Bacterial Degradation of PCB 70 and its Hydroxy Derivatives is an Environmentally Friendly Way to Destroy Pops

Tatyana D. Kir'yanova[1], *Darya O.* Egorova[1,*], *Tatyana I.* Gorbunova[2] and *Marina G.* Pervova[2]

[1]Institute of Ecology and Genetics of Microorganisms, Perm, Russia
[2]I.Ya. Postovskii Institute of Organic Synthesis, Yekaterinburg, Russia

Abstract. One of the problems of our time is the environmentally safe destruction of polychlorinated biphenyls (PCBs) and their hydroxylated derivatives. The aim of the study was to investigate the features and prospects of the decomposition of PCB 70 (2,5,3',4'-tetrachlorobiphenyl) and hydroxylated chlorobiphenyls derived from it by *Rhodococcus wratislaviensis* strain CH628. As a result of the application of methods of periodic cultivation, gas chromatography and light spectrometry, it was found that the efficiency of destruction of PCB 70 1 g of cells of strain CH628 was 90 mg PCB/day, and the same indicator for a mixture consisting of hydroxy derivatives obtained from PCB 70 was 56 mg PCB/day. It was shown that the strain uses all components of the mixture of hydroxy-PCB 70 as a growth substrate, but with different degradation rates. When cultivated in a mineral medium with PCB 70 or a mixture of hydroxy-PCB 70, strain CH628 forms biofilms. The analysis of the obtained results shows that the use of the *Rhodococcus wratislaviensis* CH628 strain will make it possible to develop a technology for the environmentally safe destruction of PCB 70 and hydroxy-PCBs derived from it.

1 Introduction

The active development of the chemical industry in the 20th century led to the synthesis and production on a global scale of organic compounds hazardous to the environment. As a result of the implementation of the UN Environment Program in 2001, an international convention was adopted in Stockholm. According to this document, the compounds included in the list of "persistent organic pollutants" (POPs) must be withdrawn from production and use, and subject to complete destruction [1, 2]. Russia ratified the Stockholm Convention in 2011 (No. 164-FZ of June 27, 2011) [3].

Along with pesticides, the list of POPs includes polychlorinated biphenyls (PCBs) [1]. In total, there are 209 PCB congeners that differ in the position and number of substituents (chlorine atoms) in the molecule. Environmental pollution with PCBs is dangerous for all

* Corresponding author: daryao@rambler.ru

components of flora and fauna, since they can accumulate in plant and animal tissues, causing a toxic effect on all organ systems [4]. During the production of PCBs, significant amounts of them got into the environment and accumulated in storage areas [5]. According to the Stockholm Convention, PCBs must be completely destroyed [6]. However, due to the unique structure of the molecule, PCBs are resistant to chemical and physical attack. Destruction is associated with high economic costs, and the technological cycle is potentially dangerous for nature [1, 2, 4]. In addition, PCBs, exposed to biological and photochemical factors in the environment, can transform into hydroxylated derivatives, which are also toxic to living objects [7, 8].

One of the solutions to the problem of destruction of PCBs and their hydroxy derivatives is bacterial destruction [9, 10]. During aerobic bacterial decomposition of PCBs, the PCB molecule is oxidized, followed by its splitting to pentadienoic and (chloro)benzoic acids [9]. However, most degrading bacteria are not able to oxidize PCB congeners containing more than 3 chlorine atoms in a molecule, and hydroxy-PCBs have a toxic effect on the enzymatic systems of PCB degrading strains [7, 8].

In this regard, of particular interest are studies of natural aerobic bacterial strains that exhibit destructive activity towards PCB congeners with 4 or more substituents in the molecule (both chlorine atoms and hydroxy groups can act as substituents).

The aim of this study was to investigate the possibility of destroying PCB 70 (2,5,3',4'-tetrachlorobiphenyl) and hydroxy derivatives derived from PCB 70 using an aerobic bacterial strain *Rhodococcus wratislaviensis* CH628.

2 Materials and methods

The *Rhodococcus wratislaviensis* CH628 strain used in this study is capable of degrading a wide range of POPs compounds (PCBs, DDT, hexachlorocyclohexanes), as well as mixtures of modified PCBs obtained from commercial PCB products [11].

PCB70 was obtained as a result of chemical synthesis [12]. Hydroxy derivatives of PCB70 were obtained as a result of the interaction of the PCB congener with alkali in 2-aminoethanol as described previously [12]. The hydroxy-PCB70 mixture consists of hydroxy-trichlorobiphenyls (64.44%), dihydroxy-dichlorobiphenyls (32.51%) and hydroxy-dichlorobiphenyls (3.05%).

The main medium for the experiments was the K1 mineral medium of composition (g/l): 3.18 K2HPO4, 0.35 NaH2PO4, 0.5(NH4)2SO4, 0.01 Ca(NO3)2, 1.5 MgSO4 x 7H2O. “Hunter” mineral solution composition (g/l): 2.50 EDTA, 10.95 ZnSO4 x 2H2O, 5 FeSO4 x 7H2O, 1.54 MnSO4 x 2H2O,0.39 CuSO4 x 5H2O, 0.24 Co(NO3)2 x 6H2O , 0.17 Na2B4O7 x 10H2O, pH 7.3.

The biodegradation of PCB70 and hydroxy-PCB70 mixtures was studied under batch culture conditions. The culture of the strain *Rhodococcus wratislaviensis* CH628 (OD600=1.0, 1 ml) preliminarily grown to the middle of the exponential phase in K1 mineral medium with biphenyl was placed in 4 ml glass vials (Sigma-Aldrich, Germany) closed with Teflon-containing caps. 0.25 g/L of PCB 70 or a mixture of hydroxy-PCB70 was added to each vial and incubated on an Environmental Shaker-Incubator ES-20/60 circular shaker (BioSan, Latvia) at 120 rpm for 14 days at a temperature of 28°C . The biodegradation process was stopped by freezing. Bacterial growth was monitored in non-frozen samples by changing the optical density of the culture at 600 nm on a UV-Visible BioSpec-mini spectrophotometer (Shimadzu, Japan).

The content of PCB70 and hydroxy-PCB70 in the samples was evaluated under GC-FID conditions on a Shimadzu GC 2010 gas chromatograph (Shimadzu, Japan) as described in [11]. The calculation of the content of PCBs or PCB-OH remaining after biodegradation in each test sample was carried out by the method of internal normalization, calculating the

contribution of individual compounds to the total peak area. Based on the obtained calculated peak areas, the content of the original PCBs or PCB-OHs was estimated after the biodegradation process.

The degradation efficiency was calculated taking into account the initial concentration of the substrate, the biomass of the strain, and the period of time spent on the complete destruction of the substrate. The specific rate of degradation of the components of the hydroxy-PCB70 mixture was carried out according to the formula

$$y = (\ln X1 - \ln X2)/ 3 \quad (1)$$

where X1 is the area of the peaks of the compound at the initial moment of the experiment, X2 is the area of the peaks of the compound after 3 days of the experiment.

Plotting and statistical data processing were performed using the standard MS Office 2019 software package.

3 Results and discussion

Rhodococcus wratislaviensis CH628 strain degrades a number of compounds included in the POPs group. It was also previously found that it is able to decompose mixtures obtained by chemical modification of a commercial mixture of PCB Sovol. Based on this fact, we studied the activity of the strain *R. wratislaviensis* CH628 to tetrachlorinated biphenyl containing two substituents in each ring of the molecule (2,5,3',4'-tetrachlorobiphenyl = PCB 70) and a mixture of hydroxy- and dihydroxy-(di-tri)chlorobiphenyls obtained by chemical modification of PCB 70.

It was found that the strain *R. wratislaviensis* CH628 completes the decomposition of PCB 70 in 10 days, while the destruction of a mixture of hydroxy-PCB 70 takes 14 days (Fig. 1, 2).

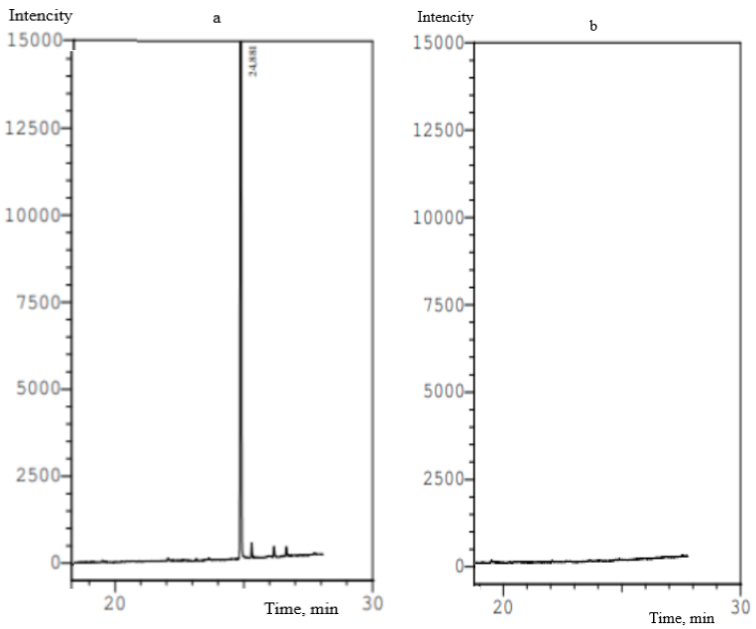

Fig. 1. GC-FID chromatogram of extracts of the culture medium of strain *R. wratislaviensis* CH628 in the experiment on the destruction of PCB 70: (a) 0 days, (b) 10 days.

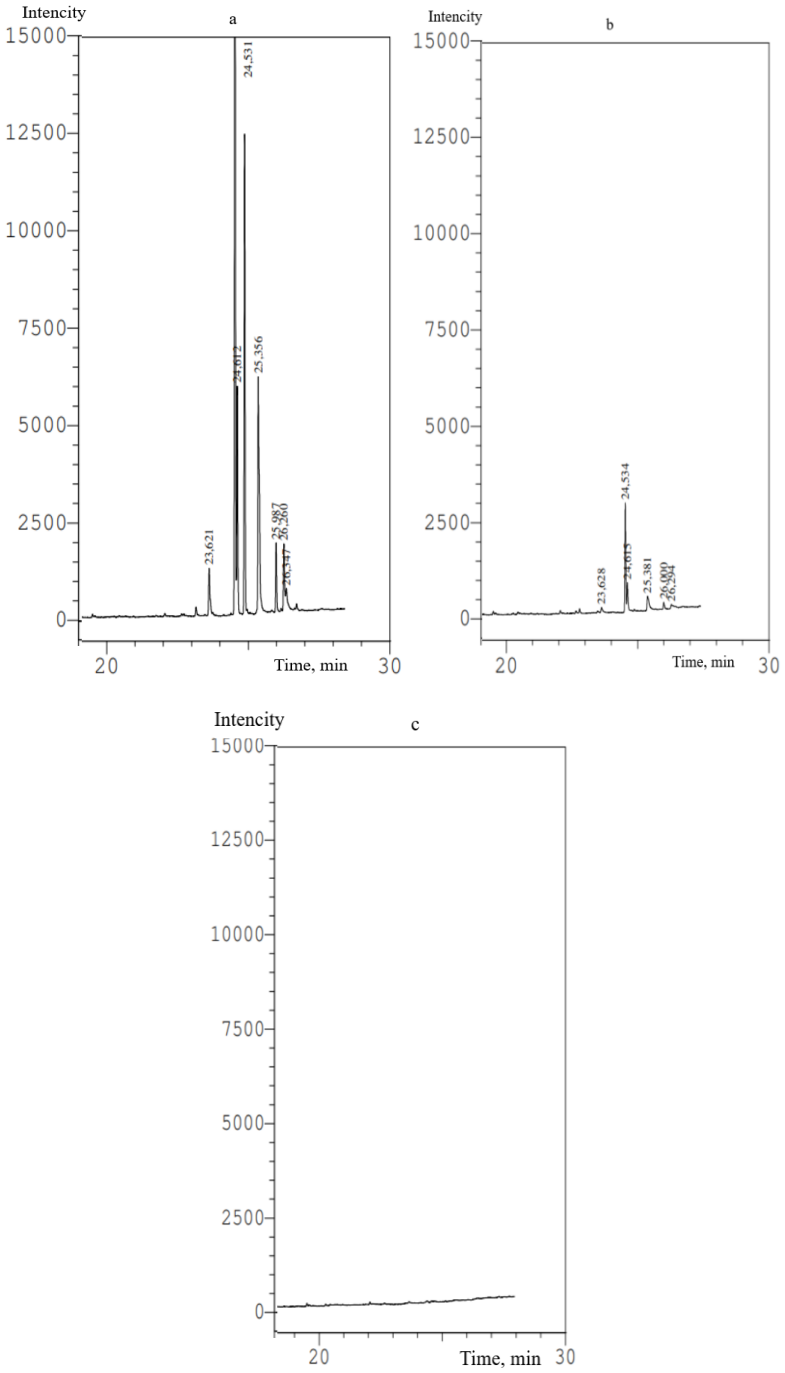

Fig. 2. GC-FID chromatogram of extracts of the culture medium of strain *R. wratislaviensis* CH628 in the experiment on the destruction of a mixture of hydroxy-PCB 70: (a) 0 days, (b) 10 days, (c) 14 days.

Calculations showed that the efficiency of destruction of PCB 70 per 1 g of cells of strain CH628 was 90 mg PCB/day, and the same indicator for a mixture of hydroxy derivatives of PCB 70 was 56 mg PCB/day. It was also found that strain CH628 decomposes all compounds included in the mixture of hydroxy-PCB 70. At the same time, the most active degradation process occurs in the first 3 days of the experiment. The specific degradation rate was 0.303 day-1 for hydroxy-trichlorobiphenyls, 0.309 day-1 for hydroxy-dichlorobiphenyls, and 0.725 day-1 for dihydroxy-dichlorobiphenyls.

As a result of the studies, the accumulation of toxic compounds that are metabolites of aerobic bacterial decomposition of PCB 70 and a mixture of hydroxy derivatives of PCB 70 was not detected.

The optical density of the plankton culture of the strain during cultivation in a mineral medium in the presence of PCB 70 or a mixture of hydroxy-PCB 70 as the only carbon source decreased slightly (Fig. 3).

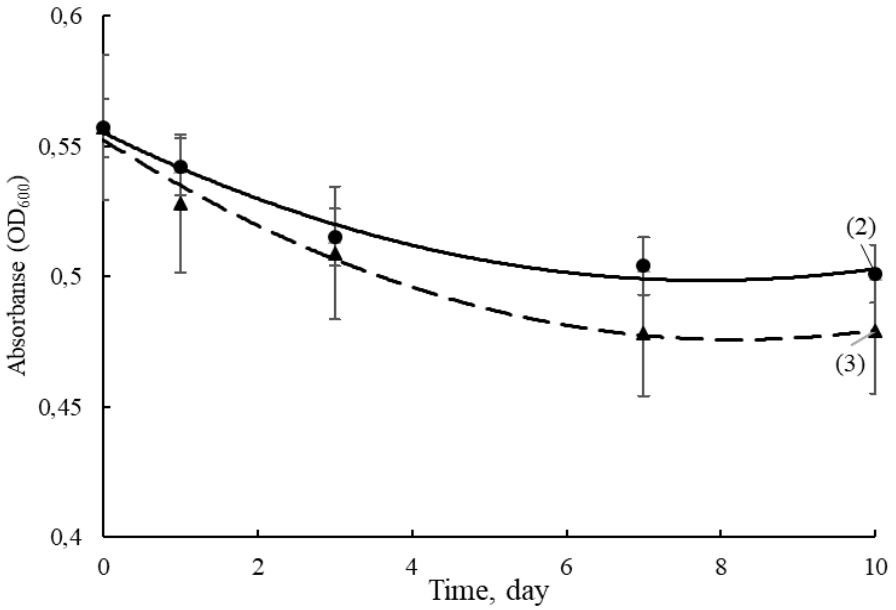

Fig. 3. Dynamics of changes in the optical density of the planktonic culture of the *R. wratislaviensis* strain CH628 when cultivated on PCB 70 and a mixture of hydroxy-PCB 70.

The resulting curves are described by the equations

$$A = 0.0009x^2 - 0.0145x + 0.5553, \; R^2 = 0.977 \quad (2)$$

$$A = 0.0011x^2 - 0.0187x + 0.5526, \; R^2 = 0.984 \quad (3)$$

It can be seen from the obtained equations that when the strain *R. wratislaviensis* CH628 is cultivated on a mixture of hydroxy-PCB 70 (Equation 3), the density of the plankton culture decreases faster than when the substrate is PCB 70 (Equation 2). This fact may be due to the potentially higher toxicity of hydroxy derivatives of chlorobiphenyls to aerobic bacterial cells [7, 8].

It is interesting to note that during cultivation in a mineral medium with PCB 70 or a mixture of hydroxy-PCB 70, cells of strain CH628 formed a biofilm. Thus, the decrease in the optical density of the plankton culture cannot be considered as an indicator of the negative effect of the substrate on the cells of strain CH628, since the process of culture growth continued.

The preservation of cell viability during the degradation of PCB 70 and a mixture of its hydroxy derivatives makes it possible to consider the bacterial method of decomposition of these substrates as promising and environmentally safe.

4 Summary

As a result of the study, it was found that the strain *Rhodococcus wratislaviensis* CH628 effectively destroys PCB 70 (2,5,3',4'-tetrachlorobiphenyl) and hydroxylated derivatives of PCB 70 obtained as a result of chemical modification. The use of a culture of strain CH628 allows complete decomposition in 10 days 250 mg/l PCB 70, and for 14 days - 210 mg/l hydroxy-PCB 70 mixture. At the same time, the strain is active against all components of the hydroxy-PCB 70 mixture.

Acknowledgments

Synthesis of a mixture of hydroxylated derivatives of PCB 70 and analysis by gas chromatography was carried out within the framework of state order AAAA-A19-119012290115-2, analysis of the growth parameters of the strain was performed within the framework of work under state order AAAA-A19-119112290009-1. The equipment used in the work was Center for Shared Use Spectroscopy and Analysis of Organic Compounds.

References

1. M. D. Erikson, R. G. Kaley II, Environ. Sci. Pollut. Res., **18(2)**, 135-151 (2011).
2. R. Gioia, A. J. Akidele, S. A. Adebusoye, Environ. Sci. Pollut. Res., **21**, 6278-6289 (2014).
3. Federal Law of June 27, 2011 "On Ratification of the Stockholm Convention on Persistent Organic Pollutants", Rossiiskaya Gazeta N 138, 29 June, 2011.
4. J. K. Sharma, R. K Gautam, S. V. Nanekar, Environ. Sci. Pollut. Res., **25**, 16355-16375 (2018).
5. J. Borja, D. Taleon, J. Auresenia, S. Gallardo, Proc. Biochem., **40**, 1999–2013 (2005).
6. Stockholm Convention on Persistent Organic Pollutants, http://chm.pops.int.
7. R. Tehrani, B. Van Aken, Environ. Sci. Pollut. Res., **21**, 6334–6345 (2014).
8. L. Passatore, S. Rossetti, A. A. Juwarkar, A. Massacci, J. Hazardous Materials, **278**, 189–202 (2014).
9. D. H. Pieper, Appl. Microbiol. Biotechnol., **67(2)**, 170-191 (2005).
10. D. O. Egorova, T. I. Gorbunova, M. G. Pervova, T. D. Kir'yanova, V. A. Demakov, V. I. Saloutin, O. N. Chupakhin, J. Hazard. Mat., 400 (2020).
11. T. I. Gorbunova, M. G. Pervova, V. I. Saloutin, O. N. Chupakhin, Russ. J. Org. Chem., **55(7)**, 988-990 (2019).
12. T. I. Gorbunova, M. G. Pervova, V. I. Saloutin, O. N. Chupakhin, Russ. J. Gen. Chem., **90**, 2255-2257 (2020).