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CHEMISTRY

An Interdisciplinary Approach to the Problem of Neutralization of Man-Made Polychlorinated Biphenyls

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Currently, biological methods of degradation of manmade polychlorinated biphenyls (PCBs) are becoming increasingly important in connection with the selection of new efficient PCB-degrading strains. The most successful studies in the field of biodegradation of PCBs are associated with the use of low-chlorinated PCB congeners [1, 2]. It has been found that the biodegradability of PCB congeners increases with decreasing number of chlorine atoms in the initial substrates. At the same time, interest persists in the search for surfactants capable of transferring more hydrophobic medium- and high-chlorinated PCB congeners into an aqueous medium in order to create stable emulsions available for efficient operation of bacterial strains [3, 4].

The same approach is applicable to PCB derivatives obtained by chemical modification of basic substrates. For example, nucleophilic substitution for chlorine atoms in PCBs can lead to water-insoluble derivatives with a reduced content of chlorine atoms. The transfer of these compounds into an aqueous medium by means of surfactants and selection of an optimal degrading strain enables their subsequent biodegradation.

One of the promising biodegradative strains for PCBs is *Rhodococcus wratislaviensis* KT112-7 (*R. wratislaviensis* KT112-7) isolated from soils subject to anthropogenic load [5].

This study deals with the synthesis of 2-aminoethanol-based PCB derivatives, as well as with their preparation for biodegradation by *R. wratislaviensis* strain KT112-7, as an example of a combination of methods of organic synthesis and microbiology for neutralization of manmade PCBs.

Previously, it has been shown that the reaction of a Sovol technical grade PCB mixture, consisting mainly of tetra-, penta-, and hexachlorobiphenyls (a total of 35 congeners) [6], with 2-aminoethanol (2-AE) in the presence of KOH at 140°C for 6 h yields mono-and di(aminoethoxy) PCB derivatives, as a result of the nucleophilic substitution for one and two chlorine atoms, respectively [7]. The major reaction products are unreacted tetra-and pentachlorobiphenyls (more than 60%) [8], which points to the low conversion of PCB congeners of a Sovol mixture under the reaction conditions.

In the present paper, we report a somewhat different result obtained when the reaction of a Sovol mixture and 2-AE occurred at lower temperature (110–115°C) for longer time (7.5 h) (Scheme 1).

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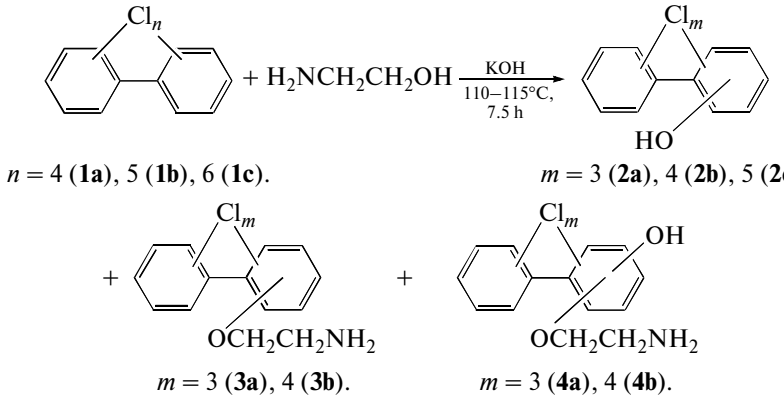

Scheme 1.

Quantification of the products of the mixture was performed using the internal normalization technique on the basis of peak areas in chromatograms recorded by the GC/MS method in the total ion current scanning mode (table, Fig. 1). We found that, for the mixture under consideration, the overall peak area of the unreacted tetra- (**1a**) and pentachlorobiphenyls (**1b**) is 17.9% (of the total area of the product peaks), which is evidence of the higher conversion of the Sovol PCB mixture under the selected conditions as compared with the results reported in [7, 8].

The resulting mixture of products **1a**, **1b**–**4a**, **4b** is an amorphous, viscous brown mass, which is water insoluble despite the plenty of products with hydrophilic HO groups (**2a**–**2c**, **4a**, **4b**). To transfer the mixture of compounds **1a**, **1b**–**4a**, **4b** into an aqueous medium for subsequent microbiological degradation by *R. wratislaviensis* strain KT112-7, two procedures were used. The first procedure consisted in dissolving the weighed sample of compounds **1a**, **1b**–**4a**, **4b** in acetone, and then the resulting solution was introduced into a bottle with the bacterial culture. The second procedure consisted in selecting a surfactant suitable for formation of a stable emulsion of compounds **1a**, **1b**–**4a**, **4b** in an aqueous medium.

The first procedure resulted in 100% degradation of the mixture of products **1a**, **1b**–**4a**, **4b** in 14 days. Gas chromatography with flame ionization detector (GC-FID) has demonstrated that *R. wratislaviensis* KT112-7 first degrades derivatives with an aminoethoxy group (**3a**, **3b**; **4a**, **4b**) since their characteristic peaks 100% disappeared in the chromatogram even on the fourth day of the experiment. Then, unreacted tetra- (**1a**) and pentachlrobiphenyls (**1b**) and polychlorinated hydroxybiphenyls (**2a**–**2c**) were degraded. As an example, Fig. 2 shows the change in the content of the components of the mixture (**1a**, **1b**–**4a**, **4b**) after degradation on the 7th and 14th day.

The dynamics of removal of the mixture of compounds **1a**, **1b**–**4a**, **4b** is represented by a concave curve with the correlation coefficient of 0.99, and the specific degradation rate is 0.033 (mg/mL)/day (Fig. 3). In most cases, the dynamics of removal of difficultly available substrates in the course of bacterial degrada-

GC/MS data and quantification of products **1a, 1b**–**4a, 4b**

Compound	Molecular formula	Molecular ion	Characteristic ion	Relative content, %
		m/z		
1a	$C_{12}H_6Cl_4$	290	292	16.2
1b	$C_{12}H_5Cl_5$	324	326	1.7
2a	$C_{12}H_6Cl_3OH$	272	272	2.9
2b	$C_{12}H_5Cl_4OH$	306	308	56.0
2c	$C_{12}H_4Cl_5OH$	340	342	4.1
3a	$C_{12}H_6Cl_3(OCH_2CH_2NH_2)$	315	279	4.3
3b	$C_{12}H_5Cl_4(OCH_2CH_2NH_2)$	349	313	10.7
4a	$C_{12}H_5Cl_3(OCH_2CH_2NH_2)(OH)$	331	295	2.7
4b	$C_{12}H_4Cl_4(OCH_2CH_2NH_2)(OH)$	365	329	0.7

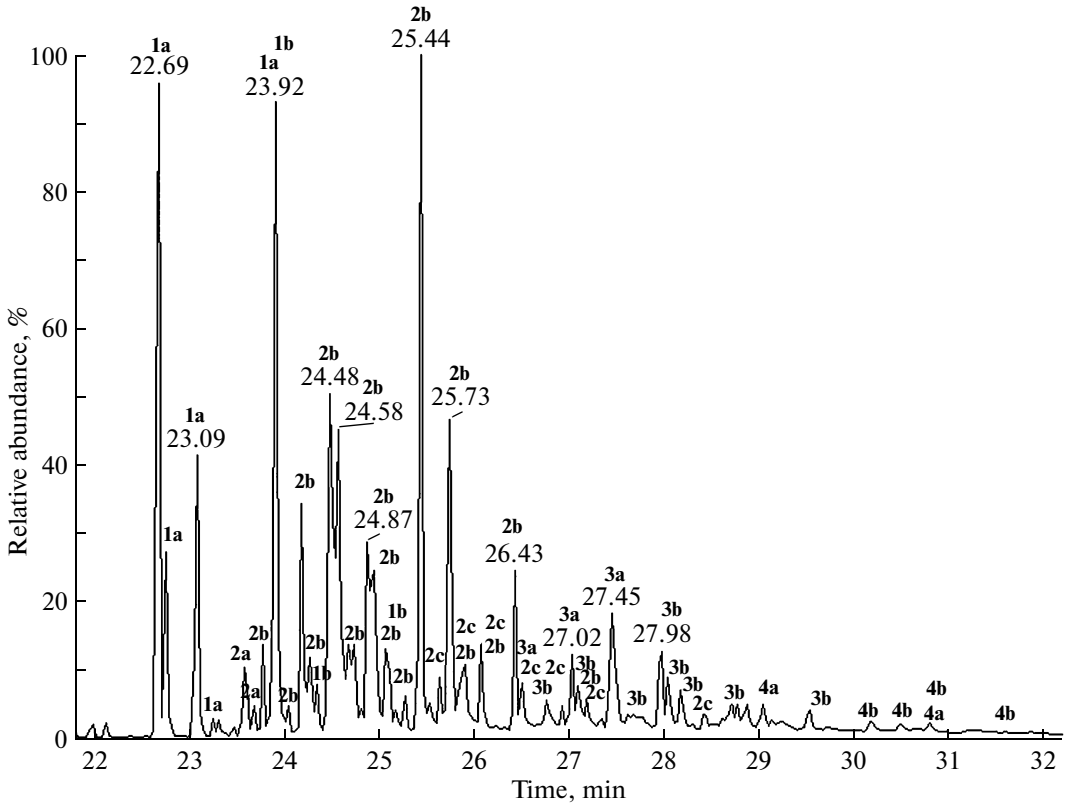

Fig. 1. GC/MS chromatogram of the reaction products of a Sovol PCB mixture with 2-AE. The numbers of compounds correspond to those in Scheme 1.

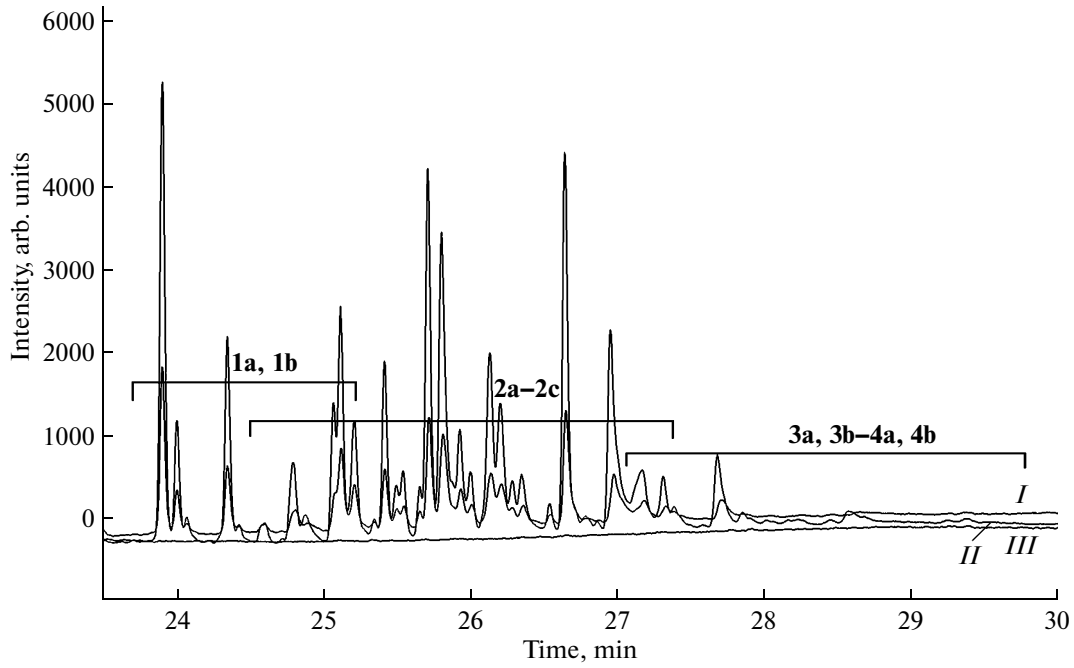

Fig. 2. GC-FID chromatogram of analysis of extracts of compounds **1a**, **1b–4a**, **4b** after microbiological degradation by *R. wratislaviensis* strain KT112-7. Time, days: 0 (*I*), 7 (*II*), and 14 (*III*).

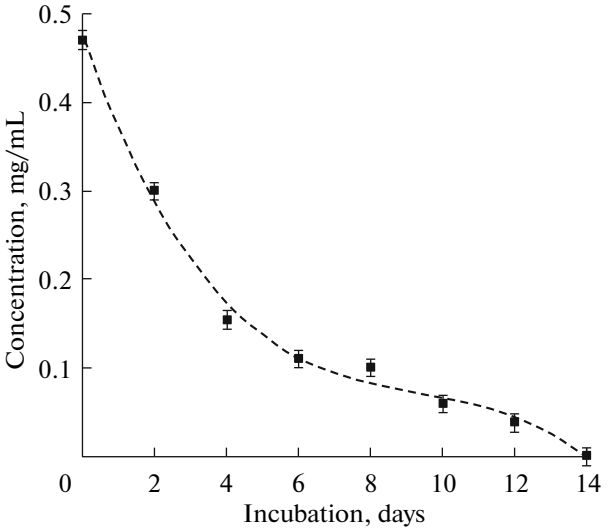

Fig. 3. Dynamics of degradation of products **1a, 1b–4a, 4b** by *R. wratislaviensis* strain KT112-7 (first procedure) $y = -0.0004x^3 + 0.0114x^3 - 0.1143x + 0.4728$, $R^2 = 0.9945$.

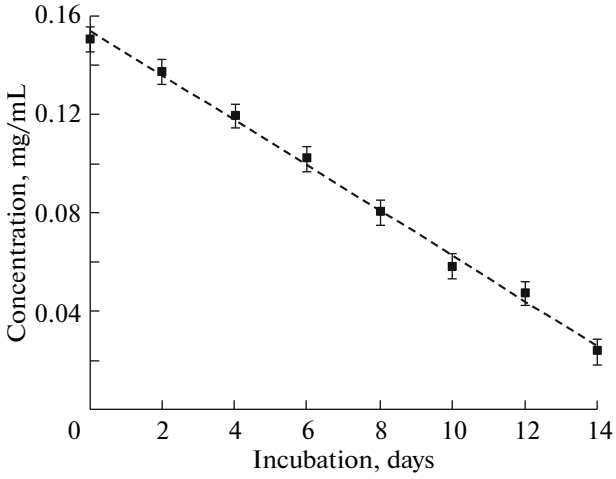

Fig. 4. Dynamics of degradation of products **1a, 1b–4a, 4b** by *R. wratislaviensis* strain KT112-7 (second procedure) $y = -0.0092x + 0.1537$, $R^2 = 0.9956$.

tion is described by an exponential curve, and the medium contains trace amounts of degradable compounds [9, 10]. No examples of microbiological degradation of compounds similar to the mixture of products **1a**, **1b–4a**, **4b** have been found in the literature.

It has been demonstrated by high-performance liquid chromatography (HPLC) and spectrophotometry that the products of bacterial degradation of the mixture of compounds **1a**, **1b–4a**, **4b** contain chloro- and hydroxy-substituted benzoic acids, as well as catechol. As known, these compounds are intermediates of the microbial degradation of chlorobenzoic acids [11].

These results enable the suggestion that upon degradation of products **1a**, **1b–4a**, **4b** by *R. wratislaviensis* strain KT112-7, environmentally toxic compounds do not accumulate.

Second procedure. No information is available on the use of surfactants for emulsifying (suspending) PCB derivatives in water, whereas the data concerning the transfer of PCBs themselves into aqueous media are rather extensive. Special attention has focused on the use of commercially available surfactants. No priority among ionic and nonionic surfactants has been established, and the amounts of surfactants used (by weight) always exceed (by one-to-two orders of magnitude) the amounts of PCBs subject to biodegradation.

It has been shown [4] that ionic surfactants (sodium alky(alkylene) sulfonates Hostapur SAS 60, Nansa LSS 38/AS) wash less PCB congeners from soil than nonionic surfactants ((alkylphenoxy)polyethoxyethanols Igepal CO-630 and Igepal CO-080; carboxylate esters Sorbax PMO-20 etc.) but promote their higher biodegradation. It has been found [12] that, among three nonionic surfactants, Tween 80 (polyoxyethylene sorbitan monooleate) is more efficient for PCB biodegradation than Tergitol NP and Triton X-100 (Igepal analogues).

In this study, we also used commercially available surfactants for creating a stable emulsion of PCB derivatives **1a**, **1b–4a**, **4b** in water. We found that surfactants, such as neonols (oxyethylated nonylphenols AF 9-4, AF 9-6, AF 9-9, AF 9-10, AF 9-12), sulfanol (sodium alkyl sulfonate), and Berol LFG 61 (a mixture of hexylglucoside and 2-ethylhexanol ethoxylate), taken in a 20–30-fold excess are inefficient for the transfer of the mixture of compounds **1a**, **1b–4a**, **4b** into water. The only experimentally found positive solution is using a mixed surfactant consisting of sulfanol and Berol LFG 61 (1 : 2.5 (w/w)). A 8.2-fold weight excess of the surfactant mixture over the weight of the mixture of compounds **1a**, **1b–4a**, **4b** is required, and the resulting stable emulsion is a slightly turbid liquid.

Upon the degradation of the resulting emulsion by *R. wratislaviensis* strain KT112-7, the concentration of the mixture of **1a**, **1b–4a**, **4b** in the cultural medium decreases by 85% in 14 days (Fig. 4). It turned out that, in addition to the PCB mixture, the surfactants are also degraded. The dynamics of removal of the mixed surfactant is described by an exponential curve (the correlation coefficient 0.94), whereas the dynamics of removal of compounds **1a**, **1b–4a**, **4b** corresponds to a straight line (correlation coefficient 0.99). It is likely that the change in the tendency of removals of the analytes is due to the appearance of an additional source of carbon for bacterial cells in the form of the surfactants present in the emulsion. The rate of surfactant

degradation by the by *R. wratislaviensis* strain KT112-7 is 0.09 (mg/mL)/day, which is one order of magnitude higher the degradation rate of compounds **1a**, **1b**–**4a**, **4b** in the emulsion (0.009 (mg/mL)/day). This result is evidence that, for *R. wratislaviensis* KT112-7, the sulfanol–Berol LFG 61 mixture as the surfactant is a more available substrate than the mixture of products **1a**, **1b**–**4a**, **4b**.

An analogous effect has been reported for degradation of di-, tri-, tetra-, penta-, and hexachlorobiphenyls by *Burkholderia xenovorans* LB400, *Ralstonia eutropha* H850, and *Rhodococcus globerulus* MB1 in the presence of Triton X-100 [13], which has a negative effect on the PCB-degrading strain growth. This seems to be a reason for the decrease in their PCB-degrading activity. In our case, the inhibition of biodegradation of compounds **1a**, **1b**–**4a**, **4b** is presumably caused by the low activation of enzymatic systems responsible for degradation of PCBs and their derivatives because of the potent activation of the enzymatic potential of *R. wratislaviensis* strain KT112-7 with respect to the surfactant mixture.

Thus, our findings show the possibility of selecting efficient surfactants for different types of derivatives of manmade PCBs. The importance of these studies is caused by a tendency of rapid development of chemical methods of processing of PCBs yielding products more available for microbiological degradation than high-chlorinated PCB congeners.

EXPERIMENTAL

Identification and quantification of compounds 1a, **1b**–**4a**, **4b** were carried out by gas chromatography/mass spectrometry using a Trace GC Ultra DSQ II GC/MS system equipped with a Thermo TR-5ms quartz capillary column (length, 30 m; i.d., 0.25 mm; film (5% phenyl polymethylsiloxane) thickness, 0.25 μm) and a quadrupole MS detector. Total ion current scanning in the mass range 20–1000 amu was carried out in the regime of electron impact ionization at 70 eV. The column was kept for 3 min at 40°C and then heated to 280°C at a rate of 10 K/min. The evaporator temperature was 250°C, the detector temperature was 200°C, and the intermediate chamber temperature was 200°C. Helium was used as a carrier gas, the split ratio was 1 : 50, and the flow rate through the column was 1.0 mL/min.

Synthesis of compounds 1a, **1b**–**4a**, **4b.** A portion of 5.1 g (0.09 mol) of KOH and 30 mL of 2-AE were placed into a round-bottom flask equipped with a reflux condenser, a dropping funnel, and a thermometer. The flask was mounted on a magnetic stirrer. The reaction mass was stirred at 110–115°C until a homogeneous mixture was formed, and then 9.8 g (0.03 mol) of a Sovol PCB mixture was added dropwise. The resulting reaction mixture was stirred for another 7.5 h at the same temperature. After that, the reaction mass was cooled to room temperature, and 75 mL of toluene, 100 mL of water, and HCl_{conc} to pH 5–6 were added under stirring. The toluene layer was separated and analyzed by GC/MS. Then, toluene was evaporated, and 9.2 g of a viscous brown product was obtained. The yield was 88% as calculated for (aminoethoxy)tetrachlorobiphenyl (3b).

For $C_{14}H_{11}Cl_4NO$ (3b) anal. calcd. (%): C, 47.90; H, 3.16; Cl, 40.39; N, 3.99. Found (%): C, 52.64; H, 3.18; Cl, 37.17; N, 1.46.

Preparation of stable emulsions of products 1a, **1b**–**4a**, **4b in water.** A weighed portion of compounds **1a**, **1b**–**4a**, **4b** (0.0300–0.0350 g) was placed into a closed beaker with 100 mL of distilled water and heated to 60°C under vigorous stirring, and a weighed portion of a surfactant (0.0010–0.0020 g) was introduced. The mixture was stirred for another 5–10 min and then cooled and visually inspected. If brown drops of products **1a**, **1b**–**4a**, **4b** were deposited onto the bottom of the beaker, the mass was again heated to 60°C, and a next portion of the surfactant (0.0010–0.0020 g) was added. The procedure was repeated until the surfactant weight exceeded 20-fold the weight of products **1a**, **1b**–**4a**, **4b**. If the procedure did not lead to the formation of a stable emulsion of PCB derivatives **1a**, **1b**–**4a**, **4b**, the result was considered negative. If a stable emulsion was formed when a less than 20-fold excess of the surfactant was introduced, the result considered positive. On completion, to verify the constancy of the composition of products **1a**, **1b**–**4a**, **4b**, a 50-mL portion of the emulsion was sampled, acidified with HCl_{conc}, and extracted with $CHCl_3$ (2 × 15 mL); the resulting extract was analyzed by GC-MS.

Bacterial degradation of a mixture of products 1a, **1b**–**4a**, **4b** was performed by using washed cells. The bacterial culture was pre-grown in 250-mL flasks containing 50 mL of Raymond's mineral medium [14] with biphenyl as a carbon source (1 g/L) on a temperature-controlled rotary shaker (120 rpm) at 28°C to the optical density $OD_{600} = 1.0$. The cells washed two times in Raymond's mineral medium taken in a concentration of 1.5×10^9 CFU/mL (1 mL, $OD_{600} = 2.0$) were transferred into bottles with Teflon-lined stoppers.

A mixture of products **1a**, **1b**–**4a**, **4b** was introduced into bottles with the bacterial culture (1) as an acetone solution to a final concentration of 0.47 mg/mL and (2) as an aqueous solution with the surfactant to a final concentration of 0.15 mg/mL. Incubation was carried out on a temperature-controlled rotary shaker (120 rpm) at 28°C for 14 days. Samples for analysis of

the efficiency of bacterial degradation were taken at intervals of 24 h, as well as immediately after the introduction of the product mixture.

Analysis and monitoring of the biodegradation of products 1a, 1b–4a, 4b were carried out using a Shimadzu GC 2010 gas chromatograph with a flame ionization detector, a ZB-5 quartz capillary column (length, 30 m; i.d., 0.25 mm; stationary phase film thickness, 0.25 μm (polymethylsiloxane, 5% of grafted phenyl groups). The column was kept for 3 min at 40°C and then heated to 280°C at a rate of 10 K/min. The evaporator temperature was 250°C, and the detector temperature was 300°C. Nitrogen was used as a carrier gas, the split ratio was 1 : 30, and the flow rate through the column was 1.0 mL/min.

Intermediate products of bacterial degradation of compounds 1a, 1b–4a, 4b were determined spectrophotometrically on a Shimadzu UV-Visible BioSpec-mini spectrophotometer and by high-performance liquid chromatography on a Shimadzu LC-10ADvp chromatograph with a Supelco C18 column, as described in [15].

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