BIODEGRADATION OF XENOBIOTICS

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Abstract

The compounds as 1, 2-dibromoethane, 1, 2-dichloroethane and phenol are ones of the most dangerous pollutants in the environment. 1, 2-Dibromoethane (DBE) is a synthetic organic chemical that is mainly used as a gasoline additive. It is also one of the widely used pesticide fumigants. 1,2-Dichloroethane is one of the most commonly used chlorinated industrial products and falls into the environment by using it as a chemical intermediate in the synthesis of a number of chlorinated hydrocarbons. Phenol is a waste product from the plastics, petroleum and pharmaceutical industries. There are different methods for treating wastewater containing the listed xenobiotics. Applied physicochemical methods are often economically ineffective and may cause other toxic products to occur. For this reason, microbiological treatment methods are preferred. We tested three different bacterial strains: Pseudomonas putida, Bradyrhizobium japonicum and Xanthobacter autotrophicus GJ10. In our studies for a period of 14 days with pre-adapted culture of Pseudomonas putida strain, we have achieved a degradation of 0.26 g/l of phenol in shaking flasks and a fed-batch process.. Over a period of 6 days, 1.2 g/l of 1,2 dibromethane was degraded using the Bradyrhizobium japonicum strain, and for 3 days Xanthobacter autotrophicus GJ10 degraded 0.9 g / l of 1,2-dichloroethane.

Keywords: *bio-degradation, Pseudomonas putida, Bradyhizobium japonicum, Xanthobacter autotrophicus GJ10, 1, 2-dibromoethane, 1, 2-dichloroethane, phenol*

INTRODUCTION

The compound 1, 2-dibromoethane is used as an additive in gasoline mixtures and in particular in aviation fuels [1, 2]. Before 1983, it was used as herbicide and pesticide in agriculture, but because of its high toxicity it was banned by the Environmental Protection Agency. Biodegradation of 1,2-dibromoethane has been the subject of many researches [1, 3-6]. . Henderson et al. (2008) proved that the rate of degradation of 1, 2-dibromoethane is higher under anaerobic conditions. Hatzinger et al. (2011) argues that in the presence of gaseous ethane and anaerobic conditions biodegradation of 1, 2-dibromoethane increases significantly. McKeever et al. (2012) confirmed it. Kyunghwa Baek et al. (2014) stimulate the degradation of 1, 2-dibromoethane by addition of phenol.

1,2-Dichloroethane is a widespread pollutant in groundwater. Its high water solubility (8000 mg/l) and its half-life (more than 50 years) strengthen its danger. Due to potential toxicity and carcinogenicity, haloparasic hydrocarbons present in the environment pose a serious threat to plants, animals and humans. The development of reliable and effective strategies for the replenishment of 1, 2-dichloroethane is essential. As a result of the combination of physical, chemical and biological processes in groundwater, its concentration may naturally decrease. Biodegradation can result in complete mineralization of 1, 2-DCA to CO_2.

Microorganisms capable of metabolizing 1,2-dichloroethane are *Pseudomonas sp*. DCA1, *Xanthobacter autotrophicus GJ10* and *Ancylobacter. aquaticus AD25*. This metabolism is shown in Fig. 1 [7, 8].

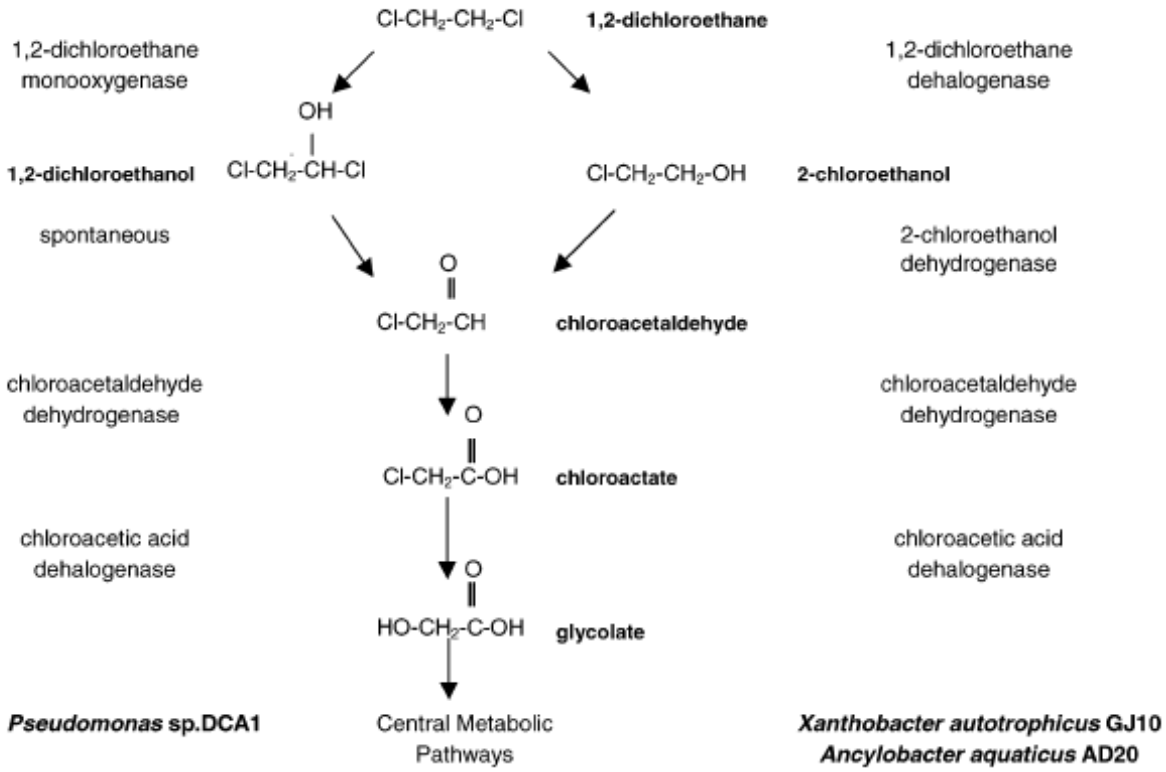


Fig. 1. Metabolic pathways of 1, 2-DCA degradation by *Pseudomonas* sp. *DCA1*, *X. autotrophicus GJ10*, and *A. aquaticus AD25*[7]

Phenol and its derivatives are classified as one of the most dangerous organic pollutants [9]. Discharges into the environment are disastrous. In it, they can basically fall in two ways. From industry and for natural reasons. Every year, different chemical industries, coal mines, woodworking plants produce large amounts of phenol in their waste water. They can naturally fall into the environment, for example, in case of forest fires. Various techniques have been employed to reduce the concentration of phenol and its derivatives to acceptable and harmless values, including activated carbon sorption, biodegradation, adsorption and ion exchange. The *Pseudomonas putida* strain successfully uses phenol and its derivatives as a source of carbon and energy [10]. This microorganism is gram-negative rod-shaped bacteria.

Two metabolic pathways are known in the literature: aerobic and anaerobic. The mechanism of aerobic phenol degradation is shown in **Fig. 2**.

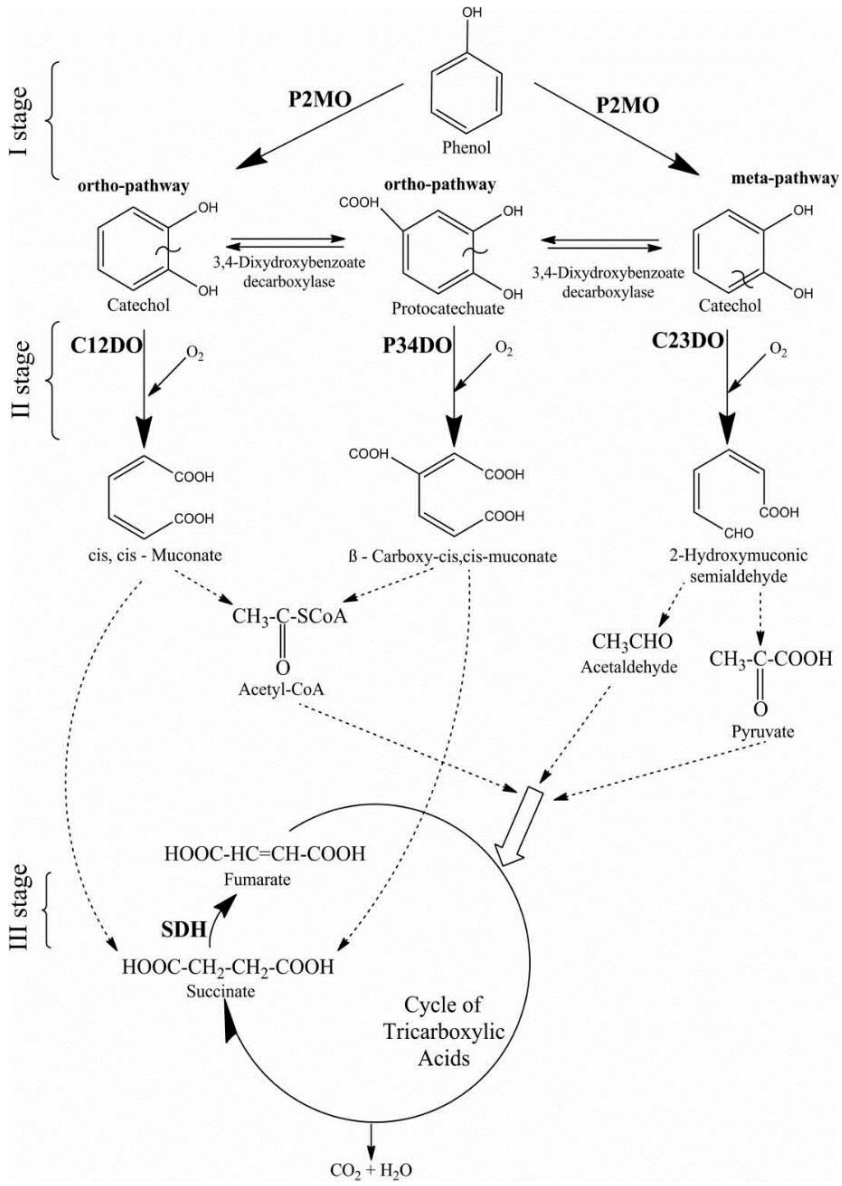


Fig. 2. Phenol biodegradation pathways and key enzymes in different stages [11].

An anaerobic phenol metabolism [12] in a denitrifying *Pseudomonas* sp. is shown in Fig. 3

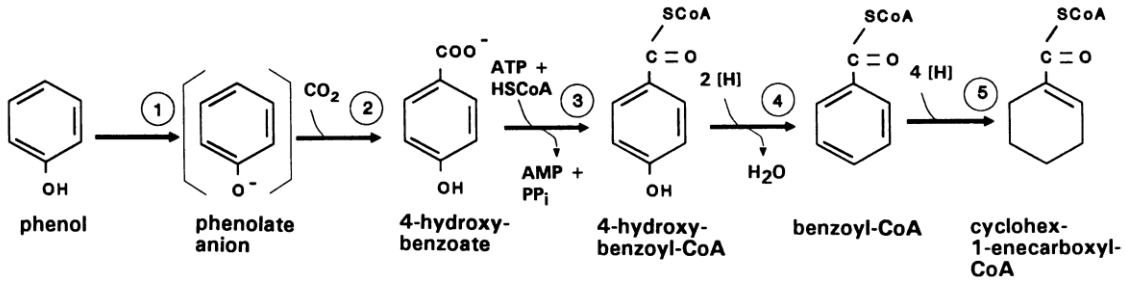


Fig. 3. Intermediates and enzymes involved in the initial steps of anaerobic phenol metabolism in the denitrifying *Pseudomonas* strain K 172. (1) and (2), Phenol carboxylase system; (3), 4-hydroxybenzoate-CoA ligase (AMP forming); (4), 4-hydroxybenzoyl-CoA reductase (dehydroxylating); (5), benzoyl-CoA reductase (aromatic ring reduction).

The purpose of our research is to prove *Pseudomonas puddy's* ability to break down phenol, *Bradyrhizobium japonicum* to break down 1, 2-dibromoethane and *Xanthobacter autotrophicus GJ10* to degrade 1, 2-dichloroethene.

MATERIALS AND METHOD

Strains of bacteria and culture media

The strains *Bradyrhizobium japonicum 273.* and *Pseudomonas putida* (NBIMCC 1046) are obtained from the Bulgarian National Bank for Industrial Microorganisms and Cell Cultures. The *Xanthobacter autotrophicus GJ 10* strain was obtained from the Department of Biochemistry of the University of Groningen (The Netherlands) through the Bulgarian National Bank for Industrial Microorganisms and Cell Cultures. These strains are inoculated into the appropriate media:

- *Bradyrhizobium japonicum 273 :*): 1 g/l of yeast extract, 0,2 g/l of NaCl, 0,2 g/l of $MgSO_4$, 0,5 g/l of K_2HPO_4, 10 g/l of Glucose, 1 g/l of Tap water. The medium pH was adjusted to 7.2 before being autoclaved.
- *Pseudomonas putida*: peptone - 10 g / l, meat extract - 10 g / l, NaCl - 5 g / l. The inoculum develops up to 24 hours at 300C per shaker and 100 rpm.
- *Xanthobacter autotrophicus GJ10* is cultured in a mineral media (MMY) containing (per liter): 5.37 g $Na_2HPO_4.12H_2O$, 1.36g KH_2PO_4, 0.5g $(NH_4)\ 2SO_4$, 0.2g. $MgSO_4\ .7H_2O$ and 0.015 g, 0.25 yeast extract, 2 g. $C_{12}H_{22}O_{11}$ supplemented with 1 ml of microelement solution containing (per liter): 2.5 mg of $CaCl_2.2H_2O$, 1 mg of $FeSO_4.7H_2O$, 0.05 mg of $ZnSO_4.7H_2O$, 0.05 mg of H_3BO_4, 0.02 mg of $CoCl_2.\ 6H_2O$, 0.015 mg of $Na_2Mo_4.2H_2O$ and 0.02 mg of $NiCl_2.6H_2O$.

Analyzes

Biomass. The biomass is determined spectrophotometrically on a Specol spectrophotometer (Carl Zeiss, Jena, Germany). During the experiments, the optical density of the respective sample is measured at wavelength $\lambda = 610$ nm.

Concentration of phenol. Phenol is determined photometrically by a standard colorimetric method based on the formation of a red color dye by treating the phenol-containing sample with 3.5% 4 amino antipyrine, 20% ammonium persulfate and the presence of buffer (50 g of NH_4Cl in 900 ml of distilled water), with pH loaded with ammonia to 9.3. The sample is centrifuged and then treated with the above mentioned reagents and, after 15 minutes of waiting, its light absorbance at wavelength $\lambda = 540$ nm is measured. The concentration of phenol is calculated by calibration straight line.

Concentration of 1,2-dibromoethane and 1,2 - dichloroethane

Bromides and chlorides were analyzed by the colorimetric method of Bergman & Sanik [26]. An aliquot of centrifuged sample (2.5 ml) was mixed with 1 ml of Fe (III) solution (8 g (NH4)Fe(SO4).12H2O in 100 ml of 6 M HNO3) and 3 ml saturated solution of 1.5 g Hg(SCN)2 in 500 ml of 98% ethanol. The mixture was shaken and after 10 min was filtered through 0.2 μm filter. The light absorption was measured at wavelength 460 nm using a spectrophotometer VWR UV-1600 PC [27]. The concentrations of bromide and chlorides ions were calculated from the optical density, using a calibration curves.

RESULTS AND DISCUSSION

All experiments were carried out in 500 ml flasks with a 250 ml medium on a rotary shaker at 30 ° C and a stirring speed of 100 rpm. In each flask the amount of inoculum is 25 ml.

The results of the removal of 1,2-dibromoethane from the *Bradyrhizobium japonicum 273* strain are presented in Fig. 4. At an initial concentration of 0.05 g / l of 1,2-dibromoethane for 160 hours, we do

not have substrate inhibition and we have a stoichiometric value that is 0.042, i.e., we have a complete 100 percent separation of the two bromide atoms.

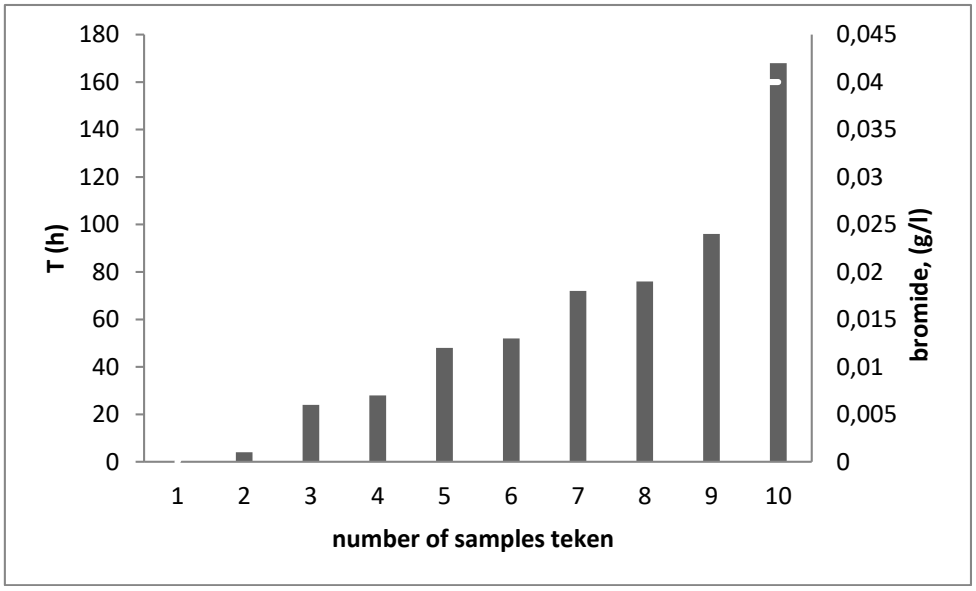


Fig. 4. Biodegradation of 0.05g/l 1, 2-dibromoethane from the *Bradyrhizobium japonicum 273* strain.

Fig. 5 presents the biodegradation results of 0.043 g / l of 1, 2-dichloroethane from the *Xanthobacter autotrophicus GJ10* strain. Within 74 hours, we have 100 separations of the chloride atoms.

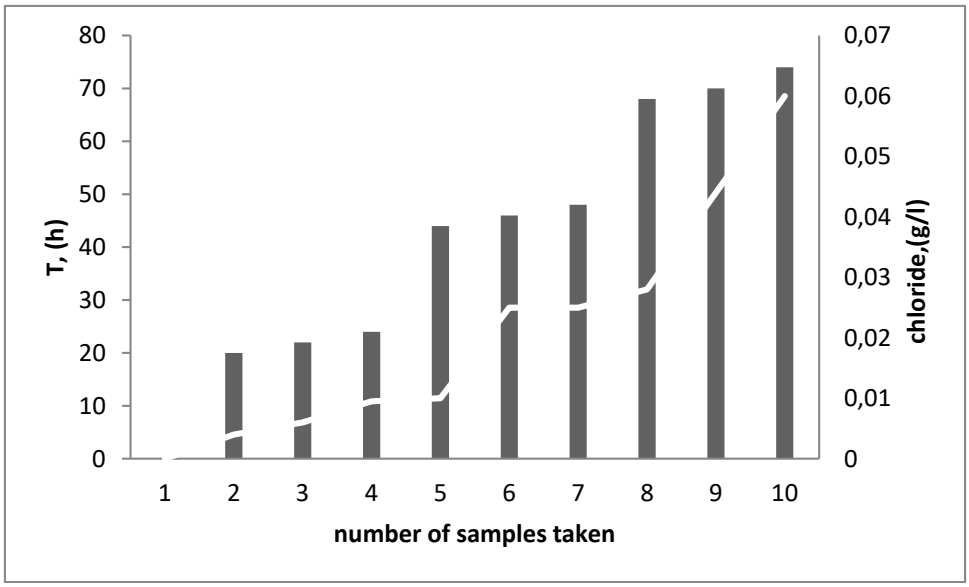


Fig. 5. Biodegradation of 0.043 g/l 1,2- dichloroethane from the *Xanthobacter autotrophicus GJ10* strain.

The biodegradation of the phenol is accomplished by gradual adaptation of the *Pseudomonas putita* strain and is shown in Fig. 6. From which it is seen that for 172 hours we have a lag phase, i.e. the adaptation process lasted for 7 days. Then for 14 days we have a total degradation of 0.26 g / l of phenol.

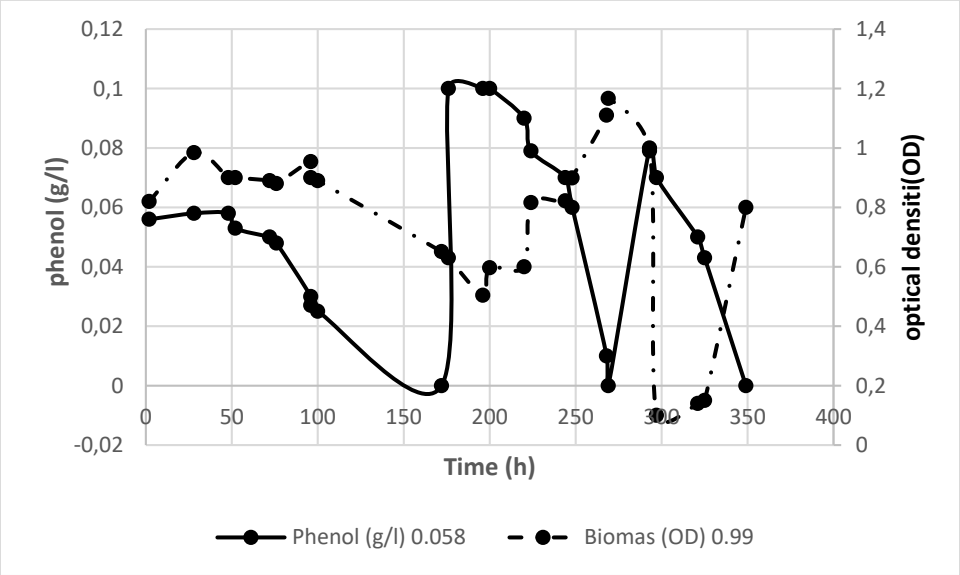


Fig. 6. Biodegradation of 0.26 g/l phenol from the *Pseudomonas putida* strain.

In order to examine how far it is possible to maintain the time adaptation of *Pseudomonas putida*, we conducted an experiment, the results of which we presented in **Fig. 7.** For the purposes of the experiment, we used a culture that we adapted for 7 days and worked for a total of 14 days. Then it was stored it in glycerol Eppendorf tubes. The graph shows that the adaptation has been preserved and the length of the lag- phase at initial phenol concentration 0.06 g/l is 60 minutes, followed by a complete degradation of the phenol for 7 hours. Accordingly, the biomass in the first hour falls, followed by an exponential growth phase.

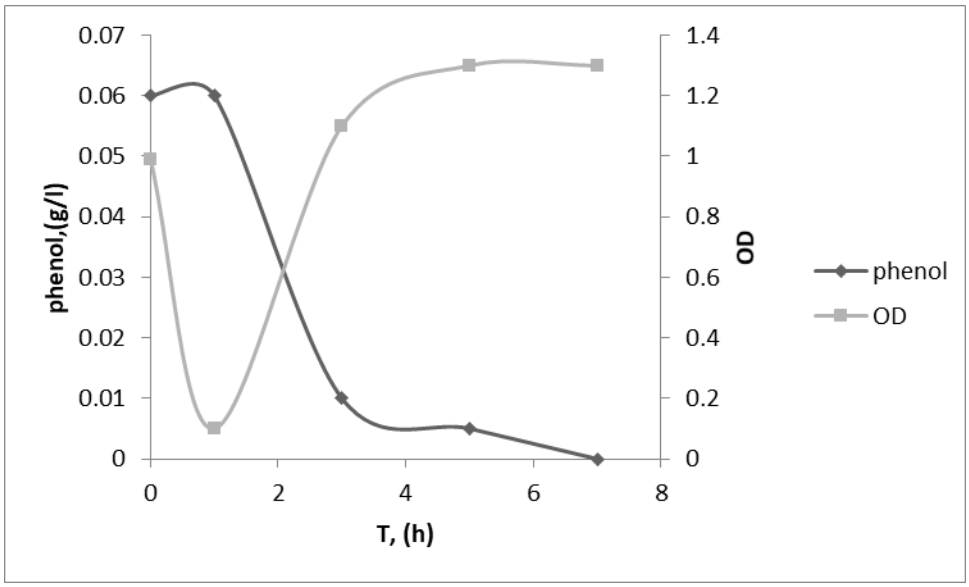


Fig. 7. Biodegradation of phenol from a pre-adapted culture *Pseudomonas putida* strain.

CONCLUSION

From the conducted research, we can draw the following conclusions:

- At a concentration of 0.05 g / l of 1,2-dibromoethane, the *Bradyrhizobium japonicum 273* strain achieves 100 percent separation of the bromide atoms, i.e. we have a complete degradation of the pollutant.
- The *Xanthobacter autotrophicus GJ10* strain successfully decomposes 1,2-dichloroethane at an initial concentration of 0.043 g / l.
- After an adaptation period of 7 days, the *Pseudomonas putida* strain successfully degrades 0.26 g / l phenol for 14 days.
- The *Pseudomonas putida* strain retains its adaptation, resulting in the lag-phase being reduced significantly from 7 days to 1 hour.

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