MICROBIAL DECHLORINATION OF POLYCHLORINATED BIPHENYLS

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Abstract: Polychlorinated biphenyls (PCBs) are organic xenobiotics contaminating environment for at least 50 years. They could be eventually eliminated by various organisms under different conditions. The degree of chlorine substitution per biphenyl molecule influences biodegradability which decreases with increasing chlorination.
Our work is focused on the PCBs biodegradation under anaerobic conditions. The suitable high chlorinated biphenyls are converted via reductive dechlorination to the chlorinated biphenyls with lower extent of chlorine, which could be eventually fully mineralized by aerobic bacteria.
Microbial consortium was isolated from sediment of Strážský Creek (located near by plant producing PCBs in the past). This consortium was able to dechlorinate polychlorinated biphenyls under anoxic conditions. The effectiveness of this process was tested under different cultivation condition – different energetic sources (Aroclor 1248 or Aroclor 1260 or Delor 103 or Delor 106), addition of potential electron donors (pyruvate, lactate or acetate with hydrogen) and further if there is necessary to add yeast extract into fresh low sulphur cultivation media.
Our microbial consortia so far do not need supplementation by non-contaminated sediment to maintain dechlorination activity. Addition of yeast extract is non essential, but needs to be further proved in serial transfers.
In all cases (except acetate without yeast extract) dechlorination proceeds at *meta*- and flanked *para*-position.

Key words: Polychlorinated biphenyls, reductive dechlorination, gas chromatography

1. Introduction

Polychlorinated biphenyls (PCBs) are a class of organic compounds with 1 to 10 chlorine atoms attached to biphenyl molecule (it is possible to derive 209 different congeners). PCBs were commercially produced as complex mixtures containing multiple isomers at different degrees of chlorination. Their unique character (nonflammable; good heat transfer fluid; electroisolation properties; low vapor pressure; low water solubility and high solubility at organic solvents and fatty acids) caused their robust production and wide usage.

Their commercial and industrial production started at the end of 20s last century. PCBs were produced by catalytical chloration of biphenyl (at Czechoslovakia they were manufactured at CHEMKO Strážské until 1985). PCBs production was banned in the 1970s (in most countries of west world) due to the high toxicity of most PCB congeners and mixtures to the organisms.

Listed properties caused food chain accumulation and difficulties during degradation and removing. PCBs are classified as persistent organic pollutants. They may be destroyed by chemical, thermal, and biochemical processes. It is extremely difficult to achieve full destruction, and there is the risk of creating extremely toxic dibenzodioxins and dibenzofurans through partial oxidation.

Chemical analogs of PCBs (as chlorophenols; chlorobenzenes or dioxines) are in nature produced during forest fires or volcano eruptions (BORJA *et al.,* 2005). It was evidenced that PCBs are transformed by microorganisms (faster at laboratory conditions than in nature). It gives us possibilities how to decrease current concentration in the environment. The halftime differs through environmental kind (aerobic, anaerobic; water, soil, atmosphere, sediment), type of organisms participated on degradation (bacteria, fungi, plants, animals) and amount and character of other contaminating compounds.

Anaerobic bacterial degradation of PCBs is not so well-known as the aerobic degradation. But at short time were serious results achieved and it was demostrated that anaerobic degradation plays an important function through PCBs biological decontamination (MALTSEVA *et al.,* 1999). Organisms growing on organohalides get energy for their metabolism and reproduction by reductive dechlorination (MOHN and TIEDJE, 1992) (halogen from the organohalide molecule is used as a terminal electron acceptor). Above mentioned mechanism (dehalogenation) transforms the high chlorinated biphenyls to the low chlorinated biphenyls which could be consumed during aerobic degradation. It should be very interesting but technically very difficult to try to combine anaerobic and aerobic degradation.

Mechanism of anaerobic degradation was first described at the end of 90s last century. It was observed at laboratory conditions that degradation ability should be primed by presence of some congeners or amended by exogenic compounds which should be used as an energy source or as a proton donor (dibromobiphenyls, lactate, malate, pyruvate, acetate, butyrate and others) (BEDARD *et al.,* 1998; WU *et al.,* 1999). ADRIAN and coworkers (2009) finally describe pure culture (*Dehalococcoides* sp. strain CBDB1) extensively dechlorinating commercial PCB Aroclor 1260.

2. Materials and methods

PCB mixes and Aroclors were purchased from AccuStandard (New Haven, The U.S.A.). Delor was provided by Mr. Kočan from the Slovak Republic. Diethylether declared as GC grade was supplied by Merck (Darmstadt, Germany).

Sediment (fractioned under 1 mm^2) was resuspended in special low sulphur purely synthetic, carbonate buffered mineral medium containing Ti(III) citrate as reducing agent and vitamins (ADRIAN *et al.,* 2000). Set of samples was amended by 4-4 dibromobiphenyl (final concentration 350 μmol/l), other was amended by 2,6-dibromobiphenyl and others were retained without addition and cultivated at two different temperatures (4 °C and 28 °C). Brominated biphenyls were sorbed on silica mesh 250 nm (providing homogenic distribution of PCBs and increasing internal area). Microbial activity was measured indirectly by changing concentration of every PCB. Growing cultures were sampled (every 3 weeks) under anoxic condition (QUENSEN *et al.*, 1990).

The taken media were shaked out to the diethylether (volume distribution 1:12.5). Proper assesment of concentration of PCB from diethylether was provided on gas chromatograph (HP 6890N) with automatic injector (HP 7683) and micro-electrone capture detector. For separation of congeners capillary column (DB-XLB, length 30m, inside diameter 0.25 mm and thickness of stationary phase 0.25 μm) was used. Mobile phase was nitrogen and the final flow was 45.1 ml/min. Sample (1 μl) was inject on column using splitless technique. Measurment was proceded by using isobaric mode (0.993 bar). Temperature program was following: the initial temperature was 50 °C and immediately increased to 160 °C (rate 30 °C/min) and then immediately increased to 300 °C (rate 2.5°C/min) and there was terminated. Detector temperature was 340 °C and final flow of mobile phase was 60 ml/min (makeup gas + column flow). Data were collected by using software GC Chemstation. However we are still not able fully quantify the whole process (it is the reason why we show data at not very well-arranged shape – chromatograms).

3. Results and discussion

Our first experiment should manifest presence of dehalogenating organisms and a possible way of accelerating their metabolism. After dechlorination activity was documented and extensive advance (samples cultivated at 28 °C) we transferred our microbial consortia in a fresh media (portion of primary culture was 10 vol. %). Commercial mixture PCBs (Aroclor 1248, or Aroclor 1260, or Delor 103, or Delor 106 at finally concentration 50 μg/ml) sorbed on silica was added as an energy source. We added different proton sources for microbial consortia – pyruvate, or lactate, or acetate amended by hydrogen.

Table 1. Sum of detected PCBs (%) in different time of cultivation. Indicator PCBs: 28, 52, 101, 118, 138, 153, 180

	Sum of detected PCBs [%]					**Sum of indicator PCBs [%]**				
	0. Day	**182. Day**	**285. Day**	**473. Day**	**586. Day**	**0. Day**	**182. Day**	**285. Day**	**473. Day**	**586. Day**
Acetate	100	100	100	100	100	100	95	104	111	107
Acetate with yeast extract	100	100	100	98	97	100	101	103	86	80
Lactate	100	95	94	94	94	100	64	59	59	58
Lactate with yeast extract	100	97	96	95	95	100	74	67	65	62
Pyruvate	100	98	95	95	94	100	79	62	65	63
Pyruvate with yeast extract	100	95	94	94	94	100	65	58	60	58

More detailed will be discussed the microbial consortia of the 4th transfer containing only 0.04 vol. % of origin sediment cultivated on Aroclor 1260 PCB mixture supplemented by pyruvate, lactate or acetate with hydrogen. We study if there is necessary to supplement fresh bacterial media for our dechlorinators by more complex carbon source - yeast extract.

PCB dechlorination activity was measured as described above. Sum of reminder PCBs (%) as well as sum of indicator PCBs during incubation period is documented in Table 1. Decreasing concentration of PCBs 138, 153 and 180 which are well present at originally used PCB mixture – Aroclor 1260 were mainly responsible for so huge non-expecting decreasing concentration of indicator PCBs. On the other hand we observed a bit increasing concentration of remaining indicator congeners.

We also analyzed change of chlorine distribution on biphenyl molecule. Documentation is at Table 2.

Table 2: Distribution of chlorines per biphenyl molecule (%).

	Particular amount of *ortho*-, *meta*-, and *para*- chlorines (%)						
	0. Day	**Acetate**	**Acetate with yeast extract**	**Lactate**	**Lactate with yeast extract**	**Pyruvate**	**Pyruvate with yeast extract**
		586. Day	**586. Day**	**586. Day**	**586. Day**	**586. Day**	**586. Day**
ortho	39.5	39.3	41.0	43.1	42.5	42.8	43.2
meta	39.8	39.7	39.1	38.4	38.4	37.8	37.8
para	20.7	21.0	19.9	18.5	19.1	19.4	19.0

At all cases (except acetate) there is examined decreasing amount at *meta*- and *para*- substitution. Dechlorination pathways were studied and it was set up our microbial communities are removing *meta*- and flanked *para*- chlorines from biphenyl molecule.

4. Conclusions

Our subcultures transformed nona, octa and hepta chlorinated biphenyls through hexa to tri, tetra and penta chlorinated biphenyls.

Dechlorination proceeds at *meta*- and flanked *para*- position. We observed decreasing concentration of indicator PCBs 138, 153 and 180 and on the other hand increasing concentration of indicator PCBs 28, 52, 101 and 118.

Addition of yeast extract in fresh media seems not to be necessary for most of tested conditions (exception of acetate) – need to be further confirmed at next transfer. Our consortia do not need additional supplementation with non-contaminated sediment as many others.

Incubation time at laboratory condition seems to be too long, but when dechlorination proceeds it is going relatively fast. We presume, that as our consortia

adapt to our condition (temperature, composition of media, non-presence of sediment) the lag period would be much shorter.

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