Inżynieria i Ochrona Środowiska 2017, 20(4), 447-455
Engineering and Protection of Environment
https://ios.is.pcz.pl/
p-ISSN 1505-3695
e-ISSN 2391-7253
DOI: 10.17512/ios.2017.4.2

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Toxicity Analysis of Sewage Sludge Treated with Polyelectrolytes Using Luminescent Bacteria

Analiza toksyczności osadów ściekowych kondycjonowanych polielektrolitami z wykorzystaniem bakterii luminescencyjnych

The aim of this work was to evaluate the ecotoxicity of municipal sewage sludge, after conditioning with polyelectrolytes, coming from a selected wastewater treatment plants (WWTPs). Microtox M500 and *Aliivibrio fischeri* (luminescent bacteria) were used for the assessment of toxicity. The most common presentation of inhibition result of chosen test is EC_{50} value, which is equal to concentration causing 50% reduction in light. Ecotoxicity studies were made for water extract of municipal sewage sludge conditioned with polyelectrolytes (polyacrylamide). Samples came from two selected municipal wastewater treatment plants located in the Silesian region, where technology is based on mechanical and biological treatment of sewage (referred to as A and B). At WWTP - A all sample solutions were classified as toxic. Sludge after dewatering on the press had a highest toxic effect 64.32 TU after 15 min of exposition. For sewage sludge taken from drying tunnel toxic effect 12.12 TU (after 15 min). In WWTP - B sample of sludge before fermentation chamber were toxic: 14.03 TU (after 15 min), raw sludge and sludge after the press weren't classified as toxic. This study revealed new insights into the acrylamide problem. Furthermore, it showed successful use of Microtox assay to measure acrylamide toxicity in the sewage sludge.

Keywords: **sewage sludge, polyelectrolytes, ecotoxicity, *Aliivibrio fischeri***

Introduction

The process of sewage treatment is always accompanied with the formation of sludge, which is defined as organic-mineral phase separated from wastewater.

Polyelectrolytes, which main component is polyacrylamid, are commonly used in wastewater treatment plants for sludge conditioning and in the water treatment stations. The main application of polyelectrolytes in potable water production is coagulation and flocculation process. Water production processes are usually followed by sedimentation and filtration, flotation may be an additional water treatment process. Sewage sludge obtained from different separation processes in WWTP (Wastewater Treatment Plant) has very high water content (about 97÷99%) and must be next put to the treatment stages mainly to conditioning and dewatering to minimize weight and transportation costs [1, 2]. Polyacrylamide is a polymer

formed from acrylamide monomers. While polyacrylamide itself is relatively non-toxic, its monomer (acrylamide) is a extremely toxic compound. Polyacrylamide used in wastewater treatment may contaminate effluents with acrylamide. The effect of environmental conditions on polyacrylamide was conducted, and it was shown that degradation of polyacrylamide under outdoor conditions may depolymerise to basic form of acrylamide monomer [3, 4]. The widespread use and indiscriminate discharge of acrylamide and polyacrylamide has led to their presence in terrestrial and aquatic ecosystems [5]. This phenomenon may be dangerous for human and animal health, because acrylamide was classified as a cancerogenic substance, which causes mutagenic effects [6, 7].

Acrylamide is an organic compound having conjugated double bonds and an amide fragment in its structure. It is well soluble in water and in polar solvents such as methanol. It is produced on an industrial scale by catalytic hydrolysis of acrylonitrile. Today it is used, among others, for the synthesis of modified polyacrylamides, which are used in the industry such as for the production of plastics, dyes, adhesives, cosmetics, masonry as well as coagulants for drinking water treatment, sewage treatment and sewage sludge conditioning. It is hard to assess the overall and particular health risk of acrylamide in humans, but it is clear that acrylamide is toxic for human and animals. Authors of numerous studies suggested that acrylamide toxicity may be connected with the creation of a hemoglobin adduct in animals as well as in humans [5]. This adduct is considered a surrogate of toxicity since if acrylamide can react with hemoglobin particles it should also be able to react with other large molecules such as important proteins and DNA. Glycidamide, which is the acrylamide metabolite, may also react with hemoglobin. Because its genotoxic, neurotoxic and potentially cancerogenic effect, acrylamide has been classified by the International Agency for Research on Cancer (IARC) as a carcinogen and is listed in the Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on the classification, labeling and packaging of substances and mixtures, as a substance having H-350 carcinogenic category 1A, 1B, H-340 mutagenic effects, H-361 suspected to be harmful to fertility.

Living organisms at different levels of the organization, allow to assess the toxicity and biological activity of the investigated samples. Using bioindication analysis the acute and chronic toxicity may be assessed, which allows to know the total toxicity of all the harmful substances contained in wastewater and sewage sludge. To determination of toxicity intensity Microtox Acute Testing System was chosen.

Microtox is an in vitro testing system which uses bioluminescent bacteria to detect toxic substances in different substrates such as water, air, soils and sediments. This system combines the advantages of biological testing with the speed and ease of use of laboratory instruments. The instantaneous system capability combined with the reliability of data over time makes it an ideal system for environmental testing, industrial process monitoring and other similar applications. The Microtox Acute Testing System consists of the Microtox Model 500 Analyzer,

a PC, data acquisition and processing software, Microtox reagents, test solutions and accessories. The Microtox Model 500 is a temperature-controlled photometer that allows the temperature of reagents, luminescent bacteria and samples to be lowered to the required temperature and to measure the light emitted by the bacteria under constant control of the measurement process [8, 9]. The Microtox test is based on the use of *Aliivibrio fischeri* luminescent bacteria (formerly known as *Vibrio fischeri* and *Photobacterium fischeri*), whose glow is a natural result of their metabolic processes [10]. These gram-negative bacteria are found in water habitats with significant salinity and is found predominantly in symbiosis with various marine animals, such as the Hawaiian bobtail squid [11, 12]. Representatives of the *Aliivibrio* genus have the shape of rods, with a diameter of 0.5÷0.8 μm and a length of 1.4÷2.6 μm. Bioluminescence of *Aliivibrio fischeri* is caused by chemical reactions accompanied by glow. These are usually the reactions of luciferin oxidation [12, 13]. Any change in these processes caused by contact with the sample causes changes in the light emission of those organisms. These changes are directly proportional to the relative toxicity of the sample and can be used to calculate a percent of glowing inhibition. The most common presentation of inhibition result of chosen test is EC_{50} value, which is equal to concentration causing 50% reduction in light [14].

The aim of this study was to evaluate the ecotoxicity of municipal sewage sludge conditioned with polyelectrolytes (cationic polyacrylamide), taken from a selected WWTPs.

1. Materials and methods

1.1. Tested samples

Ecotoxicity studies were made for water extract of municipal sewage sludge conditioned with polyelectrolytes (polyacrylamide). Samples came from two selected municipal wastewater treatment plants located in the Silesian region (referred to as A and B).

In wastewater treatment plant A, sludge from the primary settling tank is recirculated into the denitrification chamber. Excess sludge from the biological treatment process is next put to aerobic stabilization, additionally after dewatering on the belt press sludge is liming. In the spring and summer season, the sewage sludge is dried in a solar dryer. The characteristics and structure of sludge from sewage treatment plant A makes the hydration level equal 80% after mechanical dehydration.

In the B sewage treatment plant two types of sewage sludge are produced: raw sludge retained in the preliminary settling tanks and recirculated excess sludge. Excessive sludge is mechanically and gravity thickened, and raw sludge is gravitationally thickened. Sludge stabilization takes place by mesophilic methane fermentation in a separate closed fermentation chamber, after which, sludge are directed to open fermentation chambers. The sludge dewatering is carried out on a belt press.

In the fermentation process biogas is released. This biogas is captured and used primarily for combustion in co-generators in which electricity and waste heat are generated. In both WWTPs polyelectrolytes were used to sludge conditioning, name and characteristic features of these substances are presented in Table 1.

Table 1. **Characteristic of polyelectrolytes using in WWTP (A and B)**

Polyelectrolyte characteristic	WWTP - A [15]	WWTP - B [16]
Name of product	FLOPAM FO 4800	ACEFLOCK 80502
Ingredient	cationic polyacrylamide	cationic polyacrylamide
Form	powder	granulate powder
Color	white	whitish
pH	2.5÷4.5 (5 g/L)	2.5÷4.5 (5 g/L)
Solubility	10 g/L	10 g/L

1.2. Standards and chosen methods

The pH was measured in accordance with the PN-EN 12176 standard - Sewage sludge characteristics of the Elmetron company. The ecotoxicity analysis was carried out in accordance with the PN-EN ISO 11348-3: 2008 standard.

Toxicity was assessed by analyzing the liquid phase obtained after aqueous extraction (carried out with deionized water) of the solid samples tested. As part of the work, the conditions for conducting the water extraction were selected, i.e. the time and method of sample preparation. The entire test was carried out in accordance with the SPT protocol (Solid Phase Test). Individual stages of sample preparation were carried out using a 2% NaCl solution. In order to control the quality of the conducted measurements, a series of repetitions was performed and tests were carried out on control samples containing no impurities.

1.3. Bacteria preparations

Dried frozen bacteria were used as test indicators. After hydration these bacteria were immediately ready for use as measuring biosensors, acting as a short-term acute test. Bacteria were supplied in chilled vials, which should be revived by adding to the reconstitution solution. Each vial contains about 100 million lyophilized organisms. Each lot is suitable for at least two hours and up to three hours. After that time, the response may begin to varies. Because the bacterial reagent is sensitive to pH it is very important to check pH value of samples before test. The pH value should be within the range 6.0 and 8.0. When the pH is higher than 8.0 or lower than 6.0, and the sample has buffering capacity the effect can be very uncertain. The most appropriate pH value is roughly 7.0. The pH value was tested for each sample and was in the range 6.5÷7.5. Control sample was placed in testing cuvette and mixed with osmotic solution. Tested samples were mixed with diluent, next background signal of bacteria was measured using Microtox model 500 (Fig. 1), after that toxic sample solution was transferred to the cuvette with revived bacteria and test was started.

Fig. 1. Microtox model 500

1.4. Microtox reagents and protocol

Microtox Acute Toxicity Test package includes reagents and solutions needed to carry out standardized, repeatable tests. Microtox kit contains the following components:

- Bacteria: a freeze-dried culture of *Aliivibrio fischeri* that is reconstituted prior to testing. It is highly recommended that the revived bacteria solution should be used within three hours of reconstitution.
- Osmotic Solution: solution that is made up of 22% Sodium Chloride (NaCl) and Ultra-Pure Water.
- Reconstitution Solution: consists of Ultra-Pure Water.
- Diluent is a nontoxic solution that is made up of 2% NaCl in Ultra-Pure Water.

The Microtox test gives the opportunity to carry out screening tests as well as basic tests. A Screening Test is used to find a toxic effect range for which the EC_{50} can be determined. The Basic Test is used to assess the degree of toxicity selected on the basis of a sample screening test. For the toxicity of the samples, a series of dilutions is made and their amount depends on the estimated toxicity of the sample. The toxic effect value and EC_{50} are determined for each dilution.

2. Results

Earlier studies on wastewater, sewage sludge and effluent test using *Aliivibrio fischeri* showed that bacteria could be used as a sensitive indicator for effluent toxicity study [9, 11, 12, 14, 17, 18]. Chosen *Aliivibrio fischeri* was used to test the toxicity of sewage sludge treated with polyacrylamide. Samples were taken from: drying tunnel (WWTP - A), raw sludge (B), sludge before fermentation chamber (B), sludge from the press (A & B), filtrates from the press. Toxic effect was measured after 5 and 15 minute of exposition. The results showed the following. At A WWTP the highest *Aliivibrio fischeri* toxicity effect in sludge from the press was reported as 64.32 TU (after 15 min). For sewage sludge taken from drying tunnel toxic effect was 14.27 and 12.12 for 5 and 15 min respectively. Results of toxicity test of chosen sample from A WWTP, given in TU and EC_{50} (half maximal effective concentration), are presented in Table 2.

Table 2. **Results of toxicity tests of sludge samples from municipal WWTP - A**

Place of sampling	Sewage sludge (water extract)			
	EC_{50}, %	TU	Toxicity class	
WWTP - A			According to Sawicki [19]	According to Persoone [20]
Drying tunnel 5 min	7.01	14.27	1. Class Significant toxic effect, low-toxic sample	3. Class High acute toxicity
Drying tunnel 15 min	8.25	12.12	1. Class Significant toxic effect, low-toxic sample	3. Class High acute toxicity
Sludge after the press 5 min	3.64	27.44	2. Class Significant toxic effect, toxic sample	3. Class High acute toxicity
Sludge after the press 15 min	1.555	64.32	2. Class Significant toxic effect, toxic sample	3. Class High acute toxicity

At WWTP - B the *Aliivibrio fischeri* toxicity effect in the water extract of sludge after the press was reported as 1.34 and 1.23 TU (after 5 and 15 min). For raw sewage sludge toxic effect was equal 0,36 and 0,43 for 5 and 15 min respectively. The highest toxicity values were reported for sludge before fermentation chamber and were equal: 15.29 and 14.03 TU (after 5 and 15 min). Results of toxicity test of chosen sample from WWTP - B, given in TU and EC_{50} (half maximal effective concentration) are presented in Table 3.

Table 3. **Results of toxicity tests of sludge samples from municipal WWTP - B**

Place of sampling	Sewage sludge (water extract)			
	EC_{50} %	TU	Toxicity class	
WWTP - B			According to Sawicki [19]	According to Persoone [20]
Sludge after fermentation chamber 5 min	275.00	0.36	0. Class No significant toxic effect	1. Class No significant toxicity
Sludge after fermentation chamber 15 min	231.10	0.43	0. Class No significant toxic effect	1. Class No significant toxicity
Sludge before fermentation chamber 5 min	6.54	15.29	1. Class significant toxic effect, low-toxic sample	3. Class High acute toxicity
Sludge before fermentation chamber 15 min	7.13	14.03	1. Class significant toxic effect, low-toxic sample	3. Class High acute toxicity
Sludge after the press 5 min	74.53	1.34	0. Class No significant toxic effect	2. Class Significant toxicity
Sludge after the press 15 min	80.84	1.23	0. Class No significant toxic effect	2. Class Significant toxicity

Conclusions

According to the aim of investigation it was observed that tested sewage sludge samples may to be overall classified as toxic under laboratory conditions. The sludge from the press have not shown acute toxicity in B WWTP, when in A plant its toxicity was significant 64.32 TU (after 15 min). The lowest toxicity of al samples was observed in B WWTP, it was noted for raw sewage sludge and toxic effect was equal 0.36 and 0.43 for 5 and 15 min respectively.

The conducted research allowed to formulate the following conclusions:

1. At WWTP-A all sample solutions were classified as toxic (according to Sawicki and Persoone). Sludge from the press had a higher toxic effect 27.44 TU after 5 min and 64.32 TU after 15 min. For sewage sludge taken from drying tunnel toxic effect was 14.27 and 12.12 for 5 and 15 min respectively.
2. Investigated samples from WWTP - B showed no toxicity effect for raw sludge and sludge from the press, so it weren't classified as toxic. Only samples showing toxic effect were sludge before fermentation chamber, and it were equal: 15.29 and 14.03 TU (after 5 and 15 min).
3. Performing acute toxicity testing of raw sewage becomes very important in the aspect of operation of sewage treatment plants and minimization of environmental risk, as well as for the control of sewage flowing into the treatment plant in case of toxic sewage discharge to the sewage network.

This study revealed new insights into the acrylamide problem. Furthermore, it showed for the first time the successful use of Microtox test to measure acrylamide toxicity in the sewage sludge, including raw sludge and sludge from the press. Newly developed assay might be suitable for analyzing acrylamide and other micropollutants toxic effect in numerous water and sewage samples.

Acknowledgements

The financial support by Czestochowa University of Technology, Institute of Environmental Engineering (BS/MN-401-306/14 and BS/PB-401-303/12) is gratefully acknowledged.

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Streszczenie

Celem niniejszej pracy była ocena ekotoksyczności komunalnych osadów ściekowych, kondycjonowanych polielektrolitami, pochodzących z wybranych oczyszczalni ścieków. Do oceny toksyczności zastosowano analizator Microtox M500 i bakterie *Aliivibrio fischeri* (bakterie luminescencyjne). W celu prezentacji stopnia inhibicji wybranego testu stosuje się wartość EC_{50}, która jest równa stężeniu powodującemu 50% zmniejszenie światła. Badania ekotoksyczności zostały wykonane z wykorzystaniem ekstraktów wodnych z próbek osadów ściekowych kondycjonowanych polielektrolitami. Badane próbki pochodziły z dwóch wybranych komunalnych oczyszczalni ścieków zlokalizowanych na terenie województwa śląskiego, których technologia oparta jest na mechanicznym i biologicznym oczyszczaniu ścieków (określanych dalej jako oczyszczalnie A i B). W oczyszczalni ścieków A wszystkie

badane próbki zostały sklasyfikowane jako toksyczne. Osady po odwodnieniu na prasie miały największy efekt toksyczny 64,32 TU po 15 minutach ekspozycji. Dla osadów ściekowych pobranych z tunelu suszarniczego efekt toksyczny wynosił 12,12 TU (po 15 min). W oczyszczalni ścieków B próbki osadów pobranych przed komorą fermentacyjną wykazywały toksyczność 14,03 TU (po 15 min), surowe osady i osady po prasie nie zostały sklasyfikowane jako toksyczne. Badania te ujawniły nowy aspekt problemu związanego z zanieczyszczeniem środowiska osadami deponowanymi na składowiskach odpadów. Ponadto wykazano, iż z powodzeniem można wykorzystać test Microtox do pomiaru toksyczności osadów ściekowych.

Słowa kluczowe: osady ściekowe, polielektrolity, ekotoksyczność, *Aliivibrio fischeri*