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Influence of additional carbon sources on chlorophenols degradation by strain *Pseudomonas* sp.

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Streszczenie

Wpływ dodatkowego źródła węgla na degradację chlorofenoli przez szczep *Pseudomonas* sp.

Szczep *Pseudomonas sp.* wykazywał zdolność do rozkładu wybranych chlorofenoli, jednak nie obserwowano wzrostu hodowli bakteryjnej w trakcie prowadzenia badań. W podjętych badaniach sprawdzono wpływ obecności dodatkowego źródła węgla na proces degradacji dichlorofenoli i pentachlorofenolu przez szczep *Pseudomonas* sp. Wykazano wzrost szybkości degradacji dichlorofenoli w obecności glukozy lub ekstraktu drożdżowego w porównaniu z hodowlami bez dodatkowego źródła węgla. Odmienne wyniki uzyskano w badaniach nad szybkością degradacji PCP w obecności dodatkowego źródła węgla. Dodanie ekstraktu drożdżowego nie zmieniło szybkości degradacji pentachlorofenolu w porównaniu z hodowlą bez dodatkowego źródła węgla. Obecność glukozy lub cytrynianu sodu do pożywki hamowało rozkład pentachlorofenolu.

Abstract

The influence of additional, readily metabolised, carbon sources on the degradation of dichlorophenols (2,4-dichlorophenol, 2,5-dichlorophenol and 3,4-dichlorophenol) and pentachlorophenol, by a strain *Pseudomonas* sp., was examined in a mineral salts medium. The presence of glucose and yeast extract brought about the increase of dichlorophenols degradation rate in comparison with the bacterial cultures without an additional carbon source. Different results were obtained when the degradation of pentachlorophenol in the presence of additional carbon sources was examined. The addition of yeast extract didn't change degradation rate of pentachlorophenol by a strain *Pseudomonas* sp. compared to the bacterial cultures without an additional carbon source. The presence of glucose or sodium citrate inhibited the pentachlorophenol decomposition. The addition of any supplementary

carbon source to the bacterial culture with any tested chlorophenol caused the increase of the viability of a strain *Pseudomonas* sp. cells.

1. Introduction

Chlorophenols belong to one of very poorly biodegradable organic compounds [1, 2, 3]. However there are bacterial strains which use chloroarenes as sources of carbon and energy [4, 5, 6, 7]. Radehaus et al. observed the growth of *Pseudomonas* RA2 in the presence of 150 mg/l of pentachlorophenol [4]. But in many studies there was shown an inhibition of bacterial growth in the presence of chlorophenols, although these bacterial strains were able to decompose the derivatives of chlorophenols [4].

The medium composition influences essentially the pentachlorophenol degradation. The presence of different components in the medium can stimulate or inhibit the degradation of xenobiotic compounds [8, 9]. Hendriksen et al. [10] showed that the addition of acetone, glucose or methanol increased the dechlorination rate of chloroaromatic compounds. However Topp et al. [9] observed the partial repression of pentachlorophenol metabolism after the addition of glutamate in combination with glucose or cellobiose. Alike Karns et al. [11] and Hubner et al. [12] observed an inhibition of dechlorination after the addition of succinate, glucose or lactate. Probably that inhibition was caused by repression of the synthesis of enzymes, which were responsible for chlorophenols degradation, in the presence of alternative carbon sources [11, 12, 13]. There is little known about dichlorophenol degradation in cometabolic conditions [8].

In the bacterial cultures, where chlorophenols were the only sources of carbon and energy, there was no growth of *Pseudomonas* sp. cells. It could suggest that chlorophenols were not a sufficient source of carbon. In this study there was made an attempt to show the influence of additional carbon sources (yeast extract, glucose or citrate) on dichlorophenols and pentachlorophenol degradation by a strain *Pseudomonas* sp.

The knowledge about cometabolic degradation of chlorophenols can be very useful in biodegradation and protection of natural environment. Most often in the polluted environment, apart from chlorophenols, are observed some other compounds. Therefore it is very important to know how various natural and anthropogenic substances can influence together on the metabolism of microorganisms and their potential ability to dangerous compounds` degradation.

2. Materials and methods

2.1 Media and culture conditions

A strain *Pseudomonas* sp. was isolated from the mixed bacterial population of IP-70 Gamlen Industry agent that was assigned for removal of chlorinated phenols (unpublished data).

The incubation was carried out with agitation (125 rpm) at 30°C in 250-ml shake flasks containing 100 ml of mineral salts medium enriched with an appropriate chlorophenol. The medium composition was as follows: 3.78 g $Na_2HPO_4 \cdot 12H_2O$; 0.5 g KH_2PO_4; 5.0 g NH_4Cl;

0.2 g $MgSO_4 \cdot 7H_2O$; 0.1 g yeast extract, per litre of distilled water, pH was adjusted to 7.1 – 7.2. Dichlorophenols were dissolved in distilled water, and PCP was dissolved in 10 mM NaOH, and added to the cultures sterilely.

Yeast extract, glucose or citrate as the additional carbon sources were used. Yeast extract was added to the mineral medium at concentration of 1g/litre, and glucose and sodium citrate were added at 0.5 mM concentration. Degradation of tested dichlorophenols and pentachlorophenol were examined in the presence of glucose and yeast extract. Additionally the degradation of PCP was carried out in the presence of citrate. As a control, the bacterial cultures with chlorophenols and without the supplementary carbon sources were carried out.

2.2 Analytical methods

The determination of dichlorophenols and pentachlorophenol concentration in bacterial cultures was accomplished by spectrophotometric method [14]. The ultra-violet absorbence of each chlorophenol was measured at its characteristic wavelength that was determined on the ground of the analysis of UV spectrum. Culture medium without aromatic compound was used as the background medium.

Viable counts were determined by spreading on plates containing nutrient agar [15].

2.3 Chemicals

In the study the following chlorophenols were used: 2,4-dichlorophenol (2,4-DCP) which was purchased from Sigma Co, USA and 2,5-dichlorophenol (2,5-DCP), 3,4-dichlorophenol (3,4-DCP) and pentachlorophenol (PCP) which were purchased from Fluka Chemie AG, Switzerland. Glucose and sodium citrate were obtained from POCH Gliwice, Poland, and yeast extract from DOFCO Laboratories, Detroit, Mich., USA.

3. Results

3.1 Degradability of different chlorophenols by a strain *Pseudomonas* sp.

Pseudomonas sp., adapted earlier to 0.12 mM pentachlorophenol, was adapted secondarily to the degradation of 0.5 mM 2,4-, 2,5- and 3,4-dichlorophenol. During 24 hours of the incubation a *Pseudomonas* sp. degraded 0.32 mM 2,4-DCP, 0.28 mM 2,5-DCP and 0.27 mM 3,4-DCP. A *Pseudomonas* sp. decomposed 0.1 mM PCP during 28 days of the incubation. As an example the course of 2,4-dichlorophenol degradation by a strain *Pseudomonas* sp. is shown in Fig. 3.1.

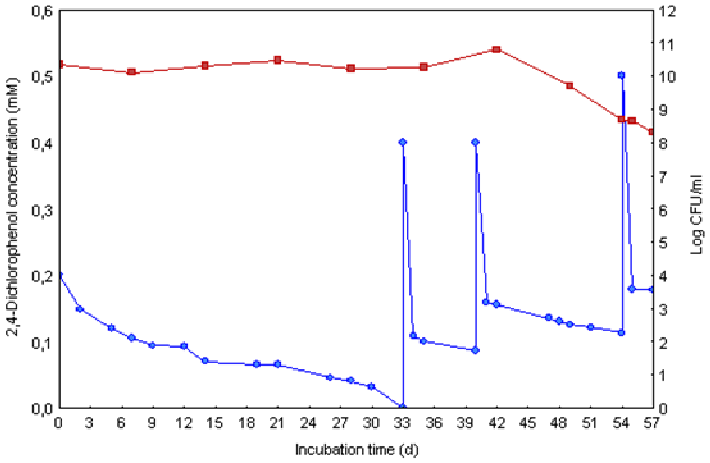

FIG. 3.1 The postadaptation of a strain *Pseudomonas* sp. to degradation of 0.5 mM 2,4-dichlorophenol (• 2,4-DCP; ■ Log CFU).

3.2 Effect of different carbon sources on chlorophenols biodegradation

The presence of the additional carbon sources had the stimulating effect on the dichlorophenols degradation. A strain *Pseudomonas* sp. degraded 0.5 mM 2,4-dichlorophenol completely in the presence of glucose and 0.42 mM 2,4-DCP in the presence of yeast extract during 5 hours of incubation. Whereas in the control culture only 0.3 mM 2,4-DCP was degraded during the time span of incubation (Fig. 3.2).

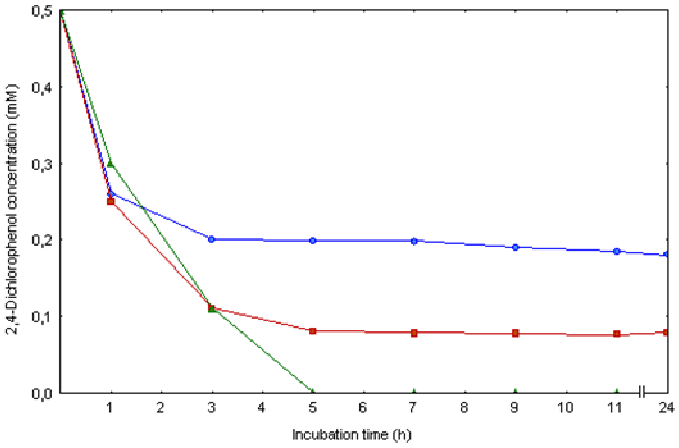

FIG. 3.2 The degradation of 0.5 mM 2,4-DCP in the presence of the additional carbon sources by a strain Pseudomonas sp. (• 2,4-DCP; ■ 2,4-DCP+yeast extract; ▲ 2,4-DCP+glucose).

The examined strain degraded 0.47 mM 2,5-dichlorophenol, 0.44 mM 2,5-DCP and 0.28 mM 2,5-DCP in the presence of glucose, of yeast extract and in the control culture, respectively, during 7 hours of incubation (Fig. 3.3).

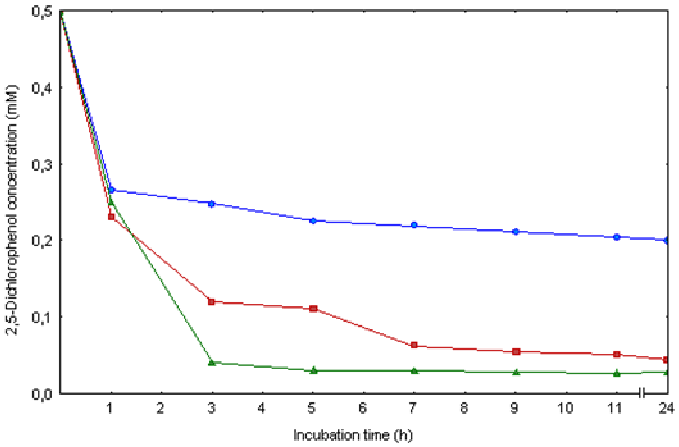

FIG. 3.3 The degradation of 0.5 mM 2,5-DCP in the presence of the additional carbon sources by a strain *Pseudomonas* sp. (• 2,5-DCP; ■ 2,5-DCP+yeast extract; ▲ 2,5-DCP+glucose).

In the culture carried out in the presence of 0.5 mM 3,4-dichlorophenol and glucose a strain *Pseudomonas* sp. degraded dichlorophenol completely during 7 hours and in the presence of yeast extract during 9 hours (Fig. 3.4). Whereas in the control culture only 0.24 mM 3,4-DCP was degraded during 24 hours of incubation (Fig. 3.4).

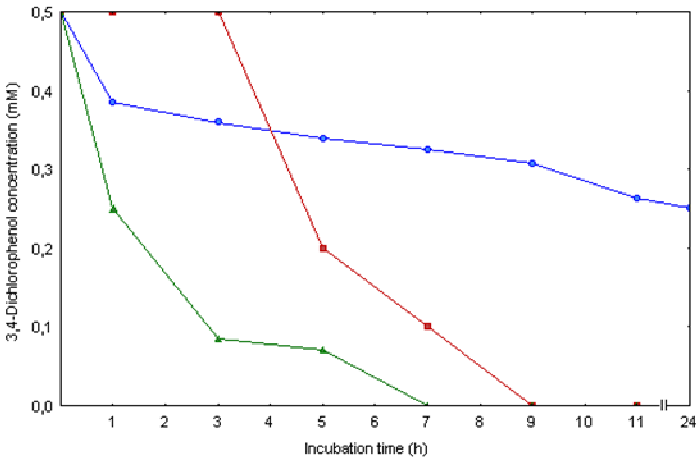

FIG. 3.4 The degradation of 0.5 mM 3,4-DCP in the presence of the additional carbon sources by a strain *Pseudomonas* sp. (• 3,4-DCP; ■ 3,4-DCP+yeast extract; ▲ 3,4-DCP+glucose).

Different results were obtained in the studies on degradation of pentachlorophenol by a strain *Pseudomonas* sp. The fastest degradation of 0.1 mM PCP was observed in the cultures without additional carbon sources. The addition of yeast extract didn't influence on the pentachlorophenol degradation rate in comparison with the control culture. In both cultures 0.1 mM PCP was completely degraded during 28 days of incubation (Fig. 3.5). Whereas the presence of glucose or citrate inhibited the degradation of PCP by a strain *Pseudomonas* sp. After 35 days of the incubation time only 0.052 mM PCP and 0.058 mM PCP were degraded in the presence of glucose and sodium citrate, respectively (Fig. 3.5).

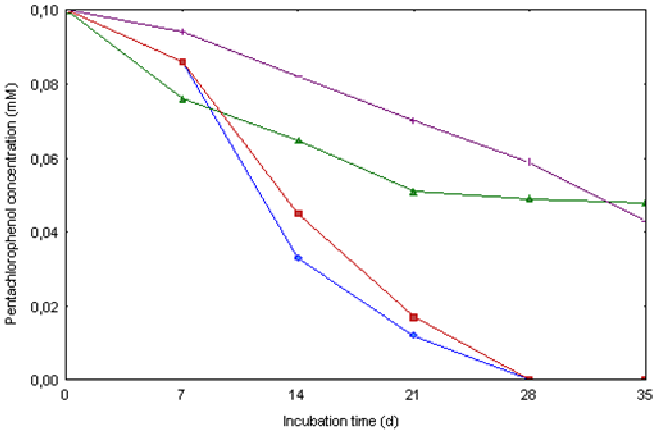

FIG. 3.5 The degradation of 0.1 mM PCP in the presence of the additional carbon sources by strain *Pseudomonas* sp. (• PCP; ■ PCP+yeast extract; ▲ PCP+glucose; + PCP+sodium citrate).

Studying the degradation of different chlorophenols in the presence of additional carbon sources, the viability of the *Pseudomonas* sp. cells was also checked. In all bacterial culture there was an increase in viability of the *Pseudomonas* sp. cells in the presence of supplementary carbon sources. During the degradation of 2,4-DCP the largest increase in viability of the *Pseudomonas* cells was observed after the addition of yeast extract (Fig. 3.6).

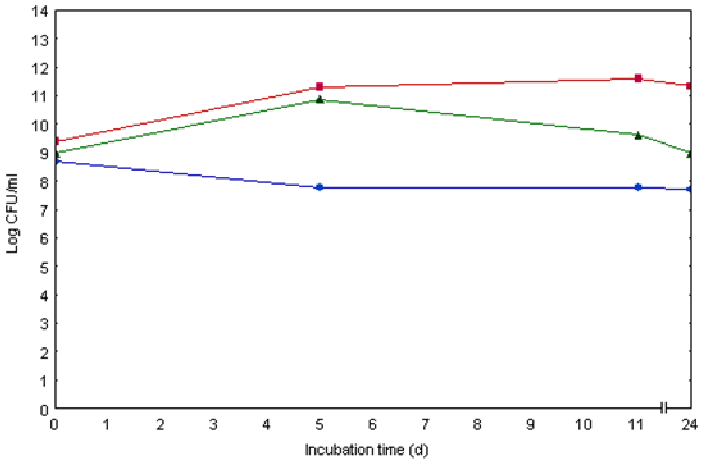

FIG. 3.6 Viability of the *Pseudomonas* sp. in the bacterial culture under 0.5 mM 2,4-dichlorophenol and additional carbon sources (• 2,4-DCP; ■ 2,4-DCP+yeast extract; ▲ 2,4-DCP+glucose).

In the residual bacterial cultures the largest viability was observed in the presence of glucose (Fig. 3.7, 3.8, 3.9).

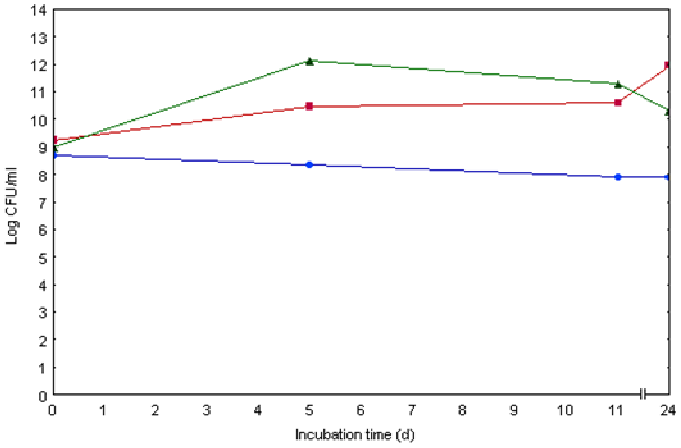

FIG. 3.7 Viability of the *Pseudomonas* sp. in the bacterial culture under 0.5 mM 2,5-dichlorophenol and additional carbon sources (• 2,5-DCP; ■ 2,5-DCP+yeast extract; ▲ 2,5-DCP+glucose).

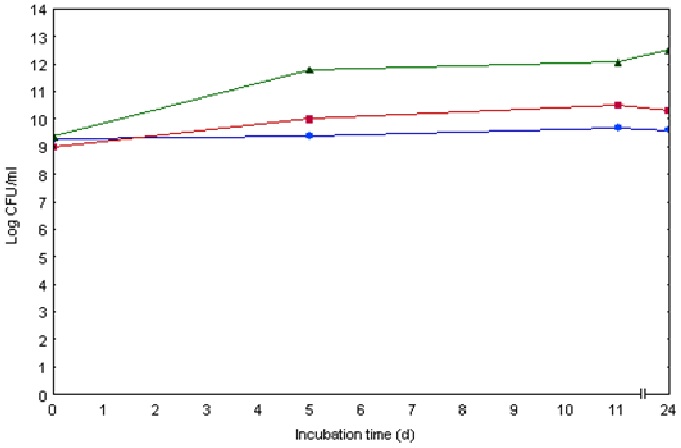

FIG. 3.8 Viability of the *Pseudomonas* sp. in the bacterial culture under 0.5 mM 3,4-dichlorophenol and additional carbon sources. (• 3,4-DCP; ■ 3,4-DCP+yeast extract; ▲ 3,4-DCP+glucose).

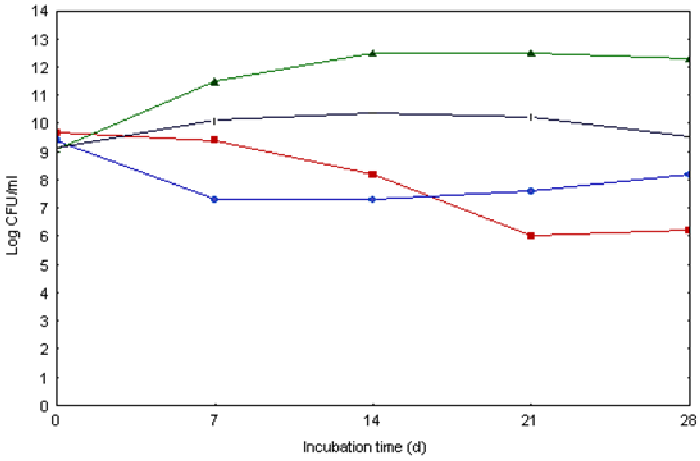

FIG. 3.9 Viability of the *Pseudomonas* sp. in the bacterial culture under 0.1 mM pentachlorophenol and additional carbon sources. (• PCP; ■ PCP+yeast extract; ▲ PCP+glucose; + PCP+sodium citrate).

4. Discussion

The aromatic structure is characterised by a specific type of unsaturation, which causes that the aromatic ring is stable in many reactions. The presence of chlorine substituents on the aromatic ring increases the stability of aromatic structure, what next causes that it is more difficulty degraded by microorganisms. Along with an increase of the number of chlorine substituents on the aromatic ring there is an increase in lipophilicity and electrophilicity of these compounds [14, 16, 17, 18].

In our research strain *Pseudomonas* sp. degraded all tested chlorophenols, but there were no growths of bacterial cultures during degradations. From among tested dichlorophenols 2,4-dichlorophenol was degraded the most rapidly, 2,5-dichlorophenol - less rapidly, and 3,4-dichlorophenol was degraded the most difficult (Fig. 3.2, 3.3, 3.4).

The observed rapidest degradation of 2,4-dichlorophenol is in agreement with the results of others who show that the *ortho*-position (2 and 6) of the OH group on the aromatic ring is preferential in the chlorophenols degradation by isolated bacterial strains. The presence of the chlorine substituents in the *ortho*-position on the aromatic ring decreased the toxic effect of chlorophenol. Microorganisms more readily degraded the chlorophenols with the chlorine atoms substituted at the *ortho*-position [14, 16, 19, 20].

The observed slower degradation of 3,4-dichlorophenol by a strain *Pseudomonas* sp. was probably caused by the configuration of substituents in the *meta-para*-position that is more toxic than *ortho-meta*-configuration of substituents [19, 21].

The high toxicity of the aromatic compound can be due to not only the occupation of the appropriate position of the aromatic ring by chlorine substituent, but also it can be due to the presence of increased numbers of chlorine substituents on the aromatic ring [16, 20]. According to McCarthy et al. [22] pentachlorophenol is characterised by the largest toxicity. PCP is known as an uncoupler of oxidative phosphorylation and an inhibitor of cell divisions, and its metabolite- tetrachloro-p-hydroquinon causes single-stranded breaks in DNA [22]. The weak degradation of pentachlorophenol by a strain *Pseudomonas* sp. (Fig. 3.5) is in accordance with the observations described above.

It has been shown [9, 10] that the presence of easily metabolised carbon sources: glucose, yeast extract, amino acids or simple organic acids facilitates the chlorophenols degradation.

In the present studies as the additional carbon sources were used yeast extract as a source of easily available amino acids, glucose as a simple sugar and citrate as an simple organic acids - intermediate of a tricarboxylic acid cycle. Biochemical characterisation of a strain *Pseudomonas* sp. showed that the tested carbon sources were degraded by this strain [23]. Our studies indicate that in the presence of glucose or yeast extract the dichlorophenols degradation is carried out more rapidly in comparison with the bacterial culture without the supplementary carbon source (Fig. 3.2, 3.3, 3.4). It is in agreement with the results of others that show faster degradation of chlorophenols in cultures under easily metabolised carbon sources [9, 10, 24]. It is interesting that the dichlorophenols were degraded in the presence of glucose easier than in the presence of yeast extract. Likewise the growth of the *Pseudomonas* sp. cells in the bacterial cultures with glucose was higher than in the cultures with yeast extract for all tested dichlorophenols apart from 2,4-dichlorophenol (Fig. 3.6, 3.7, 3.8). During the degradation of 2,4-DCP the larger viability of the *Pseudomonas* sp. cells was found out in the presence of yeast extract (Fig. 3.6). In the bacterial cultures with glucose as the additional carbon source and in the control cultures 0.1 g/l of yeast extract was added as a source of vitamins and microelements, which are necessary. It could be assumed that the addition of 1 g/l of yeast extract would facilitate the dichlorophenols degradation not only as the additional easily metabolised carbon source, but also just as the source of vitamins and microelements [25]. The lower growth of the *Pseudomonas* sp. cells and slower degradation of dichlorophenols in the presence of 1g/l of yeast extract in comparison with the cultures with 0.5 mM glucose suggest, that although yeast extract was good source of vitamins and microelements, it was not as sufficient source of carbon as glucose.

The degradation of pentachlorophenol by a strain *Pseudomonas* sp. was inhibited in the presence of glucose or citrate in the culture medium. Simultaneously there was an increase in viability of the *Pseudomonas* sp. cells at the day 14, and thereafter the viability started to decrease slowly (Fig. 3.5). Yeast extract had no influence on PCP degradation by a strain *Pseudomonas* sp. (Fig. 3.5). It can be suggested that the weak degradation of pentachlorophenol in the presence of citrate can be due to the change of activity of enzymes, which are involved in its degradation. Ampe et al. [26] showed in their studies on phenol catabolism that the presence of organic acids, such as e.g. acetate, lactate, fumarate, malate, citrate or gluconate inhibited the degradation of aromatic compounds. They suggest that the organic acids work as the repressors of transcription of degrading enzymes- phenol hydroxylase and catechol-2,3-dioxygenase. The same results were obtained by McFall et al.

[13], who confirmed that succinate and other a tricarboxylic acid cycle intermediates inhibited the degradation of chlorinated phenols. Succinate, citrate and fumarate repressed the *clcABD* operon, which encodes enzymes of chlorocatechol pathway [13]. According to McFall et al. [13] fumarate inhibited the expression of the clc transcript. This organic acid, similar on the structure to 2-chloromuconate (an inducer of transcription), probably binds in the inducer binding pocket of regulatory protein (ClcR) and works as an anti-inducer of transcription [13]. In our studies there was no catabolic repression of the less chlorinated phenols what can be due to induction of the other catabolic pathways for the degradation of these substrates. The increase of the numbers of chlorine substituents along with simultaneous increase of the toxicity of chlorophenols could activate other metabolic pathways. Simultaneously there could be a repression of catabolic pathways, which enabled the utilisation of other carbon sources.

Radehaus et al. [4] showed that the presence of glucose in the culture medium didn't increase ability to the degradation of increased amounts of pentachlorophenol, whereas the consumption of glucose increased in the presence of pentachlorophenol. Sato and Lee [27] observed that in the presence of glycine, glutamate and glucose the degradation rate of PCP was lower. In their opinion the addition of supplementary carbon sources facilitated the proliferation of microorganisms, which were unable to degrade PCP, and depress the proliferation of the PCP-degrading microorganisms [27, 28, 29]. The different results were obtained by Topp et al. [9, 30], who worked with a strain *Flavobacterium* sp. They observed that the presence of additional carbon sources facilitated the metabolism of PCP. In their opinion it was due to both, the increase of PCP-degrading biomass and the increased resistance of a *Flavobacterium* sp. cells to the toxicity of PCP. Glutamate in combination with glucose inhibited the degradation of pentachlorophenol [9]. Summing up, it is difficult to explain unequivocally why PCP was degraded less rapidly in the presence of the additional carbon source, whereas at the same conditions the degradation of dichlorophenols was stimulated. It can be suggested that the differences in the pentachlorophenol and dichlorophenols degradation are due to larger toxicity of PCP and the damages of metabolism of microorganisms that are able to degrade PCP [4, 22].

5. References

[1] Czaplicka M.: Sci. Total Environ., 2004, **322**, 21-39.

[2] Häggblom, M.M.: J. Basic Microbiol., 1990, **30**, 115-141.

[3] Häggblom M.M., Valo. R.J: Wiley-Liss Inc. New York, 1995, Pp 389-434.

[4] Radehaus P.M., Schmidt S.K.: Appl. Environ. Microbiol., 1992, **58**, 2879-2883.

[5] Vaillancourt F.H., Bolin J.T., Eltis L.D.: Crit. Rev. Biochem. Mol. Biol., 2006, **41**, 241-267.

[6] Sahinkaya E., Dilek F.B.: J. Biotechnol., 2007, **127**, 716-726.

[7] Wang S.G., Liu X.W., Zhang H.Y., Gong W.X., Sun X.F., Gao B.Y.: Chemosphere, 2007, **69**, 769-775.

[8] Ziagova M., Liakopoulou-Kyriakides M., 2007, **68**, 921-927.

[9] Topp, E., Crawford R.L., Hanson R.S.: Appl. Environ. Microbiol., 1988, **54**, 2452-2459.

[10] Hendriksen H. V., Larsen S., Ahring B.K.: Appl. Environ. Microbiol., 1992, **58**, 365-370.

[11] Karns J. S., Duttagupta S., Chakrabarty A.M.: Appl. Environ. Microbiol., 1983, **46**, 1182-1186.

[12] Hubner A., Danganan C.E., Xun L., Chakrabarty A.M., Hendrickson W.: Appl. Environ. Microbiol., 1998, **64**, 2086-1093.

[13] McFall S., Abraham B., Narsolis C.G., Chakrabarty A.M.: J. Bacteriol., 1997, **179**, 6729-6735.

[14] Kiyohara H., Takizawa N., Uchiyama T., Ikarugi H., Nagao K.: J. Ferm. Bioengin., 1989, **67**, 339-344.

[15] Różalski A.: Lodz University Press, 1998, 2nd ed.

[16] Beltrame P., Beltrame P.L., Carniti P., Guardione D., Lanzetta C.: Biotechnol. Bioenginer., 1988, **31**, 821-828.

[17] Pitter P.: Acta Hydrochim. Hydrobiol., 1985, **13**, 453-460.

[18] Steiert J.G., Crawford R.L.: Trend in Biotechnol., 1985, **3**, 300-305.

[19] Fava F., Armenante P.M., Kafkewitz D.: Letters in Appl. Microbiol., 1995, **21**, 307-312.

[20] Steiert J.G., Pignatello J.J., Crawford R.L.: Appl. Environ. Microbiol., 1987, **53**, 907-910.

[21] Utkin J., Dalton D.D., Wiegel J: Appl. Environ. Microbiol., 1995, **61**, 346-351

[22] McCarthy D.L., Claude A.A., Copley S.D.: Appl. Environ. Microbiol., 1997, **63**, 1883-1888.

[23] Peladan F., Monteil H.: Path. Biol., 1988, **36**, 187-192.

[24] Haigler B.E., Pettigrew Ch.A., Spain J.C.: Appl. Environ. Microbiol., 1992, **58**, 2237-2244.

[25] Boothe D.D.H., Rogers J.E., Wiegel J.: Appl. Microbiol. Biotechnol., 1997, **47**, 742-748.

[26] Ampe F., Leonard D., Lindley N.D.: Appl. Environ. Microbiol., 1998, **64**, 1-6.

[27] Sato K., Lee S.B.: Soil Sci. Plant Nutr., 1996, **42**, 905-910.

[28] Sato K.: Soil Sci. Plant Nutr., 1996, **42**, 911-916.

[29] Sato K.: Soil Sci. Plant Nutr., 1997, **43**, 937-941.

[30] Topp E., Hanson R.S.: Appl. Environ. Microbiol., 1990, **56**, 541-544.