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Remediation potential of bacterial mixed cultures for polychlorinated biphenyls (PCBs) biodegradation

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Abstract: Remediation of polychlorinated biphenyls (PCBs) in minimal mineral water media in the presence of bacterial mixed cultures consisting of several individual strains is proposed. Starting from the fact that the properties and features of bacterial strains in mixed cultures can be supplemented and compensated, two-, three- and seven-membered mixed cultures (MC) were performed. The strains used for the construction of the MC were isolated from the waste canal of a former PCB producer. The highest biodegradation of 70 % of the sum of seven defined PCB congeners was achieved by two-membered MC containing the strains *Rhodococcus* sp. and *Stenotrophomonas maltophilia* added in the biomass ratio of 1 : 3 and 3 : 1. PCB biodegradation by a seven-membered MC was lower (58 %) but provided several benefits over the less-membered mixed cultures or the individual strains: similarity to naturally occurring microflora, easier preparation of the inocula, certain and repeatable results. Periodical reinoculation of the water media resulted to PCB biodegradation increase to 65 %. Seven-membered MC was applied to the historically PCB contaminated sediment as well, where a 59 % degradation of the sum of seven PCB congeners was determined.

Keywords: bacteria, biodegradation, bioremediation, mixed cultures, PCBs

Introduction

Polychlorinated biphenyls (PCBs) are dangerous hydrophobic substances containing the biphenyl molecule with varying degree of chlorination. Long-term use and improper storage led to their release into the environment (Wu et al., 2018). The water cycle had spread them across all ecosystems; they were detected even in the cryoconite of alpine glacier (Weiland-Bräuer et al., 2017). PCBs are able to penetrate into the food chain and deposit in adipose tissues (Louis et al., 2016). In higher organisms, they are responsible for a wide range of diseases resulting from their endocrine-disrupting property (Bergman et al., 2001; Fénichel and Chevalier, 2017; Pinson et al., 2017). Usually, some organisms that came in long term contact with PCB contamination — plants, lower soil animals, microbes — have adapted to their presence. For example, Leigh et al. (2006) demonstrated the biodegradation ability of bacterial strain isolated from the root system of plants growing on a PCB contaminated site. Especially microorganisms are able not only to exist in the presence of PCBs but also to decompose them (Chen et al., 2015; Stella et al., 2017; Horváthová et al., 2018). This fact serves as the basis for many bioremediation techniques. Bioremediation can be described as the conversion of organic pollutants (e.g. chlorinated hydrocarbons) by microorganisms into energy, cell mass and biological waste products (Nikolopoulou and Kalogerakis, 2010; Santisi et al., 2015) and can be implemented as bioaugmention of the contaminated sites with propagated biomass (Horváthová et al., 2018) as biostimulation of naturally occurring microflora provided by (bio) surfactants (Lászlová et al., 2018), plant terpenes (Murínová and Dercová, 2014) and additional nutrients, or as phytoremediation by specialized plants and rhizoremediation by plant roots (Liu et Schnoor, 2008; Slater et al., 2011). There is wide evidence of successful bacterial bioremediation provided by individual bacterial strains as well as by mixed cultures either alone or in combination with other microorganisms or an additional process. It is possible to use: 1) individual bacterial strains, 2) mixed cultures obtained by enrichment from a contaminated environmental sample, 3) artificially prepared mixed cultures made from several individual strains, 4) integrated approach combining biodegradation of PCB by individual strains/ mixed cultures with a physico-chemical method for their elimination, e. g. use of nanoscale zerovalent iron (Cecchin et al., 2017), ozonation (Dudášová et al., 2017), or sorption on activated carbon (Ghosh et al., 1999). Soil/sediment microorganisms form a wide synergic or antagonistic community, where the product of metabolism of one group provides a substrate for another group of microbes (Kwon et al., 2008; Murínová et al., 2014). This is the reason why the application of microbial, especially

bacterial, mixed cultures emerges as a promising bioremediation technique. An example of the integration of different (bio)remediation techniques is the removal of 36.46 μmol kg^{-1} of tetrachlorinated congener PCB 61 in 140 days from the original quantity of 50 μmol kg^{-1} of dry sediment using a bio-electrochemical reactor with poised potential of –0.50 V, acetate as carbon source and an addition of Tween 80. During the experiment, several bacterial strains were isolated and identified, which contributed to the degradation of PCB 61 as an aerobic bacterial mixed culture (Yu et al., 2017). Bacterial mixed culture was successful also in the biodegradation of polycyclic aromatic hydrocarbons (PAHs). Strains enriched from manufactured gas plant site applied as bacterial mixed cultures were able to degrade >95 % of LMW and 90 % of HMW PAHs (Kuppusamy et al., 2016). Eio et al. (2014) completely degraded bisphenol-A up to 50 mg L^{-1} using sufficiently adapted non-specific bacterial mixed culture enriched by activated sludge obtained from the wastewater treatment plant. Our previous research focused on bioremediation of PCB contaminated sediment showed that suitably selected combinations of two or three bacterial strains are able to degrade a significant amount of PCBs naturally occurring in the sediment. Biodegradation of 80 % of the sum of seven PCB congeners was achieved using a two-membered mixed culture and biodegradation of 70 % was achieved when a three-membered mixed culture was applied (Horváthová et al., 2018). This research is focused on the bioremediation of defined minimal mineral media artificially contaminated by technical PCB mixture Delor 103 (equivalent to Aroclor 1242 containing 59 PCB congeners) by bacterial mixed cultures consisting of two, three and seven individual bacterial strains isolated from the sediment originating from the waste canal of the Chemko Strážske plant — a former PCB producer.

Materials and Methods

Preparation of cell suspensions

Strains used for the preparation of mixed cultures (MC) were isolated from the waste canal of the Chemko Strážske plant in Eastern Slovakia and characterized using molecular techniques (Dudášová et al., 2014) as *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia*, *Rhodococcus* sp., *Ochrobactrum anthropi*, *Pseudomonas mandelii*, *Brevibacterium* sp. and *Starkeya novella*. Mixed cultures composed of two (MC2), three (MC3) and seven (MC7) of these strains were investigated to degrade PCBs. MC2 and MC3 were prepared by inoculating liquid Culture broth no. 2 (Biolife, IT) by individual bacterial strains from stored agarised Culture broth no. 2. After 48 h of cultivation on a rotary shaker (180 rpm) at 28 °C, cells were harvested by centrifugation and cell suspensions were prepared by stirring the biomass pellet in distilled water. Concentration of the cell suspension required for the biodegradation experiment was calculated from the optical density at 620 nm (OD_{620}) by calibration curves (not shown). MC7 was prepared by inoculating Culture broth no. 2 with each individual strain from the slant agars. After 48 h of cultivation on a rotary shaker, 10 % (v/v) cell inoculum was used.

Determination of cell concentration

Measuring of OD_{620}. Concentration of bacterial biomass was determined by withdrawing and suitably diluting a sample from the flask and calculated from the calibration curve.

Sowing on the plate agar. The sample was serially diluted (10^7) and sowed on Plate count agar (Sigma-Aldrich, USA) in Petri dishes with a microbiological L-shape spreader. After 48 h of inverse cultivation in the cultivation box at 28 °C, the number of CFU mL^{-1} was determined using a handy colony counter (BIO KOBE, JP).

Biodegradation of Delor 103 mixture in artificially contaminated minimal mineral medium (MM medium)

Biodegradation experiments took place in 500 mL Erlenmeyer flasks closed with cotton stoppers with 100 mL of the MM medium of the following composition: 1 g L^{-1} of $(NH_4)_2SO_4$, 2.7 g L^{-1} of KH_2PO_4, 5.2 g L^{-1} of $NaHPO_4 \cdot 2\ H_2O$, 0.2 g L^{-1} of $MgSO_4 \cdot 7\ H_2O$, 0.01 g L^{-1} of $FeSO_4 \cdot 7\ H_2O$ and 0.03 g L^{-1} of $Ca(NO_3)_2 \cdot 4\ H_2O$ (Lachema Brno, CZ), and the technical mixture Delor 103 (Chemko Strážske, SR) in the final concentration of 100 mg L^{-1}. To examine the biodegradation ability of the individual bacterial isolates, MC2 and MC3 cell suspensions prepared from 48 h inocula of the individual strains were added in the final concentration of 1 g L^{-1}. When MC2 was used, the cell suspensions were added in the biomass ratio of 1 : 3, 1 : 1 and 3 : 1, and in the biomass ratio of 1 : 1 : 1 for MC3. In the biodegradation of PCBs by MC7, 10 % (v/v) 48 h inoculum of seven bacterial isolates was added. The flasks were closed with cotton stoppers and placed on the rotary shaker for 14 days (biodegradation provided by MC2, MC3) and for 21 days (MC7) at 28 °C and rotation speed of 180 rpm. Flasks with PCBs bioremediation by MC7 were reinoculated with fresh 48 h inoculum after seven and 14 days of cultivation. All experiments ran in three parallel measurements. For the determination of PCB biodegradation in each set-

ting, abiotic control (only MM medium with 100 mg L^{-1} of Delor 103 mixture) was used. Seven PCB congeners monitored during the biodegradation represented 30.4 % of the total weight of Delor 103 mixture. At the beginning and at the end of the bioremediation with the individual strains, biomass concentration using calibration curves was calculated after withdrawing and diluting a sample from each flask and measuring the OD_{620}.

Biodegradation of PCBs in historically contaminated river sediment

The following experiments took place in 250 mL Erlenmeyer flasks with 10 g of dried and sieved sediment drenched with 100 mL of MM medium and 10 % (v/v) 48 h inoculum of mixed culture consisting of seven individual strains (MC7). The flasks were stationary cultivated at 28 °C for 21 days with occasional shaking. After the cultivation time, the sediment was harvested by centrifugation, dried and PCBs were extracted with n-hexane (Mikrochem, SR). Sediment used in the experiments was collected from the waste canal of the Chemko Strážske plant using an Uwitec sampler (Austria) according to the Slovak Technical Norm. The total amount of seven monitored PCB congeners was 22.7 mg kg^{-1} (Horváthová et al., 2018).

Analysis of PCBs

After the cultivation, non-degraded PCBs were extracted from the MM media in a two-stage extraction with n-hexane. First, 10 mL of n-hexane were added to each flask and these were placed in an ultrasonic bath for 5 min to release the residual PCBs from the glass and from the bacterial cell structures. The whole flask content was then moved to a separating funnel for intensive shaking (2 min). After the liquid phases settled, water phase was released to the second separating funnel for the second extraction (2 min). n-Hexane phases were combined and dehydrated by filtration via an anhydrous Na_2SO_4 (Centralchem, SR) column. Residual PCBs in the sediment were extracted to n-hexane with a Soxhlet extraction apparatus. The extracts were purified by ultrasonic bath with copper (45 min), filtered through cotton wool and poured into a Florisil column. PCB extracts were subsequently analyzed by gas chromatography with an electron capture detector (GC ECD) under the same conditions as in Murínová et al. (2014). The total biodegradation of seven selected PCB congeners: (PCB 8 (2,4´-), PCB 28 (2,4,4´-), PCB 52 (2,2´,5,5´-), PCB 101 (2,2´,4,5,5´-), PCB 118 (2,3´,4,4´,5-), PCB 153 (2,2´,4,4´-5,5´-)), were evaluated based on the peak area according to Mills et al. (2007).

Statistical analysis

All experiments were done in three parallel measurements. The obtained data were processed using the software package MS Excel One way ANOVA (analysis of variance) for statistical evaluation. Differences in the biodegradation of the sum of seven PCB congeners observed in the applied degradation approaches (single strains, two-, three- and seven-membered mixed cultures) are greater than expected. There is a statistically significant difference if the P-value is below 0.05. P-values obtained from statistical analysis of an appropriate group of results meet these conditions.

Results and Discussion

Biodegradation of Delor 103 mixture by individual bacterial strains, two- and three-membered mixed cultures

When examining the biodegradability of Delor 103 by the individual bacterial strains in artificially contaminated MM medium, the following biodegradations were obtained: *O. anthropi* 60 %; *A. xylosoxidans* 41 %; *S. maltophilia* 40 %; *Brevibacterium* sp. 33 %; *S. novella* 19 %, *Rhodococcus* sp. 18 % and *P. mandelii* 17 % from the initial amount of the sum of seven selected PCB congeners (Tab. 1). Comparable results were obtained with an endophytic bacterial strain isolated from the leaves of *Salix matsudana* f. *pendula*. This strain, identified as *Enterobacter* sp., was able to degrade Aroclor 1242 mixture (equivalent to Delor 103) with the removal ratio of 43.2 % after seven days in liquid minimal medium (Cai et al., 2018). PCB-degrading strains isolated from the surface sediment sampled from Svalbard Islands by Papale et al. (2017) were able to remove Aroclor 1242 at 15 °C within a month in microcosm with the efficiency varying between 7 % and 70 %. From our isolates, *O. anthropi* (O), *A. xylosoxidans* (A), *S. maltophilia* (S) and *Rhodococcus* sp. (R) were selected for the assembly of two- and three-membered mixed cultures (MC2 and MC3). Although our strains were isolated from the same environment, there are some differences in the biodegradation of the sum of seven PCB congeners as well as in that of each congener. Biodegradation of individual PCB congeners by all above mentioned strains, except for *S. maltophilia,* decreased with the increasing amount of chlorine on the PCB molecule. Only *S. maltophilia* was able to degrade various PCB congeners with the same efficiency (data not shown). This variability was the basis for the preparation of the mixed cultures. It was assumed that the application of multiple bacterial strains simulates the

site microbiological composition better than the individual bacterial strains and that it is ultimately beneficial. To determine whether the biodegradation took place in growth or non-growth conditions, OD_{620} was measured immediately after the experiment setting and after 14 days, before the extraction of non-degraded PCBs. No increase in OD_{620} was measured for any individual strain so it was assumed that biodegradation provided by all strains runs at non-growth conditions (Tab. 1). The largest changes in biomass concentration were observed during the biodegradation with *S. maltophilia* and *S. novella* (81 % and 57 % decrease of biomass concentration). On the contrary, the smallest decrease of the biomass concentration was observed in the biodegradation with *A. xylosoxidans* (29 %).

Two-membered mixed cultures — MC2 consisted of two individual bacterial strains added in the biomass ratios of 1 : 3, 1 : 1 and 3 : 1. First MC2 (Fig. 1a) consisting of the strains *Rhodococcus* sp. and *A. xylosoxidans* added in the biomass ratio of 1 : 3 with an excess of *A. xylosoxidans* degraded 61 % of the sum of seven PCB congeners studied. This result is favorable considering that individual strains *Rhodococcus* sp. and *A. xylosoxidans* degraded only 18 % and 41 % of the sum of seven PCB congeners. Independently of the added biomass ratio, biodegradation of individual PCB congeners by this MC decreased with the increasing degree of PCB chlorination. Biodegradation of hexachlorinated PCB 138 was by approximately 40 % less effective than that of dichlorinated congener PCB 8. These differences in the biodegradation of variously substituted PCB congeners were diminished by the second MC2 (Fig. 1b) consisting of the strains *S. maltophilia* and *Rhodococcus* sp, which degraded 70 % of the sum of seven PCB congeners at the added biomass ratio of 1 : 3 and 3 : 1. At the biomass ratio of 1 : 1, the biodegradation was by more than 10 % less efficient. The difference in the congener analysis was not as significant as with the first MC2. All, lower and higher, chlorinated PCB congeners were removed in a comparable extent. Moreover, biodegradation of PCBs by this MC2 was more efficient than application of the relevant individual strains. The lowest efficiency of PCB biodegradation was observed with the third MC2 (Fig. 1c) containing *O. anthropi* and *A. xylosoxidans*. Biomass inoculated in the ratio of 1 : 1 degraded the sum of seven PCB congeners to 59 %, which is comparable with the biodegradation achieved with the individual strain *O. anthropi* (60 %). In this MC2, the highest differences were observed in the biodegradation of PCBs considering the three different biomass ratios.

Three-membered MC3 (Fig. 1d) were not as efficient as expected. Combinations OAS (*O. anthropi*, *A. xylosoxidans*, *S. maltophilia*), ORA (*O. anthropi*, *Rhodococcus* sp., *A. xylosoxidans*) and ORS (*O. anthropi*, *Rhodococcus* sp., *S. maltophilia*) with the biomass ratio of 1 : 1 : 1 removed only 29 %, 39 % and 19 % of the seven PCB congeners despite the presence of the best PCB-degrading strain *O. anthropi*. This result is probably due to a strong antagonism between the strains, and a change in the added biomass ratio should be applied in further experiments. Recently, biodegradation of bacterial mixed cultures also in the historically PCB contaminated sediment has been studied under laboratory conditions. MC2 containing the strains *Rhodococcus* sp. + *A. xylosoxidans*, and *Rhodococcus* sp. + *S. maltophilia*, both in the biomass ratio of 1 : 1 degraded approximately 80 % of the sum of seven PCB congeners, which is much more than the results achieved with the relevant individual strains in the sediment. Biodegradation was higher due to the presence of naturally occurring synergic sediment microflora which contributed to the biodegradation (Horváthová et al., 2018).

Tab. 1. Biodegradation of the sum of seven defined PCB congeners by individual bacterial strains (14 days), biomass concentration at the beginning of biodegradation and after 14 days, before the extraction of residual PCBs, expressed as percentage decrease.

Bacterial strain	Biodegradation of the sum of 7 PCB congeners (%)	Initial biomass concentration ($g\ L^{-1}$)	Final biomass concentration ($g\ L^{-1}$)	Decrease of biomass concentration (%)
O. anthropi	60	0.92	0.58	37
A. xylosoxidans	41	0.88	0.62	29
S. maltophilia	40	0.85	0.16	81
Brevibacterium sp.	33	0.81	0.43	47
S. novella	19	0.85	0.37	57
Rhodococcus sp.	18	0.83	0.39	53
P. mandelii	17	0.80	0.49	39

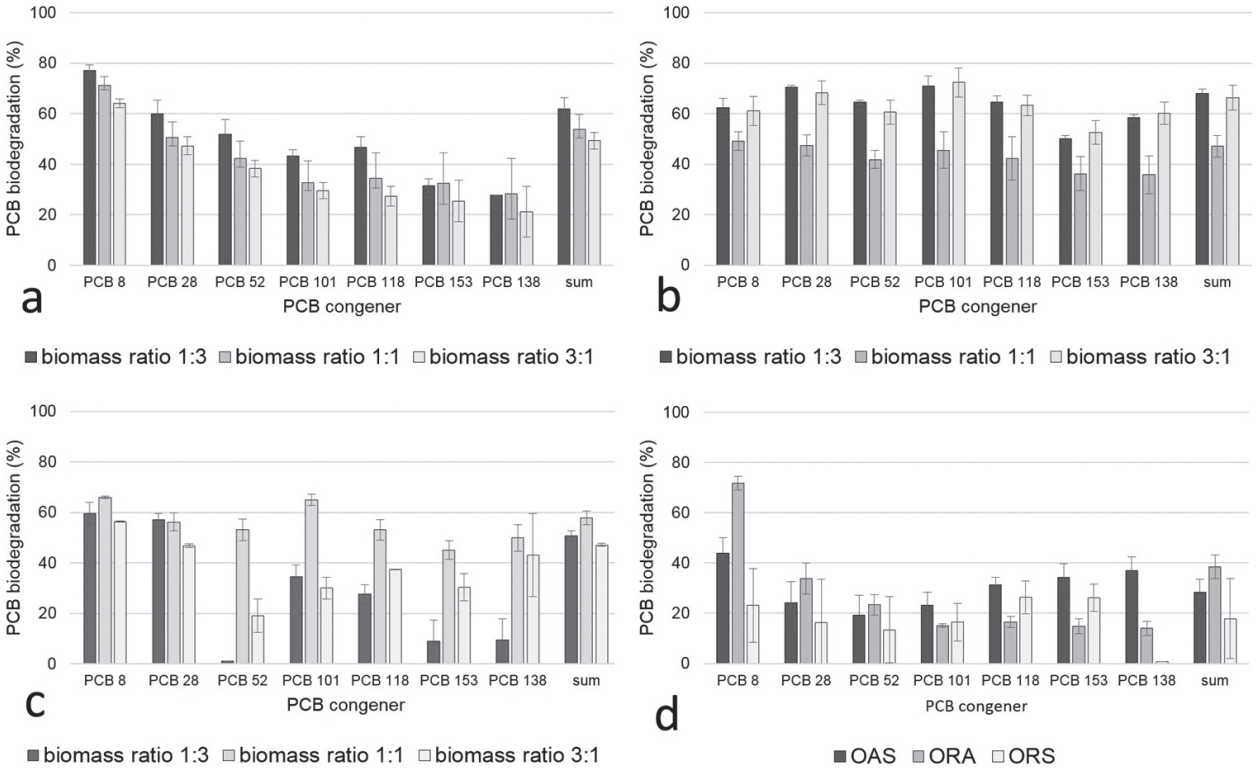

Fig. 1. Biodegradation of seven PCB congeners in minimal mineral medium (MM medium) artificially contaminated with Delor 103 by two-membered mixed cultures (MC2): *Rhodococcus* sp. + *A. xylosoxidans* (a), *Rhodococcus* sp. + *S. maltophilia* (b), *O. anthropi* + *A. xylosoxidans* (c) at the biomass ratio of 1 : 3, 1 : 1 and 3 : 1, and three-membered mixed cultures (MC3) (d) with biomass ratio of 1 : 1 : 1, where O means *O. anthropi*, A means *A. xylosoxidans*, S means *S. maltophilia* and R means *Rhodococcus* sp. Cultivation conditions: 100 mL of MM medium, 100 mg L^{-1} of Delor 103 mixture, rotary shaker — 180 rpm, 28 °C, 14 days. Abiotic control: MM medium + Delor 103 (100 mg L^{-1}).

Biodegradation of PCBs by mixed culture consisting of seven bacterial isolates (MC7)

PCB biodegradation experiments with MC7 were performed using two different sources of contamination: a) Delor 103 mixture added to the MM medium — artificial contamination with PCBs and b) historically PCB contaminated dried sediment flooded with the MM medium (Fig. 2). In the artificially contaminated MM medium, 58 % biodegradation of the sum of seven PCB congeners was observed after 21 days. Three flasks with Delor 103 biodegradation by MC7 were reinoculated after seven and 14 days of cultivation. Addition of 10 % (v/v) of fresh bacterial inocula resulted in an increased PCB biodegradation (65 % of the sum of seven PCB congeners). Generally, this is not the best result achieved by MC but the use of MC7 provides several advantages over the other ones.

Despite MC7 containing only bacterial strains, it is still more similar to the natural consortium occurring on a contaminated site. Also, MC7 is easier to prepare. It is not necessary to make cell suspensions of each individual strain and set the equivalent volume of the suspension by measuring OD_{620}, as mentioned in section 2.1., inoculating the culture broth by one inoculation loop of each strain is sufficient. During the cultivation in the liquid broth, bacterial growth is probably not uniform, the strains stabilize on basis of synergy and antagonism. The use MC7 provides stable and repeatable results in comparison with the use of single strains and two- and three-membered MCs which is fundamentally important for the scale up. In mixed cultures, some species of bacteria possibly beneficial in the degradation of PCBs by removing potentially inhibitory intermediates can be present (Clark et al. 1979). Moreover, MC7 is able to survive longer in the MM medium with Delor 103 (100 mg L^{-1}) than in that with individual strains. Biomass concentration expressed as CFU · ml^{-1} in a sample taken from the flask where Delor 103 biodegradation by MC7 took place increased from 9×10^9 to 19×10^9, OD_{620} of the sample after 21 days had the same value as at the beginning of the bioremediation process. As seen in Tab. 1, biomass concentration (g L^{-1}) of all individual strains decreased. However, heterogenous bacterial cultures are more resistant to PCBs, therefore they are better applicable. Our MC7 was tested

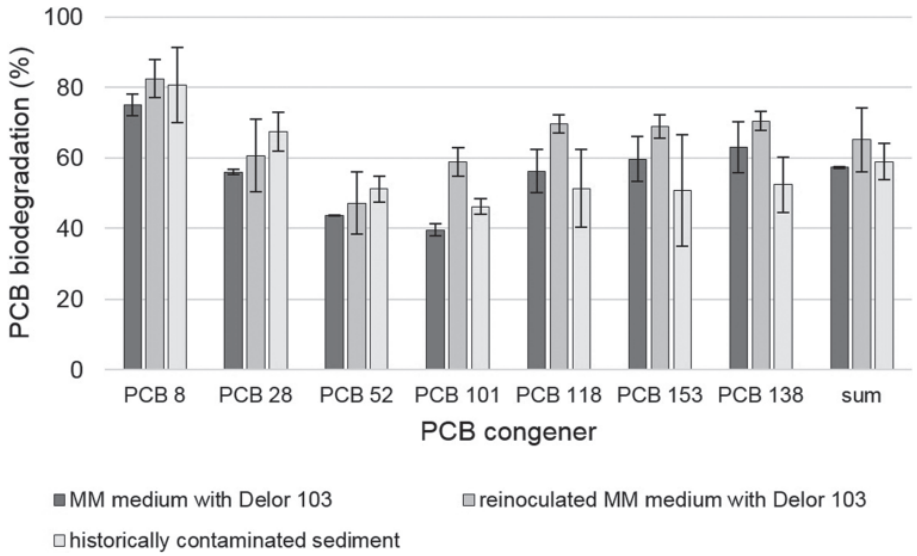

Fig. 2. Biodegradation of seven PCB congeners in the mimimal mineral medium (MM medium) artificially contaminated with Delor 103 and in historically PCB contaminated sediment by mixed culture consisting of seven bacterial strains (MC7): *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia*, *Rhodococcus* sp., *Ochrobactrum anthropi*, *Pseudomonas mandelii*, *Brevibacterium* sp. and *Starkeya novella*. Cultivation conditions in MM medium: 100 ml of MM medium, 100 mg L^{-1} of Delor 103 mixture, 10 % (v/v) 48 h inoculum of seven strains, rotary shaker — 180 rpm, 28 °C, 21 days, reinoculation after 7 and 14 days of cultivation. Abiotic control: MM medium + Delor 103 (100 mg L^{-1}). Cultivation conditions in the sediment: 100 ml of MM medium, 10 g of dried and sieved historically contaminated sediment, 10 % 48 h bacterial inoculum, stationary cultivation with occasional shaking, 28 °C, 21 days. Abiotic control: non-treated sediment.

for biodegradation of historic PCB contamination in the sediment from the former PCB producer surroundings. Microcosm bioremediation with real contaminated sediment as the PCB source was provided by flooding of the sediment with the MM medium, inoculation with MC7 and stationary cultivation under occasional shaking as mentioned in section 2.2.2. The biodegradation was similar to that achieved in the artificially contaminated MM medium, 59 % biodegradation of the sum of seven PCB congeners, and the decrease of the concentration of each congener was proportional to the total degradation of PCBs. Petrić et al. (2007) isolated two mixed cultures from polluted areas. Both cultures showed degrading activity comparable with that presented in this paper, e.g. 56 to 60 % of the mixture of 50 PCB congeners (containing di- to heptachlorinated congeners) were reduced after 14 days in PAS medium (phosphate-buffered mineral salts medium) supplemented with biphenyl. Both MCs contained the strain *Rhodococcus erythropolis* with its well-known PCB-degradation ability (Chung et al. 1994, Pham et al. 2015). The mixed cultures isolated by enrichment from the marine sediment also exhibited PCB-degrading activity. Strains forming the mixed culture mainly belong to the *Rhodococcus* and *Sphingomonas* species and despite their different PCB-degrading capability, all of them are able to degrade PCB congeners with two to four chlorine substitutions. Mixed cultures degraded the industrial mixture Aroclor 1242 in a seawater mineral salts medium within the range of 40—61 % (Kolar et al., 2007).

Conclusion

This work was focused on biodegradation of PCBs (technical mixture Delor 103) by mixed cultures consisting of two (MC2), three (MC3) and seven (MC7) bacterial strains recently isolated from the waste canal of the former producer of PCB based technical mixtures. Foremost, to consider the use of mixed cultures, biodegradation of Delor 103 mixture by the individual strains in water minimal mineral medium (MM medium) was performed. Then, the most efficient strains — gram negative *O. anthropi*, *S. maltophilia*, *A. xylosoxidans* (60, 41 a 40 % biodegradation of the sum of seven PCB congeners) and gram positive *Rhodococcus* sp. (18 % biodegradation) were used to construct the two- and three-membered mixed cultures. Biodegradation of the PCBs in MM medium contaminated with Delor 103 by three-membered mixed culture (MC3) with the biomass ratio of 1:1:1 was not significant and among two-membered (MC2), combinations, *Rhodococcus* sp.+ *A. xylosoxidans* and *Rhodococcus* sp. + *S. maltophilia* were

more efficient than the relevant single strains. The biodegradation of 61 % and 70 % with biomass added in the ratio of 1:3 was achieved. Finally, the mixed culture consisting of seven bacterial strains was constructed. MC7 degraded 58 % of the sum of seven PCB congeners in the artificially contaminated MM medium and 59 % in the historically contaminated sediment. When the MM medium was periodically reinoculated with MC7, a 65 % biodegradation of PCBs was observed. The application of the seven bacterial isolates mixed culture provides a promising remediation alternative to that of the relevant individual bacterial strains. Simple preparation of inocula tailored to the microbial conditions in the real contaminated site and repeatability are the most significant benefits for future remediation research.

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