ADAPTIVE MECHANISMS OF THE BIOFILM COMMUNITIES IN TECHNOLOGIES WITH GRADUALLY AND SHOCK CONCENTRATION INCREASE OF XENOBIOTIC POLLUTANTS – FUNCTIONAL AND FISH ANALYSIS

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Abstract: The main governing factors in the biodetoxification technologies are the concentration of xenobiotic pollutants in dependence of critical one as well as the way of its inflow in the system – gradually or shock loading.
The adaptive changes in two biofilm communities from lab-scale biodetoxifcation technologies were estimated and compared on the base of functional and microbiological parameters: The processes were studied in two analogous models – case studies. *Case study* 1 - bioremediation of sediments from Iskar river with shock phenol loading in concentration three times higher than critical one, that is about 250 mg/g sediment; *Case study* 2 – laboratory sand biofilter - treating amaranth polluted wastewater. The inoculation material was specially treated activated sludge, taken from Sofia WWTP (wastewater treatment plant). The biofilter was supplied with highly specialized adaptive algorithm for gradually increase of xenobiotic concentration (from 5 to 50 mg/L amaranth).
Two detoxification technologies have been compared according to: key technological and microbiological parameters, the micro-distribution and the role of unculturable microorganisms as well as the microbial relationships in genus *Pseudomonas.* The special attention has been paid on the analysis of the polyphosphates as an indicator for the alternative energetic sources – polyhydroxyacetate and poyhydroxybutyrate. In this study new fluorescent method was used.
The results showed that the technology with shock xenobiotic load reached the effectiveness of xenobiotic elimination 28%, while the technology with adaptive algorithm – 89%. In the same time the amount of the culturable phenol-degrading bacteria increased with 1.72 x 10^5 CFU/g, while the amaranth-degrading bacteria decreased with 1.67 x 10^6 CFU/g in the course of the adaptation processes. The analysis of polyphosphates (metachromatin) proved that the microbial community, functioned in the shock loading, used higher quantity of alternative energetic sources in comparison with this, governed by the adaptive algorithm.
As general conclusion the adaptation mechanisms towards xenobiotic biodegradation were different on the base of ways of inflow of the toxicants in the technology: 1/ After the shock xenobiotic loading in concentration three times higher than critical the biofilm developed the most simple mechanism – the multiplying of the microorganisms, but the biodegradation effectiveness was low; 2/ After application of the purposely constructed adaptive algorithm, microbial communities developed complex mechanisms of azo-detoxification, included the increased role of unculturable bacteria and synergistic relationships between them. This leaded to the increased effectiveness of xenobiotic detoxification.

Keywords*:* Detoxification, Adaptive algorithm, *Pseudomonas*, FISH, Polyphosphates

INTRODUCTION

A widely discussed and studied problem is "How the xenobiotic substances getting into the environment and provoke the biodegradation mechanisms of the microbial communities, polluted by xenobiotics"? Research databases are filled with various suggestions for removal of such contaminants. In both the physico-chemical and biotechnological solutions the focus is placed mainly on the facilities. Meanwhile the biotechnologies for toxicants biodegradation are more special type of technology - they operate on the basis of the activity of microorganisms most often involved in communities (biofilms, activated sludge). They have organization and behavior subject to the biological logic rather than the engineering one. Therefore, to achieve maximum effectiveness and sustainability of biotechnology it is necessary to know the specific features of the biological systems (microbial communities), performing biodegradation.

The key working factor of the biotechnologies based on biofilms and activated sludge is the ability of communities to adapt to biodegradation of toxic pollutants until reaching the highest concentration, that is specific for the given system (called critical concentration) [7, 40, 45]. The adaptation is the most significant factor for the functioning of an effective and sustainable detoxification technology [3, 6, 13, 15, 24, 31, 39, 46]. To manage this process, however, it is necessary to know the mechanisms of its undergoing.

When we are studying naturally formed microbial communities such as biofilms, a critical step is to limit the destruction of the relationships between microorganisms. This is important because the functioning and biodegradation in this type of community are usually defined precisely by the specific relationships. It is known that only about 1% of the microorganisms in naturally formed microbiocenoses can be tested with standard culture techniques [4, 9, 45].

This is overcomed by the application of molecular methods based mainly on PCR and *in-situ* hybridization. The fluorescence *in-situ* hybridisation /FISH/ is one of the most widely used methods for studying the biofilms, involved in water purification and detoxification processes [1, 8, 11, 18, 19, 26, 34, 35, 37, 38]. This molecular techniques gives information about the quantitative and qualitative composition and spatial distribution of microorganisms in communities [23, 29, 34, 46].

Another fluorescence *in-situ* technique, particularly suitable for the study of biofilms, is coloring of the polyphosphate granules with DAPI (4`,6-diamidino-2-phenylindole) [4, 16, 20, 27]. It is known that when DAPI bonds to the polyphosphate granules the maximum of emission of the dye changes, and its color instead of blue looks yellow-green [14]. In the biological purification of waters with microbial elimination of phosphates the technologies are based on the accumulation of polyphosphates (metachromatin) in the bacterial cells. These bacterial inclusionsare directly connected with the synthesis and using of poyhydroxybutyrate and polyhydroxyacetate granules. These granules can be used as alternative sources of energy. In the extreme difficult conditions of availability of high concentration of xenobiotics the using of the alternative sources of energy by microorganisms can play indicative role for an adaptation to the biodegradation of the xenobiotics. The reason is that the xenobiotics affect negatively the electron transport and oxidative phosphorylation and inhibit the synthesis of ATP [5, 32]. By investigation of the amount and localization of polyphosphates and the related use of reserve carbon sources it is possible to detect the degree of adaptation of the microbial community to xenobiotic biodegradation. The aim of this study was to investigate the key differences in adaptation mechanisms of 1/ sediment biofilm, subjected to *shock loading* of toxic pollutant (phenol)2/ biofilm community, that is purposefully adapted to the increased xenobiotic concentration (amaranth).

MATERIALS AND METHODS

The article contains a comparative analysis of two case studies from the practice of the Laboratory of Environmental Biotechnology of Faculty of Biology at the Sofia University “St. Kliment Ohridski”. The purpose of first case study was an implementation of adaptation algorithm in lab-scale biofilter of water contaminated with the azo-dye Amaranth. The second case study was a situation of shock loading with phenol of lab-scale bioremediation sites, contained the polluted sediments. The laboratory simulated risk situations is analysed by means of multi-layered analysis combining technological parameters, parameters of the systems analyzed by highly specialized scientific techniques such as fluorescence analysis of the metachromatin and FISH (Fluorescence *In-Situ* Hybridization) analysis.

Experimental design

Case study 1: We constructed a model bioremediation site for river sediments. The site has a capacity of 2 dm^3 with a mixture of 450 g sediment from the reservoir of HPP (Hydroelectric power plant) "Lakatnik" ("Middle Iskar" cascade, Petrovilla Group) and 450 g draining quartz sand. For simulating a situation of shock loading the system with xenobiotic we selected phenol as toxic xenobiotic. The phenol-containing xenobiotics are broadly spread in the environment as well as they are very appropriate model for the investigation of adaptive mechanisms of the microbial communities. The site functioned 214 hours under the following regime of shock loading of xenobiotic: at 2nd hour – adding 250 mg/kg; at 49th hour – adding 500 mg/kg; at 72nd hour – new 500 mg/kg. The simulated risk situation is a heavily loaded sediments with phenol. The critical concentration for phenol biodegradation is below 250 mg/kg. So the microbial communities in this situation is in condition of heavy intoxication.

Case study 2: We constructed a model sandy down-flow biological filter with a volume of 192 cm^3. The mode of operation was semi-continuous, and the period of operation was 26 days. As inoculation material to form a biofilm we used activated sludge from WWTP “Kubratovo” (WWTP of Sofia city), kindly provided by “Sofiyska voda” AD. The activated sludge was preliminary disintegrated by ultrasound disintegrator UD-20 automatic (3 × 10 sec. with frequency 22 kHz and vibration amplitude 8 μm). The treatment process is designed as a lab-scale detoxification unit for purifying water polluted with azo dyes as a toxic xenobiotic. In the experiment we used synthetic wastewater with the azo-dye Amaranth. We applied the approach of purposely designed adaptation of the microbial community with a step-by-step increase of the concentration of the azo dye from 10 to 55 mg/L. The initial concentration of the toxicant, the step of its increase and the period of transition to a higher concentration are optimized for each specific system, considering the rules for biodegradation of xenobiotics.

The key features of the two described case studies and simulated risk situations are shown in Table 1.

Table 1. Key characteristics of the two model detoxification technologies

			Case study 1	***Case study 2***
				
BIODESIGN	***Type of the purification process***		Bioremediation of river sediments	Biodetoxification of wastewater
	Biological system		**Biofilm**	**Biofilm**
	Model facility		Bioremediation site	Biofilter
	Volume of the bioreactor		2000 cm^3	190 cm^3
XENOBIOTIC	***Type of the xenobiotics***		Phenol	The azo-dye amaranth
	Way of input of the xenobiotics		Shock Loading	Step- by-step increase of the concentration
	Critical concentration of the xenobiotic		250 mg/L	55 mg/L
PROCESS	***Duration of the process***		214 hours	623 hours
	Early phase of the process	*Duration*	0-48 hours	0-191 hour
		Specificity	The biodegraders are inhibited by the high concentrations of the xenobiotics	The system adapts to biodegradation of step by step increase of concentrations of the xenobiotic
	Late phase of the process	*Duration*	48-214 hours	191-623 hour
		Specificity	The biological system adapts to biodegradation of phenol after intoxication	The biological system is adapted to biodegradation of Amaranth in high concentrations

Materials

Quartz sand: As an inert carrier in the biofilter we used quartz sand with a particle size of 0.08-0.16 cm, provided by "Bistritsa" Drinking Water Treatment Plant (Sofia City, Bulgaria).

Xenobiotics: Amaranth (Fluka Chemical Corp.), Phenol (Merck, Millipore)

Synthetic wastewater: 1/ mineral solution, g/L (NaH_2PO_4 - 3.5; K_2HPO_4 - 5.0; $(NH_4)_2SO_4$ - 2.5; $MgSO_4.7H_2O$ - 0.3; $FeSO_4$ - 0.05; $CuSO_4$ - 0.01; $ZnSO_4$ - 0,005; $CoCl_2$ - 0.005; $MgCl_2$ - 0.005; $CaCl_2$ - 0.005; Na_2MoO_4 - 0.005); 2/ trivial organic substrate (3% nutritive solution made of: NaCl, 5 g/L; peptone, 10 g/L; yeast extract, 5 g/L); 3/ certain concentration of the azo-dye amaranth (10 to 55 mg/L) [12].

Methods

Determining the concentration of xenobiotics: 1/ Amaranth - spectrophotometric determining at λ = 520 nm (Ultrospec3000, Pharmacia Biotech); 2/ Phenol - The residual phenol was measured by a spectrophotometric method with 4-Aminoantipyrine at 540 nm [41].

Effectiveness and rate of elimination of xenobiotics

The effectiveness of xenobiotic elimination is calculated by the following formula:

$$\text{(Eq. 1)} \quad \text{Eff} = \frac{C_{in} - C_{eff}}{C_{in}} \cdot 100 \text{ , \%} ,$$

where C_{in} is the concentration of Amaranth in the influent in mg/L, C_{eff} is the concentration of Amaranth in the effluent in mg/L.

(Eq. 2) $\text{Eff} = \frac{C_{in} - C_{res}}{C_{in}} \cdot 100$, %,

where C_{in} is the input concentration of phenol in mg/kg, C_{res} is the residual concentration of phenol in mg/kg.

The rate of elimination of xenobiotics is calculated by the following formula:

(Eq. 3) $V = (C_{in} - C_{eff}).Q$, mg/h,

where C_{in} is concentration of amaranth in the influent in mg/L, C_{eff} is the concentration of Amaranth in the effluent in mg/L, Q is the flow in mL/h.

(Eq. 4) $V = \frac{Ct_1 - Ct_2}{(t_2 - t_1)}$, mg/g.h,

where Ct_1 is the concentration of phenol in the moment t_1 and Ct_2 is phenol concentration in the next time t_2.

Microbiological analyses: The amount of the microorganism of the key groups in both biofilm communities is defined by the standard culture techniques [21]. The used culture media are: Nutrient agar (for aerobic heterotrophs), Glutamate Starch Pseudomonas Agar (for *Pseudomonas* sp.), Nutrient agar with 50 mg/L amaranth (for azodegradants), synthetic mineral medium with agar and 250 mg/L phenol (for biodegraders of phenol).

FISH analysis: The samples of both detoxification processes are fixed in 4% paraformaldehyde and stored in 1:1 ethanol / PBS. The immobilization, permeabilization and dehydration of the samples were carried out according to Nielsen [22]. For the study of microorganisms from g. *Pseudomonas* we used Cy3-labeled oligonucleotide probe with nucleotide sequence (5`-GCT GGC CTA GCC TTC-3`) [36]. For hybridization we used concentration of 20% formamide. We performed control hybridization with non-sense probe NON338 (5`-ACT CCT ACG GGA GGC AGC -3`) [25]. For general visualization of the samples we used the fluorescent dye DAPI (4`,6-diamidino-2-phenylindole) (AppliChem GmbH). The pictures presented in this article were taken with epifluorescent microscope Leica Microsystems DFC310FX, at 400X magnification.

Metachromatin analysis: For analysis of metachromatin we used the ever more widely applied fluorescence method for staining polyphosphate granules with elevated concentrations of DAPI. In this study staining of the samples is carried out with 10 μg/mL of the dye. When DAPI reacts with metahromatin its maximum emission shifts from 475 nm (blue color), 525 nm (yellow) [2, 30].

Statistical analysis: Presented results are average values by three repetitions, treated by Student and Fisher. The standard deviations added with guaranty – 95%.

RESULTS AND DISCUSSION

The key differences in adaptation mechanisms described in the two studied cases are demonstrated by the data obtained in the microbiological and fluorescence analyses on the base of the main technological parameters.

The amount of the aerobic heterotrophs was increased during the detoxification in both technologies (Fig. 1). In the bioremediation site the amount of microorganisms from this group increased by 7%, while in the biofilter the increase was about 5 times. Simultaneously we also found an increase in the rate of elimination of xenobiotics - 2.6 times for the biofilter and 1.8 times for the sediments. These data showed an expansion of biofilms and development of the biodegradation potential in both studied communities. The obtained results confirmed that these tendencies were stronger in the community, subject to targeted adaptation in comparison to the biofilm community placed in conditions of toxic shock. This is confirmed by the effectiveness of both processes. In bioremediation for the early stage it was 26%, and in the late one - 30%. In the technology of the biofilter the effectiveness was much higher - 88% for the early phase and 90% for the late phase.

The data from the fluorescence analyses confirmed the results obtained by the conventional methods. On Fig. 2 and Fig. 3 we can see larger fragments of the biofilm in the late phase, which proves the increase of biomass in this period.

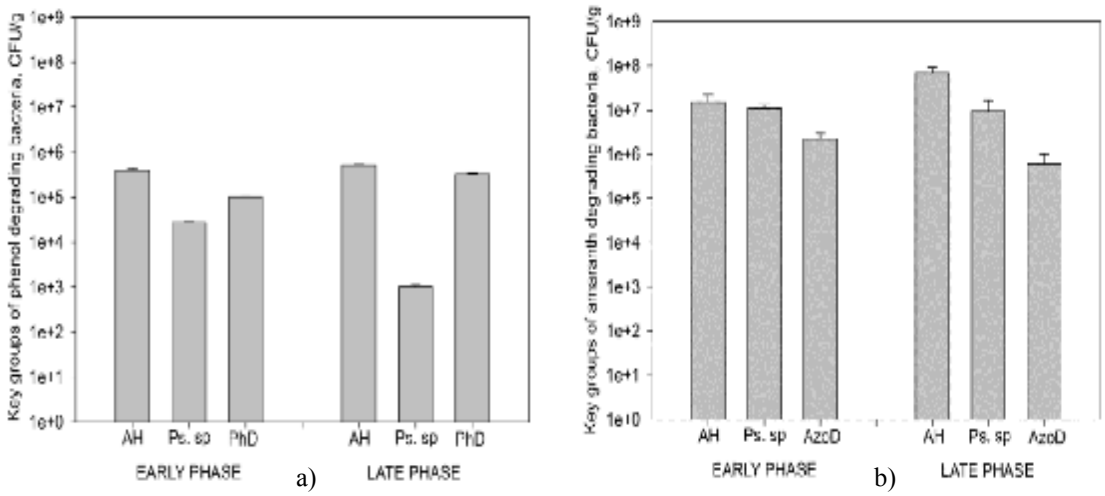

Fig. 1 Key groups of microorganisms in the biodegradation of model xenobiotics, studied by the conventional culturing techniques: a) key groups in the biodegradation of phenol (AH - aerobic heterotrophs; Ps. sp - *Pseudomonas sp*.; PhD – biodegraders of phenol); b) key groups in the biodegradation of Amaranth (AH - aerobic heterotrophs; Ps. sp - *Pseudomonas sp.;* AzoD - biodegraders of Amaranth)

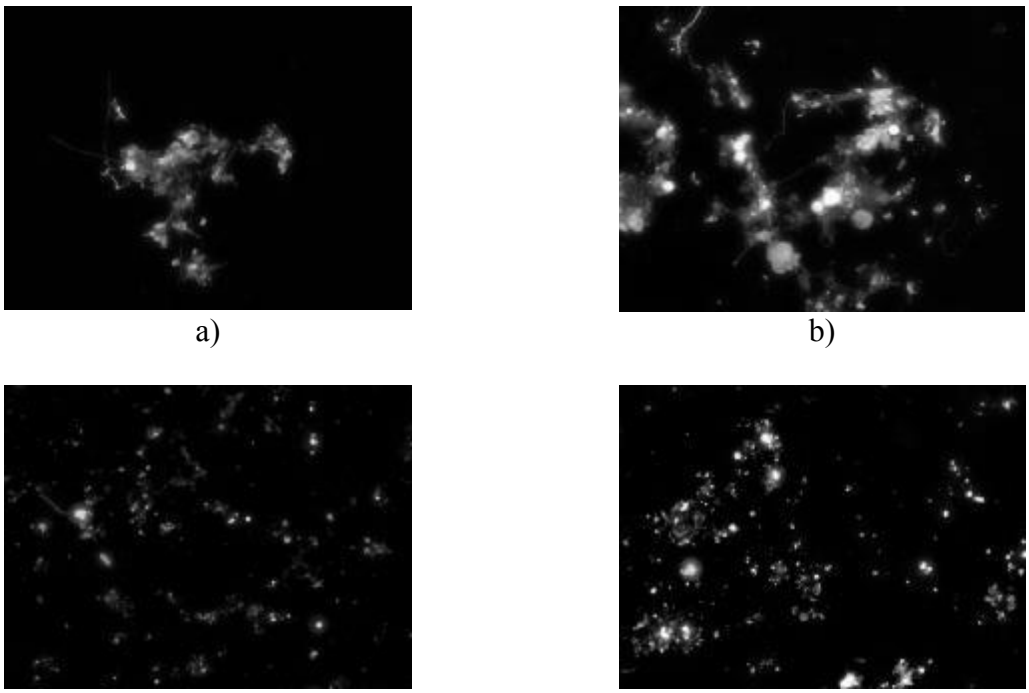

Fig. 2. FISH analysis of microorganisms from g. *Pseudomonas* in the two studied biofilm communities (red signal - bacteria from g. *Pseudomonas,* blue and blue-green signal - DAPI staining – all bacteria in the community): a) case study 1 with amaranth toxicant, early stage of the process; b) case study 1 with amaranth toxicant, late phase of the process; c) case study 2 with the phenol toxicant, early stage of the process; d) case study 2 with the phenol toxicant, late stage of the process. Photos are made at 400X magnification.

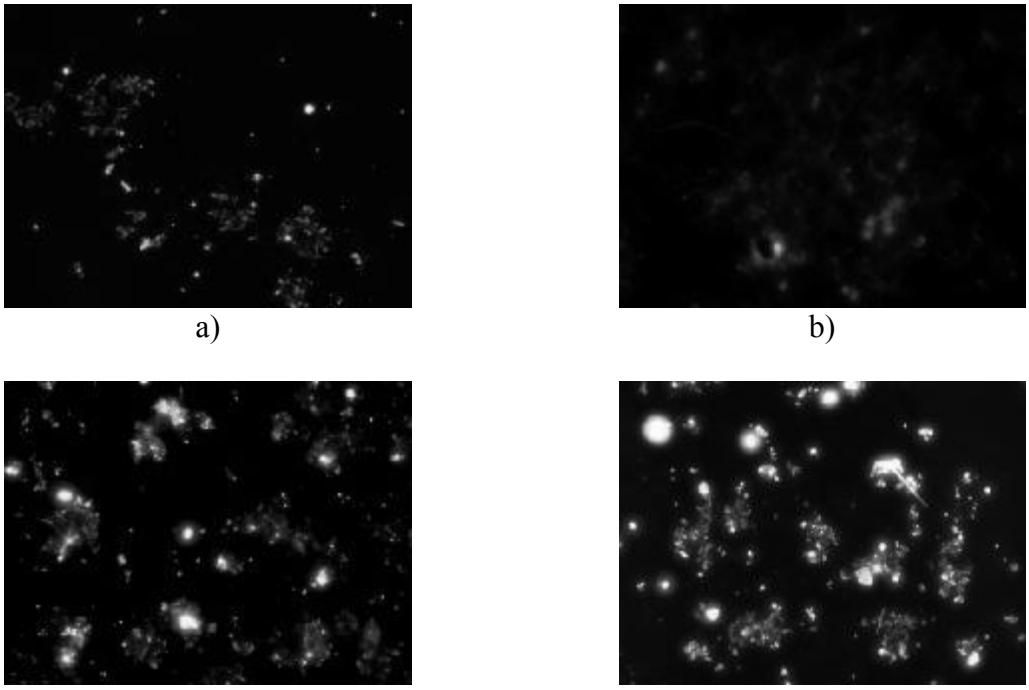

Fig. 3 DAPI staining in both studied biofilm communities: a) case study 1 with amaranth toxicant, early stage of the process; b) case study 1 with amaranth toxicant, late stage of the process; c) case study 2 with phenol toxicant, early stage of the process; d) case study 2 with phenol toxicant, late stage of the process. Photos are made at 400X magnification.

For the microorganisms from g. *Pseudomonas*, which are key for biodegradation of xenobiotics, we founded a decrease in their number in both studied cases. In the biofilter *Pseudomonas* sp. decreased from 1.09 x10^7 CFU/g in the early stage to 1.92 x10^6 CFU/g in the late phase, while in the bioremediation site the reduction was more significant – from 2.02 x10^4 CFU/g in the early stage to 7.66 x10^2 CFU/g in the late phase. The registered negative effect on both systems was based on various adaptation changes in the biofilm communities.

By means of the FISH analysis for the microorganisms from the g. *Pseudomonas* we found: 1/ in the sediment community from the late phase the fluorescent signal was weaker compared to the early stage of the process (Fig. 2c and 2d), i.e. the amount of culturable and non-culturable micro-organisms from g. *Pseudomonas* in-situ decreased; 2/ in the biofilm amaranth-degrading community during the late phase of detoxification not only the target microorganisms were more, but they aggregated in areas with high metabolic activity (Fig. 2b). With such activity of the microorganisms they contain a multitude of ribosomes, which cause the strong fluorescence. On the fluorescent image in Fig. 2b) those areas had pink colour precisely because of the overlaying of the strong red signal (FISH) on the strong blue one (DAPI). Since the cultivation analyses showed a decrease of microorganisms from g. *Pseudomonas* by 82%, we concluded that the increase founded by FISH was at the expense of non-culturable microorganisms from the target group.

Another interesting result from the viewpoint of adaptation was the accumulation of microorganisms. In microbial ecology it is known that this is indirect proof of established cooperative relationships (synergistic, symbiotic, co-metabolic) [17, 33, 28, 22, 10]. Such relationships were built in the highly adapted communities, in which the high degree of biodetoxification (90% efficiency of elimination of amaranth) is supported not only on the basis of an increased number of bacteria, but also of their synergistic cooperation [40, 43]. The establishment of such complex interactions was the reason why the quantity of the biodegraders couldn't be fully estimated by using culturing techniques [43]. So, despite their quantitative increase and their metabolic activation, by cultivation techniques we registered reduction, in this specific case – of the

amount of bacteria from g. *Pseudomonas* and azodegraders (Fig. 1).

For phenol-degrading microorganisms we founded an increase by 3.3 times ($7.5x10^4$ CFU/g during the early phase to 24,67 $x10^4$ CFU/g during the late phase) (Fig. 1). This was accompanied by an increase of the effectiveness of elimination of phenol from 26% to 30%. However, the effectiveness of removal of phenol was significantly lower than that of the Amaranth. This was related to the shock loading of the biofilm in the sediments and the absence of targeted adaptation of the system.

The comparison of both detoxification systems displayed the following differences: 1/ under shock loading the effectiveness of biodegradation was significantly lower in comparison to the system with applied targeted adaptation; 2/ under shock loading the system relies mainly on the adaptation mechanism in which a larger number of biodegraders decompose a larger amount of the toxicant; 3/ in targeted adaptation with step-by-step increase of the concentration of toxicant the system developed more complex mechanisms of adaptation including an increase of non-culturable microorganisms and microbial cooperative relationships.

In the presence of xenobiotics their toxic effect on the microbial communities was generally expressed as an inhibition of the metabolic processes in the cells, which was associated with changes of the rate of transformation according to the equation ATP $\rightleftarrows$ ADP+Pi. Because of this our interest concerned the amount of polyphosphates that the microorganisms accumulated as reserve substances. These substances were linked to the depletion and accumulation of the reserve energy sources polyhydroxybutyrate and polyhydroxyacetate. In the purification of water these processes are exploited for the purpose of accumulation of orthophosphates from wastewater in the cells of microorganisms (poly-P bacteria). Therefore specialized literature offers a variety of methods for assessing the metachromatic granules (polyphosphates). In this study we used fluorescence method to assess the amount and localization of the metachromatic granules as an indicator of the using the alternative energetic source as a proof of adaptation towards xenobiotic biodegradation.

The results obtained by the fluorescent method for testing polyphosphates based on staining with DAPI, showed that in gradual adaptation to biodegradation of the Amaranth, the microorganisms does not accumulate in their cells significant amounts of polyphosphates. This was demonstrated in Fig. 3 where the blue fluorescence signal of the biofilm was well visible. The community that developed in the conditions of a toxic press was more dependent on the reserve sources of energy. Therefore, the accumulation of polyphosphates was greater (green signal in Fig. 3c and 3d). It was also founded that in the adaptation of the communities the amount of polyphosphates increased while the process continued, as shown in Fig. 3d). The high amount of polyphosphates in the cells was indicative of the use of reserve sources of energy like polyhydroxybutyrate and polyhydroxyacetate. Therefore with the activation of the biofilm community after the period of severe intoxication, i.e. in its adaptation to these conditions, the microorganisms depended to a greater extent on reserve sources of energy.

CONCLUSIONS

In the present article we made a comprehensive assessment of the adaptive changes in two types of biofilm communities that were formed in different simulated risk situations - the situation of shock xenobiotic load with phenol and situation of purposeful adaptation to the biodegradation of amaranth. The focus was on the results, showing the key differences in the adaptive mechanisms of the two different biofilm microbial communities and related with this effectiveness of biodegradation. The obtained results showed: 1/ the effectiveness of elimination of xenobiotic in the case study with target adaptation was 3.2 times higher compared to that in the option with shock loading; 2/ in targeted adaptation of the biofilm, community developed to the extent that biodegradation of the toxicant was realized by means of complex mechanisms involving an increase of the amount of non-culturable biodegraders and the formation of cooperative microbial relationships; 3/ at concentrations of the toxicant exceeding the critical ones the microbial community was dependent to a larger extent on reserve sources of energy and adaptation took place mostly on the basis of an increase the number of culturable biodegraders.

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АДАПТАЦИОННИ МЕХАНИЗМИ НА БИОФИЛМНИТЕ СЪОБЩЕСТВА В ТЕХНОЛОГИИ СЪС СТЕПЕННО И ШОКОВО ПОВИШАВАНЕ НА КОНЦЕНТРАЦИЯТА НА КСЕНОБИОТИЧНИТЕ ЗАМЪРСИТЕЛИ-ФУНКЦИОНАЛЕН И FISH АНАЛИЗ

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Резюме: При биодетоксикацията на ксенобиотици в различни биоремедиационни технологии основен фактор за ефективността и ефикасността на пречистването е концентрацията на токсиканта в зависимост от критичните й стойности за дадената биологична система, начинът и пътищата за постъпване и натрупване в средата – степенно /кумулативно/ или шоково /взривно/.Настоящото изследване е функционално и микробиологично сравнение на адаптивните изменения на биофилм в две лабораторни моделни биодетоксикационни технологии: *Case study 1* –биоремедиационна площадка за биодетоксикация при шоково натоварване с фенол в концентрация три-кратно надхвърляща критичната (около 250 mg/g седимент); *Case study 2* –пясъчен биофилтър, третиращ води замърсени с азо-багрилото амарант. Като инокулационен материал е използвана специално обработена активна утайка от пречиствателната станция за отпадъчни води на гр. София. В този случай е предложен адаптационен алгоритъм със степенно увеличаване на концентрацията на токсиканта до достигане на критичната за системата (от 5 до 50 mg/L амарант).

Двете детоксикационни технологии са сравнени по: ключови технологични и микробиологични параметри, микроразпределение и роля на некултивируемите микроорганизми, а също и междуорганизмови взаимоотношения в р. *Pseudomonas* (изследвани чрез FISH). Обърнато е специално внимание на анализа на полифосфатите като индикатор за резервните енергитични източници – полихидроксибутират и полихидроксиацетат (флуоресцентен метод).

Получените данни показват, че при системата с фенол средната ефективност на елиминиране на ксенобиотика е 28 %, докато при системата с адаптационен алгоритъм е 89 %. Култивационните техники показаха увеличение на фенол-разграждащите микроорганизми с 1,72 x10^5 CFU/g и намаляване на амарант-разграждащите с 1,67x10^6 CFU/g в хода на адаптивните детоксикационни процеси. Чрез анализ на количеството на метахроматина беше установено, че системата, функционираща в условия на токсичен шок, е по-зависима от наличието на алтернативни енергитични източници в сравнение с биофилмните съобщества със степенна адаптация към биодеградация.

Резултатите показват, че механизмите на адаптация към ксенобиотична биодеградация се различават в зависимост от пътищата и начините на навлизане на ксенобиотици в системите: 1/ При шоково натоварване с ксенобиотик в концентрации трикратно надхвърлящи критичната се проявява най-простият механизъм на адаптация чрез увеличаване броя на микроорганизмите, а ефективността на биодетоксикация е ниска; 2/ при прилагането на целево конструиран адаптационен алгоритъм микробното съобщество проявява висока ефективност на елиминиране на ксенобиотика и развива комплексни механизми на азо-детоксикация, включващи повишена роля на некултивируемите микроорганизми и на синергетичните взаимоотношения между тях.

Ключови думи*:* детоксикация, адаптационен алгоритъм, *Pseudomonas*, FISH, полифосфати

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