemical and physical analytical methods were based on Polish Standard system (Dach et al., 2014; Boniecki et al., 2013). Analytical procedures were developed within several scientific projects financed by the EU 6th Framework Program and national founds realised in the years 2006–2012.

1.2. Digestion under meso- and thermo-philic conditions

1.2.1. Methane production set-up

The experiment of biogas production was conducted through anaerobic digestion in the set of multichamber biofermentors (Fig. 1). This biofermentor is commonly used for testing biogas efficiency for large amount of biomass samples.

The batch experiment with sewage sludge methane fermentation was carried out according to the German norms: DIN 38 414/S8 and VDI 4630. Those methodologies are commonly used in German biogas laboratories as well as in other countries in central Europe. At the beginning of the process 300 g of sludge were placed in reactors and 700 g of inoculum (mesophilic or thermophilic) were added to initialize fermentation process. Aerobic digestion experiments of analysed sewage sludge samples were carried out in glass reactors constructed in the

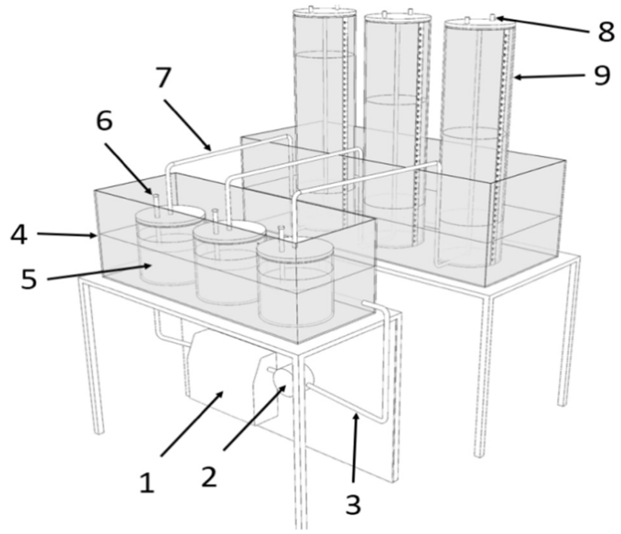

Fig. 1 – Schematic of biofermentor for biogas production (3-chamber section): (1) Water heater with temperature regulator, (2). water pump, (3) insulated conductors of calefaction liquid, (4) water coat with temperature 39°C, (5) biofermentor with charge capacity 2 L, (6) sampling tubes, (7) biogas transporting tube, (8).gas sampling valve and (9) biogas volume-scale reservoir.

Laboratory of Ecotechnologies. General rules for biofermentor work were based on the fermentation of organic substrate samples which were put in the chambers with 2 dm^3 capacity. Without the presence of oxygen (this parameter was checked daily) and an additive of proper fermentation inoculum (mesophilic or thermophilic) the conditions present within the fermentation chamber allowed the creation of an ideal condition for methane fermentation of the samples. Glass chambers with samples were placed in water with a regulated temperature of 39 ± 1°C (mesophilic conditions) and 54 ± 1°C (thermophilic). Biogas produced in each separate chamber was transferred to cylindrical store-equalising reservoirs, filled in with liquid resistant for gas solubility. The samples were tested in 3 replications.

1.2.2. Solid samples

The pH was measured using laboratory multi-meter CP-411 (Elmetron). Additionally, dry matter and organic dry matter were determined. It was necessary to calculate biogas production efficiency in typically used units—m^3/Mg of fresh matter (FM) and dry matter.

During the experiment the following standard methodology established by Polish Norms (PN) was used: for dry matter (total solids, TS) PN-75 C-04616/01, pH-PN-90 C-04540/01, conductivity PN-EN 27888:1999, organic dry matter (- volatile solids, VS) PN-Z-15,011-3 and ammonia PN-73 C-04,576/02.

1.2.3. Gas samples

The volume of the produced biogas was measured every 24 hr. Gas composition was checked out from at least each 1 dm^3 of the produced biogas (at the beginning of the experiment it was once a day, and after the culmination point, when the production slowed down, once every three days). The concentration measurements of methane, carbon dioxide, hydrogen sulphide, ammonia and oxygen in the produced biogas were carried out with the use of the absorption sensors working in an infrared and electrochemical sensor line. The Mg-72 and MG-73 head types for gas concentration measurement were used (ALTER S.A.). The ranges of detected gaseous compounds were: 0–100% CH_4, 0–100% CO_2, 0–25% O_2, 0–2000 ppmV H_2S and 0–2000 ppmV NH_3, respectively (Janczak et al., 2013). The volume of biogas production and the methane content of biogas were calculated in Excel. According to the graph, it was possible to determine if the sample was working properly during the experiment. The gas monitoring system was calibrated each week using calibration gases provided by Messer Company, using the following concentration of gas calibration: 65% of CH_4, 35% of CO_2 mixed together, 500 ppmV of H_2S and 100 ppmV of NH_3. For O_2 sensor calibration typical synthetic air was used.

1.2.4. Analysis of acrylamide monomer in inoculum and digested dairy sludge samples

Standard of pure acrylamide (≥99.8%) and acetone (high performance liquid chromatography (HPLC) grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium dihydrogen phosphate and *o*-phosphoric acid were purchased from POCh (Gliwice, Poland). Water for the HPLC mobile phase and standard solutions was purified by a Milli-Q system (Milipore, Bedford, MA, USA).

Determination of acrylamide monomer was made using three types of samples: dairy sludge, inoculum and a trial mixture of both substrates used in the experiment of anaerobic digestion. The reactant mixture was subjected to two variants stabilised by anaerobic fermentation conditions: mesophilic 39 ± 1°C (variant 1) and thermophilic 54 ± 1°C (variant 2). Substrates for fermentation were analysed by downloading them from the reactor three times in triplicate, at the start of the process (the first day of the experiment), during the process (10 day) and at the end of the process (36th day — mesophilic fermentation, 29th day — thermophilic fermentation). The thermophilic fermentation process was completed seven days before due to the fact that the process of biogas production was shorter. Each sample (2 g) was put into a plastic tube and the residual monomer was analysed using the same extraction procedure.

Acrylamide was extracted from examined samples with acetone: water (4:1, *V/V*) using 20 mL of solvent per 2 g of sample according to Mroczek et al. (2014). After homogenisation (1 min) (homogeniser H 500, Pol-Eco, Wodzislaw Sl, Poland) samples were transferred into a thermostat controlled water bath at 60°C for 60 min (Memmert GmbH & Co. KG, Schwabach, Germany). Next, the aqueous layer (10 mL) was filtered through 0.45 μm chromatographic filters (Chromafil, Macherey-Nagel, Germany) and collected for chromatographic analysis.

Preparation of liquid chromatographic conditions was based on previous reports described by Michalak et al. (2013) and Wang et al. (2008, 2013) with modifications. Chromatographic separation was performed by HPLC, using a liquid chromatograph Waters 2695 (Waters, Milford, USA) equipped with a photodiode array detector (PAD model Waters 2996) set at 220 nm. The column used was an Agilent PLRP-S 100 A, 5 μm 150 × 4.6 mm (Agilent Technologies, Santa Clara, USA). Empower™ 1 software was used for data processing. Sodium dihydrogen phosphate (0.1 mol/L in water) solution adjusted to pH 3.0 with *o*-phosphoric acid, after filtration through a 0.45 μm HV membrane (Milipore, Bedford, MA, USA) was used as the mobile phase with a flow rate of 0.8 mL/min. The mode of the HPLC instrument was isocratic with the injection volume of 10 μL. Standard stock solutions (1.0 mg/mL) were prepared by dissolving 10 mg of acrylamide in 10 mL Milli-Q water and stored at 4°C until further use. All working solutions were prepared daily by serial dilution also in Milli-Q water. The acrylamide detection limit was 1.0 ng/g. Positive results (on the basis of retention time) were confirmed by HPLC analysis and compared with the relevant calibration curve (the correlation coefficient for acrylamide was 0.9967).

2. Results and discussion

2.1. General remarks to digestion process

The characteristics of the thickened dairy sludge and the inoculums used in the experiment are shown in Table 1.

The inoculum was used in the methane fermentation experiment as one of two substrates constituting the feed to the digester, added in order to accelerate the production of biogas. The pH value, with an optimum range between 6.8 and 7.5 and ammonium nitrogen concentration lower than 2.7 g/L of prepared mixture, were among the physical and chemical parameters characterising the anaerobic digestion process (Wellinger et al., 1991). While fermentation pulp was characterised by similar parameters of pH and organic dry matter content. Significant differences were observed when comparing the dry matter of the inoculum and examined sludge equal to 5% and 11% respectively.

It is evident from the results in Table 2 that different temperature conditions did not influence the biogas efficiency of substrate. On the other hand it is worth highlighting that 90% of the cumulative biogas and methane production was reached one week earlier in thermophilic conditions. It means that the dynamics of the methanisation process was over 20% faster (Fig. 2).

2.2. Determination of acrylamide monomer content

The running of the experiment confirmed the ability of the monitoring and determination of the monomer content in the sediment matrices generated by the food industry and in the substrate matrix stabilised anaerobic fermentation technology using HPLC. Analytical methods for AMD include those based on gas chromatography (GC), HPLC, mass spectrome (MS) and a combination of these. GC–MS technique was widely used much earlier than liquid chromatography–mass spectrometry (LC–MS). US EPA method 8032 A uses liquid extraction and GC-ECD for determinations in water (US EPA Method, 1996), while a method by the German Health Agency (BGVV) uses HPLC with UV detection for migration analysis of acrylamide monomer from packing material (Results of a BGVV information seminar, 2002). A HPLC method using the reverse-phase mode for determination of acrylamide monomer in polymeric products employed for water treatment applications was recommended also by Skelly and Husser (1978) and Tseng (1990). An attempt to summarize the state-of-the-art knowledge of analytical methods of acrylamide in various matrixes was conducted in a report of Keramat et al. (2011). Recommended analytical procedures have to be not only simple and easily automated but also to be capable of determining very low levels of acrylamide without interferences from other components in the sample (Tseng, 1990).

Analyses of AMD content were conducted in the substrates used for methane fermentation process in the dairy sludge and in the inoculum.

The results demonstrated that the inoculum used for the experiment was free from AMD while the dairy sludge contained 64 ± 0.5 mg/kg in structure. The results obtained confirm that the only source of AMD in the experiment conducted is sludge obtained from the dairy.

At the beginning of process 300 g of sludge was placed in reactors and 700 g of inoculum (mosophilic or thermophilic) was added to initialize fermentation process.

Analysis of AMD was made for sediment samples taken successively after the first day of the experiment, the 10th day of the experiment and on the last day of the study to assess the impact of methane fermentation on the changes in the content of AMD in the pulp digestate (Fig. 2).

In Fig. 3 an average summary of the results of the acrylamide monomer content in sedimentary samples of the fermentation process meso- and thermophilic is provided. The obtained data confirms that over time the running process of anaerobic digestion, regardless of the temperature, affected the reduction of residual acrylamide monomer in the analysed samples. Both fermentation mixtures, containing the inoculum and dairy sludge, at the beginning reached 27.4 mg/kg of AMD (300 g of sludge and 700 g of inoculum).

Subsequently for mesophilic fermentation in the first day of the experiment the level of AMD was 23 mg/kg, followed by 6 mg/kg on the 10th day of the process, and 2 mg/kg on the last day of the study. In the case of thermophilic fermentation the level of AMD was 16 mg/kg at the beginning of the experiments, 5 mg/kg in the middle of the process (10th day) and reducing the level of AMD to 0 mg/kg on the last day of the study.

2.3. Discussion

The comparison of the influence of mesophilic and thermophilic fermentations effectiveness on the reduction of AMD in sludge samples was made. It can be assumed that the method more effective for treatment and stabilization of sewage sludge from the food industry is a thermophilic fermentation method. The degree of acrylamide monomer reduction was 100%, while for mesophilic fermentation 91%. This means that the use of the methane fermentation process for the treatment of sewage sludge from the food industry (to allocate

Table 1 – Sludge and the inoculum characteristics used in the experiment.

Sample	pH	TS (%)	VS (% TS)
Sludge	7.66	10.8 ± 0.1	70.8 ± 0.4
Mesophilic inoculum	7.98	4.8 ± 0.1	71.1 ± 0.1
Thermophilic inoculum	8.02	8.2 ± 0.1	69.3 ± 0.2

Data are presented as mean values ± SD, n = 3. TS: total solids; VS: volatile solids.

Table 2 – Comparison between mesophilic and thermophilic cumulative biogas and methane production calculated after the last day of the fermentation process.

	Cumulative methane (m^3/Mg FM)	Cumulative biogas (m^3/Mg FM)	Cumulative methane (m^3/Mg TS)	Cumulative biogas (m^3/Mg TS)
Mesophilic conditions	15.1	26.2	195.6	312.4
Thermophilic conditions	15.1	25.7	195.6	335.2

FM: fresh matter.

the fertiliser) in which acrylamide is present eliminates the risk of the migration to the plant. Szczerbina (2005) published data of the decomposition process (depolymerisation) of polyacrylamide, which depends on the access of light, and does not depend on the pH of the environment, but still insight into the potential risk to the environment remains a key research problem. Scientists have been looking for years for acrylamide in plants grown in a hydroponic base (*i.e.*, mushrooms, tomatoes) or soils with the addition of polyacrylamide gel *i.e.*, beans, corn or potatoes (Loren et al., 1999; Friedman, 2003; Castle et al., 1991; Castle, 1993). Unfortunately, their long struggle over the specified topic does not provide information on the level of residual monomer in the plant, because the AMD content is at a level, below the limit of detection. However, research conducted by Mroczek et al. (2014) confirmed the migration risk of AMD to plant material like butterhead lettuce leaves. The experiment was conducted as a simple test in butterhead lettuce hydroponic cultivation, where the polyacrylamide flocculants were intentionally added to the medium in order to assess the degree of mobility of AMD.

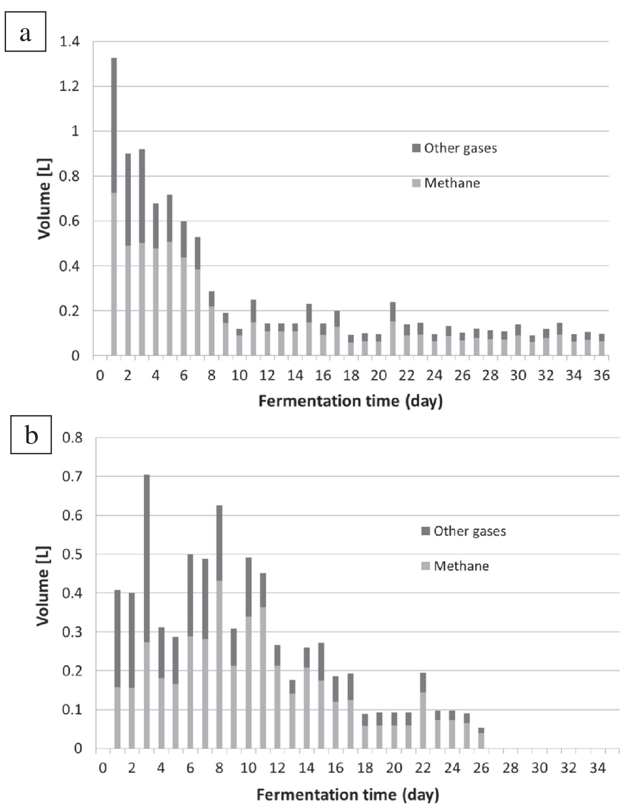

Fig. 2 – Daily biogas production under mesophilic (a) and thermophilic (b) conditions.

However, researchers noticed that there must be a previously unknown mechanism for its decomposition of acrylamide in plants, resulting in its low levels in plant tissues (Friedman, 2003; Stevens et al., 2003).

In studies which have been made for the treatment of sewage sludge from the food industry meso- and thermophilic anaerobic fermentation methods were used, which effectively reduces the level of monomer in the substrate. This may be due to the fact that bacteria are capable of enzyme synthesis, which catalyses the reactions of both the synthesis and biodegradability of acrylamide. PAM biodegradation has been studied by several researchers so far, but the results seem contradictory. For example Kay-Shoemake et al. (1998) and Grula et al. (1994) showed that PAM could be utilised as a nitrogen source rather than a carbon source via inducible amidase activity and converted in polyacrylates. Bao et al. (2010) and Nakamiya and Kinoshita (1995) confirmed that the amide group of PAM could serve as a nitrogen source. El-Mamouni et al. (2002) indicated that PAM is highly recalcitrant to microbial degradation, suggesting that this recalcitrance is linked to the high molecular weight.

Schink (1997) and Krause et al. (2008) state that anaerobic digestion is a complex and multi-step process, involving fermenting bacteria which hydrolysed complex organic compounds into oligomers and monomers. For example in *Rhodococcus* the enzymatic synthesis of acrylamide from acrylonitrile occurs, involving amidase, glutamine synthetase, and the nitrile hydratase (Friedman, 2003; Stevens et al., 2003). The latter enzyme, nitrile hydratase, is used for the production of acrylamide on an industrial scale (Friedman, 2003; Van Vliet et al., 2003). Moreover, successive scientific reports provide information that the degradation of acrylamide under favourable conditions by *Pseudomonas* sp. and *Xanthomonas maltophilia* was immobilised (Nawaz et al., 1993). It should be noted that the methane fermentation process during the various phases of the

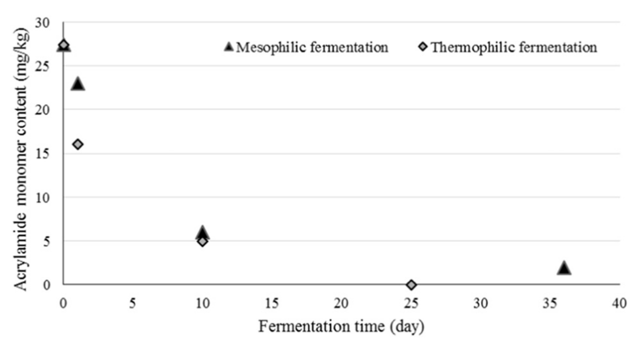

Fig. 3 – The content of acrylamide monomer in samples substrate of the meso- and thermophilic fermentation.

process involved a number of different groups of microorganisms. Among them there is aerobic bacteria *Pseudomonas* sp. involved in the decomposition of the substrate at the stage of hydrolysis and acidogenesis (Ziemiński and Frąc, 2012).

There have been also several studies on the biodegradation of AMD in aqueous natural environments, including rivers, lakes, seawater, sewage effluents and aquatic sediments. A lag period of hours to weeks, presumably allowing for enrichment of AMD decomposing bacteria or induction of enzymes in existing population, has been commonly observed. Both fungal and bacterial species commonly found in the soil are capable of degrading PAM (Bavernik et al., 1996).

Shanker et al. (1990) and Buranasilp and Charoenpanich (2011) studied the microbial degradation of AMD by soil microorganisms. The authors concluded that, AMD was degraded into ammonia and acrylic acid.

3. Conclusions

The results obtained confirm that the technology of biogas production based on anaerobic fermentation, regardless of the temperature level of the processes carried out in the range of 39 to 54°C, affects the biodegradability of acrylamide monomer contained dairy sludge. As it is known that AMD adversely affects the peripheral nervous system of organisms as a highly toxic and potent carcinogenic compound in practice, this means that the methane fermentation technology reduce the risk of migration of residual acrylamide monomer to plants from soil fertilised with digestate from biogas plant. Research proved that anaerobic digestion is a solution that could be used in every industry that deals with AMD in order to reduce the risk of it spread in environment. Since there are differential kinds of chemicals used in sludge dewatering it is purposeful to continue research to extend the knowledge of its possible biodegradation during sludge stabilization processes. The related microorganism community should be investigated.

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