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MICROBIOLOGICAL REDUCTION OF MONOETHANOLAMINE WASTE TOXICITY

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Abstract. Aims of the investigation were to evaluate toxicity of monoethanolamine waste to various microorganisms and plants; and to search for means accelerating waste biodegradation. The results of the investigation show toxic effects of MEA waste on bacteria, fungi and plant seeds at rather low concentrations. Some microorganisms are able to adapt to the pollutant in the mixed substrates of the MEA and peat. During the experiment, density of the bacterial and fungal populations in the MEA and peat mixed substrates increased, followed by the intensive acidifying process of the substrates and by the degradation of pollutant. Strong negative effect of MEA waste on peat microbial communities was determined during the first day of the experiment; respiration rate significantly decreased as compared with peat control. Inhibition rate depended on the MEA waste content in the mixture, peat pH and incubation time. After 3–4 days of the incubation, respiration rate of the MEA mixture with acid peat at lower MEA concentration exceeded that of the control by almost five times. Respiration rate of the MEA mixture with neutral peat gradually increased after 4 days of incubation, and was stimulated by the addition of NPK fertilizers. Introduction of yeasts complex and the fungus *Trichoderma harzianum* accelerated the process of degradation, moreover, the phytotoxicity of the mixed substrate decreased significantly.

Keywords: microorganisms, monoethanolamine, pollutants, degradation.

1. Introduction

Monoethanolamine (MEA) – carbon organic compound with a hydrogen group on the one carbon atom and an amine group on the other – is released into biosphere almost exclusively from anthropogenic sources. MEA is used to remove CO_2, SO_2, NO_2 and H_2S from natural gas and other gases, in the synthesis of surface active agents, in polishes, hair waving sols, in emulsifiers; as softening agents for hides, dispersing agent for agricultural chemicals, for gas scrubbing at petroleum refineries and in the production of antibiotics (Lam *et al.* 1999; Sorensen 1999; Zaretskii *et al.* 2008). The role of MEA addressed in this work is the use in the removing of CO_2 from hydrogen in ammonia processing plants.

Reaction of MEA with carbon dioxide yields different products and composed waste can contain more than 50 organic components. The main components, including residual MEA, are nitrogen- and oxygen-containing compounds the percentages of which were as follow, %: N-ethylolethylenediamine – 37.9, MEA – 20, piperazine and its derivatives – 20, 2-aminoheptane – 5, DEA (dimethilamine) – 4, saturated hydrocarbons – 1, and esters of fatty acids – 1. In the waste, some heavy metals, especially Fe and Ni, are found as well (Khitrin *et al.* 2002). During processing, a part of MEA compounds get into the atmosphere and effluent water. This pollutant prevails in water. Emission of MEA from water to the atmosphere is impossible because of physical and chemical properties of the chemical. For example, 77.98 μg of MEA L^{-1} was detected in rainwater samples but only 0.43 ng – in m^{-3} of air at Columbus Islands (Hirzel 1996).

The influence of various industrial wastes on the environment may be forecasted according to their toxicity data (Klein 2000; Baltrėnas *et al.* 2006). Monoethanolamine is toxic to various organisms. Threshold of the toxic limit values for the aquatic microorganisms is ranging from 1.6 (e.g. *Microcystis aeruginosa*) to 6.3 (e.g. *Pseudomonas putida*) mg L^{-1} MEA at pH 7. For members of the genus *Nitrosomonas,* MEA at 100 mg L^{-1} concentration caused an inhibition of ammonia oxidation by 16%. The toxic threshold limit value of 0.75 MEA L^{-1} was found for the green algae *Scendesmus quadricauda* after 8 days of exposure in the cell growth inhibition test. Chlorophyll mutations of the sowed barley (*Hordeum vulgare*) plants were estimated after incubation of seeds for 5 hours in 30 mM L^{-1} MEA solution (Hirzel 1976).

Depending on the concentration and the contact duration, MEA causes pronounced irritation that may lead to burns in contact with human skin. The contact with products containing MEA can cause asthma, rhinitis, inflammation of airways, chronic bronchitis, and allergic dermatitis. The 100% exhibition of asthmatic symptoms was found in people working with MEA (Chang-Yeng, Malo 1995; Hirzel 1996). Monoethanolamine undergoes moderate biodegradation and is not expected to be persis-

tent in the environment. Bioremediation methods based on the occurrence and activity of microorganisms are usually used for the detoxification of pollutants (Exner 1994; Klein 2000, Sabate *et al.* 2004; Baltrėnas, Zagorskis 2008). This process relies on the ability of microorganisms to break down the complex hazardous waste (O'Niell, Nzengung 2004; Sabate *et al.* 2004; Arbique 2006). Test results indicate that MEA has been successfully biodegraded or transformed into simple compounds under aerobic and anaerobic conditions (Wong *et al.* 2004). Bacterial degradation studies using uncontaminated soil amended with 1300 mg L^{-1} MEA showed very rapid degradation of MEA with more than 99% degradation occurring in less than 3 days with quantitative conversion to ammonia, followed by slower conversion to nitrite and nitrate (Hawthome *et al.* 2005). Hazardous waste can also be made into useful chemicals by applying various microorganisms, thus reducing its possibility to pollute environment. Ohtaguchi and Yokoyama (1997) developed MEA conversion biotechnology suitable for acetic, formic acids and acetaldehyde production using *Escherichia coli* or *Clostridium formicoaceticum*, and confirmed availability of ecotechnology for resolving the above-indicated problem. The available results of ecotoxicology of MEA in the environment are considered to be sufficient for evaluation of MEA waste relevance to the environment. Cleaning of environment from pollutants is a relevant problem impacting on the safe pattern of life.

Bioremediation involves the use of microorganisms to treat contaminated soil in an environmentally friendly manner. MEA and their breakdown products may be degraded in soil and groundwater using indigenous microorganisms (Mrklas *et al.* 2004). In the process of biodegradation, chemical components are transferred or bounded to complexes by the action of microorganisms. Optimization of biodegradation and bioremediation technologies requires to determine factors essential to the development and activity of microorganisms and to evaluate the reduction of the pollution level using various tests.

Aims of the investigation were to evaluate MEA waste toxicity to microorganisms and plants and to find out the effectiveness of microbiological ways in acceleration of the waste degradation.

2. Methods

The methanolamine solution is used in an ammonia production plant for depuration of hydrogen from CO_2. A pitchy fraction, consisting of more than 50 various organic compounds and heavy metals, is formed during the technological process as waste. The initial pH of the newly produced waste, chosen for the investigations was 11.5.

2.1. MEA waste toxicity to microorganisms

The effect of the MEA waste at various concentrations on the growth of microorganisms was studied using a method of paper discs, which is based on the diffusion of the tested compound to the medium. Sterile discs of the filter paper were soaked in appropriate for experiment concentrations of MEA waste. Discs were laid on media with microorganisms (3 bacterial, 5 fungal and 5 yeast strains were used) in Petri dishes and incubated at 26 °C temperature. The sterile zones around the discs with MEA were measured after 3 days of the incubation (Bilaj 1982).

2.2. MEA phytotoxicity

The seeds of the winter wheat "Širvinta 1" and the winter rye "Duoniai" were used for toxicity tests. The seeds were sorted, washed with sterile water and laid (200 seeds) in Petri dishes lined with 3 layers of the filter paper for maintenance of humidity. The experiment was carried out at 23–25 °C temperature. The germination energy of the seeds was estimated after 3 days of the incubation; seeds viability as well as length of seedling shoots and roots – after 6 days of incubation (Bilaj 1982). The MEA waste for phytotoxicity tests was diluted with water in ratios: 1:1; 1:2; 1:5; 1:10; 1:20; 1:40; 1:60; 1:80; 1:100; 1:120.

2.3. Abundance of microorganisms and pH of mixed substrates

Two types of peat (pH 3.1 and 6.3) were used for detoxification of MEA waste. Waste was mixed with either of peat substrates in ratios 1:10, 1:20 and 1:20, enriched with NPK (nitrogen (N): phosphorus (P): potassium (K) as 10: 10: 20) fertilizers. Mixed substrates were kept under conditions of 60% humidity and 18.5–19.5 °C temperature. The pH of substrates was measured in 1 N KCl solution (Thomas 1996). Bacteria and micromycetes were grown on standard media – nutrient agar (NA, Oxoid) and malt agar (MA), respectively. Abundance of microorganisms in the substrate mixtures was evaluated as a number of colony forming units (cfu g^{-1} of dry soil), determined by the suspension dilution method. Phytotoxicity of the mixed substrates was tested after 4 and 14 days of incubation according to the method mentioned above.

2.4. Respiration rate

The rate of CO_2 release from the soils provides a useful parameter of biological activity in them. Thus the measurement of the CO_2 evolution from the MEA waste mixed with peat at an appropriate ratio was applied. Tested substrates were incubated at 60% humidity and 18.5–19.5 °C for 42 days. Respiration rate of the treated substrate mixtures was measured after 1, 4, 14 and 42 days of incubation. Respiration rate was determined using the alkali traps method (Luo, Chou 2006) by absorbing released from substrate CO_2 into a sealed headspace chamber for 24 hours using 1.0 M NaOH solution. At the end of the absorption period, the total mass of CO_2 in the alkali traps was determined by titrating NaOH solutions with diluted HCl (0.5 M) to a set pH value (reaction starts at pH 8.3 and finishes at pH 3.7). The rate of substrate respiration was calculated as the total amount of CO_2 trapped over and absorption period (h^{-1}) from the kg of the investigated substrate.

Table 1. Sensibility of microorganisms to various MEA waste concentrations (sterile zone, mm)

Microorganisms	Concentration of MEA							
	100%	75%	50%	25%	10%	5%	1%	0.1%
Bacillus megaterium	8	6	3	3	2	1	0	0
Escherichia coli	10	6	4	2	0	0	0	0
Proteus mirabilis	8	5	4	3	3	1	0	0
Acremonium roseum	17	5	3	2	0	0	0	0
Cladosporium herbarum	12	8	4	2	0	0	0	0
Fusarium culmorum	5	2	0	0	0	0	0	0
Penicillium expansum	5+10*	10*	8*	6*	4*	2*	0	0
Trichoderma harzianum	2+20*	10*	10*	10*	4*	3*	0	0
Rhodosporidium diobovatum	10	13*	8*	0	0	0	0	0
Aureobasidium pullulans	10	9	8*	6*	6*	8*	8*	6*
Rhodotorula rubra	8	6	0	0	0	0	0	0
Candida lipolytica	10	8	6	4	0	0	0	0
Geotrichum fermentans	15	10	10	8	0	0	0	0

* – fungistatic effect

2.5. Introduction of microorganisms into MEA-peat mixed substrates

The peat (pH 6.3) was mixed with MEA waste at a ratio 1:20. The suspensions of microorganisms for inoculation of the substrates were prepared from the 3–7 days cultures. Petri plate with the grown culture was poured with the distilled water to remove cells or conidia of microorganism. Obtained suspension was diluted up to concentration of 10^8 cells/conidia ml^{-1}. The following microorganisms were used for substrata inoculation:

1. Yeast complex;
2. *Trichoderma harzianum* Ko-2;
3. Yeast complex + *Trichoderma harzianum* Ko-2.

The abundance of microorganisms in the prepared substrates was estimated after 24 h, 1 month and 2 months since the inoculation. The humidity and pH of substrates were observed during all incubation period. The changes of the toxicity of the degrading pollutant were estimated using the phytotoxicity test.

3. Results and discussion

3.1. MEA waste toxicity to microorganisms

MEA waste as a mixture of organic components is ecotoxic and harmful to the environment. Its toxicity was determined according to resistance of various microorganism strains. Bactericidal effect of MEA waste was relatively high – the growth of bacteria was not inhibited only when MEA content in solution was ≤ 1% (Table 1). *Esherichia coli* were the most resistant to MEA at concentration up to 25%. Higher MEA concentrations (from 25% to 100%) inhibited all bacteria alike. The more sensitive bacterial strains, *Bacillus megaterium* and *Proteus mirabilis,* were inhibited by MEA waste at 5-fold lower concentrations (≤ 5%).

The effect of MEA on the growth of fungi was weaker than on the bacteria. The most fungicidal effect was determined against *Acremonium roseum* and *Cladosporium herbarum.* The sterile zone reached 12–17 mm around stock (not diluted) MEA decreasing to 2 mm at 25% MEA concentrations. Effect of MEA waste on fungi *Fusarium culmorum*, *Penicillium expansum* and *Trichoderma harzianum* was observed at 100% and 75% MEA concentrations (diameter of sterile zone was 5–1 mm). *Penicillium expansum* and *Trichoderma harzianum* cultures lost green pigmentation (sporulation was inhibited).

Aureobasidium pullulans was the most sensitive to MEA among the treated yeasts and yeast-like fungi. Actually 0.1% MEA concentration had fungistatic effect. *Candida lipolytica* and *Geotrichum fermentans* were inhibited by MEA at 75–25% concentrations. The growth of *Rhodosporidium diobovatum* and *Rhodotorula rubra* were inhibited starting from the 25% MEA concentrations.

3.2. MEA waste phytotoxicity

Plant seeds are often used for ecotoxicity tests. The viability of rye and wheat seeds selected for current tests was 90–92%. The MEA waste attenuated with water in ratios from 1:1 to 1:40, inhibited seed germination of test-plants completely. The seeds softened and became tarnish; therefore the lower concentrations of MEA (MEA: water ratio from 1:60 to 1:120) were tested. After 6 days only few seeds germinated, moreover, roots didn't develop in 1:60 treatment. Higher dilutions of MEA waste (1:80, 1:100 and 1:120) were more favourable to the seed germination (Table 2).

Table 2. Effect of MEA waste on viability of seeds (%)

Dilution of MEA with water	Germination energy		Viability	
	Wheat	Rye	Wheat	Rye
1:80	11.9	2.2	11.9	2.2
1:100	17.4	13.3	17.7	13.3
1:120	39.1	20.0	42.5	25.0

The growth of shoots and roots was rather weak in tested MEA dilutions. Thus in the most favourable variant for germination (dilution 1:120) shoots of wheat were 3.5±0.2 cm and roots – 0.9±0.1 cm long; of rye – 2.8±0.2 and 0.7±0.1 cm respectively, after 6 days (Fig. 1). The obtained results show that winter wheat "Širvinta 1" were more sensitive to MEA; ends of roots were brown and its development stopped.

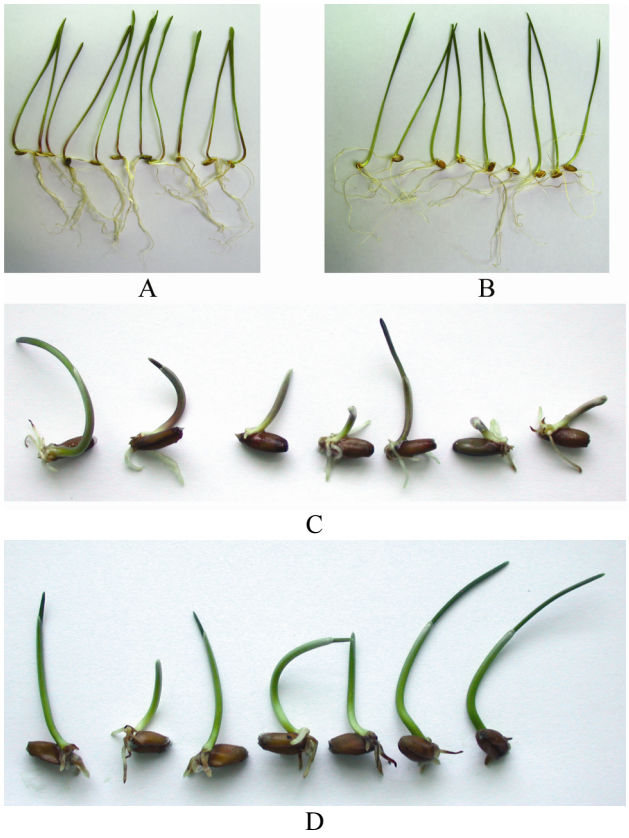

Fig. 1. The influence of MEA waste on shoots growth: A – rye (control); B – wheat (control); C – rye (MEA dilution with water 1:120); D – wheat (MEA dilution with water 1:120)

3.3. Changes of pH and abundance of microorganisms in MEA-polluted peat substrates

The appliance of peat to reduce MEA toxicity was tested. Two types of peat (with pH 3.1 and 6.3) were used for preparing of mixed substrata. Changes of pH during the experiment are presented in Table 3. The acid peat in mixture with MEA reduced substrata pH to neutral or weak alkaline (pH 7.3–8.99) and persisted till the end of the experiment (pH > 8).

In the substrata mixed with peat, which pH was 6.3, initial pH was > 9, then gradually decreased to pH 6–7 (except for the mixture variant 1:10, where pH was > 8). Enrichments of the substrates with the NPK fertilizers did not influence pH dynamics. The obtained results showed that acidic peat was more suitable for neutralization of MEA waste.

The acidity of substrata influenced on viability and biodegradable activity of microorganisms. The amount of bacteria in substrata mixed with acid peat (especially in variants 1:20 and 1:20+NPK) was greater as compared with the control variant after 4 days and increased up to 14 days of incubation (Fig. 2, A). The number of bacteria and increase of their abundance during incubation period in mixture 1:10 was significantly less, whereas in substrata with neutral peat, the number of bacteria in all treated variants exceed the control variant only after 14 days (Fig. 2, B). In the variant with addition of NPK fertilizers the number of bacteria was the greatest.

Table 3. Changes of pH_{KCl} of peat and peat: MEA mixed substrata

Peat	Mixed substrata (MEA: peat)	Time of pH measurement		
		Initial	After 4 days	After 14 days
pH 3.1	Control (peat)	3.1	3.21	3.25
	1:10	8.99	8.45	8.70
	1:20	7.31	6.67	8.39
	1:20 + NPK	7.23	6.48	8.41
pH 6.3	Control (peat)	6.3	6.03	5.94
	1:10	9.79	8.95	8.30
	1:20	9.24	8.64	6.40
	1:20 + NPK	9.21	8.66	7.02

The acid peat was more suitable for the development of micromycetes. Their number in the variant with MEA and peat mixed at a ratio 1:20 and added NPK, reached about 590 $\times 10^5$ cfu g^{-1} substrate after 14 days (Fig. 3, A).

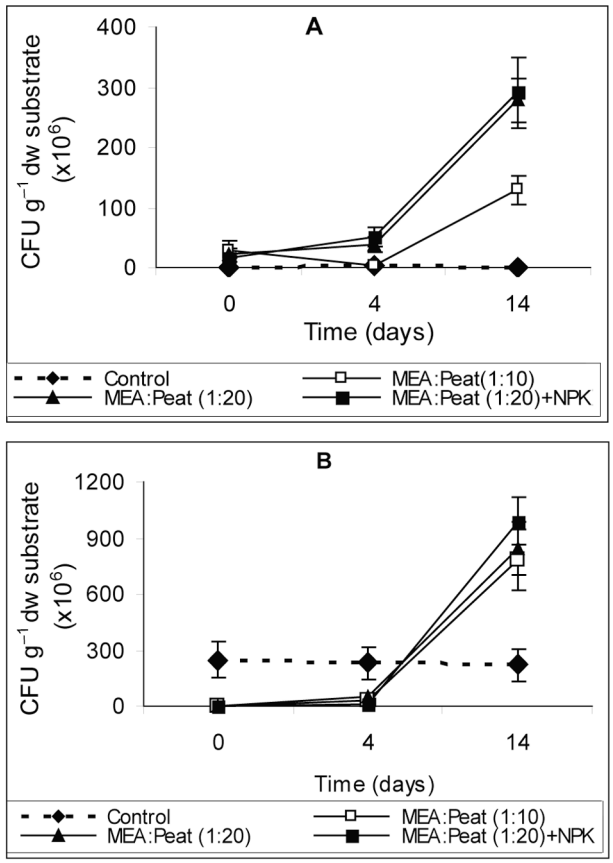

Fig. 2. The number of ammonifying bacteria in MEA and peat (A – pH 3,1; B – pH 6,3) mixed substrata

At the same time in mixture with neutral peat the amount of fungi was 17×10^5 cfu g^{-1} substrate (Fig. 3, B). In the control variant the number of fungi was greater than in mixed substrata. The addition of NPK fertilizers positively influenced the amount of fungi.

The appliance of acid peat for the neutralization of MEA pitch solution was suitable for development of both bacteria and micromycetes. The concentration of H^+ ions decreased permanently during the experiment in substrata with neutral peat. These variants were more suitable for the development of bacteria.

The toxicity of MEA mixed with peat was evaluated according to germination of wheat and rye. In substrata with acid peat in the variant 1:10 after 4 days seeds didn't start to germinate (Table 4). After 14 days, viability of seeds of rye and wheat in this variant was 8.16 and 12.51%, respectively. Viability of seeds in the variants 1:20 and 1:20+NPK was 95.8–100% and 87.5–100%, respectively.

In substrata mixed with neutral peat, germination of wheat seeds was successful only in variant 1:20. NPK affected the viability of seeds. The obtained data confirmed that neutral peat mixed with MEA at a proportion 1:20 decreases the toxicity of MEA.

It may be concluded that despite of the fact that acid peat was suitable for the pH reduction of MEA waste, results of the ecotoxicity tests showed that neutral peat is more suitable for the elimination of MEA waste toxicity and acceleration of its biodegradation process.

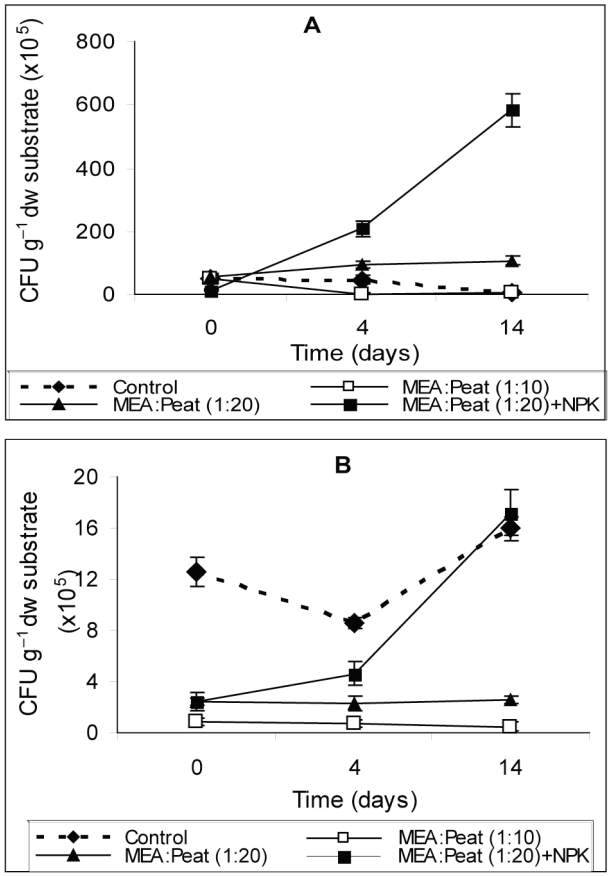

Fig. 3. The number of micromycetes in MEA and peat (A – pH 3,1; B – pH 6,3) mixed substrata

3.4. Respiration rate of peat and MEA: peat substrates

Soil respiration is one of the key ecosystem processes that release carbon from the soil in the form of carbon dioxide. It provides a useful parameter of biological activity in the soil. Respiration rate is commonly used to evaluate impact of pollutants on activity of microbial communities in soil. The possible effect of MEA waste on biological activity of peat was expected. Values of the respiration rate, determined in the control acid peat (pH 3.1) and the MEA mixture with acid peat at the ratios of 1:10 and 1:20, differed significantly (P = 0.01) after the first day of incubation. Microorganisms' community in acid peat substrates reacts simultaneously to the addition of the pollutant, therefore, after the first day of incubation, significant differences between the control peat and the contaminated peat biological activity was observed (Fig. 5). Respiration rate of the MEA: peat mixture (ratio 1:10) after the first day of incubation was very low (0.7 mg $C\text{-}CO_2$ kg^{-1} h^{-1}) and was almost 38.2 times less than of the control peat (26.9 mg $C\text{-}CO_2$ kg^{-1} h^{-1}). Lower contamination of the acid peat (at the ratio of 1:20) determined a reduction of the substrate respiration rate only 2.7 times as compared with control; the rate was 15 times higher than that of MEA: peat mixture at the ratio of 1:10. Marked reduction of the control peat respiration rate was determined after 2 days of incubation (3.4 mg $C\text{-}CO_2$ kg^{-1} h^{-1}) and decreased to 0.8 mg $C\text{-}CO_2$ kg^{-1} h^{-1}, whereas the respiration rates of the MEA-contaminated acid peat (both at the ratio 1:10 and 1:20) increased up to the 3rd day of incubation and only then sharply decreased (Fig. 4). Due to the fact that no other organic substrate

Table 4. Viability of wheat and rye seeds (%) in MEA and peat mixed substrata

Peat	Mixed substrata (MEA: peat)	Rye		Wheat	
		After 4 days	After 14 days	After 4 days	After 14 days
pH 3.1	Control (peat)	89.79±2.8	95.92±1.3	88.76±0.9	100±0
	1:10	0	8.16±0.3	0	12.51±0.6
	1:20	100±0	97.96±0.2	95.83±0.2	100±2.7
	1:20 + NPK	94.54±3.1	91.84±0.4	100±0	87.50±2.7
pH 6.3	Control (peat)	100±0	95.92±1.4	98.12±0.3	95.83±0.2
	1:10	0	0	0	0
	1:20	0	30.61±2.7	0	97.92±0.2
	1:20 + NPK	0	44.89±3.3	0	50.0±3.1

(except humus) was present in the control peat, activity of indigenous microorganisms decreased, followed by the decrease of the respiration rate. The mean concentration of the CO_2 in the peat air must be always higher than that in ambient air because of the biological activity of microorganisms, and because of restricted diffusion to the atmosphere. Due to MEA peculiarity to absorb CO_2, its additions into peat could initiate CO_2 absorption. Thus, it can be considered as an important fact during the first days of incubation, determining the lower CO_2 mass from MEA-contaminated peat. Respiration rate values either of the control peat and MEA-contaminated peat after 4 days of incubation were similar and varied only from 0.76 to 4.5 mg C-CO_2 kg^{-1} h^{-1}. Negative correlation between the respiration rate of the control acid peat and the MEA-contaminated suggest that microorganisms adapted to the pollutant and were able to degrade it in some degree. Degradation process could be energy consuming and influencing the respiration process in contaminated peat. However, the results of the respiration rate of MEA-contaminated peat showed advantage only during days 1–4 of the incubation period (Fig. 4).

MEA additions into peat can affect peat microbiota directly or indirectly through changes in peat solution, particularly through a decrease of pH due to dissolution and dissociation of CO_2 (Šantrůčková *et al.* 2005). Comparable effects were investigated in soil polluted with MEA. The change in soil pH could explain the decrease of microbial biomass and activity observed immediately after an increase of CO_2 concentration in the soil (Šantrůčková *et al.* 2005). The negative effect was, however, only temporary and was followed by an increase in biological activity as well as during our investigation. The increase of CO_2 in MEA: peat mixture (ratio 1:10) could be possible and that fact could determine the low CO_2 concentration in the ambient air from which CO_2 was alkali trapped (Fig. 4). Such statement could be explained also by the low but gradual increase of the released CO_2 from MEA: peat (ratio 1:10) substrata. It is

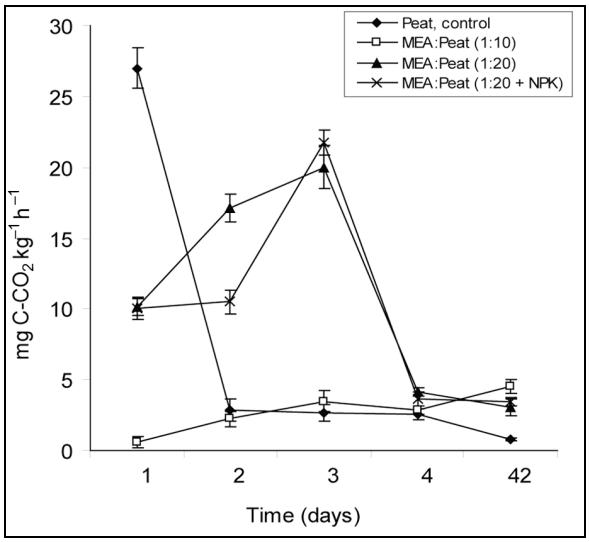

Fig. 4. Respiration rate of the acid (pH 3.1) peat, MEA: peat mixture (ratio 1:10 and 1:20) and MEA: peat (ratio 1:20) enriched with the NPK fertilizers

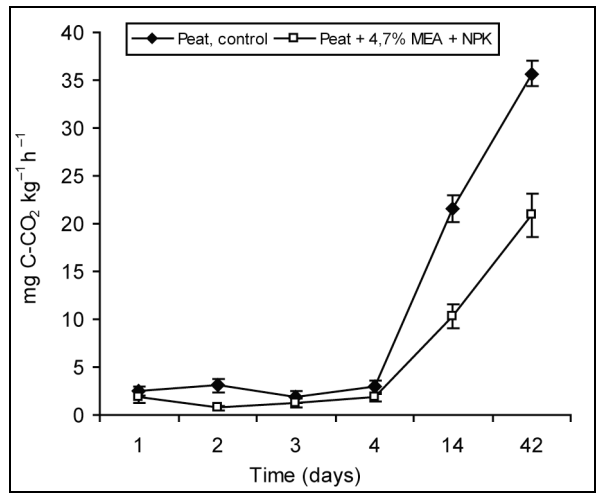

Fig. 5. Respiration rate of the peat (pH 6.3) (control) and MEA: peat mixture (ratio 1:20) enriched with the NPK

likely that peat microorganisms, being adapted to the higher CO_2 concentration in the peat environment during first days of incubation, will employ mechanisms that enable them to utilize CO_2 as an additional source of carbon and survive the unfavourable period. The important role of carboxylase in intermediary metabolism of a large variety of aerobic organisms including fungi and bacteria has been demonstrated (Jones 1994).

The aerobic slurry experiments suggested initial phosphate limitation, as biodegradation rates increased by one order of magnitude after phosphate addition (Mrklas *et al.* 2004). Enrichment of the acid MEA: peat mixture (1:20) with the NPK fertilizers did not influence respiration, except the differences determined after 2 days of incubation, when the NPK affected respiration rate (Fig. 4). It is documented that N can reduce soil respiration in N saturated tropical forest soil (Mo *et al.* 2008).

Respiration of peat with pH of 6.3 both of control and MEA-contaminated and enriched with the NPK, followed the non-linear pattern as well (Fig. 5) with the highest rates after 42 days (36.1 and 22.4 mg C-CO_2 kg^{-1} h^{-1}, respectively), and the lowest during days 1–4 of incubation. In the control variant, the mean respiration rate was almost two times higher than in the MEA-amended and NPK enriched cages. Surprisingly, the dynamics of the respiration rates determined for the substrates composed of the peat (pH 6.3) (Fig. 5) were reverse to those obtained for the substrates composed of the acid peat (pH 3.1) (Fig. 4). The amount of CO_2 released from the substrate is controlled by several factors. The temperature, moisture and nutrient content were the same for all microcosms; however, pH could produce extremely disparate rates of respiration.

3.5. Acceleration of MEA degradation by introduction of microorganisms

According to the obtained data, the MEA: peat mixture in proportion 1:20 was used for the further experiment. The initial pH of peat was 6.04, of MEA waste – pH 11.5, of mixed substrata – 8.27. The dynamic of microorganism number, pH and humidity during experiment are given in the Table 5.

The MEA waste is rather alkaline, therefore the addition of peat creates more favourable conditions for the growth and biodegradable activity of microorganisms. Thus, one month after the inoculation of substrata with microorganisms, in separate variants pH decreased to 5.5–5.8; two months after the inoculation, pH decreased to 5.3 (in the control variant pH changed to 7.4 and 6.1, one and two months after inoculation, respectively). Perhaps, the substrata acidification trends show MEA degradation due to the activity of microorganisms. Decreasing alkalinity in the control could be related to an evaporation of ammonia.

Tested substrata were periodically watered and mixed up; therefore water content comprised 30–33% during the experiment. After 1 month of incubation, humidity of the control variant was higher because of MEA property to keep moisture.

The microorganisms introduced into mixed substrata survived: their number after 24 hrs was $24.4–45.7\times10^6$ cfu g^{-1} d. substrates. In the variant with introduced complex of microorganisms 31.1×10^6 cfu of yeasts and 24.4×10^6 cfu of *Trichoderma harzianum* in g^{-1} of substrate were isolated. Their total number exceeded a number of microorganisms in variants where yeasts or fungus were introduced separately.

One month after the inoculation, the number of microorganisms decreased about 10 times and was $2.2–9.7\times10^6$ cfu g^{-1} dry substrates. After 2 months, reduction was not especially significant – $2.1–6.1\times10^6$ cfu g^{-1} of microorganisms were found. The lowest amount of yeasts was isolated from the substrata, inoculated with complex of yeasts and fungus. The highest number of fungus *T. harzianum* was isolated from the variant inoculated with this fungus. Consequently introduced microorganisms adapted and developed in MEA and peat mixed substrata. The study of other researches proposed biodegradation pathways of MEA in natural soil using indigenous microorganisms (Mrklas *et al.* 2004).

The toxicity of MEA waste decreased due to the degradation of MEA waste process under the action of microorganisms. That is confirmed by the obtained results of rye viability changes during the experiment (Table 6).

At the beginning of experiment the germination energy and viability were rather low due to the MEA waste toxicity. Introduced yeasts buffered this negative influence. After 1 month MEA toxicity significantly decreased: germination activity and viability of rye became similar to that of the control variant. Yeasts and fungus *T. harzianum* Ko-2 complex have positive effect on the germination energy and separately introduced yeasts and fungus – on the viability of seeds. The similar data were obtained after 2 months, while germination activity increased even more.

There are comparable data about the application of microorganisms in bioremediation processes. Juhanson *et al.* (2007) detected the enhanced degradation rates of pollutants (of oil shale chemical industry) in field experiment after the addition of bacteria into substrata. MEA pollutants were successfully remedied *in situ* during a 138-day period, and soil toxicity was significantly reduced (Lee, Portier 2007). It allows confirming that under the action of introduced microorganisms, the toxicity of MEA waste mixed with peat significantly decreased.

Some suggestions for the remediation of polluted soil or water sites *in situ* may be proposed appealing to the obtained results under laboratory conditions. The concentration of MEA pollutants should not exceed 1000 mg L^{-1}. It is desirable to regulate pH near neutral in liquid waste. The inoculation of indigenous active microorganism strains accelerates the process of biodegradation of amine-related waste.

Table 5. The changes of introduced microorganism number cfu g^{-1} d. substrate ($\times10^6$) and physical rates in peat and MEA pitch mixture (1:20)

Variants	After 24 h			After 1 month			After 2 months		
	cfu	pH	Humidity %	cfu	pH	Humidity %	cfu	pH	Humidity %
Control	–	7.42	63	–	7.42	38	–	6.10	30.4
Yeast complex	45.7±6.3	5.78	63	5.2±1.3	5.78	31	3.5±1.5	5.27	31.4
Trichoderma harzianum Ko-2	31.1±4.8	5.77	63	9.7±0.8	5.77	33	6.1±0.8	5.34	30.0
Yeast complex + *Trichoderma harzianum* Ko-2	31.1±4.8 + 24.4±2.1	5.51	63	2.2±0.8 + 7.4±1.5	5.51	32	2.1±0.7 + 5.0±1.2	5.27	30.4

Table 6. The effect of MEA waste in peat with inoculated microorganisms on rye germination energy and viability (%) during the experiment

Variants	After 24 h		After 1 month		After 2 months	
	Germination energy	Viability	Germination energy	Viability	Germination energy	Viability
Control	20	72	37	94	38	92
Yeast complex	40	90	39	94	45	98
Trichoderma harzianum Ko-2	20	76	30	98	40	96
Yeast complex + *Trichoderma harzianum* Ko-2	22	84	43	96	47	94

4. Conclusions

1. Bacteria were more sensitive to the toxic action of monoethanolamine than fungi. Bactericidic action of MEA waste was at 5–25%, fungicidic – at 25%, fungistatic – at 5% concentrations. The more resistant to MEA waste were *Escherichia coli* and *Trichoderma harzianum* species. Yeasts were resistant to MEA waste up to 10% concentration.

2. MEA pitch was rather toxic to seeds of wheat and rye. MEA dilutions with water from 1:1 to 1:40 inhibited the germination of seeds completely. Dilution 1:120 inhibited germination of seeds weakly but significantly depressed development and growth of shoots.

3. Neutral peat is perfect for development of active bacteria strains in polluted substrata. In MEA and peat mixed substrata (especially 1:20) the processes of biodegradation and detoxication of pollutants run successfully. The increasing amount of microorganisms showed that they are able to adapt to MEA-polluted environment.

4. Increasing rate of the respiration of the MEA mixture with acid peat showed good adaptation of microorganisms, confirming that neutral peat can be used as substrate for the MEA waste detoxification technology. Addition of NPK fertilizers didn't improve seeds germination but had positive effect on the respiration rate.

5. During a 2-months experiment in MEA and peat substrata, mixed in proportion 1:20 with addition of yeasts and fungus *T. harzianum* pH changed from alkaline to acid side. The significant decrease of phytotoxicity was estimated. The obtained data show the acceleration of MEA degradation due to activity of microorganisms.

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MIKROBIOLOGINIS MONOETILAMINO ATLIEKŲ TOKSIŠKUMO MAŽINIMO BŪDAS

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Santrauka

Darbo tikslas – įvertinti monoetilamino (MEA) atliekų toksiškumą įvairių grupių mikroorganizmams ir augalams bei parinkti būdus atliekų biodegradacijai pagreitinti laboratorinėmis sąlygomis. Nustatytas gana mažų koncentracijų MEA dervinių atliekų toksinis poveikis bakterijoms, mielėms, grybams ir augalams. Dalis mikroorganizmų durpių ir MEA mišinyje prisitaiko prie teršalų, didėja jų populiacijos tankis, mažėja substrato pH, o tai rodo MEA atliekų degradaciją, vykstančią dėl mikroorganizmų veiklos. Stiprus mikroorganizmų bendrijos aktyvumą slopinantis MEA poveikis pastebėtas pirmąją eksperimento parą; mikroorganizmų kvėpavimo intensyvumas, palyginti su kontrole, žymiai sumažėjo. Poveikio kvėpavimo intensyvumui mastas priklausė nuo MEA atliekų kiekio mišinyje, durpių pH ir ekspozicijos trukmės. Po trijų parų mikroorganizmų kvėpavimo intensyvumas nedidelės koncentracijos MEA ir rūgščių durpių mišinyje beveik penkis kartus buvo didesnis nei kontrolės. MEA dervos mišiniuose su neutraliomis durpėmis nustatyta palaipsnis kvėpavimo intensyvumo didėjimas substratą papildžius (NPK) trąšomis. Nustatyta, kad introdukavus mielių kompleksą ir mikromicetą *Trichoderma harzianum* į MEA ir durpių mišinio substratą, mikroorganizmų pradai išlieka gyvybingi ir vystosi, skatindami MEA biodegradaciją. Tai rodo žymiai sumažėjęs su durpėmis sumaišytų MEA atliekų toksiškumas augalams.

Reikšminiai žodžiai: mikroorganizmai, monoetilamino (MEA) atliekos, teršalai, toksiškumas.

МИКРОБИОЛОГИЧЕСКОЕ СНИЖЕНИЕ ТОКСИЧНОСТИ МОНОЭТИЛЕНАМИННЫХ ОТХОДОВ

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Резюме

Целью работы было оценить токсичность моноэтиленаминных (МЭА) смолистых отходов для разных групп микроорганизмов и растений, а также найти способы ускорения биодеградации этих отходов в лабораторных условиях. Установлено токсичное воздействие сравнительно небольших концентраций МЭА на бактерии, дрожжи, грибы и растения. Часть микроорганизмов в смеси МЭА и торфа приспосабливается к загрязнителям, увеличивается плотность их популяции, pH субстрата меняется в кислотную сторону, что указывает на деградацию МЭА отходов, происходящую благодаря деятельности микроорганизмов. Сильное воздействие МЭА, угнетающее активность сообщества микроорганизмов, наблюдалось в первые сутки эксперимента: интенсивность дыхания по сравнению с контрольными данными значительно уменьшилась. Степень воздействия на интенсивность дыхания зависела от количества МЭА отходов в смеси, кислотности торфа и времени экспозиции. Спустя трое суток интенсивность дыхания в смеси МЭА отходов небольшой концентрации и торфа с кислой реакцией была почти в пять раз больше, чем в контроле. В смеси МЭА и торфа нейтральной реакции установлено постепенное увеличение интенсивности дыхания при обогащении субстрата удобрениями NPK. Установлено, что при интродукции комплекса дрожжей и микромицета *Trichoderma harzianum* в субстрат смеси МЭА и торфа их пропагулы остаются жизнеспособными и размножаются, способствуя биодеградации МЭА отходов. Данный вывод подтверждает значимое уменьшение токсичности смеси МЭА отходов и торфа по отношению к растениям.

Ключевые слова: микроорганизмы, моноэтиленаминные смолы, загрязнители, токсичность.

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