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Pentachlorophenol degradation by activated sludge with phenol and glucose as growth substrates

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Abstract: Important factors affecting the effectiveness of microbiological degradation of chlorophenols include the presence of additional growth substrates, which ensure the accessibility of electron acceptors and electron donors, or the applied strains of microorganisms and their adaptation to pollution. Therefore an improvement of PCP degradation by the adaptation of activated sludge to PCP with phenol and glucose as cometabolites was examined. The activated sludge was adapted to 12 $mg \cdot L^{-1}$ of PCP and to 200 $mg \cdot L^{-1}$ of phenol, and then, the effect of the adaptation of activated sludge and the presence of additional sources of carbon and energy on the biodegradation of PCP and sorption properties were tested. The obtained results confirmed that the presence of additional sources of carbon and energy in the growth medium would improve the efficiency of PCP degradation. Among all analyzed types of research setups, the highest PCP degradation was noted in setups with phenol, regardless of the method of activated sludge adaptation or lack of adaptation. The biodegradation of PCP in the presence of glucose was less efficient than in the presence of phenol. The highest, 60% decrease in PCP concentration was obtained for activated sludge adapted to PCP in the presence of phenol.

Introduction

Long-term use of chlorophenols, including pentachlorophenol (PCP), has spread these compounds throughout the environment. PCP used to be a common component of biocides and wood preservatives. It is toxic, and humans exposed to it display many symptoms of poisoning, e.g., digestive, circulatory, and skin disorders, lacrimation, or hoarseness. People who remain in constant contact with PCP have an increased risk of cancer, mutations, abnormal blood count, and changes in the nervous system, it can also cause disorders of the fetus, (ATSDR 2001, Szewczyk and Długoński 2007). PCP has been classified by the US Environmental Protection Agency as a moderately toxic xenobiotic (US EPA 1999). The compound has also been identified as a priority hazardous substance and included in the list of priority substances under the EU Water Framework Directive (Decision 2001). PCP is highly stable in the environment, and owes its persistence to the presence of chlorine substituents attached to the aromatic ring, which affect the stability and hydrophobicity of the compound (Greń et al. 2008, McMurry 2000).

Despite restrictive regulations on the use of PCP and other chlorophenols, their amount in the environment shows only an insubstantial decrease. So far, no cheap and effective methods, whether physicochemical or biological, for the removal of chlorophenols have been developed. Therefore, on the one hand, research is conducted on adsorption on cheap materials (Kuśmierek et al. 2017), and on the other hand, biological methods for removing chlorophenols from water, sewage, and soil have been subject of scientific research for many years thanks to their effectiveness and relatively low costs (El-Naas et al. 2017, Arora and Bae 2014). However, most experiments concern the degradation of monochlorophenols and dichlorophenols by pure bacterial species (Hao et al. 2002, Hill and Nawrocki 1996, Wang et al. 2015, El-Naas et al. 2017, Arora and Bae 2014). Even though the biodegradation of aromatic compounds by mixed bacterial consortia shows many advantages, only a few studies exist on the degradation of chlorophenols by microorganisms of activated sludge (Mosca and Tomei 2015, Sahinkaya and Dilek 2005, Visvanathan et al. 2005, Zilouei et al. 2006).

PCP can be degraded via different metabolic pathways under both aerobic and anaerobic conditions by several bacteria, filamentous fungi and algae (El-Naas et al. 2017, Lamar et al. 1990, Webb et al. 2001, Szewczyk and Długoński 2009, Juteau et al. 1995). The degradation of PCP conducted by pure strains is usually inefficient and frequently results in the accumulation of toxic intermediary metabolites that do not undergo any further transformations (Orser and Lange 1994). Important factors affecting the effectiveness of microbiological degradation of chlorophenols include the presence of additional growth substrates, which ensure the accessibility of electron acceptors and electron donors, or the applied strains of microorganisms and their adaptation to pollution (Lopez-Echartea et al. 2016, Hill and Nawrocki 1996,

Kim and Hao 1999, Field and Sierra-Alvarez 2008, Sahinkaya and Dilek 2005, Szewczyk and Długoński 2007). Therefore an improvement of PCP degradation by the adaptation of activated sludge to PCP with phenol and glucose as cometabolites was the main goal of the experiment.

Materials and methods

Research material

Activated sludge was collected from an aeration tank in the Central Sewage Treatment Plant in Gliwice (Poland). Sequencing batch reactors (SBRs) in which the sludge was adapted to PCP and phenol were inoculated with sludge collected directly from the treatment plant, whereas batch reactors were inoculated with sludge collected directly from the SBR, in which the activated sludge had been adapted to PCP, or from the SBR, in which the sludge had been adapted to phenol.

Research setups

Two different types of experimental setups were prepared: SBR reactors, in which the activated sludge was adapted to PCP and phenol, and batch reactors used to assess the effect of the adaptation of activated sludge and the presence of additional sources of carbon and energy on the transformation of PCP and sorption properties of adapted and not adapted activated sludge. Table 1 presents the composition of the synthetic wastewater used for the experiments. COD was measured using the MERCK COD Cell Test No. 114541. The content of sludge dry mass was measured using standard methods (APHA 1999).

Adaptation of active sludge to PCP and phenol

Activated sludge was adapted to PCP and phenol in SBRs, with the reactor working volume of two liters. The reactors operated at room temperature. The sludge age was about 10 days. The reactors worked in one cycle per day. The cycle consisted of 23 hours of aeration, 30 minutes of sedimentation, and 30 minutes of decantation and the growth medium replacing.

Appropriate amounts of organic compounds, either PCP and glucose or phenol and glucose, were introduced into the synthetic wastewater so that the total chemical oxygen demand (COD) amounted to about 500–600 mg $O_2 \cdot L^{-1}$. The adaptation of the activated sludge was conducted through the "adaptation saw" method whereby the concentration of PCP or phenol in the growth medium was increased and the concentration of glucose was decreased at the same time. The activated sludge adaptation to PCP and phenol lasted 144 and 50 days respectively. Tables 2 and 3 present the adaptation procedure of the activated sludge to PCP and phenol.

Batch reactors

An appropriate amount of centrifuged activated sludge (5 minutes at 1200 rpm) was introduced to 500 mL Erlenmeyer flasks, with a working volume of 250 mL. The initial concentration of the dry mass of activated sludge amounted to 1.5–2.0 $g \cdot L^{-1}$. The synthetic wastewater with 15 $mg \cdot L^{-1}$ PCP or 15 $mg \cdot L^{-1}$ PCP + 200 $mg \cdot L^{-1}$ phenol or 15 $mg \cdot L^{-1}$ PCP + 400 $mg \cdot L^{-1}$ glucose was also added. The tests were carried out in triplicate.

In order to assess the sorption of PCP on activated sludge, the activated sludge was pre-sterilized twice. Then non-active sludge was introduced into the control setups.

The flasks were shaken in an orbital shaker at 150–200 rpm for 14 days. The growth medium was not replaced and no new organic compounds were added during the experiment.

PCP and phenol concentration measurement using HPLC

The concentration of aromatic compounds in the batch reactors was measured using Reverse Phase High Performance Liquid Chromatography. The measurement was performed using a Dionex UltiMate 3000 liquid chromatograph equipped with a Hypersil GOLD capillary column (250 × 4.6 mm) and a Hypersil GOLD 5 µm 10 × 4 mm precolumn. The HPLC system used in the analyses comprised a pump, an autosampler, and a DAD UV/VIS detector.

Table 1. Synthetic wastewater composition (Visvanathan et al. 2005)

Compound	Concentration in wastewater [$mg \cdot L^{-1}$]
K_2HPO_4	1730
KH_2PO4	680
(NH4)SO4	1000
$MgSO_4 \cdot 7H_2O$	100
$FeSO_4 \cdot 7H_2O$	20
$CaCl_2 \cdot 2H_2O$	30
$MnSO_4 \cdot H_2O$	30
$ZnSO_4$	5
CuSO4	5

Table 2. Activated sludge adaptation to PCP

Time [d]	PCP [$mg \cdot L^{-1}$]	Glucose [$mg \cdot L^{-1}$]
0	0	500
14	1	500
28	2	450
35	3	450
42	4	400
49	5	400
56	6	350
70	7	350
87	8	300
100	9	250
114	10	250
129	11	200
143	12	200

Table 3. Activated sludge adaptation to phenol

Time [d]	Phenol [$mg \cdot L^{-1}$]	Glucose [$mg \cdot L^{-1}$]
0	0	500
7	20	500
14	40	400
21	60	350
28	100	300
42	150	200
49	200	100

Acetonitrile and acetate buffer were used as eluents at a share of 60% and 40%, respectively. The flow speed through the column was 1 ml/min, and retention time of PCP and phenol was about 7.25 minutes and 8.9 minutes, respectively. The volume of the sample injected onto the column equaled 2 µl. Buffer composition can be found in Table 4. Wavelengths of 252, 264, and 322 nm were used for detection. LOD and recovery value were estimated respectively as 0.0905 and 98.7%.

The effectiveness of PCP removal was calculated as a percentage removal of the initial concentration. The initial concentrations were measured using HPLC.

ANOVA was performed to assess the significance of differences between PCP concentrations in each research setup on the last day of the experiment. *Post hoc* Tuckey test was conducted in order to determine the setups between which statistically significant differences occurred.

Results and their discussion

Activated sludge was adapted to PCP and phenol to obtain biomass with a better ability to degrade PCP. This strategy is applied often when xenobiotics are resistant to biodegradation. Mosca and Tomei (2015) adapted activated sludge to PCP in order to obtain a higher degradation potential. Sahinkaya and Dilek (2005) adapted activated sludge to 4-chlorophenol, and Marsolek et al. (2007) to 2,4,5-trichlorophenol. Figures 1 and 2 present the changes in the concentration of PCP and phenol as well as the changes in mixed liquor volatile suspended solids (MLVSS) and COD during adaptation.

The active sludge was adapted to PCP in 144 days, yielding biomass adapted to 12 mg·L^{-1} of PCP (Fig. 1). In turn, the activated sludge was adapted to phenol in 50 days, yielding biomass adapted to 200 mg·L^{-1} of phenol (Fig. 2). Visvanathan et al. (2005) also adapted activated sludge to 12 mg·L^{-1} PCP, whereas Bhattacharya et el. (1996) adapted activated sludge to 15 mg·L^{-1} PCP under anaerobic conditions.

Twenty-four hours after an increased concentration of PCP and phenol application into the reactor, the compounds' concentrations and COD decreased, indicating that these compounds underwent degradation. An increase in the degradation rate of PCP and phenol occurred with an increase in the concentrations of these two compounds. It indicates that the activated sludge adapted to the studied compounds. Maximal degradation rate was 4.53 mg·L^{-1}·d^{-1} for PCP and 185.18 mg·L^{-1}·d^{-1} for phenol. Both reactors showed a decrease in sludge dry mass from about 2.3 g·L^{-1} to about 1.7 g·L^{-1} during the initial stage of adaptation. In the reactor in which the activated sludge was adapted to PCP, sludge dry mass stabilized on day 70 of the experiment, whereas in the reactor in which the activated sludge was adapted to phenol, sludge dry mass stabilized on day 21.

Table 4. Acetate buffer composition

Reagent	Volume
Acetic acid	1.2 mL
water MiliQ	Fill up to 1000 mL
NH_4OH	adjust pH to 5.7
acetonitrile	110 mL

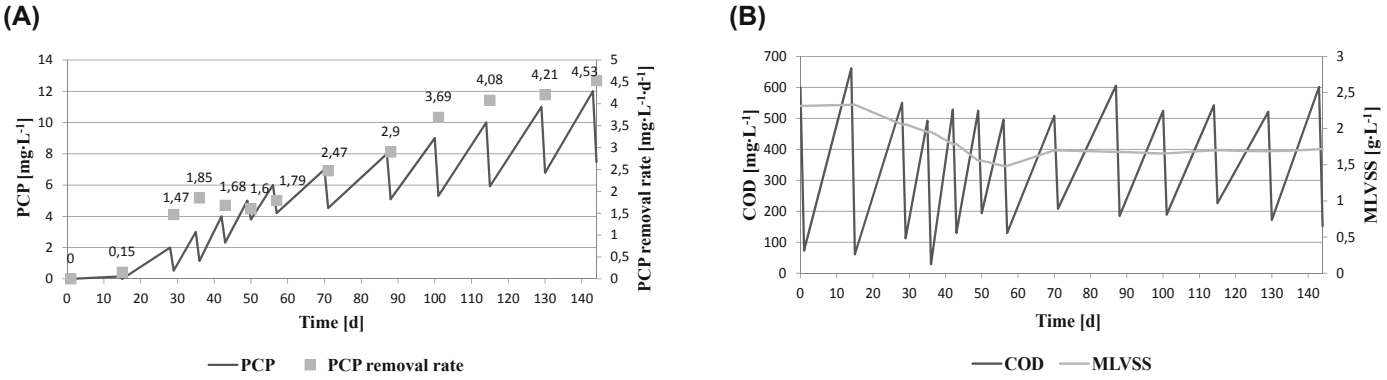

Fig. 1. PCP concentration changes and removal rates (A), COD and MLVSS variations (B) during activated sludge adaptation to PCP

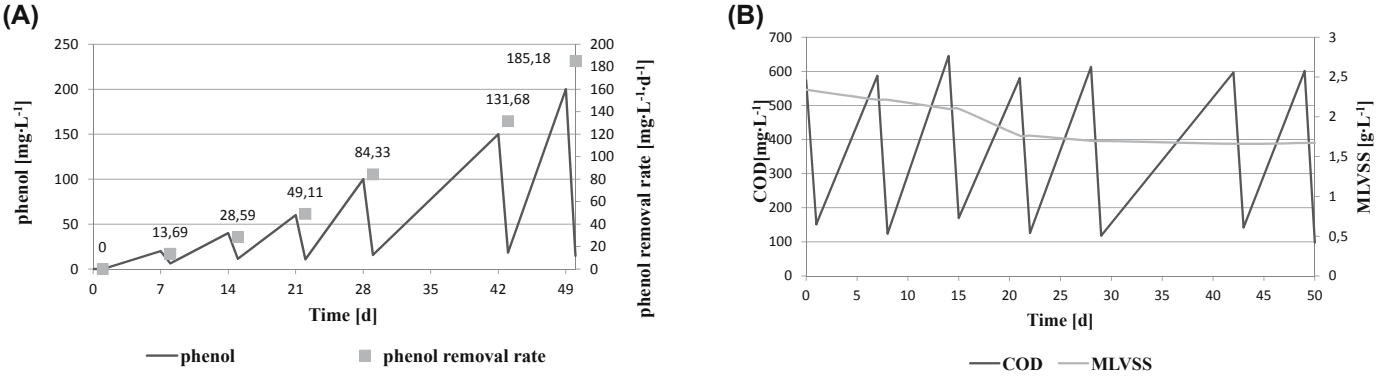

Fig. 2. Phenol concentration changes and removal rates (A), COD and MLVSS variations (B) during activated sludge adaptation to phenol

Effect of biosorption on PCP removal

In order to determine whether PCP undergoes sorption on flocs of activated sludge, and if so, to what extent, research involving inactivated sludge was conducted with nine different research setups. At the same time, in order to rule out chemical or photochemical degradation of PCP, control setups were established without activated sludge (either in the microbiologically active or inactivated forms).

The initial activated sludge dry mass in all research setups used to assess sorption amounted to 1.6–1.7 g·L^{-1}. During the experiment, PCP concentration decreased by about 10% due to the biosorption of the compound on flocs of active sludge, regardless of the type of the research setup (Fig. 3–5). This corresponded to about 0.85 mg of PCP per 1 g of sludge dry mass. The sorption of PCP on flocs of activated sludge was determined experimentally to be about 35% higher than in the literature. Visvanathan et al. (2005) conducted a study on the biosorption and biodegradation of PCP by activated sludge. Their study found that the sorption amounted to 0.63 mg PCP per 1 gram of sludge dry mass for a PCP concentration of 15 mg·L^{-1}. No significant changes in PCP concentration or COD were observed in the control setups (with no activated sludge added).

In the case of the research setups with not adapted sludge (Fig. 3A, 4A, 5A), PCP concentration decreased by about 30% on day 1, after which the concentration began to increase. This may be due to the sorption of PCP on the flocs of not adapted activated sludge followed by the desorption of the compound. Furthermore, COD decreased in all research setups, which may indicate that the introduced growth substrates underwent biosorption. Aksu and Yener (2001) conducted a study in which they showed that dried and granulated activated sludge may be used as a sorbent for the removal of phenol and monochlorophenols from aqueous solutions.

In the research setups used to assess the sorption of PCP on flocs of activated sludge adapted to PCP or phenol (Fig. 3B and 3C, Fig. 4B and 4C, Fig. 5B and 5C), PCP concentration decreased by about 10% on day 1. No significant changes in PCP concentration in the growth medium were noted during the subsequent days of the experiment.

When pentachlorophenol was the only growth substrate, the decrease in PCP concentration in the degradation setups with non-adapted activated sludge and activated sludge adapted to phenol was similar to that in the corresponding sorption setups (Fig. 3A and 3C). This suggests that PCP as a sole growth substrate does not undergo microbiological degradation by non-adapted activated sludge and activated sludge adapted to phenol. In other flasks (Fig. 3B), the decrease in PCP concentration on day 14 of the experiment was higher in the biodegradation setups than in the sorption setups. Therefore, the obtained results confirm that a decrease in PCP stems partially from biodegradation and partially from the sorption of the compound. The results also indicate that microbiological transformations of PCP took place in the research setups.

In the reactors to which both PCP and phenol were added, the biosorption of phenol on day 14 of the experiment amounted to about 85–90% (no data shown). In the biodegradation setups, a reduction in phenol concentration of about 90% was observed already on day 2 of the experiment, and no presence of phenol was observed from day 5 onwards. The obtained results may indicate that the sorption and biodegradation of phenol took place simultaneously. Moreover, the results may suggest that PCP and phenol accumulated in the biomass inside

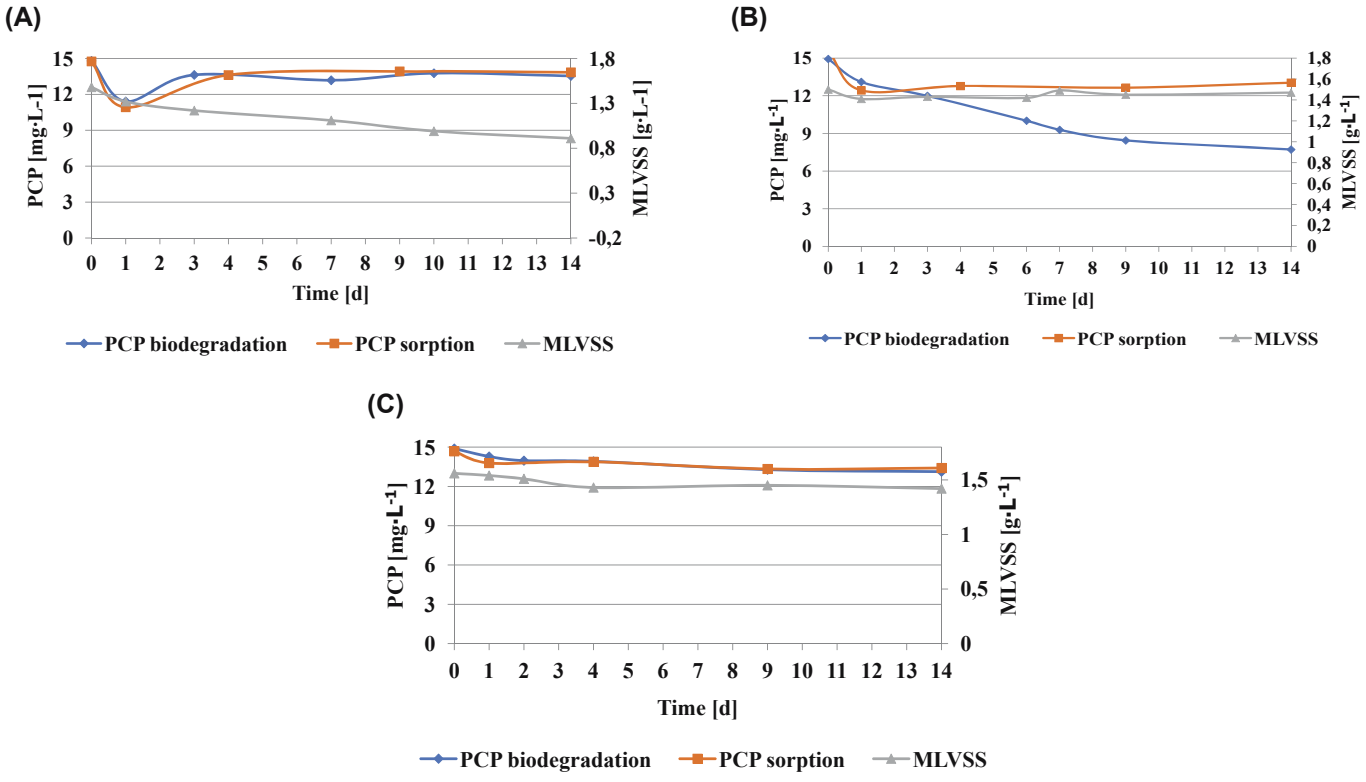

Fig. 3. Impact of activated sludge adaptation on biodegradation and sorption of PCP as a sole growth substrate and MLVSS for (A) not adapted activated sludge, (B) activated sludge adapted to PCP and (C) activated sludge adapted to phenol

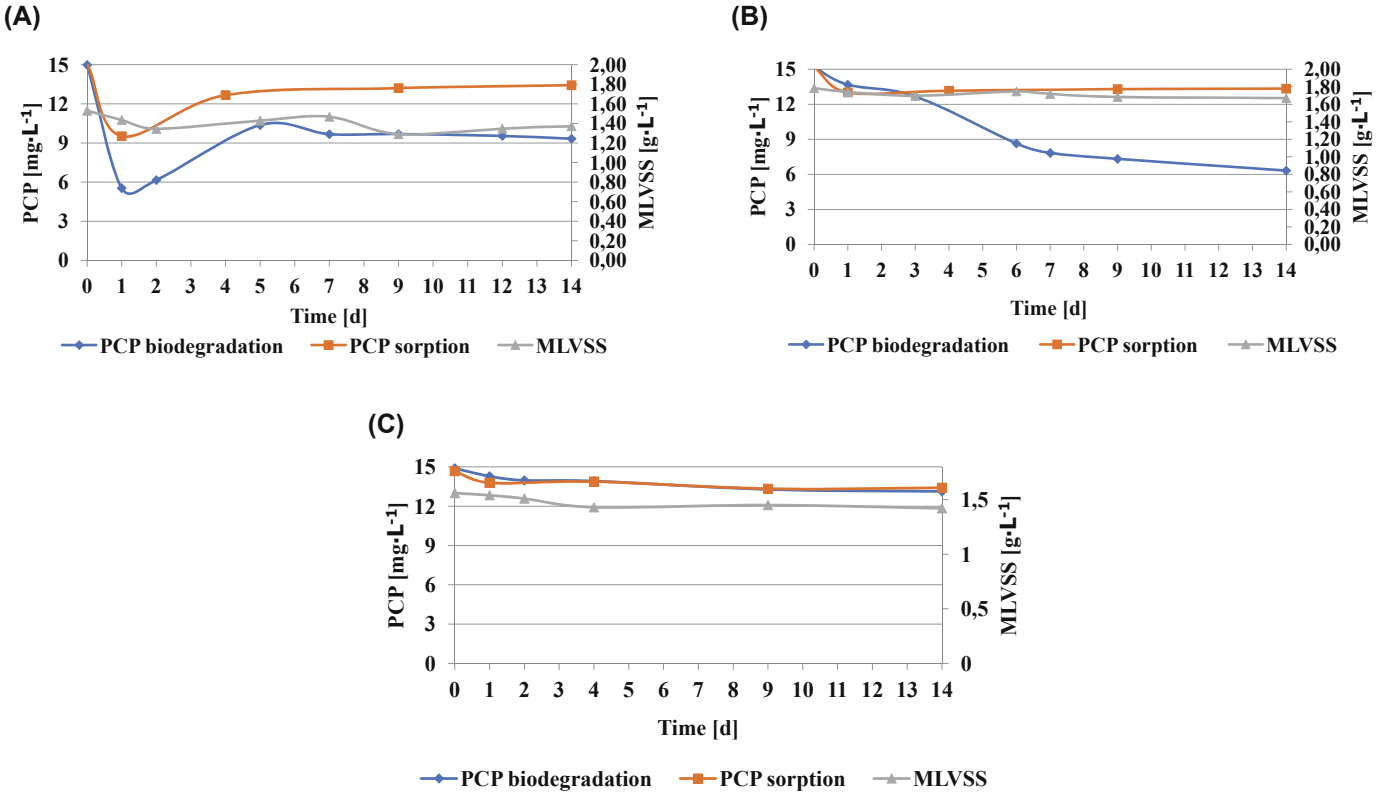

Fig. 4. Impact of activated sludge adaptation on biodegradation and sorption of PCP with phenol as the growth substrate and MLVSS for (A) not adapted activated sludge, (B) activated sludge adapted to PCP and (C) activated sludge adapted to phenol

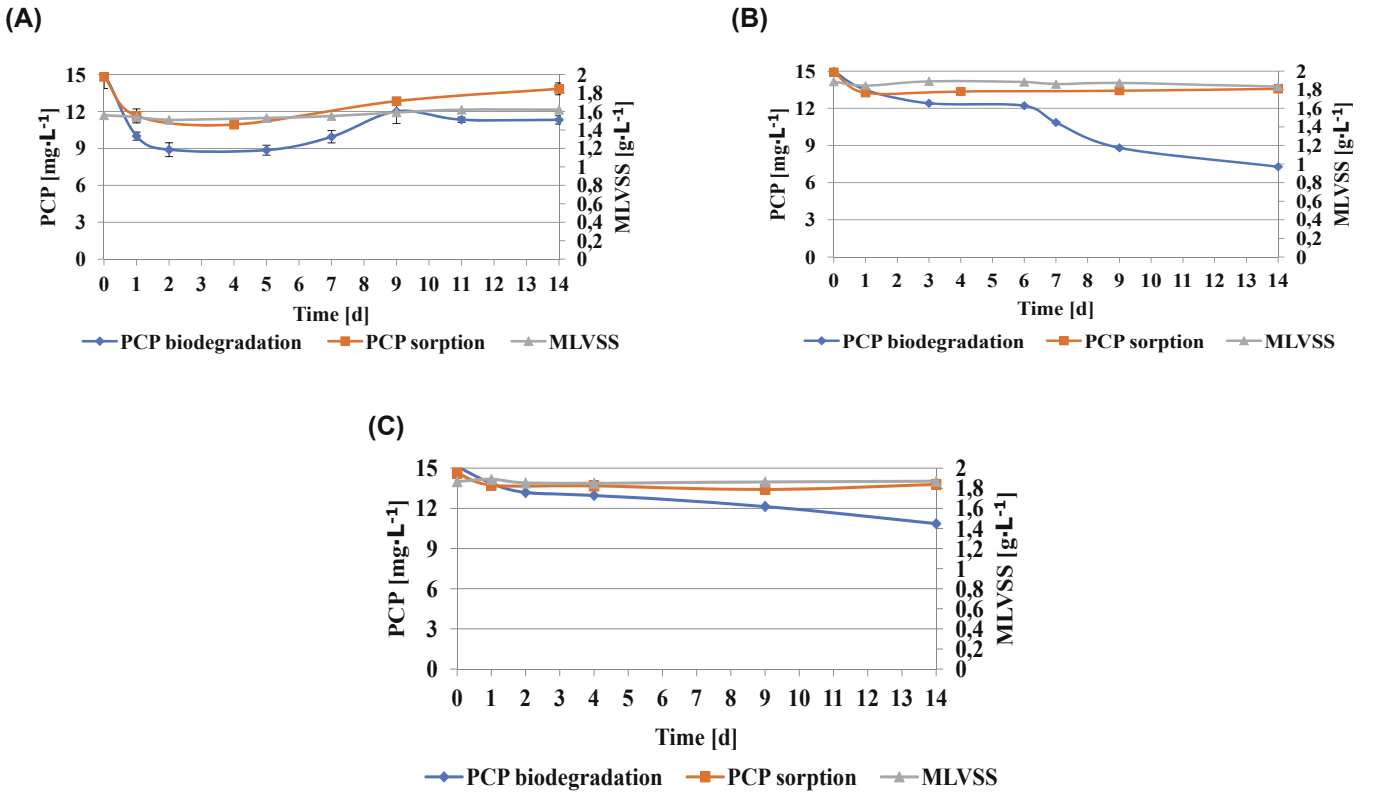

Fig. 5. Impact of activated sludge adaptation on biodegradation and sorption of PCP with glucose as the growth substrate and MLVSS for (A) not adapted activated sludge, (B) activated sludge adapted to PCP and (C) activated sludge adapted to phenol

the biodegradation setups, i.e., the two compounds initially underwent brief sorption on flocs of activated sludge, after which they underwent biodegradation.

Antizar-Ladisalo and Galil (2004) and Wang et al. (2012) also observed the biosorption of phenol and chlorophenols, including PCP. Jacobsen et al. (1996) conducted a study on the effect of sludge age on the sorption of PCP into the activated sludge. They found that sorption exceeded 50% for sludge aged less than 3 days, but was below 10% for sludge aged more than 14 days. The age of the activated sludge used in this study was about 10 days, which means that the observed sorption of about 10% is comparable to literature data.

Effect of the adaptation of activated sludge on the biotransformation of PCP

Important factors that affect the degradation of chlorophenols include the presence of additional sources of carbon and energy, the concentration of these sources, activated sludge adaptation, pH, and technology used in the experiment (Mosca and Tomei 2015, Sahinkaya and Dilek 2006).

Figure 6 presents data on the effect of the adaptation of activated sludge to PCP or phenol on the biotransformation of PCP in comparison to non-adapted sludge. A positive effect of activated sludge adaptation to PCP was found. On the other hand, no statistically significant differences occurred between non-adapted activated sludge and activated sludge adapted to phenol. In both cases, the lack or the addition of an additional source of carbon and energy did not result in differences in the PCP removal.

All the conducted statistical analyses indicate that the differences between the tested research setups were statistically significant (Tab. 5). Table 6 presents the results of the Tukey test *post hoc* analysis. Statistically significant differences were found. For the level of significance of $\alpha = 0.05$ between the setups with non-adapted activated sludge and activated sludge adapted to phenol and setups with activated sludge adapted to PCP. *Post hoc* analysis did not find an effect of the adaptation of activated sludge to phenol on the efficiency of PCP degradation. Most probably, the adaptation to phenol did not result in activation of enzymes capable of PCP decomposition

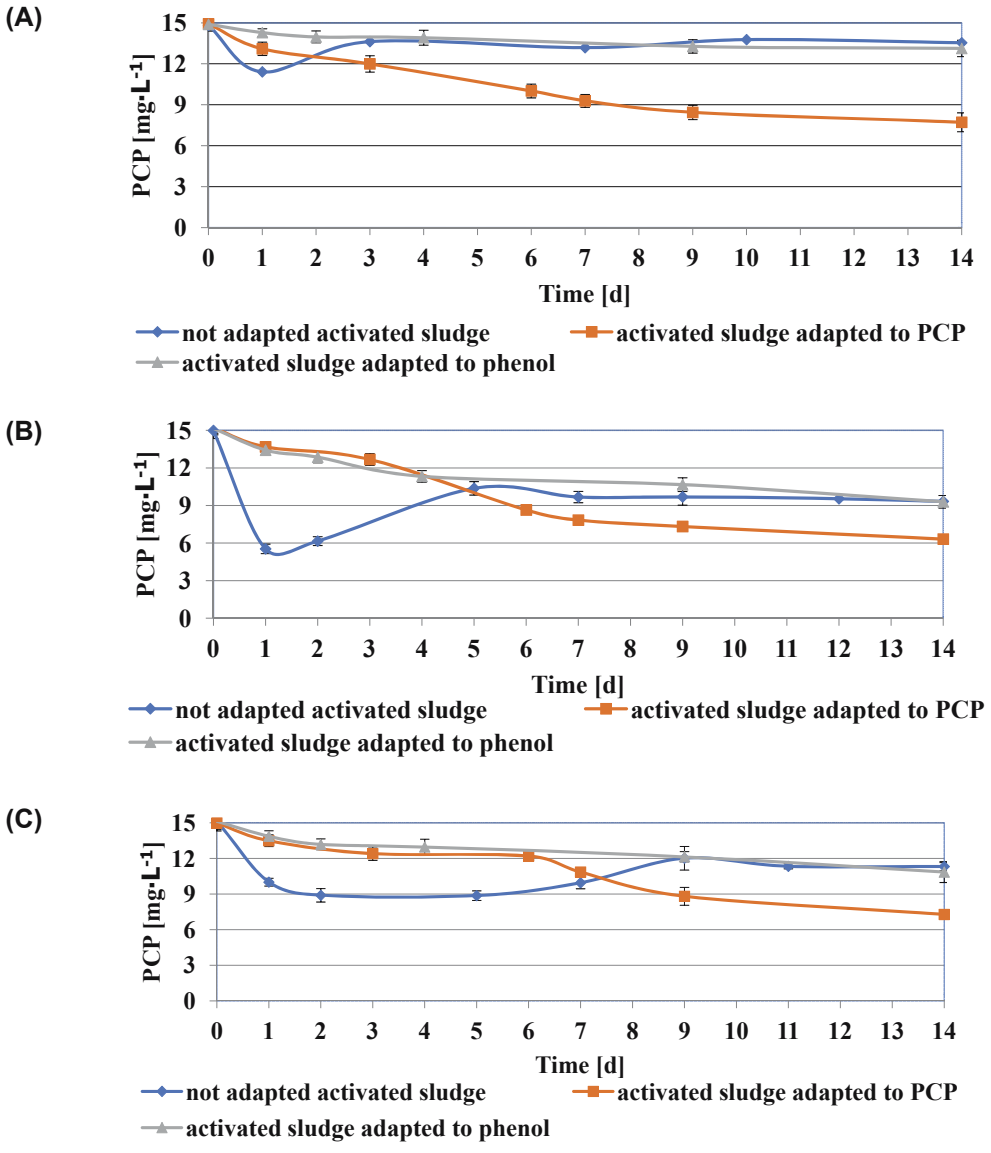

Fig. 6. Impact of activated sludge adaptation to PCP biodegradation in setups: with PCP as a sole growth substrate (A), with PCP and phenol (B), with PCP and glucose (C)

in activated sludge. Although phenol is a structural analogue of PCP, the presence of chlorine substituents in the ring results in its high stability and resistance to non-specific, phenol oxidizing enzymes. Visvanathan et al. (2005) also observed a positive effect of activated sludge adaptation to PCP on the degradation of PCP under aerobic conditions. Activated sludge adaptation is also important for the anaerobic degradation of PCP (Shen et al., 2005). Bhattacharya et al. (1996) noted a 93% degradation of PCP under anaerobic conditions for adapted activated sludge and no changes in PCP concentration for not adapted activated sludge. The effect of activated sludge adaptation was also observed for other chlorophenols under aerobic conditions. Sahinkaya and Dilek (2005) and Carruci et al. (2008) noted that 4-chlorophenol degraded only when activated sludge adapted to it was used in the research. On the other hand, Uysal and Turkman (2007) found that the adaptation of activated sludge to 4-chlorophenol had no effect on the degradation of the compound.

In all types of research setups, regardless of the presence of additional growth substrates or lack thereof, adaptation was found to affect COD. In the setups with PCP as the only growth substrate, adaptation was also found to affect sludge dry mass. When PCP was the only growth substrate in the setups with not adapted activated sludge and activated sludge adapted to phenol, a decrease in sludge dry mass and an increase in COD compared to the initial values were observed during the experiment. The decrease in sludge dry mass may have resulted from the toxic effect of PCP on microorganisms in the activated sludge, whereas the increase in COD may have resulted from the degradation of the biomass.

Table 5. One-way analysis of variance ANOVA (impact of activated sludge adaptation)

	Research setup	Mean concentration of PCP in 14th day [mg·L^{-1}]	Variance	F	TEST F	p
PCP	Not adapted activated sludge	13.54	0.12	115.01	3.68	7.98E-10
	Activated sludge adapted to PCP	7.88	0.81			
	Activated sludge adapter to phenol	13.13	0.62			
PCP + PHENOL	Not adapted activated sludge	9.33	0.19	59.92	3.68	7.03E-08
	Activated sludge adapted to PCP	6.23	0.33			
	Activated sludge adapter to phenol	9.30	0.44			
PCP + GLUCOSE	Not adapted activated sludge	11,32	0.11	85.03	3.68	6.55E-09
	Activated sludge adapted to PCP	7.28	0.15			
	Activated sludge adapter to phenol	10.86	0.78			

F – calculated value; Test F – critical value determined from tables of distribution F-Snedecora for 2 and 15 degrees of freedom and α = 0,05; p – value calculated

Table 6. The results of *post hoc* analysis of variance (Tukey test) testing the significance of differences between tested research setups (impact of activated sludge adaptation)

	Research setup	Difference of mean values	LSD	Significance of differences between mean values
PCP	Not adapted activated sludge vs activated sludge adapted to PCP	5.66	1.42	+
	Not adapted activated sludge vs activated sludge adapted to phenol	0.42		-
	activated sludge adapted to PCP vs activated sludge adapted to phenol	5.24		+
PCP + PHENOL	Not adapted activated sludge vs activated sludge adapted to PCP	3.09		+
	Not adapted activated sludge vs activated sludge adapted to phenol	0.03	1.11	-
	activated sludge adapted to PCP vs activated sludge adapted to phenol	3.06		+
PCP + GLUCOSE	Not adapted activated sludge vs activated sludge adapted to PCP	4.04		+
	Not adapted activated sludge vs activated sludge adapted to phenol	0.47	1.16	-
	activated sludge adapted to PCP vs activated sludge adapted to phenol	3.57		+

LSD – Least Significant Difference

In the research setups with added phenol or glucose and non-adapted sludge, COD decreased by about 50% on day 1 of the experiment, and increased back to about 70% of its initial value on the following days. These results correlate with the course of sorption for analogous research setups. Therefore, the changes in COD may be explained through the sorption and desorption of PCP on flocs of the activated sludge and through a partial biodegradation of the additional growth substrates. In the research setups with adapted activated sludge, COD decreased by 65–95%. The obtained results likely stem from the concurrent degradation of the co-metabolite and the growth substrate. It can be concluded that the adaptation of activated sludge to PCP and phenol decreases the toxic effect of PCP to microorganisms and allows phenol and glucose to be degraded to a higher degree compared to non-adapted sludge.

Effect of the presence of additional sources of carbon and energy on the biotransformation of PCP

Due to the fact that PCP is resistant to biodegradation and that no studies exist on the degradation of PCP as the only growth substrate by activated sludge, the aim of this study was to determine whether phenol and glucose may constitute growth substrates for a co-metabolic degradation of PCP. A co-metabolic system involves a co-metabolic substrate in addition to the growth substrate. The co-metabolic substrate is not used by bacteria as a source of carbon or energy (Cornelissen and Sijm 1996). Phenol and glucose were chosen as the growth substrates since, according to literature data, they can be applied for a co-metabolic degradation of chlorophenols (Hao et al. 2002, Huang et al. 2011). Phenol is a structural analogue of PCP and can induce the enzyme of the PCP degradation pathway. In turn, glucose is an easily assimilated source of carbon and energy and can contribute to an increase in biomass and the regeneration of cofactors, necessary for the transformation of the co-metabolite (Loh i Wang 1998).

The effect of the presence of phenol or glucose on the biotransformation of PCP was assessed. Figure 7 presents data on the effect of additional sources of carbon and energy on the microbiological degradation of PCP. Phenol addition was responsible for the best PCP removal not depending on

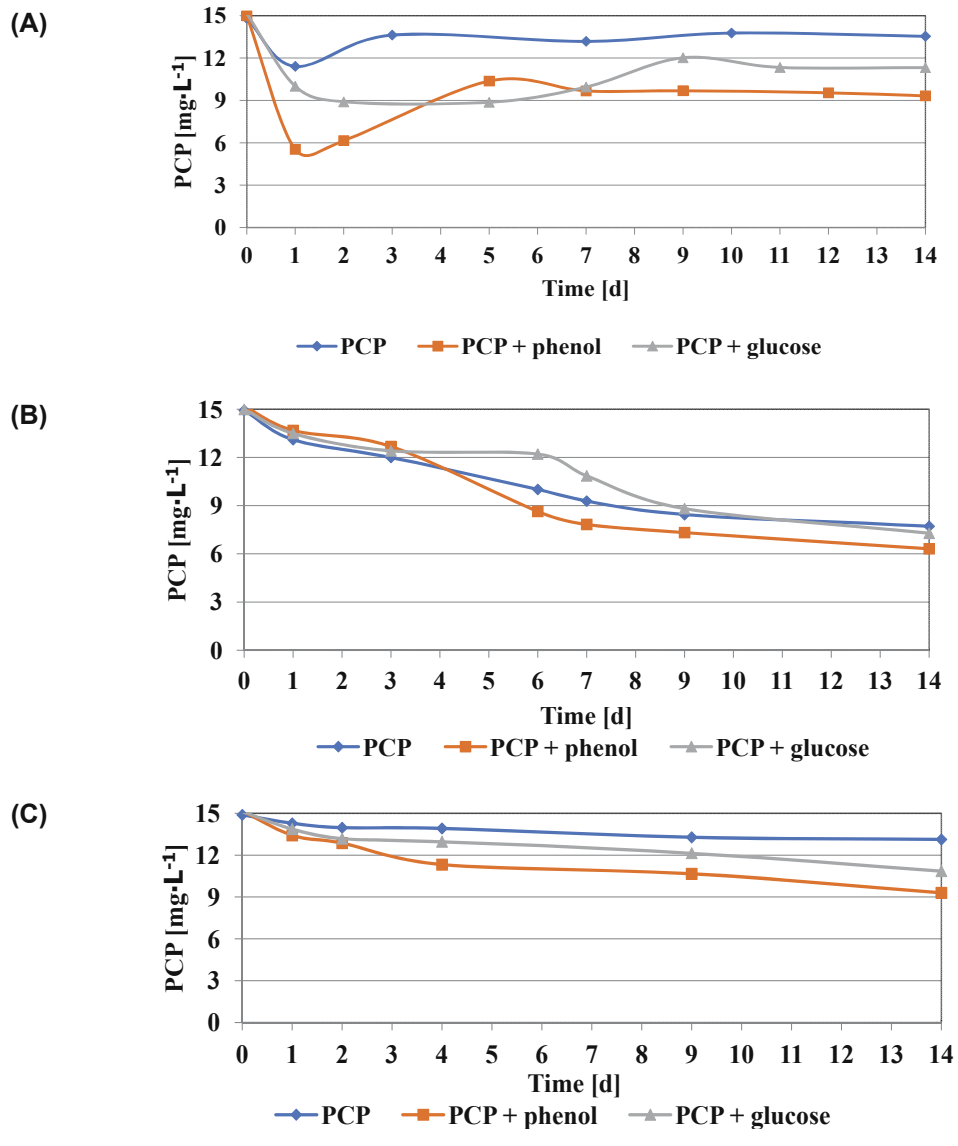

Fig. 7. Impact of the growth substrates on PCP biodegradation in the setups: with not adapted activated sludge (A), with activated sludge adapted to PCP (B), with activated sludge adapted to phenol (C)

activated sludge adaptation (Fig. 7B). Wang et al. (2015) also observed a positive effect of phenol on the transformation of 4-chlorophenol by *Pseudomonas putida* LY1, although they noted (2012) an increase in the efficiency of PCP degradation in the presence of glucose. In the presented research the glucose addition also gave positive results in PCP removal not depending on activated sludge adaptation. However its effect was weaker than the addition of phenol. The difference in PCP removal in the case of the addition of glucose and phenol decreases in the adapted sludges. The smallest difference was observed in the activated sludge adapted to PCP. Probably, for adapted bacteria, having enzymes able to degrade recalcitrant compound, the source of biodegradable carbon is less important.

ANOVA test was performed to determine the significance of differences in PCP concentration on day 14 of the experiment between the research setups. The results of ANOVA can be found in Table7.

All three one-way analyses of variance conducted in order to confirm or exclude the effect of phenol and glucose on the transformation of PCP leads to the conclusion that statistically significant differences occurred between the tested groups of setups. *Post hoc* test was conducted in order to determine the pairs of setups between which the differences were statistically significant. The results of the *post hoc* test can be found in Table 8.

The *post hoc* test found statistically significant differences between all tested pairs of research setups for both non-adapted

Table 7. One-way analysis of variance ANOVA (phenol and glucose impact)

	Research setup	Mean concentration of PCP in 14th day [mg·L^{-1}]	Variance	F	TEST F	p
Not adapted activated sludge	PCP	13.54	0.12	191.33	3.68	2.11E-11
	PCP + phenol	9.32	0.19			
	PCP + glucose	11.32	0.11			
Activated sludge adapted to PCP	PCP	7.88	0.81	9.77	3.68	0.0019
	PCP + phenol	6.23	0.33			
	PCP + glucose	7.28	0.15			
Activated sludge adapted to phenol	PCP	13.13	0.62	36.25	3.68	1.8E-06
	PCP + phenol	9.30	0.44			
	PCP + glucose	10.86	0.78			

Table 8. The results of *post hoc* analysis of variance (Tukey test) testing the significance of differences between tested research setups (phenol and glucose impact)

	Research setup	Difference of mean values	LSD	Significance of differences between mean values
Not adapted activated sludge	PCP vs PCP + phenol	4.22	0.74	+
	PCP vs PCP + glucose	2.22		+
	PCP + phenol vs PCP + glucose	1.20		+
Activated sludge adapted to PCP	PCP vs PCP + phenol	1.65		+
	PCP vs PCP + glucose	0.60	1.30	–
	PCP + phenol vs PCP + glucose	1.05		–
Activated sludge adapted to phenol	PCP vs PCP + phenol	3.82		+
	PCP vs PCP + glucose	2.27	1.55	+
	PCP + phenol vs PCP + glucose	1.56		+

activated sludge and activated sludge adapted to phenol. In the case of the activated sludge adapted to PCP, significant differences occurred between the setups containing PCP as the only growth factor and the setups containing both PCP and phenol. However, no statistically significant differences occurred between flasks containing PCP alone and PCP and glucose or between the two setups containing additional growth factors.

Conclusions

The obtained results confirmed that the presence of additional sources of carbon and energy in the growth medium would improve the efficiency of PCP degradation. Among all analyzed types of research setups, the highest PCP degradation was noted in setups with phenol, regardless of the method of active sludge adaptation or lack of adaptation. The biodegradation of PCP in the presence of glucose was less efficient than in the presence of phenol.

The conducted research on the effect of activated sludge adaptation showed that the highest degree of PCP degradation occurred in the setups with activated sludge adapted to PCP. Non-adapted and adapted to phenol activated sludge is not able use PCP as a sole source of carbon and energy.

The obtained results indicate that the highest, 60% decrease in PCP concentration was obtained for activated sludge adapted to PCP in the presence of phenol.

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Degradacja pentachlorofenolu przez osad czynny z fenolem i glukozą jako substratami wzrostowymi

Streszczenie: Długotrwałe stosowanie pentachlorofenolu (PCP) spowodowało jego powszechne występowanie w środowisku. Ludzie mający stały kontakt z PCP narażeni są na choroby nowotworowe, uszkodzenia płodu, mutacje genetyczne, zaburzenia obrazu krwi, a także zmiany w układzie nerwowym. Ważnymi czynnikami wpływającymi na efektywność mikrobiologicznego rozkładu chlorofenoli są dodatkowe substraty wzrostowe zapewniające donory i akceptory elektronów, odpowiednie mikroorganizmy i ich adaptacja do rozkładanych związków. Z tego powodu oceniono wpływ adaptacji osadu czynnego do PCP i fenolu oraz glukozy i fenolu, jako dodatkowych substratów wzrostowych, na poprawę biodegradacji PCP. Osad czynny został zaadaptowany do 12 $mg \cdot L^{-1}$ PCP i 200 $mg \cdot L^{-1}$ fenolu, a następnie efekt adaptacji osadu czynnego i dodatku źródła węgla i energii na biodegradację i sorpcję PCP był badany. Uzyskane rezultaty potwierdziły, że obecność dodatkowego źródła węgla i energii w pożywce zwiększa efektywność usuwania PCP. Najwyższe, 60 procentowe usunięcie PCP uzyskano w zaadaptowanym do PCP osadzie czynnym w obecności fenolu jako substratu wzrostowego.