

BIODEGRADATION OF PHENOLS AND HALOGENATED DERIVATIVES OF AROMATIC CARBOHYDROGENS BY BACTERIA SPECIFIC TO THE GENUS *PSEUDOMONAS* AND *ARTHROBACTER*

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Abstract

As a result of the research, active phenol-absorbing bacterial strains belonging to the genera Pseudomonas and Arthrobacter were isolated from the Azerbaijani shores of the Caspian Sea. Biodegradation of chlorinated derivatives of phenols and aromatic hydrocarbons by isolated bacteria was studied. Investigation of biodegradation products of phenols and halogenated derivatives of aromatic hydrocarbons (p-, m-, o-chlorophenol, p-, m- process, o-chlorotoluene, chlorobenzene) and formation of halogen forms of benzene and toluene using the technology of reversed-phase liquid chromatography method. as the degradation mechanism of benzene and toluene goes through the system.

Keywords: bacteria, biodegradation, oil pollution, halogenated derivatives of phenol, halogenated derivatives of aromatic hydrocarbons

I. Introduction

In the last 50-60 years, the environmental condition of the Caspian Sea has deteriorated under the influence of anthropogenic factors. In modern times, oil and oil products are considered one of the most important and dangerous pollutants of modern water ecosystems [3]. Since the distant past, the southern part of the Caspian Sea - Azerbaijan has been exposed to oil pollution.

However, all ecological contradictions existing in the Caspian Sea are not only related to hydrocarbons of petroleum origin. In addition to oil hydrocarbons, more dangerous pollutants, xenobiotics, and carcinogens enter the Caspian Sea every year. The composition of these compounds in seawater depends on the design of wastes coming in from the outside, and changes under the influence of abiotic and biological factors.

The entry of oil and oil products and sewage into the Caspian Sea strongly impacts the vital functions and gene pool of marine hydrobionts, reducing their effectiveness. In addition, separate areas of the Caspian Sea lose from self-purification and become unsuitable for fish farming [2].

Permanent pollution causes the infection of some fish with various parasites, pathogenic microbes, and fungi, which causes serious consequences. Phenol pollution gives a foul smell and taste to the water and its inhabitants, and in addition, to the fish. A high concentration of phenols has a lethal effect on hydrobionts. One of the main ways in which phenols, aromatic hydrocarbons, and their derivatives pollute the seas is wastewater from the processes of oil extraction, oil transportation, and oil and oil product processing [2,3].

The study of the role of microorganisms in the biological purification of water bodies from phenols and halogen derivatives of aromatic hydrocarbons is one of the urgent issues of the modern era [4,5,6]. Degradation of halogenated derivatives of phenols and aromatic compounds by phenol-absorbing bacteria isolated from the Azerbaijani shores of the Caspian Sea has been studied.

II. Methods

During the research, 44 active bacterial strains isolated from the Azerbaijani shores of the Caspian Sea were planted with 50, 100 and 300 mg/l halogenated derivatives of phenols and aromatic hydrocarbons (p-, m-, o-chlorophenol, tetrachloropyrocatechin, tetrabrompyrocatechin) as the sole source of energy and carbon, p-, m-, o-chlorotoluene, chlorobenzene, bromobenzene) were added.

The molecular level study of the intermediate products formed in the biodegradation of phenols and halogenated derivatives of aromatic hydrocarbons was carried out by the reversed-phase liquid chromatography method. The structural composition of biodegradation products of the compounds was confirmed by IR and NMR¹H spectral analysis.

III. Results

As a result of preliminary studies, it has been determined that bacterial strains do not develop in an environment containing toxic substances at a concentration of 300 mg/l. Therefore, all subsequent studies have been carried out in a medium containing halogenated derivatives of phenols and aromatic hydrocarbons at a concentration of 100 mg/l.

As a result of the research, it has been determined that 73% of the 44 active bacterial strains isolated from the shores of the Caspian Sea developed in an environment containing p-chlorophenol at a concentration of 100 mg/l as the only source of energy and carbon. 66% of bacterial strains grew in o-chlorophenol and 36% in m-chlorophenol.

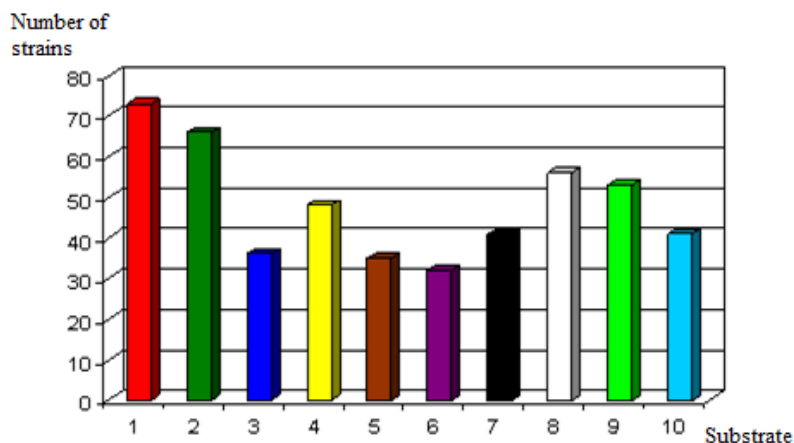
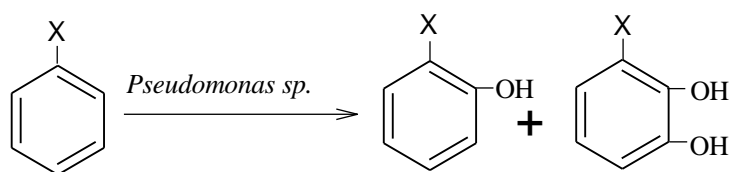


Figure 1: The number of bacteria growing in halogenated derivatives of phenols and aromatic hydrocarbons (at a concentration of 100 mg/l) in %: 1-o-chlorophenol, 2-p-chlorophenol, 3-m-chlorophenol, 4-chlorobenzene, 5-bromobenzene, 6-tetrachloropyrocatechin, 7-tetrabrompyrocatechin, 8-p-chlorotoluene, 9-m-chlorotoluene, 10-o-chlorotoluene

It was determined that 58% of the bacteria assimilated chlorobenzene as the sole source of energy and carbon, and 35% assimilated bromobenzene. 32% of the studied bacterial strains grew on tetrachloropyrocatechin, and 41% on tetrabrompyrocatechin. 56% of bacteria assimilated p-chlorotoluene, 53% m-chlorotoluene, and 41% o-chlorotoluene as the sole source of energy and

carbon. As can be seen from the picture, most of the bacteria isolated from the Azerbaijani shores of the Middle and South Caspian had the characteristic of growing in an environment containing halogenated derivatives of phenols and aromatic hydrocarbons as the only source of energy and carbon at a concentration of 100 mg/l.

During the studies, *Pseudomonas sp.*, *Arthrobacter sp.* The composition of the biodegradation products of halogenated derivatives of phenols and aromatic hydrocarbons (p-, m-, o-chlorophenol, p-, m-, o-chlorotoluene, chlorobenzene, bromobenzene, tetrachlorpyrocatechin, tetrabrompyrocatechin) was studied with the participation of strains of has been shown to follow a similar mechanism. In both cases, it was determined that the directions for purchasing biotransformation products are the same. The similarity was observed even in the proportions of the amounts of degradation products. As can be seen from the chromatographic curve in Figure 2 (curve a), chlorobenzene is mainly converted to 2,3-dihydroxy chlorobenzene (30%) and o-chlorophenol (65%) in the intermediate stage of the degradation process (peaks 1 and 2).



X=Cl, Br

During the studies, the process of degradation of p-chlorotoluene (curve b) according to the degradation of toluene with a mixed mechanism, i.e., in the direction of simultaneous oxidation of the methyl radical in the side chain and the aromatic ring, phenolic acids: 2,3-dihydroxy-p-chlorobenzoic (25%), it was determined that 2-hydroxy-p-chlorobenzoic (30%) and p-chlorobenzoic acids (20%) were obtained (peaks 1, 2 and 4). The formation of 2-hydroxy-p-chlorotoluene (15%) (peak 3) was observed in the degradation process that occurred only as a result of the oxidation of the aromatic ring.

From the results obtained during the research, it can be concluded that the decomposition of chlorine ions does not occur at the initial stage of the degradation processes of chlorobenzene and chlorotoluene. This shows their stability in the composition of transformation products under selected conditions.

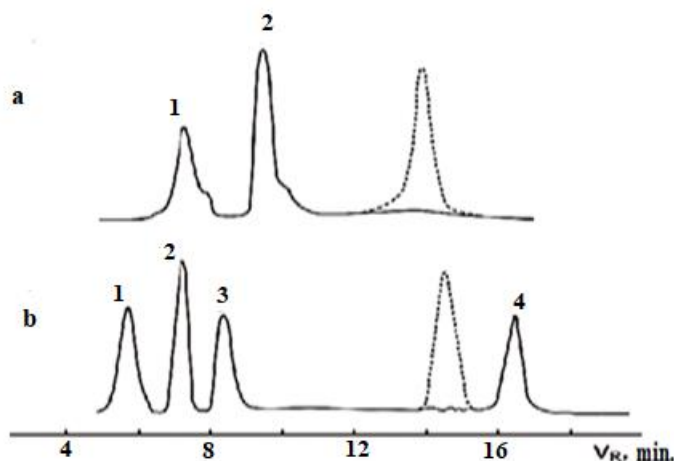
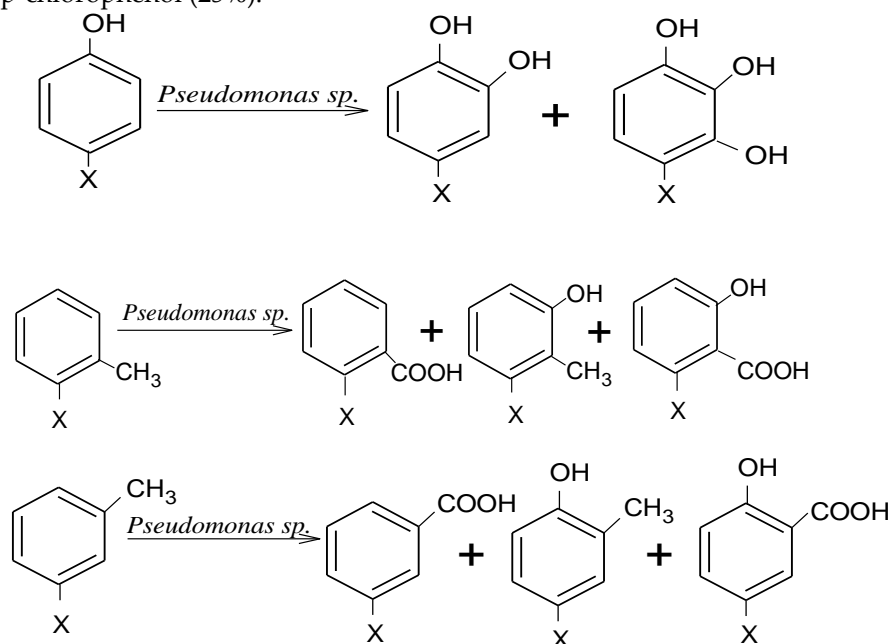


Figure 2: Chromatography curves of chlorobenzene (a) and p-chlorotoluene (b).
 a) Peaks 1-2: 2,3-dihydroxychlorobenzene and o-chlorophenol;
 b) Peaks 1-4: 2,3-dihydroxy-p-chlorobenzoic acid, 2-hydroxy-p-chlorobenzoic
 The structure of degradation products was confirmed by IR- and PMR-spectra.

In the IR spectra, in addition to absorption bands ($600-750\text{ cm}^{-1}$) characteristic of the C-Hlg bond, bands characteristic of the aromatic ring ($860, 1600-1635, 3050\text{ cm}^{-1}$) and $3465-3600\text{ cm}^{-1}$ of the hydroxyl group were found. PMR spectrum of chlorobenzene $d=7.70-9.00\text{ m.h.}$ in the field (in singlet form) and $d=6.70-6.85\text{ m.h.}$ in the area, signals characteristic of chemical shifts (in the form of multiples) of protons of aromatic ring and hydroxyl group ($3\text{H}, \text{C}_6\text{H}_3$; $4\text{H}, \text{C}_6\text{H}_4$) were found.

The degradation of o-chlorotoluene followed a similar process to the degradation of p-chlorotoluene. Thus, among the degradation products of o-chlorotoluene, 6-hydroxy-o-chlorotoluene (25%), o-chlorobenzoic acids (20%) and 6-hydroxy-o-chlorotoluene (15%) were observed. Analogous compounds were also noted in the process of degradation of m-chlorotoluene.

A faster disintegration process was observed in the degradation of p-chlorophenol. The biotransformation product of p-chlorophenol was mainly 2-hydroxy-p-chlorophenol (15%) and 2,3-dihydroxy-p-chlorophenol (25%).



A faster disintegration process is observed in the degradation of p-chlorophenol and tetrachloropyrocatechin (Figure 3). The biotransformation product of p-chlorophenol was mainly 2-hydroxy-p-chlorophenol (15%) and 2,3-dihydroxy-p-chlorophenol (25%) (curve a). During the transformation of tetrachloropyrocatechin, only one more stable substance - tetrachloromuconic acid - was fixed in the intermediate stage of the degradation process (curve b). It was determined that the degradation of these compounds, unlike the previous combinations, occurs with the breaking of the phenyl ring.

It should be noted that unlike chlorinated derivatives of phenols and aromatic hydrocarbons, the biodegradation of brominated derivatives of these compounds is complex, and the amount of biotransformation products formed corresponds to 5-7%.

Absorption bands characteristic of the aromatic ring and hydroxyl group were found in the IR-spectra of the degradation products of chlorophenol. In addition, bands related to the C-Hlg bond were also observed in the $600-750\text{ cm}^{-1}$ region. $d=7.65-9.00\text{ m.h.}$ characteristic of protons of the hydroxyl group (Ar-OH) and aromatic ring ($3\text{H}, \text{C}_6\text{H}_3$; $2\text{H}, \text{C}_6\text{H}_2$) in PMR-spectra. Singlet shape in the field and $d=7.65-9.05\text{ m.h.}$ Multiplet signals are recorded.

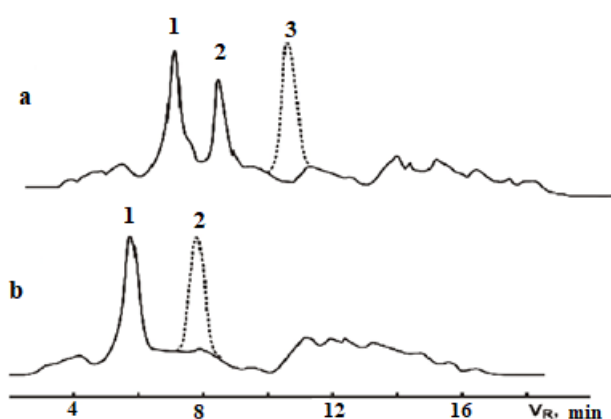
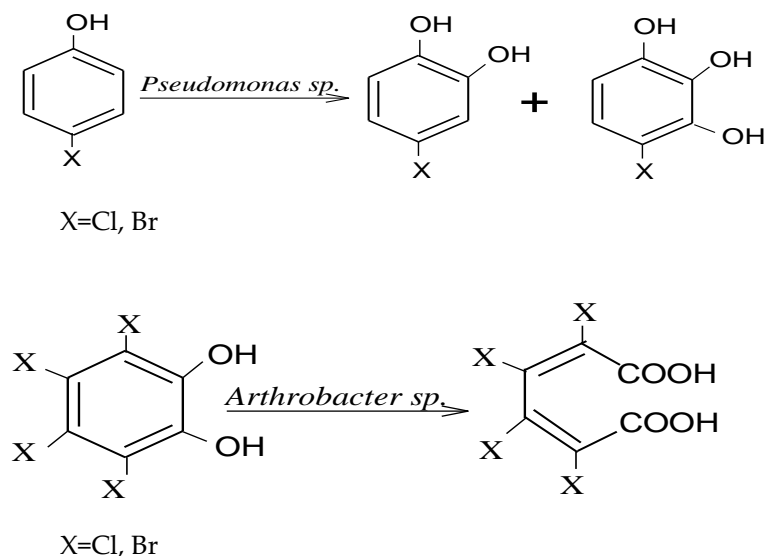


Figure 3. Chromatography curves of biodegradation of chlorophenol (a) and tetrachloropyrocatechin (b).
 a) Peak1- 3: 4-chloropyrocatechin, 2,3-dihydroxy-p-chlorophenol and starting chlorophenol;
 b) Peak 1 and 2: tetrachloromuconic acid and starting tetrachloropyrocatechin.



As a result of the research, it was determined that the isolated active phenol-absorbing bacterial strains degrade not only phenols, but also halogenated derivatives of phenol and aromatic hydrocarbons. This shows that it is possible to use those bacterial strains in cleaning the waters and soil contaminated with hydrocarbons and provides a basis for the preparation of biopreparations.

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