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REVIEW PAPER

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BIOLOGICAL CONTROL AND MANAGEMENT OF THE DETOXICATION WASTEWATER TREATMENT TECHNOLOGIES*

Detoxication technologies require the combination of theoretical and practical knowledges of xenobiotic biodegradation, wastewater treatment technologies, and management rules. The purpose of this complicated combination is to propose specialized strategies for detoxication, based on lab– and pilot–scale modeling. These strategies include preliminary created algorithms for preventing the risk of water pollution and sediments. The technologies and algorithms are essentially important outcome, applied in the textile, pharmaceutical, cosmetic, woodtreating, and oiltreating industries.

In this paper four rehabilitation technologies for pretreatment of water contaminated by pentachlorophenol (PCP) have been developed in the frame of the European and Bulgarian National projects. Emphasize is put on the biological systems and their potential of detoxication management. The light and transmission electron microscopy of the reconstructed activated sludges, the microbial, kinetic and enzymological indicators are presented and approved as critical points in the biocontrol.

The management of wastewater treatment technologies with detoxication element includes several key points:

1) Application of the highly specialized adaptive algorithm for the reconstruction of the AS (activated sludge). This algorithm is directed towards induction and realization of the biodegradation potential of the biological system, according the rules of xenobiotic biodegradation theory.

2) It is important to create specific parameters of the technologies that according to the mechanisms of the xenobiotic pollutants. As a whole this directs the modules of the technology.

3) It is necessary to pay attention on the critical concentrations of toxic pollutants independent on their complexity and chemical structure.

4) The detoxication technologies have to be equipped with purposely designed parameters, indicators for control, that are more complicated than the trivial ones.

In the presented paper the mechanisms of building up and functioning of the biological system of four PCP–detoxication technologies have been discussed. Pentachlorophenol (PCP) was chosen as a toxic pollutant model due to its wide application in various industries and persistence in the environment [1,2]. Twelve criteria based comparison according has been accomplished in order to help the specialists dealing with industrial wastewater treatment.

The four technologies are developed in the frame of International Inco–Copernicus Progect [1] by the interdisciplinary team and full description of the technologies has been made in our previous papers [2–8]. The joint element of the four technologies is that the model xenobiotic was PCP, as well as their common system developed for biological control purposes. This creates the possibility of elucidating the mechanisms of the processes and based on this to accomplish their regulation and management.

EXPERIMENTAL PART

Aerobic and anaerobic technologies have been realized in the mini lab–scale, located on the area of Department of General and Applied Hydrobiology – Sofia University, consisting of full mixed bioreactors and secondary clarifiers. The parameters of aerobic technology were – real wastewater taken from the Sofia Wastewater Treatment Plant (SWWTP) and supplied with 25 mg/l PCP, COD of influent – 2076 mgO_2/l, volume 4 l, initial concentration of AS – 3.3 g/l, retention time – 3.9 days, T – 25 °C, pH – 6.8, age of AS – 40 days. The aerobic AS was supplied from biobasins of SWWTP. **The anaerobic technology** parameters were: real wastewater taken from SWWTP, supplied additionally with 25 mg/l PCP, COD of influent – 2188 mgO_2/l, volume – 4 l, initial concentration of AS – 4.2 g/l, retention time – 3.7 days, T – 25 °C, pH – 6.8, age of AS – 67 days. Initial AS was taken from AS–thickeners of SWWTP. For the two technologies the AS initially was subjected to adaptation

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towards lab–scale for one month. The adaptation towards PCP was again one month. After that the biomanipulation procedure has been accomplished in order to increase the efficiency of PCP–detoxication – fresh laboratory selected biodegradant *Pseudomonas aureofaciens AP–9* biomass has been added into the aerobic bioreactor. Nitrogen gas was introduced into the anaerobic bioreactor in order to decrease the redox potential.

An algorithm for adaptation of AS towards PCP–biodegradation has been developed for the **two stages anaerobic/aerobic technology**. This algorithm consisted of two phases. **First**, the initial concentration – 2 mg/l PCP has been added to the bioreactor. After the biodegradation of this amount of PCP a new portion of 2 mg/l has been added again. The degradation of the portions of 2 mg/l was monitored six times at the shortening of the time interval. **Second**, the concentration of PCP gradually has been increased to 10 mg/l (see Figure 6). The rest of the anaerobic stage parameters were: real wastewater from Sofia Waste Water Treatment Plant supplied by PCP, bioreactor volume – 4 l, SBR (sequencing batch reactor), AS concentration – 10 g/l, pH – 6.8, T – 25 °C. For the aerobic stage – real water with COD – 200 mgO_2/l, retention time – 8 h, DO – 3 mg/l, and three– and di–chlorophenols have been presented in the influent.

The specific aeration system in the **hybrid technology** created zones with various redox potential with light transition among them. The other parameters were: bioreactor volume – 43 l, COD – 556–572 mgO_2/l, increased concentration of PCP in the influent from 0.5 to 37 mg/l, SS – from 4.2 to 5.2 g/l, pH – 8.5, retention time – 2 days. The hybrid bioreactor is shown in Figure 1.

Chemical and Kinetics Parameters: The residual amount of PCP in the watery phase, PCP–accumulation in cells has been determined in different points of sampling by means of HPLC [9]. PCP–removal and PCP–biodegradation has been calculated according to the previous described procedure [8]. COD and SS have been determined according APHA [10].

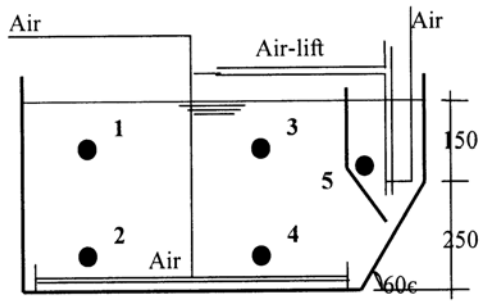

Figure 1. Technological scheme of the hybrid bioreactor and CCP (Critical control points) of sampling: CCP–1 and CCP–3 – anaerobic zones; CCP–2 and CCP–4 – aerobic zones; CCP–5 –settlement zone.

Microbiological Control: **first**, microscopic control with differentiation of aerofile zone and TEM analysis [7]; **second**, counting of the physiological and taxonomic groups of bacteria crucial for the processes (*heterotrophic aerobic (AH)*, *heterotrophic anaerobic bacteria (AnH)*, *bacteria from genus Pseudomonas (Ps)* and *Acinetobacter (Acinet)*, *sporeforming bacteria (Spor.)*, family *Enterobacteriaceae (Endo)*, *NH_3–releasing (NH3–rel.)*, *denitrifiyng (Denitr.)*, *nitrifying (Nitr.) bacteria*) was determined according to the routine microbiological practice [11]. The amount of the PCP–degrading (PCP–degr.), were determined on the solid medium according to Furukawa [12], with PCP (0.16 mMol) as a sole carbon and energy source).

Enzymological indicators: The crude cell extracts were obtained by sonic disruption of AS according to the well described methods [13]. The enzymological control–design has been developed and realized based on the following hypothesis. A broad spectre of oxygenases, dehydrogenases, specific oxydoreductases and dehalogenases has been included in the bioindication – enzyme activities that we have approved as key indicators for the catalysing of the critical steps of PCP–detoxication in our previous investigations [2,7,8]. The investigated enzyme activities and the used methods were: Phenolhydroxylase (PHH) (EK 1.14.13.7) [14], Succinate dehydrogenase activity (SDH) [15], Catechol 1,2–dioxygenase (C12DO) (EK 1.13.11.1) [16], Catechol 2,3–dioxygenase (C23DO) (EK 1.13.11.2) [17], Protocatechate 3,4–dioxygenase (P34DO) (EK 1.13.11.3) [13], DH /Dehalogenase activity/ [7]. The protein contents of the samples were determined by the method described by Herbert et al. [15]. All chemicals were supplied by Merck (Germany) and Fluka (Germany).

RESULTS AND DISCUSSION

One–stage aerobic and anaerobic technologies: The initial concentration of PCP was high – 25 mg/l and there are no specialized algorithms for adaptation of AS towards PCP. The only factor is the time of adaptation. After one month the AS of the two technologies developed resistance towards PCP at this high concentration. The biodegradation activity remains low and undeveloped. It is mainly in the frame of constitutive detoxication potential that is conjugated and depends on the increase of homogenic cells from *Pseudomonas* and *Acinetobacter* genuses.

An insignificant part of the inductive enzymes are expressed, which is the reason for the low efficiency of PCP–detoxication – for anaerobic 47% and for aerobic 55% (Figure 2).

The data in Figure 3 illustrates that, like in the aerobic technology adaptive reaction, the amounts of

aerobic heterotrophic, anaerobic heterotrophic bacteria, g. *Pseudomonas* and *Acinetobacter* and PCP–degrading bacteria are increased. The microbial density of anaerobic heterotrophic bacteria, g. *Acinetobacter* and PCP–degrading bacteria are increased at anaerobic technology.

With correspondence of microbial increase the constitutive and only partly inductive biosynthesis of the key for the detoxication enzymes are increased – PHH, C12DO, C23DO, P34DO, SDH, DH (Figure 4).

The biological manipulations in both bioreactors (adding of fresh biomass of *Pseudomonas aureofaciens* AP–9 in aerobic and decrease of redox potential in anaerobic bioreactors) have an insignificant effect on the efficiency of PCP–detoxication. That is why manipulations are not appropriate from an economical and technological point of view. Figure 5 shows the changes of the microscopic structure of AS for the aerobic and anaerobic technologies.

Two–stage anaerobic/aerobic technology: In the first anaerobic stage of this technology a special adaptive two–phase algorithm has been applied. In the first phase the PCP (low concentration – 2 mg/l) has been added six times. The time for complete biodegradation of every portion of PCP has been followed strictly. In the second phase the concentration of PCP was increased step by step. In parallel the control was applied in order to be sure that every portion PCP had been completely degraded without any accumulation in the watery phase or in AS. That

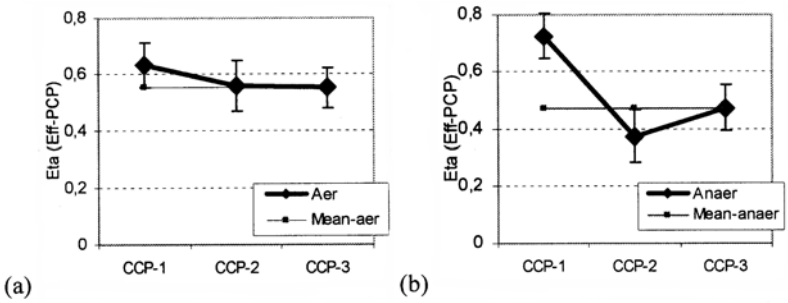

Figure 2. Comparison of affectivity of PCP–removal in aerobic (a) and in anaerobic (b) technology in three CCP (Critical control points): CCP–1 – initial phase of addition of PCP in the influent; CCP–2 – phase after one month adaptation towards PCP (25 mg/l); CCP–3 – after biological manipulation of the processes.

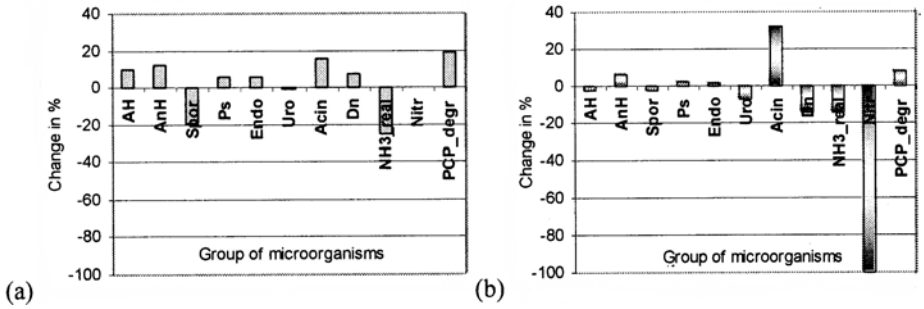

Figure 3. Comparison of change (in %) of the amount of the key microorganism groups for wastewater treatment process after one month of adaptation of AS towards PCP: (a) aerobic technology; (b) anaerobic technology

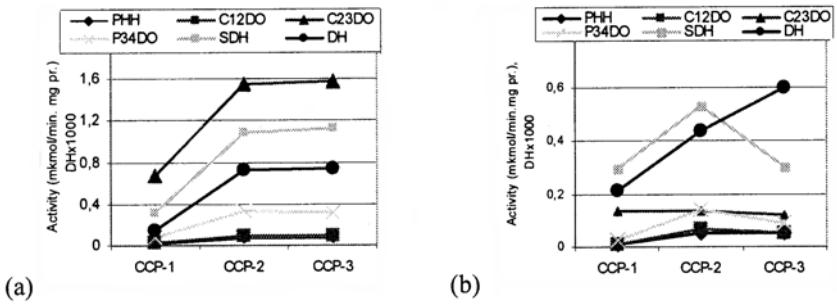

Figure 4. Dynamic of enzyme activity of AS in the course of aerobic (a) and anaerobic (b) technologies for PCP–detoxication in three CCP: CCT–1 – initial phase of addition of PCP in the influent; CCP–2 – phase after one month adaptation towards PCP (25 mg/l); CCP–3 – after biological manipulation of the processes

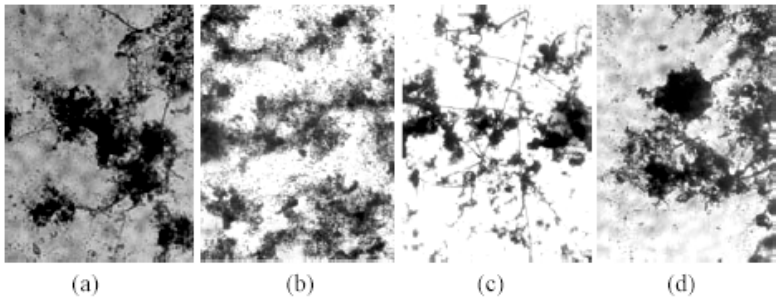

Figure 5. Changes in microscopic structure of AS in the course of the aerobic and anaerobic technology for PCP–removal: (a) Aerobic technology – initial floccular structure (400X); (b) Aerobic technology – deflocculated AS in the process of adaptation towards PCP LM (400X); (c) Anaerobic technology – AS with floccules with very high density (400X); (d) Anaerobic technology – Floccules with very high density LM (803X).

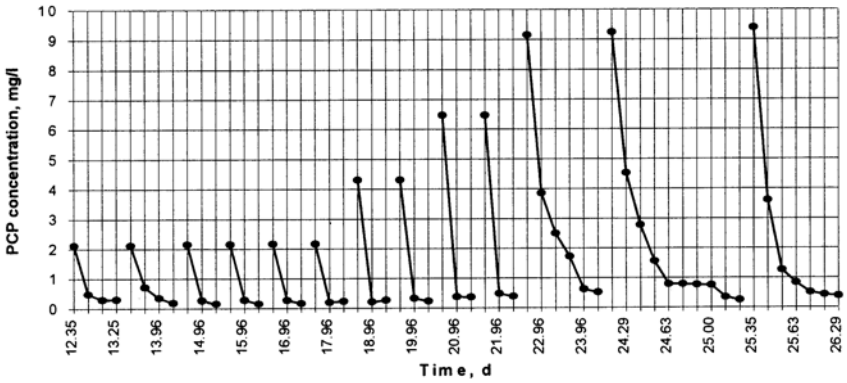

Figure 6. Algorithm of adaptation of AS towards biodegradation of PCP. The procedure was accomplished by means of increase of PCP–concentration from 2 to 10 mg/l and shortening of time for biodegradation at semicontinuous regime (batch regime with gradual adding of PCP)

way AS that can degrade 10 mg/l PCP for 4 hours was gradually created. This technological achievement allows the anaerobic reactor to be included in function of SBR. The adaptive algorithm is shown on Figure 6.

The low–chalogenated and non–chalogenated products are generated in the anaerobic stage. These products are flowed in the second aerobic stage, where they are degraded completely in harmless products of the central catabolic pathways [12,14]. So the developed and applied adaptive algorithm allows the two–stage PCP–detoxication technology that completely degrades PCP to start and the treated waters can flow in the water receiver. The scheme of this technology is shown on Figure 7.

The applied specialized biological control at the time of adaptation showed that when anaerobic AS possessed the highest PCP–biodegradation activity, PCP–degraded bacteria could not be registered by means of trivial culture methods (Figure 8a). This paradox can be explained by the results of light and transmission electron microscopy (TEM) analysis. The two phase AS has been ascertained – pellets and homogenic microbial cells (Figure 8b). In the pellets a structure similar to that of tissue can be seen (Figure 8c). The microbial cells and populations in the pellets constructed a synergetic consortium with high specialization and jointly realization of PCP–degrading function.

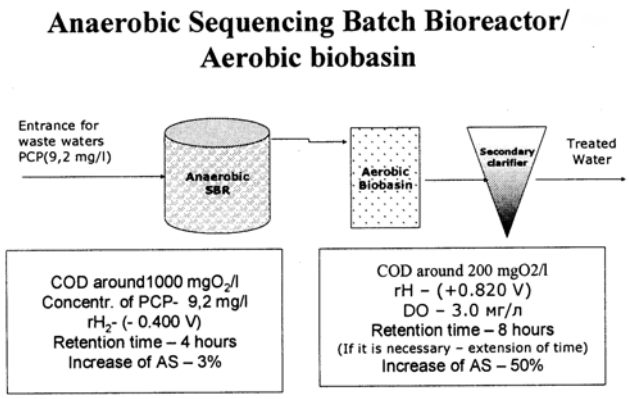

Figure 7. Scheme of the two stage anaerobic/aerobic technology for detoxication of PCP

The strongly increased dehalogenase function at the course of adaptation is illustrated by the gradual increase of dehalogenase activity (Figure 9).

So in the anaerobic bioreactor the two phases of PCP–biodegradation are localized in different biological structures. The anaerobic dehalogenation is

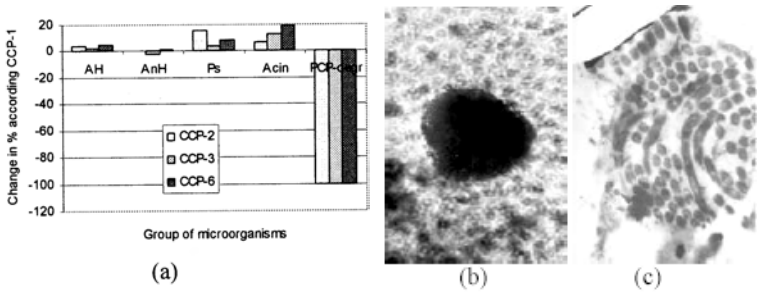

Figure 8. Changes of the amount of the key for wastewater treatment and detoxication processes groups of microorganisms in four CCP (Critical control points) – (a) changes in %; (b) two phase AS – pellet and free swimming bacterial cells LM (800X); (c) internal synergetic structure of the pellets TEM (12000X); CCP–2 – AS adapted towards 2 mg/l PCP at batch regime; CCP–3 – AY adapted towards 2 mg/l PCP at semicontinuous regime; CCP–6 – AS adapted towards 10 mg/l PCP at semicontinuous regime.

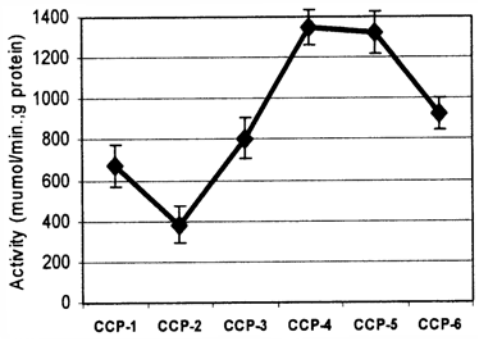

Figure 9. Dynamics of dehalogenase activity in different CCP: CCP–1 – AS in 0 moment without PCP; CCP–2 – AS adapted towards 2 mg/l PCP at batch regime; CCP–3 – AY adapted towards 2 mg/l PCP at semicontinuous regime; CCP–4 – AS adapted towards 4.3 mg/l PCP at semicontinuous regime; CCP–5 – AS adapted towards 9.2 mg/l PCP at semicontinuous regime; CCT–6 – AS adapted towards 10 mg/l PCP at semicontinuous regime.

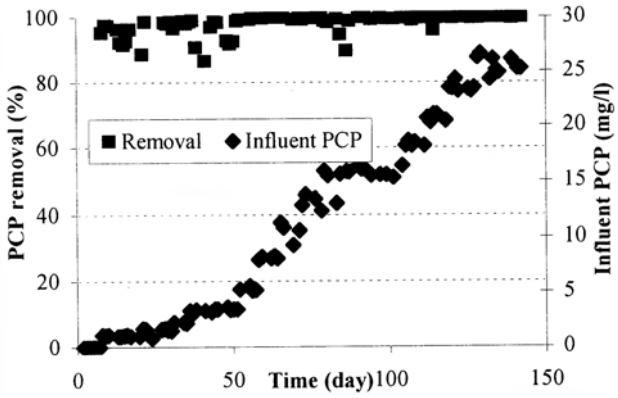

Figure 10. Elimination of PCP and PCP–concentration in the influent at the time of adaptation of AS. The figure is reproduced with permission [1, 6].

accomplished in pellets. In homogenic cells the benzene cleavage of non–halogenated and low–halogenated has been accomplished. This process in the anaerobic bioreactor is low–rate because of strong oxygen limitation. The catabolizing of the aromatic products of PCP continues in the aerobic bioreactor and the detoxication process is completely finished there. So the applied biological control allow the decoding and creating of specialized biological structures that can accomplish the detoxication with high velocity in two phases – adequate of the mechanisms of PCP–biodegradation.

Hybrid technology: Two new moments can be seen in this technology – **first**, the bioreactor with a specific aeration system is created, which allows the zones with different redox potential to be formed; **second**, the specialized long (111 days) adaptive procedure has been applied. It ensures the control according to the concentration of PCP in the influent, the residual concentration of PCP in water and in AS and time for degradation of every portion of PCP. Figure 10 shows the dynamic of PCP in the influent and efficiency of PCP–elimination at the time of adaptation.

As a result of the applied adaptive algorithm a four component biological system, highly specialized towards PCP–detoxication, has been created.

Component A: Consisted of free–swimming bacterial cells, the essential part of them are adsorbed to the filaments of AS. This skeleton–like component (background) is located throughout the bioreactor and consists mainly of bacteria from genera *Pseudomonas*, *Acinetobacter* and PCP–degrading bacteria. These three groups play an important role in the complete biodegradation of PCP as well as of low halogenated metabolic products of PCP.

Component B: Very well developed colonies consisting of homogenous bacteria, localized around filaments, belonging to the genera *Pseudomonas*, *Acinetobacter* and PCP–degrading bacteria. They carry out the same function like the bacteria of component A, but with high rate. Such structure we found for the first time in the AS structure.

Component C: Well formed floccules in which all the key microorganism groups typical for the AS can be found. In the floccules the amount of AH, AnH, PCP–degrading, 2,4,5–trichloro–degrading, 3,4–dichloro–degrading bacteria from g. *Pseudomonas*, *Aci-*

netobacter are very well presented. In this higher rate component of AS the aerobic processes of PCP–transformation and catabolism of its metabolic products – low halogenated and non – halogenated aromatic compounds are accomplished.

Component D: Very well packed floccules, with very strong density like pellets. These formations were very hard to disrupt by ultrasonic disintegration. These structures prevailed in the anaerobic part of the bioreactor. In these structures the anaerobic dehalogenation is most probably accomplished with high velocity because of the synergetic anaerobic consortium. So the different mechanisms of PCP–biodegradation are localized and divided in different components of AS. There is an internal strong specialization in the separated micronishes. These results on a biological structure level confirmed exclusively harmonious distribution of the separated mechanisms and metabolic pathways of PCP–biodegradation. The localization and the rate of these mechanisms are developed in maximal degree and its management depends on the gradient of redox potential, flows of acceptors of electrons and hydrogen ions. The factors that play an important role in this management are: 1) the specialized adaptive algorithm, ensured gradually and maximal development of the detoxication potential; 2) professional regulation of xenobiotic concentration in the influent and residual amount in the bioreactor; 3) availability of appropriate cometabolite, supporting of the biomass and the constitutive mechanisms of PCP–detoxication. Such high professional control and management transforms the hybrid technology into a "functional masterpiece", in which all evolutionary developed possibilities for PCP–transformation are expressed and ensured.

TEM structure of the essential structures of AS is shown on Figure 11.

In the course of the adaptive procedure the complicated relationships among microorganisms and between microorganisms and micro and metafauna, like cometabolic cooperation and syntrophy have been formed. The transition from aerobic to anaerobic conditions has been created in the bioreactor, which ensures transition from aerobic to anaerobic mechanisms of PCP–transformation. More zones in the bioreactor really are with mixed functions because of different density of various biological elements of AS. In the bioreactor equal distribution of the key microorganisms for wastewater treatment and detoxication processes has been ascertained (Figure 12). The bacterial functions are regulated in a

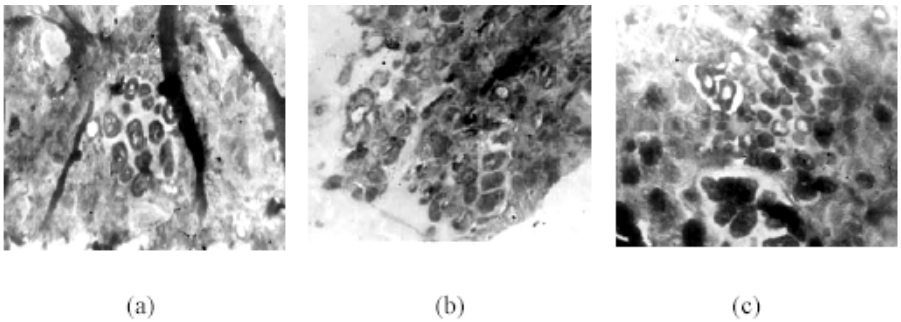

Figure 11. Typical structures of the AS in the hybrid bioreactor generated in the course of adaptation towards biodegradation of high PCP–concentration: (a) CCP–1 – Filaments and adsorbed colonies, TEM – 12000X; (b) CCP–3 – Internal structure of anaerobic pelet TEM – 12000X; (c) CCP–4 Friability in the structure of the floccules in the aerobic TEM – 12000X.

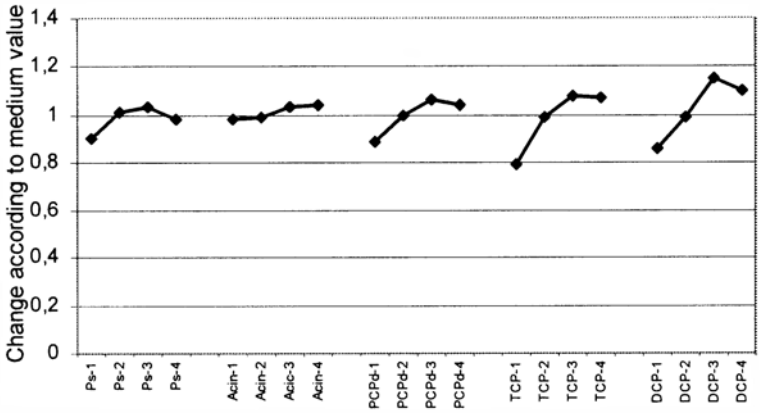

Figure 12. Change in the key groups of microorganisms for the detoxication process towards medium value for the corresponding group in the anaerobic and aerobic zones of the hybrid bioreactor

complicated way in harmony and depending on the location in the specific structures. The complex of fauna is stable and with high diversity [7]. In transition from aerobic to anaerobic zone it decreases with 60%, but in both zones it is in the position to carry out the function of the protector, distributor and participants in the biodegradation process [1,2,7]. Distribution of PCP–detoxication function is in environmental micronishes on the biological principle – compartmentalization.

The relatively equal distribution of the PCP–transformation activity in the hybrid bioreactor was proved by means of the oxygenase and succinatdehydrogenase activity. Regardless of the fact that their values varied in different zones, the total activity as a whole is high and depends on local PCP–concentration, redox potential, gradients of electrons and hydrogen (Figure 13).

The comparison of the four described technologies according to the most important advantages and disadvantages is shown in Table 1.

Logically, a question arises of the receptivity and applicability of the above mentioned biological information by a broad circle of professionals – including such building up bioreactors, realizing the scale–up, applying and financially ensuring the detoxication technologies – engineers, technologists, investors, business world. So we come to the fact that biologically decoded biological systems have to be understood

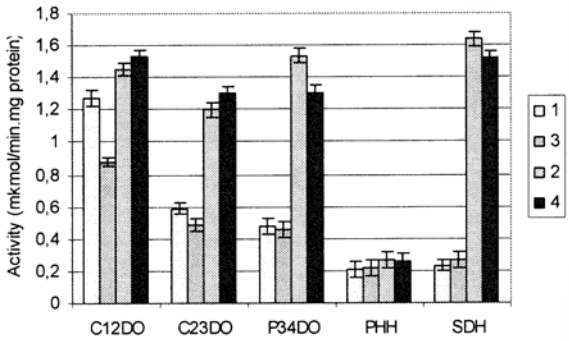

Figure 13. Oxygenase and Succinatedehydrogenase activity in anaerobic and aerobic zone of hybrid bioreactor in the five CCP: CCP–1 and CCP–3 – anaerobic zones; CCP–2 and CCP–4 – aerobic zones; CCP–5 – settlement zone.

Table 1. Comparison among four technologies for detoxication of PCP

Criteria	Aerobic technology	Anaerobic technology	Two stage Anaerobic/aerobic	Hybrid technology
Criteria 1 "*Adaptation algorithm*"	NO Specialized Adaptation algorithm	NO Specialized Adaptation algorithm	Specialized adaptation algorithm in two phases with factor PCP–concentration and time	Specialized adaptation algorithm in four phases with factor PCP–concentration and time and redox potential
Criteria 2 "*Effectivity of PCP–elimination*"	55%	47%	96%	98–100%
Criteria 3 "*Effectivity of COD–decrease*"	83%	41%	98%	95–97%
Criteria 4 "*Concentration of PCP in the influent*"	25 mg/l	25 mg/l	10 mg/l	35–37 mg/l
Criteria 5 "*Concentration of PCP in the effluent*"	11 mg/l	12.8 mg/l	0–0.2 mg/l	0–0.35 mg/l
Criteria 6 "*Structure of the activated sludge*"	Floccular, a lot of free swimming cells, turbid effluent	Floccular with thick anaerobic zones, turbid effluent	**Anaerobic stage**–two component AS – pellets and homogenic cells; **Aerobic stage** – floccular AS, clear effluent	Three component AS – filaments with adsorbed cells and colonies; pellets, floccules and transition zones
Criteria 7 "*Bacterial segment*"	Best adapted are the homogenic cells from g. *Pseudomonas* and g. *Acinetobacter*	Best adapted are the homogenic cells from g. *Acinetobacter* and g. *Pseudomonas* and anaerobic heterotrophs like PCP–degraders	Take shape peletswith syntrophic PCP–degraded function and homogenic cells from g. *Acinetobacter* and *Pseudomonas*	g. *Pseudomonas* and *Acinetobacter* homogenic and adsorbed to the filaments colonies, syntrophy in the pelets, metabolic cooperation, localization in specialized ecological nishes

Criteria	Aerobic technology	Anaerobic technology	Two stage Anaerobic/aerobic	Hybrid technology
Criteria 8 "*Segment of miro– and metafauna*"	Strongly reduced according to the quantity and quality	Strongly reduced according to the quantity and quality, presence of *Oikomonas socialis* (14 ind./cm^3) *Bodo erectus* (50 ind./cm^3)	Strongly reduced according to the quantity and biodivesity	Well formed communities of micro– and metafauna
Criteria 9 "*Mehanisms of PCP–biodegradation*"	Oxidative and hydrolytic generally in the homogenic cells and surface of the floccules	Anaerobic reductive and hydrolitic	Reductive and hydrolytic mechanisms in very limited degree oxydative	Oxydative, hydrolytic and anaerobic mechanisms with different rate in various structures
Criteria 10 "*Mechanisms of management and control*"	Easy conventional control	Easy conventional control	Two stage, complicated control	Easy conventional control
Criteria 11 "*Applicability*"	As a pre–treatment, not completely finished technology	As a pre–treatment, not completely finished technology	Completely finished technology, the treated water can flow in the water receiver	Completely finished technology
Criteria 12 "*Risk for water receiver*"	High degree of risk	High degree of risk	Low degree of risk	Low degree of risk

*At two stage technology the data for anaerobic step are discussed in details Low degree of risk

and applied by non biologists. The contemporary tendency in the biological and ecological management is the necessity of competent translation of extracted scientific biological information into the language of practice. This will discover more possibilities to use this information for the real management of risk situations. Such applicable information is proposed in the form of algorithms and criteria, described creation and application of effective detoxication biological systems. These algorithms are valuable commercial products and are an irrevocable part of the technologies.

In this aspect Table 1 shows the possibility of transfer of biological information to practice. The ascertained biological dependences are presented as criteria that can be used for choice, offering and investment in concrete detoxication technology.

CONCLUSION

The discussed results have shown that purposely designed development of biodetoxication potential has general principles, that decoded in different biological systems can be applied for the practical management of wastewater detoxication treatment processes as well as for creation, application and exploitation of highly specialized "biologically progressive" detoxication technologies.

As it became clear from the analysis of the biological systems, the application of the selective adaptive algorithm is the decisive factor for gradual development of the extensive (constitutive) and the intensive (inductive) biodegradation detoxication potential. The inclusion of this potential is the crucial mechanism in the creation and realization of one effective detoxication technology. The adaptive algorithm is the essential part of the all technology. The adequate selection and biological control of this mechanism is a guaranty for carrying out all technology as well as for real protection of the water receivers from the risk of flowing of xenobiotics.

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IZVOD

BIOTEHNOLOŠKA KONTROLA I UPRAVLJANJE TEHNOLOGIJAMA TRETMANA DETOKSIKACIJE OTPADNIH VODA

(Pregledni rad)

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Tehnologije detoksikacije zahtevaju kombinaciju teorije i prakse ksenobiotske biorazgradnje, tehnologija tretmana otpadnih voda i pravila njihovog upravljanja. Svrha ove složene kombinacije je da predloži posebne strategije detoksikacije, a razvijene na osnovu modelovanja na laboratorijskom i pilot postrojenju. Ove strategije uključuju preliminarno kreiranje algoritma za sprečavanje rizika od zagađenja vodom i talogom. Tehnologije i algoritmi imaju izuzetnu važnost za tekstilnu, farmaceutsku, drvnoprerađivačku i industriju boja. U ovom radu su, u okviru evropskih i bugarskih nacionalnih projekata, razvijene četiri tehnologije za oporavak predtretmanom vode, zagađene pentahlorfenolom (PCP). Naglasak je stavljen na biološke sisteme i njihove mogućnosti za upravljanje detoksikacijom. Fotografije svetlosne i transmisione elektronske mikroskopije, aktivirano blato, mikrobni, kinetički i eznimološki indikatori su prikazane i potvrđeni kao kritične tačke u biokontroli.

Ključne reči: Tehnologije detoksikacije • Pentahlorfenol • Aktivirano blato • Mikrobiološka i enzomološka kontrola •

Key words: Detoxication technologies • Pentachlorophenol • Activated sludge • Microbiological and enzymological control •