Microbial transformation of xenobiotics

Izabela GREŃ – Department of Biochemistry, Faculty of Biology and Environmental Protection, University of Silesia, Katowice

Please cite as: CHEMIK 2012, **66,** 8, 835-842

Introduction

The term xenobiotic (Greek *xenos* + *bioticos*, which means "strange" and "life-related" respectively) means a chemical substance that is not a natural component of a living organism exposed to it, i.e. a strange, exogenous substance or anthropogenic material. This definition covers substances strange to the target organisms, hence its use for most poisons and drugs. An important group of xenobiotics are chemical compounds produced by humans, with artificial chemical structure, to which organisms have not adjusted through prior evolution [1]. But can we always say with certainty that a given chemical structure cannot be found in the natural environment? One of the key groups of xenobiotics are aromatic compounds with chlorine substituent. At the same time, the *Amblyomma americanium* tick produces 2,6-dichlorophenol as pheromone and the 2,5-dichlorophenol in the same function was also found in grasshoppers [2]. Therefore, for the microorganisms the xenobiotics are generally defined as all substances with the structure with which the microorganism has had no direct contact during their evolution [1]. It should also be pointed out that not all xenobiotics are necessarily toxic [3]. Due to its structure, strange to the natural microorganism populations, xenobiotics released into the environment can linger there for months or years. However, this does not mean that those substances are never degraded, since not all anthropogenic substances are resistant to microbiological degradation. The horizontal gene transfer and other adaptive processes allow the microorganisms to participate in the transformation of xenobiotics processes.

Basic terminology of transformations of xenobiotics

The literature of the subject of elimination of various contaminants from the environment with the use of microorganisms exhibits a certain confusion of terms applied to the processes of transformation of xenobiotics. Biodegradation, mineralisation or transformation are used interchangeably, which is partially unjustified and at the same time causes some confusion. The term mineralisation is usually understood as the complete decomposition of an organic compound into inorganic elements, while biodegradation is a process taking place with the participation of living organisms that also involves the decomposition of organic compounds into inorganic elements, but with the simultaneous accretion of biomass. The term (bio)transformation, on the other hand, is understood as the process leading to the change of the structure of the original chemical compound to such degree that its original characteristic properties change as well. The (bio)transformation process modifies not only the physico-chemical properties of compounds, such as solubility or (bio)availability, but also the toxicity level of the given xenobiotic [3].

If the given compound undergoes biodegradation, it is then used as the source of carbon, nitrogen, phosphorus or sulphur and energy by the microorganism conducting the process, thus acting as a growth substrate. It often happens in the natural environment that those degradation processes are accompanies by transformations of other compounds, other xenobiotics. This phenomenon is defined using various terms, such as cometabolism, co-oxidation, or "accidental" or "free" metabolism. Usually the term cometabolism is reserved for partial transformation processes, i.e. transformation of xenobiotics in the presence of compounds serving as the sources of carbon and energy, and those processes are not accompanied by biomass accretion that would result from the decomposition of the growth substrate [3, 4]. At times, xenobiotic cometabolism can produce substances that are more easily oxidised than the original chemical compound. With a number of subsequent cometabolic reactions, often conducted by various microorganisms, the xenobiotic, which in the presence of a single strain would only undergo minor transformation, might become completely mineralised [5]. In 2003 Aranda et al. [6] described the *Sphingopyxis chilensis* S37 and *Sphingopyxis chilensis* S232 strains that, if both present in the culture at the same time, conducted complete mineralisation of the non-growth substrate of 2,4,6-trichlorophenol. The strains did not degrade 2,4,6-trichlorophenol on their own. Because no accumulation of intermediate products of this xenobiotic was observed during cometabolism, a new definition was proposed for this type of complete degradation process, i.e. secondary utilisation.

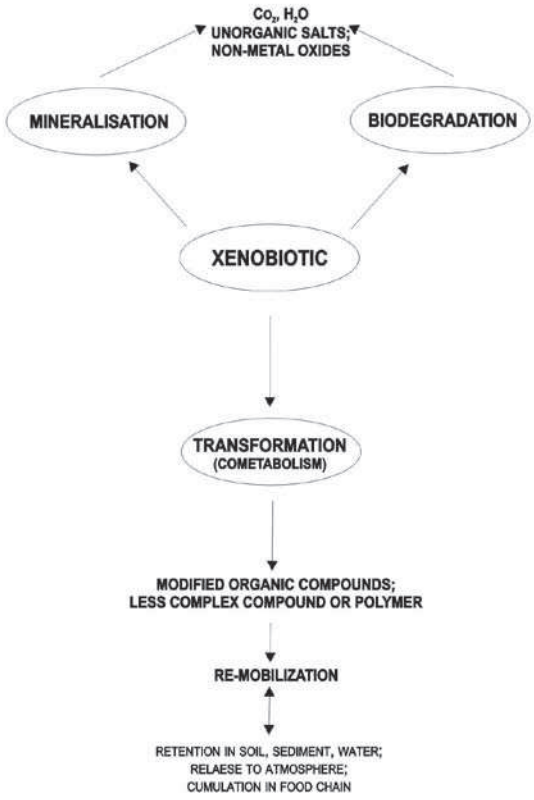

Fig. 1. Possible ways of biological transformations of xenobiotics in the environment

Usually, however, the processes of cometabolic transformations of xenobiotics do not benefit the conducting microorganisms in any measurable way, with the exception of eliminating a potentially toxic

substance from their environment, or at least reducing its toxicity due to the change of its molecular structure [3]. It is possible, though, that the toxicity of the cometabolic transformation product might turn out to be higher than that of the original compound. An example of such compound can be the cleavage product of the aromatic ring of 3-chlorocatechol by catechol 2,3-dioxygenase, i.e. acyl chloride. If the ring-cleaving dioxygenase does not have the capability to remove the chlorine substituent, then the chlorine substituent in acyl chloride is substituted by an enzyme. In consequence, the enzyme undergoes acylation and becomes inactive [7]. Another example can be the ethanol produced during vinyl acetate transformations [8]. As a result of hydrolysis of vinyl acetate the produced vinyl alcohol undergoes spontaneous isomerisation to ethanal, which, however, has strong toxic effect on microorganism cells. The condition for defence against its toxicity is the efficient system of dehydrogenases oxidising ethanol to acetic acid or temporarily reducing it to ethanol [8, 9].

The general diagram of potential development of xenobiotics' situation after release into the environment is provided on Fig. 1.

The products of xenobiotics' transformation contribute to the increase of humification processes and then either return temporarily to the environment, or are stored in the soil, the sludge, dissolve in surface and/or ground water, are released into the atmosphere or aggregate in the food chain. It should also be noted that the described biotic transformations of xenobiotics in the environment may be accompanied by abiotic chemical transformations in the soil and sediments, and abiotic photochemical transformations in the water and in the air [3].

Bioavailability of xenobiotics in the environment

The scope and rate of all transformations of xenobiotics depends on the chemical structure and concentration of the xenobiotic, type and number of microorganisms capable of degrading or transforming the xenobiotic, as well as the physico-chemical properties of the environment to which the xenobiotic is released or in which it accumulates [3, 10, 11]. The term of bioavailability is defined as the total volume of the contaminant found in the soil or bottoms in free state (not permanently bound to the matrix) which is or can be absorbed by the organism [10]. Among various environments into which xenobiotics are released the soil appears to be the most diverse system, comprising solid, liquid and gas phases. The solid phase are mineral (fragments of rocks and minerals), organic (humus, animal and plant remnants) and mineral-organic particles. The liquid phase is water with dissolved mineral and organic substances, as well as gases, retained by capillary forces between soil aggregates and lumps. The mineral and organic compounds dissolved in the water constitute the soil water retention. The soil air is saturated by vapour and contains approximately 10-times more carbon dioxide than atmospheric air, and fills the soil spaces between solid particles that have not been taken by water.

The bioavailability of a xenobiotic depends on its state (solid, liquid or gas), water solubility, capability of adsorbing and adhering to solid particles of soil or sediment [3]. It is generally accepted that only the water-dissolved fraction of the xenobiotic is available to the microorganisms and the direct contact of the xenobiotic with the microorganism's cell is the condition of the biological transformation of the xenobiotic. At the same time it should be pointed out that the vast majority of xenobiotics exhibit significant hydrophobicity and thus, after release into the environment, they are immobilised on the solid particles of the matrix by sorption, or in the structures of the organic matter by occlusion. The process of desorption, that is, releasing the contamination, is the result of a collaboration between the physico-chemical factors, such as a change of humidity, reaction or surface properties of the sorbent, and biological factors, i.e. it is caused not only by the activity of microorganisms, but also plants and animals. The released contaminants are transported by way of diffusion and dispersion, which may lead to the xenobiotic coming into direct contact with the microorganism's surface. Passing the physiological barrier of cellular membranes of microorganisms is the key stage in the process of transformation of xenobiotics, taking place with the participation of more or less specialised enzymes of xenobiotic decomposition pathways [10, 12].

However, the process of transporting the xenobiotic into the cell is not always crucial to the process of its transformation, since at times those phenomena occur with the participation of extracellular enzymes. One example of this can be the transformation of xenobiotic esters that occur with the participation of extracellular lipases and esterases [13, 14], or the transformation of chlorophenols with the participation of extracellular laccase (*p*-diphenol oxidase), isolated from the fungus *Coriolus versicolor* [15]. This enzyme transfers electrons and protons from *ortho*- or *para*-diphenols to oxygen. An example reaction of inhibition of 2,4-dichlorophenol transformation in the presence of ferulic acid and active laccase is provided on Fig. 2.

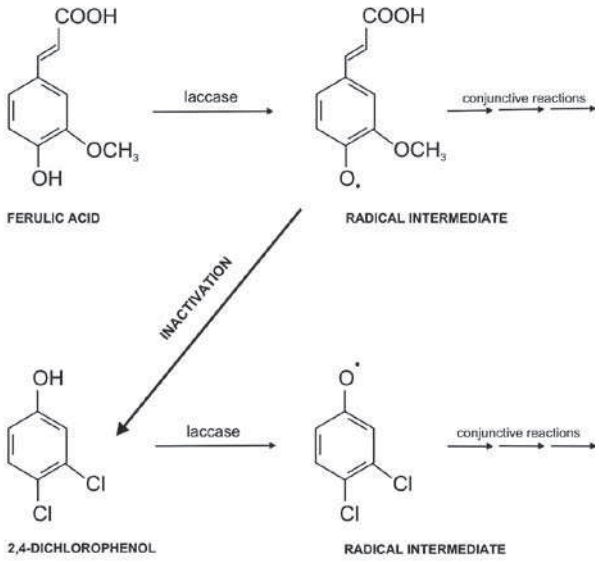

Fig. 2. 2,4-Dichlorophenol transformation in the presence of ferulic acid by laccase from *Coriolus versicolor*

The transport of xenobiotics into the microorganism cells is relatively less known and described. The literature of the subject usually provides descriptions of ATPase-dependent carriers, participating in the transfer of xenobiotics into the microorganism cells. Examples include the StyE protein, responsible for the active transport of styrene in *Pseudomonas putida* CA-3 [16], or the TfdK protein, participating in the transfer of 2,4-dichlorophenoxyacetic acid into the cells of *Ralstonia eutropha* JMP134 strain [17]. Another method of transporting xenobiotics is the functioning of porin proteins located in the outer cellular membrane, such as the XylN protein, participating in the transport of *m*-Xylene and its analogues through the outer membrane [18], or the TbuX protein which takes part in the transport of toluene in the *Pseudomonas pickettii* PKO1 strain [19].

Biochemical decomposition pathways of xenobiotics in aerobic conditions

The microbial transformation of xenobiotics can take place either in aerobic or anaerobic conditions. In the majority of cases molecular oxygen participates in the first transformation reactions of xenobiotics, regardless whether their chemical structure is aliphatic or aromatic [20, 21]. The hydroxylation reactions of xenobiotics appear to be of crucial importance to the processes of xenobiotic transformation and are often the limiting stage for their metabolism by microorganisms. Those processes involve mainly oxygenases, usually mono- or dioxygenases [22].

The products of aliphatic xenobiotic oxidation are carboxylic acid intermediates that appear to be the key intermediate metabolites involved in the process of transformation of fatty acids in the cell [23, 24, 25].

On the other hand, in the decomposition of xenobiotics with aromatic structure the activity of specific types of oxygenases depends on the original xenobiotic structure, since the main objective of upper pathways of decomposition of aromatic xenobiotics is the transformation of xenobiotic into one of the key intermediate metabolites, i.e. catechol, procatechuic acid, gentisic acid or hydroquinone (Fig. 3).

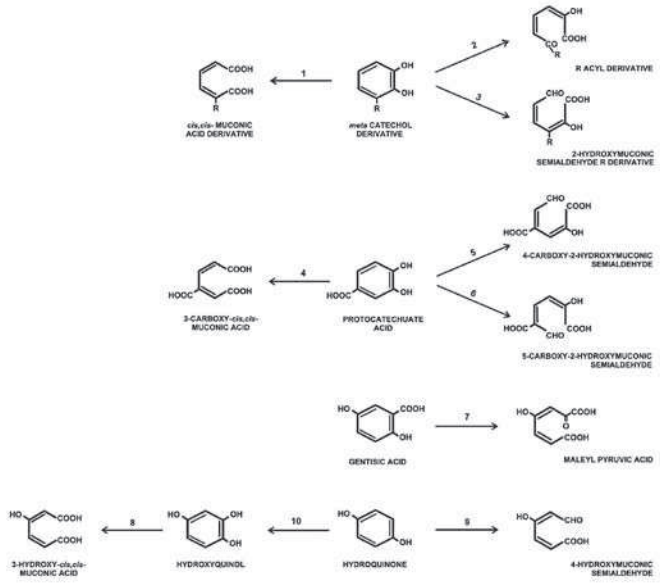

Fig. 3. Possible types of aromatic ring cleavage of key intermediates of aromatic hydrocarbons degradation in aerobic conditions, where: 1 – catechol 1,2- dioxygenase; 4 – protocatechuate 3,4-dioxygenase; 8 – hydroxyquinol 1,2 – dioxygenase are enzymes of the *ortho* pathways, and: 2 – catechol 2,3- dioxygenase; 3 – catechol 1,6- dioxygenase; 5 – protocatechuate 4,5-dioxygenase; 6 – protocatechuate 2,3-dioxygenase; 7 – gentisate 1,2-dioxygenase; 9 – hydroquinone dioxygenase are enzymes of the *meta* pathway of aromatic compounds degradation; 10 – hydroquinone hydroxylase

Their common property is the presence of two hydroxyl groups, located either in *ortho* or in *para* position. If the structure of the compound undergoing microbial transformation already contains a hydroxyl group, the transformation involves monooxygenase which transports one of the molecular oxygen atoms to the aromatic rings and reduces the other atom to water [22]. If the original structure of the aromatic compound does not have hydroxyl substituents, the situation is reversed. In that case further transformation of xenobiotics requires introducing two hydroxyl groups to the ring and the transformation is catalysed by ring-hydroxylating dioxygenase [26, 27].

The upper pathways of decomposition of xenobiotics often include also the transformation of additional substituents, such as halogens, nitro group, sulfo group or azo group. The presence of those substituents contributes to the increase of xenobiotics' "strangeness" in the environment and frequently to the increase of their resistance to microbial decomposition [20, 21, 28÷31].

The second key stage in the decomposition of aromatic structure is the destruction of this structure by the activity of aromatic ring-cleaving dioxygenases and the production of unsaturated aliphatic acids. The type of dioxygenase involved in the ring cleavage depends on the structure of the central intermediate, whereby ring-cleaving dioxygenases are divided into two main groups. Intradiol dioxygenases are a group of enzymes that cleaves the carbon-carbon bond of the aromatic ring, provided that both carbon atoms have a hydroxyl substituent and the product of this reaction is the *cis,cis*-muconic acid or its derivative. Extradiol dioxygenases on the other hand are responsible for cleaving the carbon-carbon bond of the aromatic ring if only one of the carbons in question is hydroxylated and the products of the reaction is the hydroxymuconic semialdehyde or its appropriate derivative (Fig. 3). Therefore, the substrates of their activity include not only catechol, but also compounds with two hydroxyl groups located in the *para* position and/or having a carboxyl or amine group in the position of the second hydroxyl substituent. Greater diversity of substrates for extradiol dioxygenases makes this enzyme group more versatile and useful in comparison to intradiol dioxygenases [26, 27].

Aromatic-ring-cleaving dioxygenases, together with enzymes catalysing further transformations of cleavage products of this stable structure, constitute two main pathways of decomposition of aromatic rings in xenobiotic transformations [32]. If the microorganism cells exhibit activity of intradiol dioxygenase, then the xenobiotic decomposition pathway is described as *ortho*, while the presence of extradiol dioxygenase categorises the pathway of further reactions as *meta*-type (Fig. 3). Transformations of xenobiotics on *ortho* or *meta* pathways involve mainly the reactions of hydrolysis, (cyclo)isomerisation and reduction of compounds, producing carboxyl acids which, upon further processing, become the Krebs-cycle intermediates [32]. If the additional substituent has not been eliminated prior to the cleavage of the ring, then the xenobiotic transformations on *ortho* or *meta* pathways trigger appropriate reactions, e.g. the elimination of halogen substituent during decomposition of mono- and dichlorophenols with the participation of appropriate dehydrogenases [33, 34].

The diversity of microorganisms participating in the aerobic transformations of xenobiotics is vast. The majority of those microorganisms include the following species: *Pseudomonas, Acinetobacter, Alcaligenes, Bacillus, Rhodoccocus, Micrococcus* or *Streptomyces* [21, 23-25, 28-31, 34].

Decomposition of xenobiotics in anaerobic conditions

The processes of xenobiotic transformations in the conditions of oxygen deficiency are currently intensively researched, as they are much less known. The strains isolated thus far, transforming the aromatic xenobiotics, belong mainly to bacteria reducing nitrate(V), sulphate(VI), ferrum(III), vanadium(V), chromium(VI) ions, as well as photosynthesising purple bacteria and fermentation bacteria. Among microorganisms the dominating types are *Desulfobacterium, Clostridium, Methanococcus, Thauera, Azoarcus* or *Geobacter* [35, 36].

The central intermediate of those transformations is benzoyl-CoA which is produced by way of numerous transformations. If the structure of the original compound includes a carbon substituent, then its transformations are directed so that it becomes a carboxyl group, which is then bonded to coenzyme A. An exception from this rule is the transformation of toluene which, with the participation of benzylsuccinate synthase, is condensed with fumarate. After this atypical reaction of addition a number of transformations take place, similar in nature to β-oxidation reactions, creating benzoyl-CoA (Fig. 4).

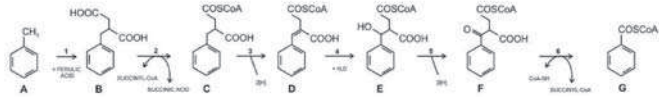

Fig. 4. Toluene transformation to benzoyl-CoA under anoxic conditions, where: 1 – benzylsuccinic acid synthase; 2 – benzylsuccinyl-CoA transferase; 3 – benzylsuccinyl-CoA dehydrogenase; 4 – phenylitaconyl-CoA; 5 – 3-hydroxylacyl-CoA dehydrogenase; 6 – benzylsuccinyl-CoA thiolase; A – toluene; B – benzylsuccinic acid; C – benzylsuccinyl-CoA; D – phenylitaconyl-CoA; E – 2-karboxymethyl-3-hydroxyphenylpropionyl-CoA; F – benzylsuccinyl-CoA; G – benzoyl-CoA

If the compound does not have an alkyl substituent, its transformation process comprises carboxylation of the ring in the

presence of carbon dioxide. Consequently, 4-hydroxybenzoic acid is produced from phenol and naphthoic acid from naphthalene. Benzoyl-CoA is the final aromatic product of transformation, since further stages of its conversion include reduction of the aromatic ring, comprising gradual saturation of unsaturated bonds and the creation of unsaturated cyclic systems. The aromatic ring opening is hydrolytic and results in the production of 3-hydroxypimelic-CoA which then, through oxidation and decarboxylation, can be finally conversed into acetyl-CoA molecules [25, 35, 36].

Condensation with fumarate is also a transformation process during anaerobic transformations of alkanes and produces alkyl derivative of succinate which, after condensation with coenzyme A, enters the β-oxidation pathway. The mechanisms of transformation of cyclic alkanes in anoxia conditions have not yet been described in detail [25, 35, 36].

Summary

The processes of biodegradation, mineralisation and transformation conducted by microorganisms are the foundation of modern struggle of human being against the rising volumes of xenobiotics released into the environment. If it had not been for the presence of the sets of genes encoding the enzymes of xenobiotic decomposition pathways, both aerobic and anaerobic, which are frequently located on the mobile genetic components of the cell, such as plasmids or transposons, life on Earth as we know it could not have survived. Constant contact with contaminants and prolonged exposure to their presence are the foundations of the struggle against xenobiotics, since they enable the evolution of new, more or less secure processes of xenobiotic transformations by microorganisms.

Literature

1. http://www.ietu.katowice.pl/wpr/Slownik_terminow.htm 10.04.2008.
2. Gribble G.W.: *Amazing organohalogens*. American Scientist 2004, **92**, 342.
3. http://www.eolss.net/Sample-Chapters/C17/E6-58-09-08.pdf 10.06.2012.
4. Horvath R.S.: *Microbial co-metabolism and the degradation of organic compounds in nature*. Bacteriological Review 1972, **36**, 2, 146.
5. Knackmuss H-J.: *Basic knowledge and perspectives of bioelimination of xenobiotic compounds*. Journal of Biotechnology 1996, **51**, 287.
6. Aranda C., Godoy F., Becerra J., Barra R., Martinez M.: *Aerobic secondary utilization of a non-growth and inhibitory substrate 2,4,6-trichlorophenol by Sphingopyxis chilensis S37 and Sphingopyxis-like strain S32*. Biodegradation 2003, **14**, 265.
7. Van Hylckama Vlieg J.E.T., Poelarends G.J., Mars A.E.: *Detoxification of reactive intermediates during microbial metabolism of halogenated compounds*. Current Opinion in Microbiology 2000, **3**, 257.
8. Nieder M., Sunarko B., Meyer O.: *Degradation of vinyl acetate by soil, sewage, sludge, and the newly isolated aerobic bacterium V2*. Applied and Environmental Microbiology 1990, **56**, 3023.
9. Greń I., Gąszczak A., Szczyrba E., Łabużek S.: *Enrichment, isolation and susceptibility profile to the growth substrate of bacterial strains able to degrade vinyl acetate*. Polish Journal of Environmental Studies 2009, **18**, 383.
10. Oleszczuk P.: *Biodostępność i bioakumulacja hydrofobowych zanieczyszczeń organicznych. Część I. Informacje ogólne*. Biotechnologia 2007, **1**, 76, 9.
11. Jacyszyn K.: *Czynniki warunkujące toksyczność*. [w] Sończuk W.: Toksykologia. Wydawnictwo Lekarskie PZWL, 2005, **40**.
12. Oleszczuk P.: *Organic pollutants in sewage sludge-amended soil. Part II fate of contaminants in soils*. Ecological chemistry and engineering 2007, **14**, 2, 185.
13. Ozcan B., Ozyilmaz G., Cokmus C., Caliskan M.: *Characterization of extracellular esterase and lipase activities from five halophilic archeal strains*. Journal of Industrial Microbiology and Biotechnology 2009, **36**, 105.
14. Smacchi E., Gobbetti M., Rossi J., Fox P.F.: *Purification and characterization of a extracellular esterase from Arthrobacter nicotianae 9458*. Lait 2000, **80**, 255.
15. Itoh K., Fujita M., Kumano K., Suyama K., Yamamoto H.: *Phenolic acids affect transformations of chlorophenols by a Coriolus versicolor laccase*. Soil Biology and Biochemistry 2000, **32**, 85.
16. Mooney A., O'Leary N.D., Dobson A.D.W.: *Cloning and functional characterization of the styE gene, involved in styrene transport in Pseudomonas putida CA-3*. Applied and Environmental Microbiology 2006, **72**, 2, 1302.
17. Leveau J.H.J., Zehnder A.J.B., van der Meer J.R.: *The tfdK gene product facilitates uptake of 2,4-dichlorophenoxyacetate by Ralstonia eutropha JMP134 (pJP4)*. Journal of Bacteriology 1998, **180**, 8, 2237.
18. Kasai Y., Inoue J., Harayama S.: *The TOL plasmid pWWO xylN gene product from Pseudomonas putida is involved in m-xylene uptake*. Journal of Bacteriology 2001, **183**, 22, 6662.
19. Kahng H-Y., Byrne A.M., Olsen R.H., Kukor J.J.: *Characterization and role of tbuX in utilization of toluene by Ralstonia pickettii PKO1*. Journal of Bacteriology 2000, **182**, 5, 1232.
20. Cao B., Nagarajan K., Loh K-C.: *Biodegradation of aromatic compounds: current status and opportunities for biomolecular approaches*. Applied And Microbial Biotechnology 2009, **85**, 207.
21. Sinha S., Chattopadhyay P., Pan I., Chatterjee S., Chanda P., Bandyopadhyay D., Das K., Sen S.K.: *Microbial transformation of xenobiotics for environmental bioremediation*. African Journal of Biotechnology 2009, **8**, 22, 6016.
22. Ullrich R., Hofrichter M.: *Enzymatic hydroxylation of aromatic compounds*. Cellular and Molecular Live Sciences 2007, **64**, 271.
23. Kwapisz E.: *Szlaki tlenowej biodegradacji węglowodorów ropy naftowej*. Biotechnologia 2006, **2**, 73, 166.
24. Guzik U., Wojcieszyńska D., Krysiak M., Kaczorek E.: *Mikrobiologiczny rozkład alkanów ropopochodnych*. Nafta-Gaz 2010, **11**, 1019.
25. Van Hamme J.D., Singh A., Ward O.P.: *Recent advances in petroleum microbiology*. Microbiology and molecular biology reviews 2003, **67**, 4, 503.
26. Guzik U., Greń I., Wojcieszyńska D., Łabużek S.: *Dioksygenazy – główne enzymy degradacji związków aromatycznych*. Biotechnologia 2008, **3**, 82, 71.
27. Vaillancourt F.H., Bolin J.T., Eltis L.D.: *The ins and outs of ring-cleaving dioxygenases*. Criticial Reviews in Biochemistry and Molecular Biology 2006, **41**, 241.
28. Wojcieszyńska D., Guzik U., Hupert-Kocurek K., Siupka P.: *Mikrobiologiczny rozkład chlorofenoli, uciążliwych odpadów przemysłu chemicznego*. Przemysł Chemiczny 2011, **90**, 8, 1.
29. Kulkarni M., Chaudhari A.: *Microbial remediation of nitro-aromatic compounds: an review*. Journal of Environmental Management 2007, **85**, 496.
30. Ye J., Singh A., Ward O.P.: *Biodegradation of nitroaromatics and other nitrogen-containing xenobiotics*. World Journal of Microbiology and Biotechnology 2004, **20**, 117.
31. Jindrova E., Chocova M., Demnerova K., Brenner V.: *Bacterial aerobic degradation of benzene, toluene, ethylbenzene and xylene*. Folia Microbiologica 2002, **47**, 2, 83.
32. Harwood C.S., Parales E.P.: *The β-ketoadipate pathway and the biology of self-identity*. Annual Review of Microbiology 1996, **50**, 553.
33. Van Pee K.H., Unversucht S.: *Biological dehalogenation and halogenation reactions*. Chemosphere 2003, **52**, 299.
34. Janssen D.B., Oppentocht J.E., Poelarends G.J.: *Microbial dehalogenation*. Current Opinion in Biotechnology 2001, **12**, 254.
35. Weelink S.A.B., van Eekert M.H.A., Stams A.J.M.: *Degradation of BTEX by anaerobic bacteria: physiology and application*. Reviews in Environmental Science and Biotechnology 2010, **9**, 359.
36. Guzik U., Wojcieszyńska D., Hupert-Kocurek K.: *Mikrobiologiczny rozkład związków aromatycznych w warunkach anoksji*. Postępy mikrobiologii 2010, **49**, 3, 217.

Izabela GREŃ, Ph.D., graduated from the Faculty of Biology and Environmental Protection of the Silesian University in Katowice (1999). She obtained her doctoral degree on the same Faculty (2004), where she is currently employed, researching the microbial degradation of aromatic compounds, focusing particularly on chlorophenols and phenolic plant compounds, as well as cometabolic processes. Izabela Greń is the co-author of a script (Polish and English), 21 articles in Polish and foreign publications, and 12 lectures and posters presented at domestic conferences.

E-mail: izabela.gren@us.edu.pl; Phone: 32 2009 576