ECOTOXICITY ASSESSMENT OF STABILIZED SEWAGE SLUDGE FROM MUNICIPAL SEWAGE TREATMENT PLANT

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Abstract

Aim of this study was to evaluate the ecotoxicity of municipal sewage sludge conditioned with polyelectrolytes, taken from selected sewage treatment plant. Using the bioindication analysis overall toxicity was assessed, which allows to know the total toxicity of all the harmful substances contained in sewage sludge, in many cases acting synergistically. To prepare a sample of sludge for the basic test, all analyses were performed with a ratio of liquid to solid of 10:l (water extract), *Daphnia pulex* biological screening test was used. A dilution series of an water extract of sludge were prepared to include within its scope the lowest concentration that causes 100% effect and the highest producing less than 10% of the effect within a specified range of the assay. The results of the test were read after 24 and 48 hours. Based on the research and analysis of test results it proved that the sewage sludge conditioned with polyelectrolytes exhibit the characteristics of eco-toxic.

Keywords: sewage sludge, conditioning, ecotoxicity, water extract, *Daphnia pulex*, bioindication analysis

1. INTRODUCTION

There are numerous processes that can be used to clean up wastewater depending on the type and extent of contamination. Wastewater can be treated in

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wastewater treatment plants which include physical, chemical and biological treatment processes.

Municipal effluent is treated in numerous ways depending on its composition, mainly to remove bioactive materials from the water. Environmental issues have brought about increasing pressure to remove all such materials including suspended solids coming from sewage treatment process.

Polyelectrolytes 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 in coagulation and flocculation. Water production processes are usually followed by sedimentation and filtration, flotation may be an additional water treatment process. Polyelectrolytes have been used in water treatment processes for at least fifty years [9]. Sewage sludge obtained from the different separation processes has very high water contents (97-99%) and must be further concentrated to minimize weight and transportation costs [1,8,14]. Polyacrylamide is a polymer ($-CH_2CHCONH_2-$) formed from acrylamide monomers. While polyacrylamide itself is relatively non-toxic, its monomer (acrylamide) is a highly toxic compound.

Concerns have been raised that polyacrylamide used in wastewater treatment may contaminate effluents with acrylamide, a known neurotoxin. In a study conducted in 1997 the effect of environmental conditions on polyacrylamide was tested, and it was shown that degradation of polyacrylamide under outdoor conditions may de-polymerise to form acrylamide [12,15]. Acrylamide was classified as a cancerogenic substance which causes mutagenic effects [6].

2. METHODOLOGY

The aim of this study was to evaluate the ecotoxicity of municipal sewage sludge conditioned with polyelectrolytes (cationic polyacrylamide), taken from a selected wastewater treatment plant. The further goal was checking whether acrylamide penetrates into the effluent and natural environment. To evaluate the ecotoxicity of sludge, a method based on organisms *Daphnia pulex* was used [13].

The bioindication analysis was conducted on the environmental samples: sewage sludge (water extracts from sewage sludge) and filtrate from the press. Bioindication analysis was performed in two stages. In the first stage screening assay was performed. Toxicity of raw samples was assessed. If the test result was not greater than the effect of the control sample, the sample was considered to be non-toxic. However, if the effect was significant - then a second stage of analysis was conducted - a test of dilutions. It allows assess the level of toxicity of the sample, so that samples can be compared with each other as well as to

analyze e.g. change in toxicity in time. The living organisms vary in their sensitivity to particular groups of toxic substances, and even within populations of the same species there can be great variations in sensitivity. Dozens of different bio-indicators were used but only some of them gained international acceptance.

In the studies was used DAPHTOXKIT F pulex bioassay. This is a test using *Daphnia pulex* shellfish. DAPHTOXKIT F procedure was performed according to the ISO 6341 standard procedure [5,7].

2.1. Characteristic of chosen wastewater treatment plant

This study was carried out on the sewage sludge derived from wastewater treatment plant, with an average daily capacity from 16,000 to 18,000 m^3/day. The wastewater was supplied with sewer system and transported by waste removal cars. The amount of wastewater transferred by cars is approximately 1% of all wastewater [16]. At the treatment plant is also treated industrial wastewater, it estimated 5.6% of wastewater influent. Chosen plant is a mechanical-biological treatment plant with increased biogenes removal. The mechanical part includes a stepped grating where solids are blocked, sand separator where the mineral fractions are stopped and the initial sedimentation tanks, where insoluble organic fraction are removed (suspension). The biological part is represented by chambers of activated sludge and biological phosphorus removal [2]. Biological treatment is based on the technology of sludge treatment in the chambers of nitrification and denitrification and biological phosphorus removal in anaerobic chambers. Biological phosphorus removal is aided by the addition of chemical precipitation of iron sulphate (PIX) [10]. Sludge dewatering process is aided with polyelectrolytes.

2.2. Polyelectrolyte

In the selected sewage treatment plant FLOPAM FO 4800 polyelectrolyte was used. Flopam consist of cationic polyacrylamide flocculant. Polyacrylamide is a water-soluble polymer with the ability to enhance flocculation of suspension solids. It is grouped in a class of compounds formed by the polymerization of acrylamide [4]. Pure polyacrylamide is a homopolymer of identical acrylamide units. Polyacrylamide can be formulated with copolymers to give specific charges and the molecular weight which give polyacrylamide its various characteristics. Because sludge is negatively charged, the Flopam with positive charge is widely used in dewatering process. Cationic polyacrylamide can have the effect of charge neutralization and absorption bridge in sludge dewatering. Polyacrylamide is toxic to aquatic wildlife, its LC_{50} for *Danio rerio* (96h) equals 10-100 mg/L, EC_{50} for *Daphnia magna* (48h) $\approx$ 50 mg/L [3]. Polyacrylamide

does not show bioaccumulation in aquatic environment, it is caused by presence of dissolved organic carbon and hydrolysis process.

2.3. Sample preparation

Before placing the organisms in the solution, standard medium was aerated, aeration time was about 15 minutes. Aeration was carried out using an air pump to the aquarium. Hatching of the test organisms began three days before the start of the toxicity test, and process consist of steps:

- the contents of one vial with ephippia was poured on the micro sieve,
- ephippias were gently rinsed with tap water to eliminate any traces of storage medium,
- ephippias were transferred on the Perti dish with 50 ml of aerated standard medium,
- Petri dish was covered and organisms were incubated for 72 hours at 20-22°C in a continuous light of 6000 lux [5].

2.4. Test solutions preparation

Sewage sludge (conditioned with FLOPAM FO 4800 polyelectrolyte) was filtered, next the filter cakes was dried, after that, dry mass of sewage sludge was calculated. Water extract from sewage sludge was done in the ratio 1:10 (to homogenization). The test range was established, if the approximate toxicity (in order of magnitude) of pollutants is known, this step can be omitted and it can be proceed the final test. Test wells were filled with medium and the test organisms were transferred into, and then incubation occurred.

The test results were read after 24 hours and 48 hours of incubation, the number of killed or immobilized organisms was recorded, in relation to other vessels active in each well. The total number of killed and immobilized organisms for each concentration of toxins was calculated.

The quality control test was performed, according to which the number of killed and immobilized organisms should not exceed 10% of the control wells.

2.5. Assessment of EC_{50} value

The term half maximal effective concentration (EC_{50}) refers to the concentration of a toxicant which induces a response halfway between the baseline and maximum after a specified exposure time. EC_{50} is commonly used as a measure of acute toxicity of environment pollutant. For better clarity in the following discussion we use the abbreviation EC_{50}. In the case of environmental samples, the ratio of the EC_{50} is quite inconvenient, since the higher the EC_{50}, the lower toxicity, which might be confusing. Thus, the EC_{50} value was converted per toxicity unit TU, which do not have mentioned drawbacks.

Toxicity unit could be calculated according to the formula [11]:

$$TU = (1/EC_{50}) \times 100 \qquad (1)$$

Toxicity intensity is divided for 5 classes:
I. No acute toxicity test - no toxic effect observed,
II. The low acute toxicity - at least one test demonstrated the effect of the test in the range of $0,4 < TU \leq 1$,
III. Acute toxicity - at least one test demonstrated the effect of the test in the range $1 < TU \leq 10$,
IV. A high acute toxicity - at least one test showed the effect of the test in the range $10 < TU \leq 100$,
V. Very high acute toxicity - at least one test showed the effect of the test in the field: $TU > 100$.

2.6. Reading of the results

Reading of the results shall be in accordance with the procedure: after 24h and 48h incubation [5]. Then the number of organisms killed or immobilized with respect the other active floating in each well should be noted and recorded in the Scorecard. The total number of organisms killed and immobilized for each concentration of toxins was calculated and the mean value of the % effect was calculated also.

2.7. Control test

To validate the performance of the tested organisms sensitivity procedure, the test with the reference solution of toxic potassium dichromate $K_2Cr_2O_7$ is recommended to perform. Test is invalid if control mortality $>10\%$ or if $> 10\%$ of controls show overstressed behavior (e.g., immobility). The chosen control group was considered to be healthy and suitable to test (mortality in the validation assay was less than 10%) [5].

3. RESULTS

So far, it has not been constructed the apparatus, by means of which it would be possible to conduct toxicity testing of chemical compounds. Only living organisms at different levels of the organization, allow to assess the biological activity of the tested material. By bioindication analysis the overall toxicity is assessed, which allows to know the total toxicity of all the harmful substances contained in sewage sludge, in many cases acting synergistically.

Earlier studies on effluent test using different *Daphnia* species showed that macro-invertebrates could be used as a sensitive indicator for effluent toxicity study [13].

Chosen *Daphnia pulex* was used to test the toxicity of sewage sludge treated with polyacrylamide. Samples of filtrates from the press, water extract (from sewage sludge) and effluents were tested. The results showed the following. The average *Daphnia pulex* toxicity in filtrates from the press, water extract and effluents was reported as 3.85-4.35, 3.2-3.6 (toxic unit) and <0.4 (TU) respectively.

Number of immobilized *Daphnia pulex* organisms, toxicity effect in percents and EC_{50} (half maximal effective concentration) values of all samples determined in this study and the corresponding TU (toxicity unit) values are presented in Table 1.

Table 1. Results of toxicity tests on *Daphnia pulex*

Type of sample	Sample conc. (%)	Number of immobilized *Daphnia pulex*		Toxicity effect (%)		EC_{50}		TU $1/EC_{50}*100$	
		24h	48h	24h	48h	24h	48h	24h	48h
Filtrates from the press	100	20/20	20/20	100	100	26	23	3.85	4.35
	50	19/20	20/20	80	100				
	25	10/20	11/20	50	55				
	12.5	4/20	5/20	20	25				
	6.25	1/20	1/20	5	5				
Sewage sludges (water extracts)	100	20/20	20/20	100	100	31	28	3.2	3.6
	50	19/20	20/20	95	100				
	25	9/20	12/20	45	60				
	12.5	7/20	9/20	35	45				
	6.25	3/20	7/20	15	35				
Effluent	100	4/20	3/20	20	15	-	-	<0.4	<0.4
	50	3/20	3/20	15	15				
	25	2/20	2/20	10	10				
	12.5	1/20	1/20	5	5				
	6.25	0/20	2/20	0	10				

The polyelectrolytes toxicity could be masked by a toxicity caused by ammonia, metals or presence of other toxic impurities. Finally, the authors believe that

more discussion should be undertaken regarding depolymerization of polyelectrolytes during water purification processes, and the rate of acrylamide penetration into effluents.

The validation studies indicated that the acute toxicity test can be considered as high sensitivity analytical tools to detect common environmental concentrations of the pollutants.

Acquired knowledge can be a valuable source of information for researchers working on the introduction of new, effective and less harmful agents used to sludge conditioning to improve the effectiveness of their drainage, as well as for exploiters sewage being prepared in connection with the legal regulations concerning the disposal of sewage sludge.

4. CONCLUSIONS

According to the aim of investigation it was observed that two tested sewage sludge samples have to be classified as toxic under laboratory conditions: filtrates and water extracts. The effluents have not shown acute toxicity. The conducted research allowed us to formulate the following conclusions:

1. Solutions of filtrates and water extracts were classified as toxic. Filtrates from the press had a higher toxicity ($3.85TU_{24h}$ and $4.35TU_{48h}$) than water extracts ($3.2TU_{24h}$ and $3.6TU_{48h}$).
2. Investigated effluent samples showed very low toxicity effect (comparable with fresh water), so it weren't classified as toxic ($<0.4TU$).
3. Acute toxicity test using *Daphnia pulex* can be considered as suitable analytical tool for micro pollutants detection and investigation.

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OCENA EKOTOKSYCZNOŚCI USTABILIZOWANYCH OSADÓW ŚCIEKOWYCH POCHODZĄCYCH Z KOMUNALNEJ OCZYSZCZALNI ŚCIEKÓW

S t r e s z c z e n i e

Celem pracy była ocena ekotoksyczności komunalnych osadów ściekowych kondycjonowanych polielektrolitami, pochodzących z wybranej oczyszczalni ścieków. Za pomocą analizy bioindykacyjnej ocenia się ogólną toksyczność, co pozwala na poznanie sumarycznej toksyczności wszystkich szkodliwych substancji zawartych w osadach ściekowych, w wielu przypadkach działających synergistycznie. W celu przygotowania próbki osadów do analizy wykonano test podstawowy przy stosunku cieczy do fazy stałej równej 10:l (wyciąg wodny), użyto do tego przesiewowego biologicznego testu oceny toksyczności na rozwielitkach *Daphnia pulex*. Serię rozcieńczeń z wyciągu wodnego z osadów przygotowano tak, aby obejmowała swoim zakresem najniższą koncentrację wywołującą 100% efektu i najwyższą wywołującą mniej niż 10% efektu w określonym zakresie testu. Wyniki badań testu odczytano po 24 i 48 godzinach. Na podstawie przeprowadzonych badań oraz ocenę wyników badań wykazano, iż osady ściekowe kondycjonowane polielektrolitami wykazują właściwości ekotoksyczne.

Słowa kluczowe: osady ściekowe, kondycjonowanie, ekotoksyczność, wyciągi wodne, *Daphnia pulex*, analiza bioindykacyjna