

Assisted Bioremediation Approaches - Biostimulation and Bioaugmentation - Used in the Removal of Organochlorinated Pollutants from the Contaminated Bottom Sediments

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ABSTRACT: The prospects of using the biostimulation and bioaugmentation treatment for the removal of the polychlorinated biphenyl (PCBs) from contaminated sediment collected from the sewage canal in the surroundings of a former PCB manufacturing plant in Slovakia is described. The eleven bacterial strains isolated in our previous work from sewage Strážsky canal sediments were able to aerobically degrade significant amounts of PCBs. Five of the bacterial isolates obtained were used in bioaugmentation treatment individually as single strains and within the eight artificially prepared consortia consisting of two or three strains. Bioaugmentation by a single strain was performed in other set of experiments combined with the addition of nonionic surfactants (Triton X and Tween 80) to increase bioavailability of PCBs and with the addition of terpenes (carvone and limonene) to induce required enzymes. The highest biodegradation of PCBs in biostimulation treatment was obtained using all three studied factors - addition of nitrogen, phosphorus, and oxygen to the indigenous microorganisms naturally present in the contaminated sediment. The highest biodegradation of PCBs in bioaugmentation experiments was obtained with the individual bacterial isolates (one Gram-positive and one Gram-negative), and with a laboratory prepared consortium consisting of three selected bacterial isolates. Addition of surfactant Tween 80 led to a higher biodegradation of PCBs than that of Triton X. Bioaugmentation treatment using the addition of single bacterial isolate and surfactant Tween 80 improved elimination of the evaluated indicator PCB congeners to the highest extent and thus appeared to be the most perspective technique for possible field bioremediation.

Key words: Bioaugmentation, Biostimulation, PCBs, Surfactants, Terpenes

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a group of 209 compounds, which were commercially produced as complex mixtures for a variety of applications such as coolants and dielectric fluids in capacitors and transformers (Laes et al., 2006). Their thermal and chemical stability, resistance to chemical corrosion, and general inertness have contributed to their persistence in the environment even if their manufacture was banned more than 40 years ago (Petric et al., 2011). PCBs are considered as one of the most widely distributed class of aromatic chlorinated chemicals in the food chain.

Degradation of PCBs is the major environmental concern regarding their contamination of soils and groundwater, and potential hazardous effects on human health. Since PCBs are hydrophobic compounds, they partition preferentially to organic milieu in the environment that serves as both long-term reservoirs and carriers that can distribute PCBs great distances from the original source point. Although adsorbed PCBs resist migration into the water fraction, PCBs enter the food chain by ingestion and desorption in microorganisms leading to eventual bioaccumulation and biomagnification of PCBs in organisms higher up in

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the food chain (Jonka et al., 2015). PCBs are absorbed by humans and animals through the skin, lungs, and the gastrointestinal tract (Borja et al., 2005).

Several methods for PCBs degradation in environmental matrices have been suggested. However, since physical and chemical treatments are generally expensive and not always efficient, current research has been focused on the development of bioremediation processes. Bioremediation is considered as an efficient and cost-effective process for the elimination of PCBs using microorganisms capable of degrading toxic compounds (Tandlich et al., 2011). The main bioremediation strategies are bioaugmentation and biostimulation, the introduction of PCB-degrading bacterial strains individually or as a consortium and introduction of nutrients, oxygen, surfactants or inducers, respectively (Mrozik et al., 2010; Megharaj et al., 2011). Biostimulation is based on the stimulation of growth of the naturally occurring pollutant-degrading microorganisms. The acceleration of microbial turnover of chemical pollutants generally depends on the supply of carbon, nutrients, on temperature, available oxygen (O₂), soil pH, redox potential, the type and concentration of the organic pollutant itself. Stimulation of microbial degradation by the addition of nutrients is the subject of various studies evaluating bioremediation of oil or petroleum-contaminated soil and sediments (Evans et al., 2004) or PBDE-contaminated sewage sludge (Stiborová et al., 2015). Bioaugmentation is based on the application of indigenous or exogenous microorganisms to polluted hazardous waste sites in order to accelerate the removal of undesired compounds. Augmentation approaches appear to have a great potential for aromatic compounds remediation (Egorova et al., 2013). The main objective of this study was to evaluate the biodegradation of PCBs using a) bioaugmentation treatment of historically PCB-contaminated sediment with the addition of the individual bacterial strains or bacterial consortia to sterile or non-sterile sediments, b) addition of selected surfactants (Triton X and Tween 80), and terpenes (carvone and limonene) to enhance bacterial degradation of PCBs, b) biostimulation treatment of sediment using the addition of nutrients, namely N and P, to enhance growth and required metabolic activity of the indigenous bacterial degraders naturally present in the contaminated bottom sediment.

MATERIALS & METHODS

Minimal mineral medium (MM medium) containing of 1 g/l (NH₄)₂SO₄, 2.7 g/l KH₂PO₄, 5.2 g/l NaHPO₄·2H₂O, 0.2 g/l MgSO₄·7H₂O, 0.01 g/l FeSO₄·7H₂O and 0.03 g/l Ca(NO₃)₂·4H₂O (Lachema Brno, Czech Republic) was prepared according to Dudášová et al., 2012. PCA agar (Plate count agar) containing yeast extract 2

g/l, dextrose 1 g/l, agar 15 g/l, and casein enzyme hydrolysate 5 g/l (Imuna, Šarišské Michaľany, SR) was prepared according to Oxoid Company (UK) manual. Growth medium was prepared using meat extract 3 g/l, peptone 2 g/l, 5 g/l NaCl, and 0.3 g/l KH₂PO₄ (HiMedia Lab., Mumbai, India). PCBs naturally present in contaminated sediment were meant to be the main carbon source.

Isolation of the eleven bacterial strains from PCB-contaminated sediments was described in our previous study (Dudášová et al., 2014) and this study deals with five of them namely *Achromobacter xylosoxidans* (Ax), *Stenotrophomonas maltophilia* (Sm), *Starkeya novella* (Sn), *Stenotrophomonas* sp. (Ss), and *Rhodococcus* sp. (Rs). Bacterial strains had calibration formula (absorbance measured at $\lambda = 620$ nm): AAx = 0.6150x + 0.022; ASm = 1.6033x - 0.0493; ASn = 1.1791x + 0.0273; ASs = 2.0918x - 0.0802; ARs = 2.8460x - 0.0301.

Sediment used in the following experiments was collected from the bottom of the Strážsky canal. This region had been historically contaminated with PCBs because of the production of commercial PCB mixtures at a factory Chemko Strážske. The sediments were sampled using an Uwitec sampler (Austria) according to the standard protocol in agreement with the Slovak technical norm (STN) in cooperation with the Water Research Institute in Bratislava, Slovakia. Sampling was provided from multiple places mainly to ensure the representativeness of sediment properties (N 48°51'33.8"; E 21°50'35.4" Garmin GPSmap 78) pH of sediment was 6.94-7.24, total organic carbon (TOC) was 21.9 g/kg, redox potential was 197-439 mV, and organic content mass was 1.05-7.06 %. The reference congeners commonly monitored in the environmental toxicology (PCB 28, 52, 101, 118, 138, 153, and 180) were used to represent the PCB contamination. The same congeners were used for evaluation of PCB degradation. Initial amounts of selected PCB congeners in the sediment used in the experiments were as follows (in mg of PCBs per kg of sediment): PCB 8 (2,4-) 0.659; PCB 28 (2,4,2-) 4.50; PCB 52 (2,2',5,6'-) 6.77; PCB 101 (2,2',4,5,5'-) 4.34; PCB 118 (2,3',4,4',5-) 11.5; PCB 138 (2,2',3,4,4',5'-) 7.27; PCB 153 (2,2',4,4',5,5'-) 8.78; PCB 180 (2,2',3,4,4',5,5'-) 6.03; PCB 203 (2,2',3,4,4',5,5',6-) 1.67; the total content of the detected PCBs was 51.7 mg/kg of sediment. Important information about bioavailability of each PCB congener was reported in our previous study (Dudášová et al., 2014).

Biostimulation treatment was carried out in 250 Erlenmeyer flasks closed with a cotton wool stopper. Twenty g of dried non-sterile sediment was mixed with 100 ml mineral medium. Two different experimental patterns were set up. The first set of experiments was performed with sediment mixed with mineral medium with

additional nitrogen (N) and phosphorus (P) source (500 mg/kg NaNO₃ and 1800 mg/kg Na₂HPO₄) (Lachema, Brno, Czech Republic). The second set of experiments used sediment mixed with mineral medium with additional nitrogen, phosphorus, and oxygen sources (500 mg/kg NaNO₃ and 1800 mg/kg Na₂HPO₄, increased oxygen concentration was provided by using 500 ml flasks, i.e. twofold bigger volume). Untreated sediment mixed with the mineral medium was used as the control. All flasks were incubated at 28 °C on a rotary shaker (180 rpm) for 21 days in the dark.

The bioaugmentation treatment was carried out in 250 ml Erlenmeyer flasks closed with a cotton wool stopper. Twenty g of dried sterile/non-sterile sediment was mixed with 100 ml mineral medium. Five selected bacterial strains isolated from the sediment sampled from Strážský canal and eight consortia were selected and constructed for bioaugmentation of sterile and non-sterile sediment contaminated with PCBs. Bacterial strains were prepared individually by incubating in a growth medium at 28 °C and 180 rpm for 48 h. After cultivation, strains were centrifuged at 3200 rpm for 20 min and the biomass suspension was added into the flasks as inoculum in concentration of 1 g/l. When the consortium of two degraders was used, the concentration of added suspension of each strain was 0.5 g/l, while in the case when consortium consisted of three degraders, suspension of each strain was used in a concentration of 0.33 g/l. Concentrations of biomass were evaluated according to absorption and calibration curves. Untreated sediment mixed with the mineral medium free of biomass was used as the control. The flasks were incubated at 28 °C in stationary position for 21 days in the dark. The biodegradation set was carried out in the sterile 250 ml Erlenmeyer flasks. Bioaugmentation of sediment using the addition of bacterial strain *S. maltophilia* was stimulated by two synthetic nonionic surfactants Triton X and Tween 80 (Sigma Aldrich, USA). Triton X and Tween 80 were used within each of three parallels at the concentration of 0.3% w/w and 0.032 % respectively which is twice the value of its critical micelle concentration (CMC). Amount of dried non-sterile sediment, mineral medium, biomass concentration and control were identical as described above.

The bioaugmentation experiment was performed in 250 ml Erlenmeyer flasks with the bacterial strain *Rhodococcus* sp. and with a consortium consisted of *A. xylosoxidans*, *Rhodococcus* sp., and *S. novella*. The bacterial degradation ability was stimulated by the addition of synthetic terpenes (carvone and limonene). The initial concentration 10 mg.l⁻¹ of carvone or limonene (Merck, Germany) was used and the terpenes were added separately into the flasks at the beginning of degradation process (1st day). The experimental pro-

cedure as well as the conditions remained the same as in the previous bioaugmentation process with the exception of using sterile sediment.

After the 21 days of degradation, whole flasks content was placed into the centrifugation cuvette. After centrifugation ($t = 15$ min, 3000 rpm), 2 g of dried sediment was placed into Soxhlet extractor (Sigma Aldrich, Germany) and extracted using 60 ml n-hexane (Merck, Germany) for 4 h. After this time, a teaspoon of powdered copper was added into the extract to eliminate sulphuric compounds from the sediment and the whole mixture was placed into an ultrasonic bath for 45 min. Copper was removed by filtration through the florisil column. n-Hexane was evaporated on a vacuum rotary evaporator and the extract was dissolved in 500 μ l n-hexane and analyzed by GC. Internal standard PCB 209 was used for determination of the extraction efficiency, with recovery of 85.3 % and 4.7 % accuracy. To ensure high quality of measurements, blanks were monitored simultaneously with each batch of 10 samples.

All determined PCB congeners were analyzed by gas chromatography (HP 5890) with an electron capture detector (ECD). The GC conditions were as follows: silica capillary column (30 m \times 0.25 mm i.d.) with non-polar stationary phase HP-5MS (0.25 μ m), a column temperature program: initial temperature 70 °C (hold 2 min) to 150 °C at 25 °C/min (hold 0 min), from 150 °C to 200 °C at 3 °C/min (hold 0 min), from 200 °C to 280 °C at 8 °C/min (hold 3 min); carrier gas: H₂ (90 kPa) with constant flow 1.5 ml/min; injection temperature: 250 °C; injection volume: 2 μ l using split injection mode (split time: 2 min), detector: ECD, 280 °C, make up gas N₂ 40 ml/min. Identification of seven PCB congeners (PCB 28, 52, 101, 118, 138, 153, and 180) was based on their retention time, while quantitative analysis of the results was performed based on peak areas corresponding to the congeners in the chromatograms. Mixture of seven indicator PCB congeners was used for calibration (Dr. Ehrenstorfer, Germany).

SPSS (version 22) and Microsoft Excel were used for statistical evaluation (Kruskal-Wallis One Way Analysis of Variance on Ranks). Level of $p < 0.05$ was considered statistically significant. The degradation of PCBs was calculated from mean values and expressed in percentage. Concentrations of all determined PCB congeners were above detection limit. All experiments were performed in triplicate. The amount of eliminated PCBs was evaluated according to the equation: $X = (M_i - M_a) / M_i \times 100 \%$, where X stands for the biodegradation of the individual PCB congeners in the system (%), M_a is the total amount of the individual PCB congeners determined in the system after 21 days of cultivation (μ g), and M_i is the initial amount of the individual PCB congeners (μ g). The final results took into

account the amount of evaporated PCBs during the experiment determined using the apparatus described in Dercová et al., 1999. This amount never exceeded 5 % of the initial amount of PCBs present in sediment.

One ml of cultivation media was diluted in sterile PCA medium and applied to Petri plates. After 48 h of incubation at 28 °C in the dark, bacterial colonies were counted as colony forming units per ml (CFU/ml) using Handy Type Colony Counter (Bio Kobe, Japan). All measurements were carried out in triplicate.

RESULTS & DISCUSSION

This study deals with PCBs biodegradation in historically contaminated sediment collected from the industrial sewage Strážsky canal located in the Eastern part of Slovakia near Michalovce District. Strážsky canal is a part of the factory Chemko Strážske, a former producer of technical PCB mixtures mainly Delor 103 (42% of chlorine). The factory manufactured altogether approximately 21500 tons of these products. Although production had been already stopped, surroundings of the industrial Strážsky canal and Michalovce District in particular are among the most heavily PCB-contaminated areas in the world, i.e. kilograms of PCBs per ton of sediment (Kořan et al., 2001; Tanyiasu et al., 2003). A huge amount of waste from this production resulted in serious contamination of soil, sediments, and water in this area (Langer et al., 2012). Contaminated sediment was chosen for aerobic degradation using biostimulation by an indigenous microbial consortium and bioaugmentation techniques performed by inoculation of sediment with the bacterial strains with a required potential to degrade PCBs in the sediment. To ensure successful biodegradation, the microorganisms must possess genes encoding biphenyldioxygenase responsible for the start of the particular degradation metabolic pathway. In our previous study (Dudášová et al., 2014), bphA gene (chromosomal DNA) was detected only in the *Achromobacter xylosoxidans*. Our preliminary results showed that all applied strains increased degradation of PCBs at certain levels, even though the bphA gene was not detected on chromosomal DNA. Considering these facts we assumed that a gene or cluster of genes coding enzymes responsible for degradation activity is located probably on plasmid

DNA. Eventually, our assumption has been confirmed, as bphA gene was detected on plasmid DNA of the above mentioned *A. xylosoxidans* strain (unpublished). The biostimulation process depends on the metabolic potential of the indigenous microorganisms to detoxify and transform the pollutant molecule, which is dependent on both accessibility and bioavailability (Antizar-Ladislao et al., 2010). As much as the diversity in sources and chemical complexities in organic pollutants exists, there is probably more diversity in microbial members and their capabilities to degrade organic compounds (Ramakrisnan et al., 2011). Although 11 bacterial strains were isolated and identified in the contaminated sediment, other non-cultivable strains can probably be also involved in the biodegradation process. Biostimulation in natural environments has been a frequently used procedure in the two last decades (Ruberto et al., 2003).

Although autochthonous microflora with a potential for the degradation is present in sediment in a sufficient amount, there are several factors that could inhibit the degradation of pollutants, e.g. low nitrogen or phosphorus availability, absence of electron acceptors or donors or lack of required enzyme inducers. Therefore, we have used two different cultivation conditions to monitor influence of biostimulation on PCB degradation using additional nitrogen and phosphorus source in the first set and additional nitrogen, phosphorus and oxygen source in the second one. The number of CFUs of the indigenous microorganisms were determined at the beginning (1st day) and at the end of experiment (21st day). Results received from the biostimulation treatment using macronutrients N and P in utilizable form are listed in Table 1. Biostimulation approach using indigenous degraders with the addition of utilizable nitrogen, phosphorus, and oxygen 1.

As reported in Table. 1, the estimated initial CFUs number of natural microflora was in the range 1×10^8 - 5×10^8 . At the end of the biostimulation experiments, a significant increase of CFU number to 53×10^8 (set using 250 ml flasks) and 64×10^8 (set using 500 ml flasks) was observed. These results on biomass were expected since macronutrients such as N and P are among the most important components responsible for bacterial

Table 1. Biostimulation approach using indigenous degraders with the addition of utilizable nitrogen, phosphorus, and oxygen

| Biotreatment | Addition | Biomass ($\times 10^8$ CFU/ml) | | Biodegradation PCBs (%) |
|----------------|---|------------------------------------|----------------------|----------------------------|
| | | 1 st day | 21 st day | |
| Biostimulation | NaNO ₃ + KH ₂ PO ₄ | 5 ± 0.35 | 53 ± 3.27 | 73.04 ± 3.90 |
| Biostimulation | NaNO ₃ + KH ₂ PO ₄ +O ₂ | 1 ± 0.25 | 64 ± 4.85 | 84.64 ± 5.60 |

growth. The obtained results also revealed that concentration of oxygen was a very important factor influencing CFU number. Table. 1 presents degradation data obtained in both sets of biostimulation experiments. The degradation of PCBs using indigenous microorganisms stimulated by the addition of N and P was significantly higher (73 and 84 %) than degradation in the control sediment without such amendment (10 %). It is also noteworthy, that the removal of PCBs was more effective in the experimental set using increased amount of N and P and increased O₂ concentration ($p < 0.05$) (Table. 1). These results are consistent with those reported in several studies, where significant improvement in biodegradation process of hydrocarbons by the addition of utilizable form of nitrogen and phosphorus was observed (Ruberto et al., 2003). Greater PCB removal in the experiments using a nutrient and oxygen addition, compared to those without the addition of O₂, is understandable, since the enzymes responsible for cleavage of aromatic rings - dioxygenases - need molecules of oxygen as a substrate (Furusawa et al., 2004). In the experiment with the addition of N and P, the highest degradation of congener PCB 101 (89 %) was observed while PCB 203 was the lowest degraded congener (13 %). The lower chlorinated PCB congeners PCB 28 and PCB 52 were degraded similarly to high extent in both experimental sets. Increase of oxygen amount (two-fold) simultaneously with the N and P amendment stimulated degradation of highly chlorinated congeners. Degradation of congener PCB 203 increased significantly from 13 % to 79 %. According to our results, it could be concluded that the presence of higher concentration of oxygen probably stimulated mainly biodegradation of highly chlorinated congeners (not shown). Biostimulation treatment eliminates a number of steps such as isolation, identification, preservation, cultivation of microorganisms, and transporting inoculum to the contaminated location that makes this procedure a less demanding method than bioaugmentation. However, biostimulation is conditioned by the presence of a sufficient number of required indigenous microorganisms with degradation potential at the particular contaminated site.

Another task was bioaugmentation treatment using the individual bacterial strains. Natural microbial degraders of chlorinated aromatic compounds are often found in areas contaminated with similar anthropogenic substances (Alvarez et al., 2011). Three bacterial strains isolated in our previous work from long term PCB-contaminated sediments of industrial sewage canal in the surroundings of former PCB producer were individually used in separate experimental settings in sterile (to eliminate interactions between exogenous and indigenous microorganisms) and non-sterile sediments

(to evaluate synergistic or competitive effects - interaction of allochthonous and autochthonous microorganisms). The ascending PCB degradation capability (%) of the individually used bacterial strains, inoculated into the sterile sediment, was detected in the following order: *Rhodococcus* sp. > *S. maltophilia* > *A. xylosoxidans* (Table. 2). Bacterial strain *Rhodococcus* sp. had the highest potential to eliminate the PCB congeners which is in agreement with several studies that described this strain as an efficient degrader of a wide range of pollutants with one or more aromatic rings (Ohmori et al., 2011). The initial and final CFUs of each used strains detected throughout degradation are presented in Table. 2. Only *Rhodococcus* sp. was able to increase the number of CFUs during 21 days of degradation. Biomass of *A. xylosoxidans* expressed in CFUs remained almost at the same level, while biomass of *S. maltophilia* decreased by 26 %. The same experiment under the consistent conditions using the individual bacterial strains *Rhodococcus* sp., *A. xylosoxidans*, and *S. maltophilia* was performed in non-sterile sediment to observe degradation potential of a single strain with the influence of other autochthonous microorganisms present naturally in sediments. Cumulative biodegradation extent of the seven PCB congeners decreased by 16.7 % in the presence of *Rhodococcus* sp. strain. The diminished PCB elimination requires a detailed study and might be probably caused by several factors such as competition for substrates and nutrition by the indigenous and inoculated bacteria, cross enzyme inhibition or predation (Thompson et al., 2005). Degradation profiles of the seven PCB congeners using three bacterial strains inoculated into the sterile or non-sterile sediments are shown in Fig. 1 and using consortia in Table 2.

Efficient biodegradation of a broad range of pollutants using consortia constructed from several autochthonous microorganisms has proven to be a successful strategy for environmental decontamination (Rahman et al., 2002). In this study we investigated the degradation potential of eight bacterial consortia, artificially prepared from the bacterial isolates. The results are presented in Table 2.

Although elimination of PCBs by each consortium containing strain *Rhodococcus* sp. was not lower than 60 %, only two consortia *Rhodococcus* sp. + *S. novella* and *Rhodococcus* sp. + *A. xylosoxidans* + *S. novella* were able to increase biomass amount during the 21 days of the process (increase by 325 % and 605 %, respectively). Moreover, these two consortia of selected bacterial strains were able to eliminate the highest amount of the seven PCB congeners among all studied consortia. High degradation potential of a consortium consisted of *Rhodococcus* sp., *A. xylosoxidans*, and *S.*

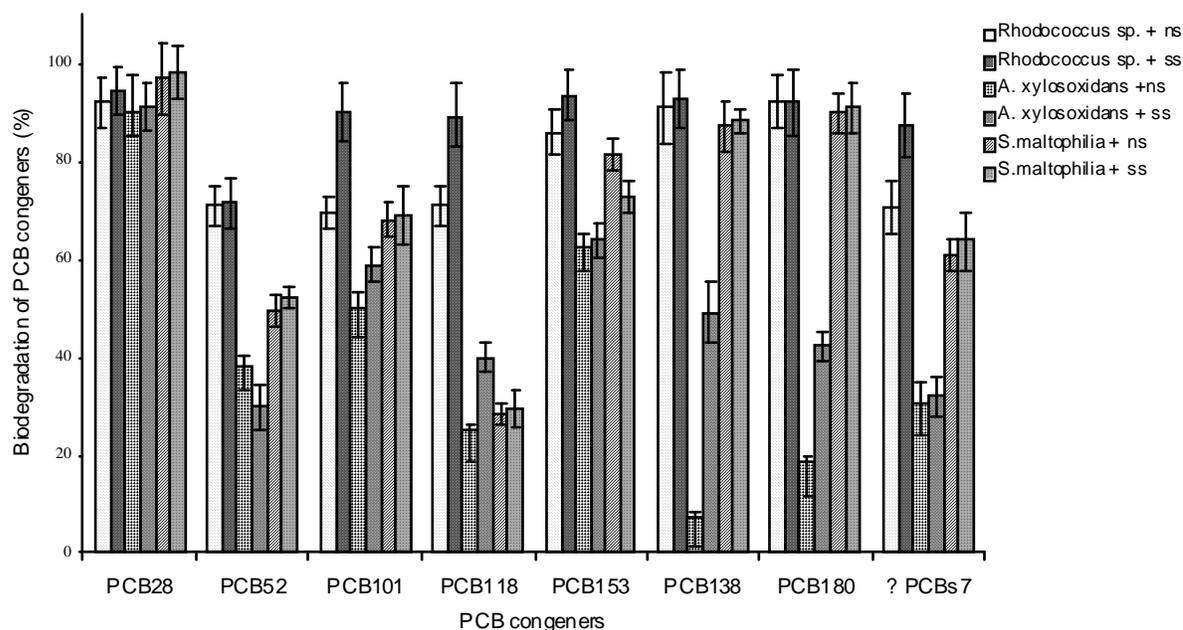


Fig. 1. Biodegradation of the individual PCB congeners using strains: *Rhodococcus* sp., *A. xylooxidans*, and *S. maltophilia* inoculated into sterile (ss) and non-sterile (ns) sediments

Table 2. Total degradation of PCB congeners using bioaugmentation treatment in the presence of the individual bacterial strains or prepared consortia

| Bacterial strain/consortium | Sediment | Biomass ($\times 10^{-8}$ CFU/ml) | | Biodegradation PCBs (%) |
|---|-------------|---------------------------------------|----------------------|----------------------------|
| | | 1 st day | 21 st day | |
| Single strains | | | | |
| <i>Rhodococcus</i> sp. | non-sterile | 120 \pm 9.12 | 120 \pm 8.15 | 70.68 \pm 5.57 |
| <i>Rhodococcus</i> sp. | sterile | 120 \pm 7.63 | 130 \pm 7.63 | 87.39 \pm 6.60 |
| <i>A. xylooxidans</i> | non-sterile | 23 \pm 1.32 | 18 \pm 1.10 | 30.93 \pm 3.81 |
| <i>A. xylooxidans</i> | sterile | 53 \pm 3.74 | 52 \pm 4.41 | 32.21 \pm 4.19 |
| <i>S. maltophilia</i> | non-sterile | 120 \pm 8.62 | 11 \pm 0.93 | 60.81 \pm 5.88 |
| <i>S. maltophilia</i> | sterile | 115 \pm 7.14 | 85 \pm 6.20 | 63.85 \pm 3.20 |
| Consortia | | | | |
| <i>A. xylooxidans</i> <i>Rhodococcus</i> sp. | sterile | 40 \pm 2.54 | 13 \pm 1.85 | 65.76 \pm 5.17 |
| <i>A. xylooxidans</i> <i>S. novella</i> | sterile | 120 \pm 10.86 | 26 \pm 1.63 | 59.31 \pm 4.21 |
| <i>Rhodococcus</i> sp. <i>S. novella</i> | sterile | 8 \pm 0.51 | 35 \pm 2.54 | 67.58 \pm 3.10 |
| <i>A. xylooxidans</i> <i>Rhodococcus</i> sp. <i>S. novella</i> | sterile | 17 \pm 1.99 | 120 \pm 9.11 | 79.00 \pm 5.67 |
| <i>S. maltophilia</i> <i>Stenotrophomonas</i> sp. | sterile | 80 \pm 4.97 | 34 \pm 2.65 | 5.74 \pm 0.92 |
| <i>A. xylooxidans</i> <i>S. maltophilia</i> | sterile | 90 \pm 7.54 | 6 \pm 0.12 | 53.11 \pm 4.33 |
| <i>A. xylooxidans</i> <i>Stenotrophomonas</i> sp. | sterile | 59 \pm 3.22 | 23 \pm 1.14 | 59.00 \pm 3.84 |
| <i>A. xylooxidans</i> <i>S. maltophilia</i> <i>Stenotrophomonas</i> sp. | sterile | 50 \pm 4.21 | 22 \pm 0.96 | 63.12 \pm 4.21 |

novella can be explained probably by synergistic effects of all used bacteria. Based on the obtained results we assumed that mainly *Rhodococcus* sp. has several valuable properties including efficient degradation capacity and sufficient survival capability in both used bacterial consortia. The lowest degradation was observed in the consortium consisting of *S. maltophilia* and *Stenotrophomonas* sp., which eliminated less than 10 % of the initial amount of PCBs. In this consortium, a significant decrease of CFUs by 57.5 % was observed. It can be explained by the presence of antagonistic effects caused most likely by competition of the used strains for nutrients. Decrease of biomass amount and low removal of PCBs could have been also caused by competition for substrate and the cross-inhibitory effect of different enzymes to the growth (Gentry, 2004). The most efficiently degraded congeners were PCB 28 and PCB 101, whereas the lowest degradation was observed for PCB 52 and PCB 180. Therefore, it can be concluded that low biodegradation of less chlorinated PCBs such as PCB 52 resulted from the fact that highly chlorinated PCB congeners were probably dechlorinated to lower chlorinated biphenyls under aerobic conditions (Furukawa, 2006).

Table 3. Total degradation of PCBs by bioaugmentation treatment using the individual bacterial strains and their consortia in combination with the addition of synthetic surfactants or terpenes. Currently there are many studies dealing with the use of synthetic surfactants in remediation processes to remove pollutants such as PAHs, and aliphatic chlorinated compounds e.g. trichloroethylene from the soil. Attention is given mainly to desorption and biodegradation processes of the unwanted compounds or the practical applications of surfactants in soil. However, some studies have shown that the presence of surfactant in-

creased biodegradation efficiency, while other studies have not confirmed a significant improvement in the efficiency of biodegradation process using surfactants (Viisima et al., 2013; Yu et al., 2013). In this work the following experiment was carried out to evaluate the effects of two synthetic surfactants (Tween 80 and Triton X, applied separately) on PCB removal in the presence of the best degrader from the used G- bacterial strains: *S. maltophilia*. In both experiments, no toxic effect of the used surfactants on microbial growth was observed. Table. 3 shows that amount of biomass during the 21 day experiment increased in all cases when the surfactants were used ($p < 0.05$).

Bioaugmentation using *S. maltophilia* without added surfactant showed a slight decrease of CFU number by 7.5×10^8 (not shown). In the experiment using *S. maltophilia* with application of the non-ionic surfactant Tween 80, the biomass revealed increased the CFU number by 272×10^8 . When the other non-ionic surfactant Triton X was used, CFU increased by 247×10^8 . In both cases of the addition of synthetic surfactants, higher biodegradation of PCB congeners ($p < 0.05$) has been detected when compared with the degradation in the absence of surfactants. Degradation profiles of the individual PCB congeners are demonstrated in Fig. 2. The most significant increase in elimination of the seven analyzed PCB congeners was observed with the use of synthetic surfactant Tween 80. When compared to a similar set without the addition of Tween 80, biodegradation was increased about 14 %. Addition of surfactant Triton X increased PCB removal by 4.8 % compared to the control bioaugmentation experiment using inoculation of the same bacteria in the absence of the surfactant. Addition of Tween 80 significantly increased the degradation of two congeners, namely PCB101 and PCB153.

Table 3. Total degradation of PCBs by bioaugmentation treatment using the individual bacterial strains and their consortia in combination with the addition of synthetic surfactants or terpenes

| Bacterial strain/consortium | Additional compound | Biomass ($\times 10^8$ CFU/ml) | | Biodegradation PCBs (%) |
|--|------------------------|---------------------------------|----------------------|-------------------------|
| | | 1 st day | 21 st day | |
| <i>Rhodococcus</i> sp. | Carvone (10 ppm) | 222 ± 16.65 | 205 ± 11.21 | 71.53 ± 4.10 |
| <i>Rhodococcus</i> sp. | Limonene (10 ppm) | 208 ± 13.94 | 104 ± 7.54 | 83.23 ± 6.61 |
| <i>Rhodococcus</i> sp. <i>S. novella</i> <i>A. xylooxidans</i> | Carvone (10 ppm) | 195 ± 9.99 | 60 ± 4.25 | 53.21 ± 3.72 |
| <i>Rhodococcus</i> sp. <i>S. novella</i> <i>A. xylooxidans</i> | Limonene (10 ppm) | 182 ± 10.85 | 172 ± 12.63 | 33.74 ± 1.10 |
| <i>S. maltophilia</i> | Tween 80 (0.3 % w/w) | 120 ± 9.29 | 392 ± 22.22 | 75.21 ± 6.64 |
| <i>S. maltophilia</i> | Triton X (0.032 % w/w) | 128 ± 8.78 | 375 ± 14.93 | 65.59 ± 5.12 |

The reason for selection of Tween 80 was conditioned by the positive references. Degradation rate of PAHs increased with increasing length of surfactant chain, related to the type of fatty acid (Tween 20, -40, -60, -80, polyoxyethylene sorbitan monolaurate, -monopalmitate, -monostearate, -monooleate, respectively). The degradation rates using Tween-20, -40, and -60 were 1.51~2.96 mg/kg/d for naphthalene and 0.98~2.44 mg/kg/d for phenanthrene (Lu Xiaoying, 2011).

Terpenes are well known inducers of PCB biodegradation (Dudášová et al., 2012). In the following experiment, the individual strain *Rhodococcus* sp. and the bacterial consortium consisting of *Rhodococcus* sp., *A. xylooxidans*, and *S. novella* with the highest degradation activities were used. Dried sediment was inoculated with the autochthonous bacterial strain or a consortium. PCB elimination was stimulated by the addition of synthetic terpenes, carvone and limonene, separately (the final concentration 10 mg/l). The obtained results (Table. 3) indicated that the number of CFUs significantly decreased in all experiments ($p < 0.05$). The addition of synthetic terpenes, carvone or limonene, at the beginning of the experiment had an evident inhibition effect on the growth of biomass. In all experiments involving synthetic terpenes, significant reduction in the PCB elimination was observed, in some cases down to 40 %, when compared to bioaugmentation using a single strain without the addition of carvone or limonene ($p < 0.05$). Fig. 3 demonstrates that the addition of synthetic terpenes decreased degradation of congeners PCB28 and PCB101

by *Rhodococcus* sp. and by the applied bacterial consortium. Decrease in degradation of congeners PCB52 and PCB153 with consortium was observed in the presence of carvone or limonene. Terpenes increased only degradation of congener PCB118 with bacterial consortium. Thus, it might be concluded that carvone and limonene have no enhancing effect on PCB degradation ability of the used bacterial strains, in contrast to our previous observations using different strains (Tandlich et al., 2001; Dercová et al., 2003).

In our previous studies we have described use of natural terpenes contained in biological matrices such as pine needles and ivy leaves in order to improve biodegradation of PCBs (Dudášová et al., 2012; Murínová et al., 2014). We established that natural terpenes enhanced biomass growth and caused induction of biphenyldioxygenase, the key enzyme starting degradation process. These observations indicated that the use of natural terpenes in the biodegradation process has big potential for enhancement of degradation efficiency. Addition of terpenes as synthetic compounds in the current work (used in sub-inhibitory concentrations) decreased degradation potential of the used bacterial strains and has not confirmed the effect previously observed for the naturally present terpenes in needles and leaves.

The comparison of degradation of PCBs using biostimulation and bioaugmentation treatments is shown in Fig. 4. The highest degradation was observed in the experiments involving stimulation with nitrogen, phosphorus, and oxygen, as well as in the experiments,

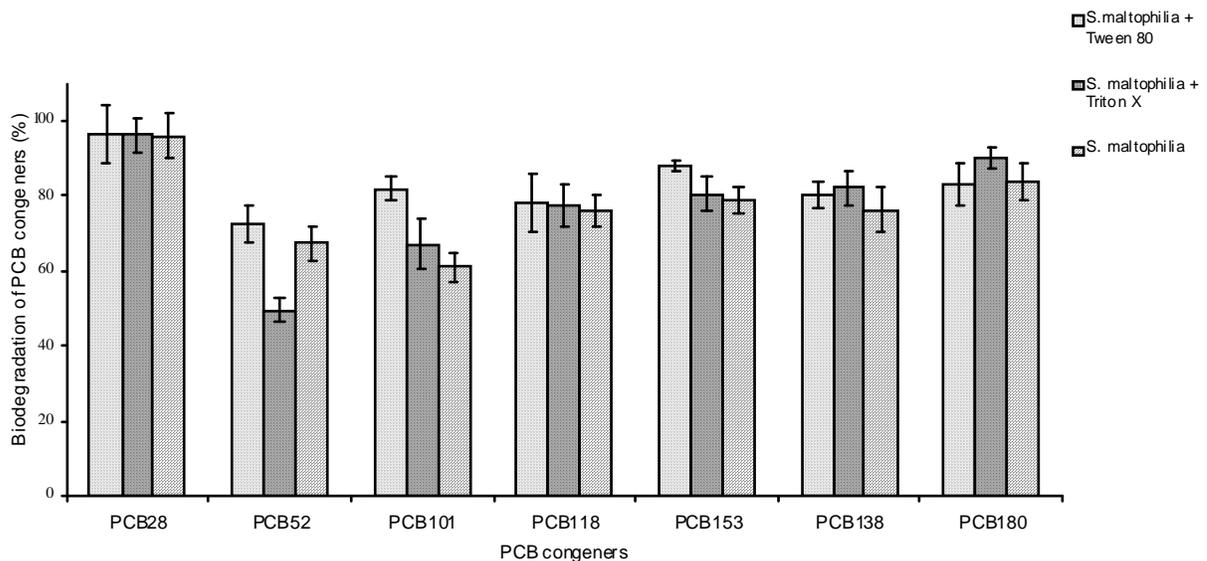


Fig. 2. Biodegradation of the individual PCB congeners using *s. maltophilia* with and without the addition of non-ionic synthetic surfactants Tween 80 and Triton X

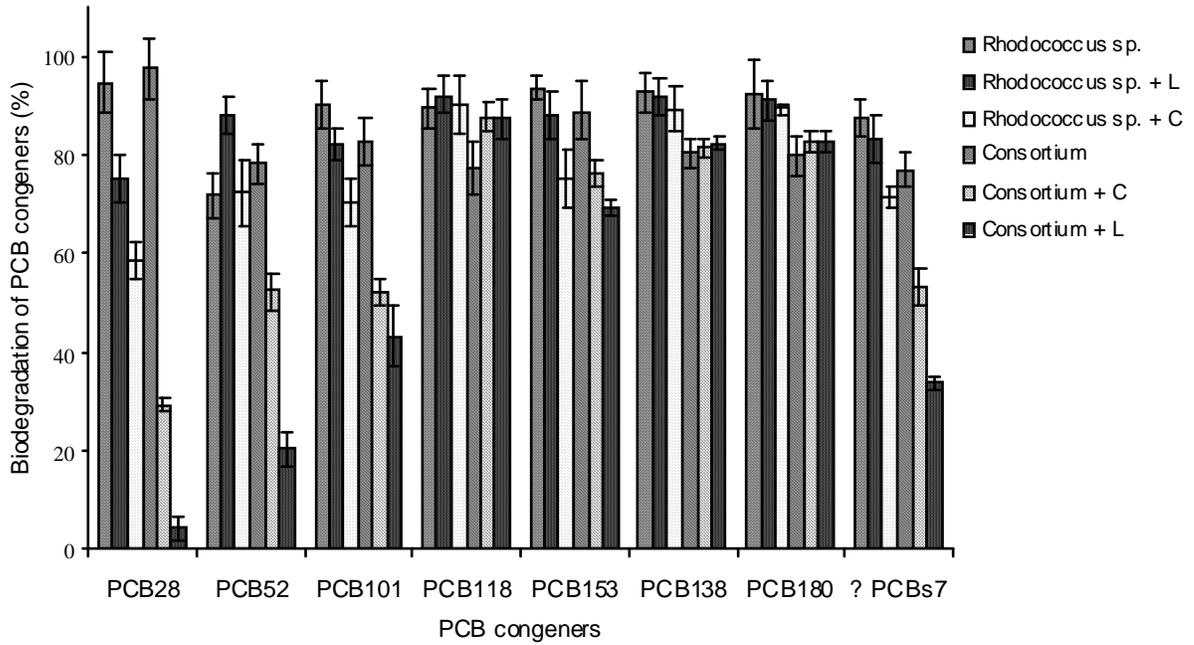


Fig. 3. Biodegradation of the individual PCB congeners by a single strain *Rhodococcus* sp. and a consortium consisting of *Rhodococcus* sp., *A. xylooxidans*, and *Stenotrophomonas novella*, as well as in combination with addition of limonene (L) and carvone (C)

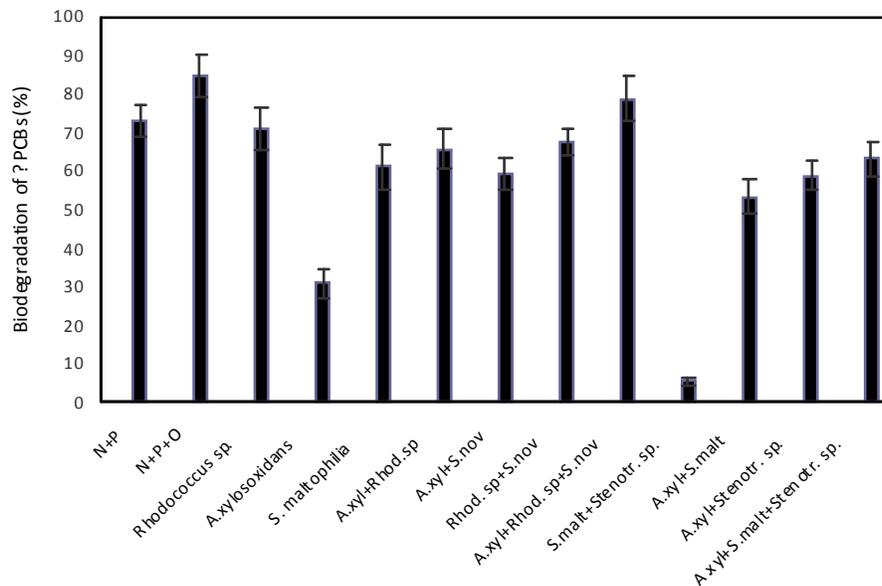


Fig. 4. Comparison of PCB degradation in bioaugmentation and biostimulation experiments using non-sterile sediment

in which bioaugmentation with the consortium *A. xylosoxidans*, *Rhodococcus* sp., and *S. novella* was applied. The lowest degradation was determined in the experiment with bioaugmentation using a consortium *S. maltophilia* and *Stenotrophomonas* sp. followed with the experiment involving bioaugmentation with *A. xylosoxidans*.

CONCLUSIONS

Based on the results obtained, it can be concluded that both biostimulation and bioaugmentation treatments with the simultaneous addition of solubilizing agent (surfactant) or inducer of required degrading enzyme (e.g. terpene) provide promising results regarding the degradation of PCBs and lead to bioremediation of the contaminated sediments under used microcosm conditions. The most efficient bioaugmentation treatment seems to be the use of a bacterial strain *Rhodococcus* sp. and *Stenotrophomonas maltophilia*. Other approaches that showed relatively effective degradation potential were bioaugmentation using artificially prepared bacterial consortium consisting of *Rhodococcus* sp., *Achromobacter xylosoxidans*, and *Starkeya novella*. The advantage of using a particular consortium when compared with a single strain should be a wider range of eliminated PCB congeners, including both lower and higher chlorinated ones. Biostimulation of autochthonous microflora by addition of increased amount of oxygen and utilizable source of nitrogen and phosphorus has shown to be comparable with bioaugmentation treatment in degradation efficiency terms. Our results indicate beneficial effects of both biostimulation and bioaugmentation approaches on biodegradation of PCBs. The efficiency of both applied techniques depends on the naturally present or inoculated bacteria, their number and viability. It should be noticed that despite the high degradation potential of the used bioaugmented strains in sterile and non-sterile sediments, their application in field remediation would require considerable energy and time consumption due to the preparation of the inoculum. From this point of view, biostimulation process appears to be a more economic approach. Moreover, in the field degradation process many other parameters will probably affect PCB degradation, mainly hydrogeological conditions, that influence mobility and bioavailability of pollutants. Regardless these facts we believe that the results obtained could be useful in designing bioremediation technologies for polluted sites contaminated with PCBs.

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