CHANGES IN ACRYLAMIDE MONOMER CONTENT DURING COMPOSTING OF DAIRY PROCESSING SLUDGE


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Abstract. This paper focused on the results of an experiment involving sewage sludge drawn from a typical dairy processing plant after application of polyacrylamide (PAM) flocculant in an approximate dosage of 3.5 kg Mg⁻¹ dry matter for dewatering and subsequently taken for aerobic fermentation in a lab-scale bioreactor with a capacity of 120 dm³ within 31 days. Since it cannot be ruled out that such sewage sludge will be used as a good soil fertilizer for edible plants, e.g. vegetables, the concentration of neurotoxic and carcinogenic residual acrylamide monomer (AMD) was monitored throughout the composting process. Analysis of AMD was conducted using the HPLC method for compost samples taken after the 1st day of the experiment, on the 10th day and on the last 31st day of the experiment. The results obtained from the composting process with measurement of temperature and air flow intensity indicate that aerobic composting resulted in a significant reduction of the AMD content in the compost, up to 23% of the initial value. It appears to be the case that intensive processes of biochemical degradation during aerobic fermentation of sludge from the dairy processing plant significantly reduces the threat of residual monomers being released into the environment from a polyacrylamide applied as a chemical sludge conditioner.

Keywords: AMD, PAM (polyacrylamide), flocculants, aerobic fermentation process, composted mixture

Introduction

Thanks to supporting wastewater treatment processes using various kinds of chemical reagents, including coagulants and flocculants in different nodes of technological installations for cleaning municipal and industrial wastewater, large quantities of sewage sludge are produced (Vanerkar et al., 2013). Raw sludge is difficult to use and dispose of not only due to its large mass, but generally its undesirable texture, and the fact that it easily ferments. Selecting the process of sewage sludge management frequently requires an individual approach and should remain in close relation to the particular conditions of the plant (Green and Stott, 2001).

In the case of sludge collected during wastewater treatment processes we deal with features that often prevent it from being used in agriculture, for example, an increased amounts of heavy metals and other highly toxic inorganic or organic chemicals.
However, sewage sludge from food processing can be used as fertilizer if there were appropriate technical means and if the value of the subsequent products were to exceed the cost of reprocessing. After adequate stabilization, such sludge is suitable for improving soil used for agricultural purposes and for efficient land reclamation of degraded soils (European Commission, 2001; Adani, at al., 2004; Ruden, 2004; Szwedziak, 2006). The high content of organic matter and the significant amount of macro- and microelements has a positive effect on the physico-chemical properties of the soil and demonstrate a soil-forming impact stimulating the accumulation of humic substances in the environment. The methods applied for this purpose include methane fermentation and sludge stabilization by composting. Sewage sludge can be composted, especially after the addition of a variety of structural materials. The purpose of this is to increase the permeability by weight, to facilitate optimum moisture enrichment of the sludge with an additional carbon source and provide an optimal C:N ratio (Su and Wong, 2003, Adani, 2004; Szwedziak, 2006; Rebollido et al., 2008).

In view of the common usage of polyacrylamide flocculants for thickening or dewatering raw sewage sludge, the key issue is to assess the risk that unwanted monomeric forms, which constitute the unpolymerized part of such a reagent, may be released into the environment. It is not explicitly excluded that the monomer acrylamide is also a transitional biodegradable polyacrylamide product, which can be hydrolyzed as a nitrogen (i.e. amide hydrolysis) or carbon (i.e. carbon chain hydrolysis) source during the anaerobic process (Mroczek et al., 2015). The monomer acrylamide is characterized by proven, high carcinogenic and neurotoxic activity (Chico-Galdo at al., 2006; Mustafa at al., 2008 Wang et al., 2010). Available literature sources focus primarily on analyzing the problem of acrylamide in foods subjected to intense heat treatment. Studies have also been conducted to analyze this threat from a different perspective, and these studies are therefore related to the application of polyacrylamides in the soil environment as hydrogels or as components of organic fertilizers (Lee and Shoda, 2008; Wan et al., 2011; Dai et al, 2013, Dai et al, 2014; Uma Rani et al., 2013 Di Maria et al., 2014). Despite the strict legal requirements in this area, the practice of sourcing plant materials, especially vegetables, from soil fertilized with stabilized sewage sludge, and thus the food use of such materials still cannot be clearly excluded (Class et al, 2007). Scientists’ queries concern the degradation mechanism for polyacrylamides, their chemical reactivity and especially the risks of the mobility and accumulation of residual monomers in edible plants (Friedman, 2003). Thus far the results of the authors’ own research confirmed that in both lettuce cultivation in hydroponic conditions and its cultivation in peat, the contamination of plant tissue with such residues can occur (Mroczek et al., 2014, 2015). At the same time, other studies (Mroczek et al., 2016) demonstrated that the methane fermentation process used for the stabilization of industrial sludge, which is thickened with polyacrylamide flocculant, significantly reduces acrylamide monomer content in post-ferment, which is recommended for organic fertilization. Together with the continuation of the above research, in this paper, a laboratory bioreactor was used for preparation, in an attempt to determine the extent of changes in acrylamide monomer content in sediment from the dairy processing plant and under the conditions of aerobic fermentation.
Materials and methods

Materials

The material tested was PAM-treated sludge collected from a local dairy processing plant producing typical milk waste and cleaning wastewater from the facilities of the factory. The sludge samples were obtained from a dairy effluent treatment plant characterized by a flow approximately 600 m³/day and a population equivalent about 23,500. The characteristics of the influent wastewater of this plant, which produces drinking milk, yoghurt, kefir and quark and working without a 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. The chemicals used during wastewater and sludge treatment included alum, iron chloride, iron sulphate and selected PAM flocculants. Following the technological procedure, a highly charged, very high molecular weight cationic product in the form of an emulsion with a residual acrylamide monomer content below 1000 ppm was employed for sludge thickening in an approximate dosage of 3.5 kg Mg⁻¹ dry matter (DM). Wheat straw was used as a co-substrate for the composting process and as a structural material.

In order to simulate the process taking place in a full-scale composting pale and to measure the parameters of such a process, a special composting chamber was constructed (Fig. 1) (Czekala et al., 2016).

![Figure 1. Design of chamber composting bioreactor used to compost dairy processing sludge and straw mixture.](image-url)

After many experiments, it was discovered that the minimum chamber volume needs to be greater than 120 dm³. Thus, the dimensions of the research reactors made of Plexiglas were 50x50x73 cm, which gave a volume of more than 180 dm³. All the reactors were covered with a 10-cm layer of Styrofoam to simulate thermal isolation (in the field the top of the composting substrate pale performs the role of an isolation layer).

To analyze the temperature of the substrates, a special measurement set-up was constructed. This consisted of nine sensors situated on a plastic stick at 5-cm intervals. The stick was then placed vertically in the middle of the reactor and connected to a computer which collected the data every eight hours. Thanks to such a technique, it was possible to verify temperature changes not only in time but also on different layers of the composting mixture being analyzed.
Other important data collected from the reactors were the speed and volume of air pumped into the chambers from below. To measure the air flow, Brooks rotameter (GT 1355/D with a range of 0.43-4.3 ln/min) was used. In order to be sure of the air volume pumped into the reactor, a gas meter was connected to the system right after the gas pump. Afterwards, when gas flowed through the chamber, the air volume was examined twice a day.

Solid samples

During the experiment, the following standard methodology established by Polish Norms (PN) was used: for dry matter (TS - Total Solids) PN-75 C-04616/01, pH-PN-90 C-04540/01 and conductivity PN-EN 27888:1999.

Analysis of acrylamide monomer in digested dairy sludge and composting samples

Chemicals

A standard of pure acrylamide (≥99.8%) and acetone (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).

Sample preparation

Acrylamide monomer was determined using three types of samples: dairy sludge, straw and a trial mixture of both substrates used in the aerobic fermentation experiment. The reactant mixture was subjected to one variant stabilised by aerobic fermentation conditions: composting. 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 (10th day) and at the end of the process (31st day). Each 2 g sample was put into a plastic tube and the residual monomer was analysed using the same extraction procedure.

Extraction of acrylamide

Acrylamide was extracted from the samples using acetone: water (4:1, v/v) using 20 ml of solvent per 2 g of the sample, in accordance with Mroczek et al. (2014). After homogenisation (1 min.) (homogeniser H 500, Pol-Eco, Wodzislaw SI, Poland), the samples were transferred to 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.

Liquid chromatographic conditions

Preparation of liquid chromatographic conditions was based on previous reports described by Michalak et al. (2013) and Wang et al. (2008, 2013) with own modifications. Chromatographic separation was performed by high performance liquid chromatography (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 100A, 5 µm 150 x 4.6 mm (Agilent
Technologies, Santa Clara, USA). Empower™ 1 software was used for data processing. A sodium dihydrogen phosphate (0.1 M 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⁻¹. The mode of the HPLC instrument was isocratic with an injection volume of 10 µl. Standard stock solutions (1.0 mg ml⁻¹) 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 in Milli-Q water. The acrylamide detection limit was 1.0 ng g⁻¹. 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).

**Statistical analysis**

Tests were performed in triplicate, and the significance of the results was tested by ANOVA analyses. P < 0.05 was considered to be statistically significant.

**Results and discussion**

After anaerobic treatment, aerobic composting is the preferred method of neutralizing sewage sludge usually mixed with different structural materials in a suitable proportion to obtain a C:N ratio between 20 and 30 in the compost. The parameters influencing the composting process include: the temperature of composting (55-60°C), moisture composting masses (40-60 %), aeration intensity (90-160 m³/t ∙ h) and the duration time of composting process (< 4 weeks) (Kosobudzki et al., 2000; Jiang, 2011). Dach (2010) reported that it is very difficult to obtain a proper C:N ratio in composting sewage sludge since typical sludge contains even 4-5 % nitrogen in dry matter, and in consequence, the C:N ratio for raw sludge is often below 10. In this study, co-composting of commercially dewatered dairy processing sludge using a commercially made polyacrylamide (PAM) based flocculant was performed in a chamber bio-reactor. The sludge, which was previously described (Mroczek et al., 2016), comprised flocculated solids recovered from dairy wastewater treatment as a spade-able solid with an approximate moisture content of 75 % and fat and protein contents of about 35% and 15%, respectively. The proportions of the substrate prepared for the composting process and selected parameters are illustrated by the data in Table 1.

**Table 1. Main ingredients of mixture composted in the lab scale experiment.**

<table>
<thead>
<tr>
<th></th>
<th>wheat straw [kg]</th>
<th>dairy sludge [kg]</th>
<th>C:N ratio</th>
<th>pH [-]</th>
<th>Conductivity [mS · cm⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixture</td>
<td>7.60</td>
<td>26.00</td>
<td>11.6</td>
<td>8.0</td>
<td>0.78</td>
</tr>
</tbody>
</table>

The proportion of dairy processing sludge and wheat straw incorporated in a mixture is likely to be limited by the porosity of the final mix and the ability of the mixing equipment to mix evenly and break up clumps of solids. Porosity can also be affected by varying other bulking agents, for example when fats are incorporated (Dai et al.,
In our present study, mixture composition did not appear to inhibit the composting process, even at a lower rate of aeration.

**Temperature and oxygen concentration profiles**

All substrates prepared in the proportions mentioned above were homogenized and placed in the composting chamber, following which the air pump was started. Fig. 2 shows the air flow in the composting chamber.

![Air flow profile at various stages of composting](image)

**Figure 2.** Air flow profile at various stages of composting.

There seems to be a strong correlation between the air flow and oxygen concentration (Fig. 3).

![Oxygen concentration with composting period](image)

**Figure 3.** Oxygen concentration with composting period.

Owing to the fact that the composting process requires an oxygen concentration higher than 5%, the air flow was increased from 2.2 dm$^3$/min to 3.5 dm$^3$/min, since it had been noticed that the initial air flow of 2.2 dm$^3$/min was not sufficient to provide an oxygen concentration at the required level.

During the composting process, the temperature in the chamber was measured at nine depths from 0 to 40 cm at 5-cm intervals Fig. 4 shows the temperature determined by the sensor placed in the middle of each chamber – at 20cm).
Table 2. Temperature changes in composed sewage sludge mixture.

<table>
<thead>
<tr>
<th>K3</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hours</td>
<td>0</td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
<td>120</td>
<td>140</td>
<td>160</td>
<td>180</td>
</tr>
<tr>
<td>Depth [mm]</td>
<td>50</td>
<td>100</td>
<td>150</td>
<td>200</td>
<td>250</td>
<td>300</td>
<td>350</td>
<td>400</td>
<td>450</td>
<td>500</td>
</tr>
</tbody>
</table>

Figure 4. Temperature changes in composed sewage sludge mixture.
It is worth noting that at the 528th hour of the process (21st day), the temperature decreased rapidly and then increased by almost 30°C within two days. This situation was caused by mixing intervention – a method widely used in large-scale composting and processing by special machines. This technique serves to improve the structure of a composting pale by creating air pores, enabling oxygen to penetrate a pale easily. As a result of mixing, temperature increases rapidly. Although this temperature rise lasts much shorter than the initial one, it can sometimes reach again the thermophilic values. For better visualization of temperature distribution in the composting chamber, a Table 2 was prepared.

It presents the changes in temperature over time at every level of the composting material measured. There was a need for high-temperature substrate hygienization during the process because a temperature exceeding 60°C was maintained for more than six days (Boniecki et al., 2013).

**Determination of acrylamide monomer content**

The running of the experiment confirmed the possibility of monitoring and determining the monomer content in the sediment matrices generated by the food industry and in the substrate matrix stabilised by aerobic fermentation technology using high performance liquid chromatography (HPLC).

Analysis of acrylamide monomer content was conducted in the substrates used for the aerobic fermentation process: in the dairy sludge and in the straw (Table 3).

**Table 3. The initial content of acrylamide monomer (± SD) in main compost substrates.**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>The content of acrylamide monomer [mg kg⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy sludge</td>
<td>64,0 ± 0.5</td>
</tr>
<tr>
<td>Straw</td>
<td>nd</td>
</tr>
</tbody>
</table>

nd – none determined

The results demonstrated that the inoculum used for the experiment was free of AMD, while the dairy sludge contained 64 mg kg⁻¹ in structure. These results confirm that the only source of acrylamide monomer in the experiment is sludge obtained from the dairy.

Analysis of AMD was conducted 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 aerobic fermentation on the changes in the content of AMD in the composted mixture (Table 4).

**Table 4. Changes of acrylamide monomer content in composted sewage sludge mixture (mean values ± SD).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AMD content [mg kg⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day</td>
</tr>
<tr>
<td></td>
<td>9,0a ±0,3</td>
</tr>
</tbody>
</table>

a, b, c – mean values, designated by small letters, are statistically significantly different at p ≤ 0.05.
Subsequently, for aerobic fermentation on the first day of the experiment, the level of AMD was approximately 9 mg kg\(^{-1}\), followed by 3 mg kg\(^{-1}\) on the 10\(^{th}\) day of the process, and 2 mg kg\(^{-1}\) on the final day of the study.

During the composting process, most of the reduction in acrylamide occurred during the first stage of the process. It seems that due to a changing microbial population and its activity, the degradation process speeds up and the breakdown of substrate organic matter leads into a residue consumed as a carbon and nitrogen source. Based on the results obtained during the composting process under conditions described, approximately 77% of the initial content of acrylamide monomer disappeared within 31 days. Furthermore, preliminary studies (Mroczek et al., 2016) also indicated that the process of methane fermentation continues regardless of the effect of temperature on AMD degradation in dairy sludge. The degree of acrylamide monomer reduction for thermophilic fermentation is 100%, while for mesophilic fermentation it is 91%. Similarly, biodegradation and removal of polyacrylamide by anaerobic hydrolysis during waste-activated sludge fermentation has been reported recently by Dai et al. (2015). Shanker (1990), Nawaz et al. (1993), Yu et al. (2015), Lima (2013) reported that acrylamide decomposition under model conditions was caused by populations of \(Pseudomonas\) sp. and \(Xanthomonas\) maltophilia. It is worth pointing out that in a consortium of various microorganisms taking part in the aerobic composting of organic wastes, \(Pseudomonas\) sp. bacteria activity is also one of most important factors for efficient hydrolysis of composted substrate (Grula, 1994; Zieminski and Frac, 2012; Costa, 2014). An efficient detoxification and rapid biological degradation of toxic pollutant acrylamide was observed using also a \(Stenotrophomonas\) acidaminiphila bacterium isolated from the soil (Lakshmikandan et al., 2014). The bacterial strain isolated from paper mill effluent and identified as Gram negative, diplobacilli \(Moraxella\) osloensis MSU11 demonstrated a potential to degrade the acrylamide present in the environment (Jebasingh et al., 2013). Many studies have been carried out on the fate of acrylamide monomer following the application of polyacrylamide (PAM) to cropland (Castle et al., 1991; Castle et al., 1993; Loren et al., 1999; Friedman, 2003; Tareke, 2004). To understand the underlying mechanisms of the biological hydrolysis of PAM, some authors suggested very complicated metabolic pathways with respect to the enzymes (Bavernik et al., 1996; Dai et al., 2016).

Generally, it is clear that organic waste in the form of sludge should only be managed in an environmentally sound manner. Due to the fact that, as in the case of sludge from food processing wastewater installations, organic waste is often recommended for agricultural purposes, and especially for organic fertilization, there is still a potential risk of transferring and accumulating residual acrylamide monomer from polyacrylamide along the food chain where humans are the final users (Mroczek et al. 2014). Further studies focused on this problem are necessary.

Conclusions

The research proved the usefulness of a new kind of temperature sensor. Temperature analysis in the entire lab chamber allows the composting process to be controlled in a much more effective way. Moreover, it gives indirect information concerning the structure of the composting layer. Wherever the structure of the composting material is too packed to provide an optimal aeration temperature, the sensors will indicate that the temperature in this fragment differs from that in other parts.
of the composting layer. Another advantage of these sensors’ location is the possibility of good visualization of the temperature distribution. This provides an opportunity to monitor the influence of weather conditions in large-scale composting processes and react appropriately to these changes (by mixing the composting layer or creating a thicker layer of composting mass).

The results obtained confirm that the technology of compost production based on aerobic fermentation affects the biodegradability of acrylamide monomer contained in dairy sludge. In practice, this means that aerobic fermentation technology reduces the risk of residual acrylamide monomer migrating to plants.

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REFERENCES

Mroczek-Krzyzelewska et al.: Changes in acrylamide monomer content during composting of dairy processing sludge


