

Anna Holda, Ewa Kisielowska*, Tomasz Niedoba**

PARTICIPATION OF MICROORGANISMS IN EFFLUENT TRANSFORMATION

1. Introduction

The significant problem of current environmental engineering is effluent treatment and disposal of secondary products created in this process, which are the effluents [1, 2].

The amount of the effluents created during treatment is about 1–3% of the flowing effluents volume, being the marginal percent of wastes created in municipal management. However, the investments and exploitive costs for their treatment may be even 50% of total treatment costs [3–5].

The amount of effluent treatment plants, both in Poland as in the whole world, is growing making the problem of effluents management very crucial. As it is known, the majority of effluents is located on dumps or lagoons, because the municipal and industrial effluents originating from the high industrial areas often contain heavy metals. This makes impossible to use them in agriculture and in degraded areas reclamation. The effluents from municipal effluent treatment plants features by high rotting, low ability to give water back by its high contents and frequent presence of causal bacteria and parasites, what often makes application of their soil-creating and fertilizing features very hard [6–14].

Because of that, it is necessary to carry research over possibility of lowering the biological and environmental harmfulness of created effluents, what will make their management efficient [5, 15, 16].

The purpose of the investigation presented in the paper is chemical and microbiological analysis of effluents located in the area of effluent treatment plant and research over the influence of microorganisms on effluents properties.

* Faculty of Mining and Geoengineering, University of Science and Technology AGH, Krakow

2. Methodology of research

2.1. Sampling of effluents

The representative samples of effluents were collected according to the norm PN-EN ISO 5667-13 and PN-ISO 5667-15 to glass containers in specified locations of effluent treatment plant: directly from the press (daily sample) and from the field located by the plant, where the effluents are transferred (3 weeks sample). The analysis was conducted after 2 hours from collecting in original state, without additional drying.

2.2. Determination of dry remnants and water contents

The dry rest and water content was determined on the basis of the norm EN 12880.

2.3. Determination of pH

The pH was determined according to the norm PN-EN 12176.

2.4. Determination of the metals in effluents samples

The contents of the following metals were determined by the ASA method (absorptive atom spectrometry): magnesium, calcium, lead, cadmium, chromium, copper, nickel, mercury and zinc.

2.5. Determination of other elements

The investigation of general nitrogen was determined by Kjeldahl method according to the norm PN-EN 133342. The ammonium nitrogen in effluent sample was determined by application of distillation method till 4 hours since the sample was collected. To this purpose, the ammonia was distilled from the water sample in weak alkaline environment and then was determined by colorimeter with Nessler agent on spectrophotometer.

The phosphor was determined by colorimeter method on the basis of norm PN-C-04537-14.

2.6. Microbiological qualitative and quantitative analysis of samples

The analyzes were performed by the Koch dilution plate method. The samples of 10 g of soil were prepared, which were then transferred to the Erlenmayer bulb with 90 ml of physiological liquid and then the samples were shaken in time of 15 minutes to make microorganisms transferred to the solution. Such prepared solutions were diluted in ratio 1:10, from which next dilutions were performed till 1:1 000 000.

To determine the amount of mezo- and psychophil bacteria amounts, the inoculation was done by the method of cast-iron plates. To the sterilized Petri plates, the samples of 1cm³ of suspension from prepared dilutions and covered with the nutritive agar MPA. The incubation was carried out in normal temperature during 72 h and in temperature of 37°C during 24 h.

The research over the titre of nitrificative bacteria was conducted by transferring of 1cm³ of inocultaion from individual dilutions on the bed according to Winogradzki and incubation during 7 days in temperature 28°C. After certain time the readings were carried out by means of factor agents.

In purpose of determination the denitrificative bacteria titre, the proceeding was analogical as for nitrificative bacteria. The pink (carmin) colour proved the presence of nitrites(III) and the lack of colour proved the presence of nitrites(V).

To determine the microscopic fungi, the inoculation was done by the grated plates method. To this purpose, the samples of 1 cm³ of the suspension prepared from dilutions on solid Czapek-Dox bed were transferred to sterilized Petri plates. Next, by application of glass stroker, the suspension was properly distributed and incubated during 72 h in temperature 28°C.

To the purpose of diagnostics, the samples coloured by simple method were prepared, taking fuxine as the pigment and coloured complexly by Gram method. The prepared samples were observed by „immersion” in magnification of 1000 times.

In case of fungi, the samples prepared from the fragments of the mushroom spawn drown in Lugol liquid and covered by glass, were observed in magnification of 400 times.

3. Results

3.1. Determination of dry rest and water contents

The water contents and dry mass were calculated from the difference between the samples masses before and after drying, determining it in g/kg.

The water contents (w_w) was calculated according to the formulae (1)

$$w_w = \frac{(m_b - m_c)}{(m_b - m_a)} \cdot f \quad (1)$$

The dry rest (w_{dr}) was calculated from the formulae (2)

$$w_{dr} = \frac{(m_c - m_a)}{(m_b - m_a)} \cdot f \quad (2)$$

where:

w_w — water contents in sample of effluent, in percentages or g/kg;

w_{dr} — dry rest of effluent sample value, in percentages or g/kg;

m_a — mass of dry evaporator or crucible, in grams;

m_b — mass of evaporator or crucible with the sample of effluent, in grams;

m_c — mass of evaporator or crucible with dry mass of effluent, in grams;

f — calculation coefficient, $f= 100$ by determining the results in percentages or $f= 1000$ by determining the results in g/kg.

The given results were presented in Table 1.

TABLE 1
Dry mass contents in sample

Sample	Dry mass surface, [% of dry mass]
1 – daily effluent	15,1
2–3 weeks effluent	17,9

3.2. Determination of pH

Table 2 contains the results of measurements.

TABLE 2
pH readings

Sample	pH
1 – daily effluent	13,03
2–3 weeks effluent	11,60

3.3. Determination of metals in effluents samples

The results of investigating the contents of individual metals in sample were presented in Table 3.

3.4. Determination of other elements

The contents of general phosphor in effluent was calculated from the following formulae:

$$X_1 = X \cdot 0,0326 \quad (3)$$

where:

X — contents of general phosphor in effluents determined as PO_4 , [g/kg];
0,0326 — coefficient re-calculated from PO_4 to P and from the unit g/kg, [m/m].

The results of conducted researches were presented in Table 4.

TABLE 3
Contents: magnesium, calcium, lead, cadmium, chromium, copper, nickel, mercury and zinc in effluents sample

Sample	Contents in % of dry mass		Contents in mg/kg of dry mass							
	Mg-general	Ca-general	Lead (Pb)	Cadmium (Cd)	Chromium (Cr)	Copper (Cu)	Nickel (Ni)	Mercury (Hg)	Zinc (Zn)	
1-daily effluent	0,98	25,42	36,20	1,56	105,64	167,30	20,56	0,25670	1154,6	
2-3 weeks effluent	0,76	4,87	32,90	1,09	37,32	142,7	14,28	0,11275	597,3	

TABLE 4

Results of contents in general and ammonium nitrogen, organic substance and phosphor in samples

Sample	Contents in % of dry mass			
	N-general	N-NH ₄	Organic substance	P-general
1 – daily effluent	4,16	0,87	61,12	1,12
2 – 3 weeks effluent	4,72	0,25	58,63	1,95

3.5. Microbiological qualitative and quantitative analysis

Results of microbiological qualitative and quantitative analysis were presented in Tables 5–8.

TABLE 5

Number of bacteria and fungi in individual samples and dilutions

Sample	Mesophil bacteria amount	Psychrophil bacteria amount	Fungi amount	Leaven amount
1	1 161 670	2 214 330	460	35 000
2	2 980 000	13 969 330	1 150	0

TABLE 6

Results of nitrificative bacteria titre determination

Sample	Phase	Dilution				Titre of bacteria
		1:100	1:1000	1:10000	1:100000	
1	I	+	+	+	+	0,00001
	II	+	+	+	+	0,00001
2	I	+	+	+	+	0,00001
	II	+	+	+	+	0,00001

TABLE 7

Results of denitrificative bacteria titre determination

Sample	Presence	Dilution				Titre of bacteria
		1:100	1:1000	1:10000	1:100000	
1	nitrates(III)	+	+	+	+	0,00001
	nitrates(V)	+	–	–	–	0,01
2	nitrates(III)	+	+	+	+	0,00001
	nitrates(V)	–	–	–	–	0

TABLE 8

Results of qualitative microbiological analysis

Sample	Mesophil bacteria	Psychrophil bacteria	Fungi
1	<i>Bacterium sp.</i>	<i>Staphylococcus sp.</i> <i>Bacterium sp.</i>	<i>Aspergillus sp.</i> <i>Trichoderma sp.</i> <i>Penicillium sp.</i> <i>Geotrichum sp.</i>
2	<i>Bacterium sp.</i>	<i>Staphylococcus sp.</i> <i>Bacterium sp.</i>	<i>Aspergillus sp.</i> <i>Trichoderma sp.</i> <i>Geotrichum sp.</i> <i>Cladosporium sp.</i> <i>Penicillium sp.</i>

4. Conclusions

The microbiological analysis indicates that the investigated effluents contain relatively reach microflora. The microorganisms — both fungi and bacteria — are numerous. Their presence is connected with the proper conditions as humidity, access to oxygen and high amount of nutritive components.

On the basis of breeding observations, the following conclusions were made:

- amount of psychro- and mesophil bacteria grows as a result of storage what is connected with very good conditions for their development;
- in process of nitrification — positive result on the nitrates(III) and nitrates(V) presence, products of I and II phase of nitrification proves the positive course of this process and

is connected with lowering of the ammonium nitrogen concentration (substrate of the nitrification process) observed after 3 weeks of observation;

- in process of denitrification — negative result on the nitrates(V) presence and high concentration of nitrates(III) proves the high amount of denitrificative bacteria what is confirmed by the chemical investigation — the amount of free nitrogen (final product of denitrification process) grows after 3 weeks of storage.

The conducted chemical analysis indicated that the effluents contain the heavy metals concentrations within the norm ranges allowing their agricultural application. However, it is significant that after 3 weeks of storage, these concentrations lowered what may be caused by biochemical activity of microorganisms, which growth confirms that observation.

The presence of microorganisms in effluents support the possibility of their transformation in purpose of their further agricultural, forest or reclamation applications — they are very important by storing and distributing the nutrients to plants. Their amount depends on conditions in certain moment and environment. Their growth and presence is supported by humidity and proper temperature for individual species, lack of light and presence or lack of oxygen (dependably on the sort of microorganisms).

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