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SORPTION OF POLYCHLOROBIPHENYLS BY THE FUNGUS *Phanerochaete chrysosporium*†

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Abstract

The pellets of the fungus *Phanerochaete chrysosporium* remove the majority of polychlorobiphenyls from contaminated tap water, especially those that are less ortho substituted and contain three or more chlorine atoms. A fluid bed process seems to be promising in this respect.

INTRODUCTION

Polychlorobiphenyls (PCBs) are stable industrial chemicals consisting of complex congener mixtures. These PCB mixtures have been considered as one of the most ubiquitous and persistent pollutants in the environment. They disperse from industrial sources, by accidental leakage or spills from electrical transformers containing PCBs, from landfills, waste deposits, or during incineration, and they contaminate the atmosphere, soil and water bodies [1]. By these routes, also water sources [2] and

†Dedicated to the memory of Professor Jože Šiftar

food [3] become contaminated. Contaminated water sources should not be used for water supply unless the contamination is decreased down to acceptable levels.

It has been found the white rot fungus *Phanerochaete chrysosporium* can degrade several persistent pollutants, among them polychlorobiphenyls [4-7]. The optimum temperature for growing this fungus is 37 to 39°C, whereas the temperature of drinking water sources in temperate regions is much lower, around 10°C and should not be increased. On the other hand, at its optimal temperature, the fungus needs several days to perform the degradation [4] and huge fermenters would be needed to accomplish the fungal degradation of PCB in raw drinking water contaminated with them. In order to avoid the discrepancies in temperature and the high residence time of water, a two-phase process would be needed to separate the sorption from the degradation process and to accomplish them each in optimal conditions for drinking water and the fungus, respectively. A prerequisite for the applicability of such a process is a sufficiently fast and effective sorption of PCBs by the fungus at the temperature of the water source.

EXPERIMENTAL

Contaminated water

was prepared by passing tap water through a generator column. The generator column consisted of a chromatographic tube, 2 cm in diameter, 25 cm long, equipped with a fritted glass filter B0 (Boral, Pula, HR) and a stopcock, and filled with Aroclor 1242 coated high density polyethylene (HDPE) beads. The beads were coated by suspending 1 mL of Aroclor 1242 and 100 mL of HDPE in excess tap water. After a week, the floating beads were transferred into the tube half-filled with water to avoid their contact with the fritted filter, and covered with glass wool. To prepare water contaminated with PCBs, tap water was passed through the column by gravity at a flow rate of up to 6 mL/min.

Preparation of pellets of the fungus Phanerochaete chrysosporium.

The organism, the medium, and the culture conditions are described elsewhere [8,9].

Sorption

Static tests

1 mg to 2 g of two-day old fungal pellets were suspended in 1 L of contaminated water and stored at 4-8°C for one week with occasional mixing.

Dynamic tests

10 mL of settled two-day old fungal pellets were placed in a conical fluidised bed apparatus with the opening of 1 cm, maximum diameter of 4 cm, and height of 8 cm. The contaminated water cooled to 13°C by tap water was allowed to rise through the fungal pellet layer suspended in it. 100 mL portions were collected and 10 mL samples were taken for analysis.

Analysis of PCBs

The water sample was extracted with hexane, purified and analysed as described elsewhere [10,11]. The results are expressed as the percentage of PCB concentration in the contaminated water before the treatment. IUPAC numbers are used for the assignation of PCB congeners [12].

RESULTS AND DISCUSSION

Preparation of aqueous PCB solution

The generator column method has been proven as a suitable technique for preparing PCB solutions for aquatic bioassay and toxicological investigation [13]. The authors prepared the PCB solutions by pumping water through a column packed with glass beads coated with Aroclor mix. In our experiments, water immediately displaced PCB from the glass beads surface and the tiny PCB droplets clogged the voids between the beads, seriously decreasing the flow rate. To avoid the clogging, hydrophobic beads, *i.e*. high density

polyethylene (HDPE) beads intended for extrusion, were used instead of glass beads. Their impregnation is simple: the HDPE beads and PCB are suspended in excess water and let to rest. PCB adheres to the beads like a pearlescent envelope. Properly loaded beads float on the top of water, whereas the overloaded ones and excess PCB sink to the bottom. The floating beads are transferred to a chromatographic column fitted at the bottom with a coarse sintered glass filter (B0 or equivalent) and partly filled with water to prevent contact of the beads with the filter. On the top of the beads a plug of glass wool is fitted. Water then readily flows through the column, except if surplus PCB is introduced that settles onto the fritted filter and clogs it. At least 10 L of contaminated water can be obtained using this column. In our case, it contained 10.8 mg PCB/L. After 20 L of water had flown through the column, the contaminated water contained only 3.2 mg PCB/L on account of lower concentrations of dichlorobiphenyls. This is consistent with the previous observation that lower chlorinated congeners are preferably dissolved in water [13] and thus eliminated from impregnated beads, where less soluble ones then predominate.

Sorption ability of the fungus

The sorption ability of fungal pellets was tested in a static as well as in a dynamic system. In the static system, fungal pellets were stored in PCB-containing water in a refrigerator for one week with occasional mixing. The percentage of PCB congener groups removal as well as that of total PCB remaining in water are presented in Table 1. From Table 1 it follows that PCBs, especially those having more chlorine atoms, have high affinity for *P. chrysosporium* pellets.

Whether this property could be of practical use, was tested in a dynamic system mimicking the fluidised bed process using two-day old fungal pellets. The settling velocity of the fungal pellets was 0.65 cm/s. In Table 2 is presented the residual concentration of individual PCB congeners in the last 100 mL of the effluent when the specified quantity of contaminated water passed through the fungal pellets' bed, as the percentage of their concentration in the inflowing contaminated water.

Table 1. Elimination of PCBs from contaminated water by *Phanerochaete chrysosporium* pellets (static tests).

PCBs removed (%)	Pellets to contaminated water ratio			
	1 : 500	1 : 5000	1 : 50 000	1 : 500 000
Dichlorobiphenyls	96-97	96-98	82-93	70-87
Trichlorobiphenyls	96-98	96-98	91-98	87-97
Tetrachlorobiphenyls	96-98	96-98	96-99	93-98
Pentachlorobiphenyls	96-98	96-98	99	97
Hexachlorobiphenyls	99	98	99	97
ΣPCB not removed (%)	2.9	2.4	7.3	12

Table 2. Residual concentration of individual PCB congeners in the last 100 mL of the specified quantity of contaminated water passed through the fungal pellets' bed, expressed as % of the inflowing concentration.

PCB congener	Volume of contaminated water passed the bed (L)			PCB congener	Volume of contaminated water passed the bed (L)			PCB congener	Volume of contaminated water passed the bed (L)		
IUPAC No.	3.4	10	20	IUPAC No.	3.4	10	20	IUPAC No.	3.4	10	20
4/10	0.0	13.4	18.1	31	1.4		3.6	66	5.2	2.7	1.2
6	0.1	7.6	12.3	33	1.6	3.6	3.4	70	4.7	2.5	1.3
7/9	0.0	4.6	10.4	40	3.7	2.8	2.1	74	4.5	2.3	1.7
8	0.3	7.5	9.9	41/64	3.6	2.8	1.7	82	5.0	8.1	1.1
16/32	0.8	5.1	5.6	42/37	3.3	2.6	1.6	85	5.2	5.8	3.5
17	1.0	4.7	5.1	44	3.4	3.0	1.8	87	5.3	3.9	2.0
18	0.5	5.0	5.7	45	2.0	4.0	2.8	97	5.3	5.1	1.2
19	0.1	6.8	8.3	46	1.7	3.3	2.7	99	5.9	4.2	2.0
22	2.0	3.7	4.1	47/48	4.7	2.9	3.0	101	5.6	2.9	0.7
25	0.0	3.5	2.9	52	2.6	2.8	1.7	108/118	5.5	6.5	1.6
28	2.0	5.0	3.8	56/60	4.9	2.6	1.4	153	3.4		1.1
								Total	1.6	6.5	7.1

If we take PCB-6 as an example, we can see that when 3.4 L of contaminated water passed through the fungal pellets' bed, then the concentration of PCB-6 in the last 100 mL of effluent was 0.1 % of that in the inflowing contaminated water. At least 99.9% of PCB-6 was thus removed by fungal pellets. When 20 L of contaminated water passed through the fungal pellets' bed, then the concentration of PCB-6 in the last 100 mL of effluent was 12.3% of that in the inflowing contaminated water and consequently at least 87.7% of PCB-6 was removed by fungal pellets. At 3.4 L of contaminated water passed through the fungal pellets' bed, the removed fraction decreases with increasing substitution of PCBs. At 20 L of contaminated water passed through the fungal pellets' bed, on the other hand, higher substituted PCBs are removed to a much higher degree than the lower substituted ones. According to Table 2, in the dynamic system, 93% of PCBs entering the system were retained by 10 ml of fungal pellets from 20 L of contaminated water. Regarding individual PCB congeners, the efficiency of removing of 2,2'(6) substituted PCBs as well as of dichlorobiphenyls rapidly diminishes. On the other hand, the efficiency of removal of tetrachloro- and higher biphenyls slightly increases with time. Since the lower substituted PCBs are less toxic and more biodegradable, this property of *P. chrysosporium* pellets is not as bad as could be considered at first sight.

CONCLUSION

The pellets of the fungus *Phanerochaete chrysosporium* efficiently remove PCBs from their saturated water solution at the temperature of tap water up to the volumetric ratio of one to several thousand, and higher ratios can be expected to be achievable in less concentrated solutions. The ability to degrade sorbed PCBs and the optimum conditions for degradation remain to be determined.

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Povzetek

Micelijske kroglice glive *Phanerochaete chrysosporium* učinkovito odstranjujejo poliklorirane bifenile iz pitne vode, predvsem tiste, ki so manj orto substituirani in bolj klorirani. Obetaven je videti postopek v fluidiziranem sloju.