DYNAMICS OF THE FUNCTIONAL STRUCTURE OF THE SEDIMENT MICROFLORA DURING THE MODEL PROCESS OF PHENOL BIODEGRADATION

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Abstract


The biodegradation of the toxic compound phenol in the sediments from the storage reservoir to HEPP “Lakatnik”, situated in the Iskar River was simulated in the laboratory model process. Phenol at a concentration of 200 mg.l\(^{-1}\) was the investigated xenobiotic. The following microbial and chemical indicators characterized the process: the amount of the key functional groups of microorganisms – aerobic heterotrophic bacteria, anaerobic heterotrophic bacteria, \(g.\)Pseudomonas, \(g.\)Acinetobacter and reduction of the residual phenol, mg.l\(^{-1}\). The dynamics of microorganisms showed greater changes after 72 hours of the process. All investigated microbial groups quickly adapted to the amount of 200 mg.l\(^{-1}\) phenol. The results confirmed that at the end of the process the phenol concentration decreased up to 69%. This result can be used in the decision-making at risk situations associated with increased concentration of phenol in the sediments.

Key words: Functional microbial diversity, phenol biodegradation, sediments

Abbreviations: AH – aerobic heterotrophic bacteria, AnH – anaerobic heterotrophic bacteria, HEPP – Hydroelectric Power Plants, Ps. – \(g.\)Pseudomonas, Ac. – \(g.\)Acinetobacter

Introduction

The modeling is widely applied approach for study of water treatment and detoxification processes. These days it is one of the scientific areas with the highest number of studies (Kalinkov et al., 2003). Some biological systems are analogous models that provide information on the mechanisms, scale and speed of biodegradation processes (Topalova, 2009).

The reservoir to HEPP “Lakatnik” is located in urbanized areas with highly developed industry in the past (Schneider et al., 2012). Sediments contain trivial and toxic organic and inorganic components. Biodegradation processes of toxic compounds occurring in sediments are mainly carried out by microbial communities. They have options for alternative ways of energy supply and powerful potential for rapid inclusion of metabolic pathways for degradation of xenobiotics.

This study simulated a potentially hazardous situation of phenol accumulation in the sediments from the storage reservoir to HEPP “Lakatnik”. The processes in the lake sediments are poorly studied and the information in the scientific literature is partial (Dube, et al., 2001). Therefore the aim of this study is to investigate phenol transformation under the influence of microbial communities in sediments of HEPP “Lakatnik”. The development of models to predict the processes in the sediments is a prerequisite for adequate response in the risk situation.

Materials and Methods

Experimental design: A model anaerobic process of phenol biodegradation of the accumulated aromatic xenobiotics in the sediments of reservoir “Lakatnik” was simulated in lab
scale. The investigated sediments and water from the reservoir to the HEPP “Lakatnik” were taken during March 2012. The model process was accomplished in bottles with a total volume 620 ml. 16 bottles with caps were used for the purposes of the experiment. In each bottles were placed 300 ml of water and 300 g sediment. Parameters of the anaerobic batch process are T °C ≈ 25; pH ≈ 7; added phenol = 200 mg.L⁻¹; working volume = 500 ml; 50 % sediments; 50 % water; stirring every time during the sampling. All bottle were placed in the dark for maximum approximation to real conditions in the sediments. The duration of the simulated process was 336 hours. At a certain hour, we were examined one bottle of control variant and variant with phenol. The phenol concentration was measured spectrophotometrically at the following times: 0 h, 24 h, 48 h, 72 h, 120 h, 192 h, 264 h, 336 h (Lurye, 1973). The anaerobic conditions were created in the bottles during the experiment because of biodegradation processes. The effect of the added phenol was determined through dynamics of the functional structure of the sediment microflora. Studied indicators of the process were: residual phenol in the system (mg.L⁻¹), the amount of aerobic and anaerobic heterotrophic bacteria; g.Pseudomonas and g.Acinetobacter. They were examined at the following hours: 0 h, 24 h, 72 h, 120 h, 192 h, 264 h, and 336 h.

**Analytical methods:** The microbial numbers were counted by plate techniques according to routine practices (Kuznetzov and Dubinina, 1989) and were presented as colony forming unit per gram dry weight sediment. The heterotrophic bacteria were cultivated on nutrient agar (Brit. Pharm.). Anaerobic heterotrophs were incubated in anaerobic jars with Anaerocult A (Merck & Co., Inc.) for creation of anaerobic conditions. G. Pseudomonas was cultivated on Glutamate Starch Pseudomonas Agar (GSP) and g. Acinetobacter – Sellers Agar. All data were average values from three iterations, treated for standard deviation by means of Excel 2007.

**Results and Discussion**

The dynamics of the sediment microflora during the process showed that the phenol biodegradation was biphasic (Figure 1). The duration of the early phase was to 72th hour.

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**Fig. 1.** Dynamics of the amount of aerobic (a), anaerobic heterotrophic microorganisms (b) g.Pseudomonas (c) and g.Acinetobacter (d) in both studied variants.
and it was characterized by relatively high oxygen levels and
the adaptation of microorganisms to phenol biodegradation.
The duration of the late phase was between 72th and 336th
hour and it was related with depletion of oxygen, fast in-
crease of the amount of microbial groups and rapid biodeg-
radation of toxic compound.

The amount of aerobic and the anaerobic heterotrophs
for the both variants had similar dynamics at the start-up of
the process (Figure 1a, b). The amount of microorganisms
of these two groups was significantly more in control vari-
ant at the beginning of the late phase. This was due to the
longer adaptation period of microorganisms in the presence
of the toxic compound. At the end of the process, the count
of aerobic and anaerobic microorganisms in the control vari-
ant was significantly reduced because of the depletion of the
substrates. It wasn’t observed only for AnH in the variant
with phenol. At the end of the late phase AH and AnH was
more in the variant with phenol. Probably the reason for this
fact was slower consumption of substrates in the presence of
phenol allowing its use in the late phase. The anaerobes were
dominated in variant with phenol of the last studied hour
of the experiment. The obtained results confirmed oxygen
depletion in the systems and adaptation of microorganisms
to the toxic compound.

The adaptation period of *g.* Pseudomonas under the influ-
ence of toxic compound (up to 72th hour) was shorter, than
that of heterotrophs (up to 192th hour) (Figure 1c, d). At the
beginning of the process only bacteria from *g.* Acinetobacter
increased significantly their amount in the version with phe-

Table 1

<table>
<thead>
<tr>
<th>Residual phenol (mg.l⁻¹) – early phase</th>
<th>Residual phenol (mg.l⁻¹) – late phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>120 h</td>
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<tr>
<td>24 h</td>
<td>192 h</td>
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<tr>
<td>48 h</td>
<td>264 h</td>
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<tr>
<td>72 h</td>
<td>336 h</td>
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<tr>
<td>Control</td>
<td>Control</td>
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<tr>
<td>5.78</td>
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<tr>
<td>22.32</td>
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<td>11.67</td>
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<td>0</td>
<td>67.66</td>
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<tr>
<td>0</td>
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<tr>
<td>Phenol</td>
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<tr>
<td>201.25</td>
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<td>197.97</td>
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<td>62.75</td>
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</table>

The concentration of the residual phenol showed that
during the late phase of the process was a maximal con-
centration decrease (to 112 mg.l⁻¹) for variant with phenol
(Table 1). The phenol biodegradation in early phase was four
times less (26.5 mg.l⁻¹) than this in the late phase. A small
amount of phenol (22.32 mg.l⁻¹ of 24 hour) was measured in
the control variant in the beginning of the process. Its bio-
degradation was completed during the early phase. Phenol at
low concentration (7.7 mg.l⁻¹) was registered in the control
at the end of the experiment. This was probably due to the re-
leased phenol after lysis of many microbial cells at the end of
the model process. The reduction of the residual phenol and
microorganisms showed that the period of the highest phenol
biodegradation coincided with the peaks in the growth of the
key microbial groups. The studied groups of microorganisms
demonstrated large potential for adaptation to the phenol in
concentration 200 mg.l⁻¹ in reservoir sediments as well as to
activate adequate biodegradation potential.

**Conclusion**

The dynamics of the density of sediment microflora dur-
ing the model process of phenol biodegradation in the sedi-
ments showed that the adaptation was biphasic (early phase
and late phase). The complex of aerobic and anaerobic het-
erotrophs adapted more difficult than *g.* Pseudomonas and
*g.* Acinetobacter. The adaptation of microorganisms proved
that the growth of the studied groups weren’t inhibited by
phenol (200 mg.l⁻¹). The period of the highest phenol biode-
gradation coincided with the peaks in the growth of the key
microbial groups. The model process showed that the microb-
ial communities in the sediment were able to de degrade
69% of added phenol (200 mg.l⁻¹) for 14 days. This result
can be used in the decision making, when the xenobiotic
transformation in sediments has been managed.
Acknowledgements

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References


