1,2-DIBROMOETHANE BIODEGRADATION CAPACITY OF BRADYRHIZOBIUM JAPONICUM STRAIN 273

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Abstract

1,2-Dibromoethane is an extremely toxic chemical owing to its cancer-causing potential. Many years after its last known application as a soil fumigant, residual 1,2-DBE is still found at remarkably high concentrations in soil because it strongly interacts with the soil matrix. Its biodegradation passes through very toxic intermediates e.g. bromoethanol and bromo-acetaldehyde and that is why the complete biodegradation is not observed at higher substrate concentrations. This work examines potentials of Bradyrhizobium japonicum 273 strain for degrading 1,2-DBE.. The experiments were carried out in shaking flasks with various initial concentration of substrate: 0,05 g/l; 0,1 g/l; 0,2 g/l and 0,3 g\l 1,2-DBE. In order to verify biodegradation, formation of biodegradation products like bromoethanol and bromide ions were measured. The concentration of biomass, generated bromoethanol and the quantity of bromide ions were determined spectrophotometrically, and were calculated from the optical density, using a calibration curve. Complete mineralization was verified by measuring stoichiometric release of the biodegradation products.

Keywords: *1,2-Dibromoethane, Bradyrhizobium japonicum 273, bromoethanol, bromide ions*

1. INTRODUCTION

The presence of man-made (anthropogenic) organic compounds in the environment is a serious public health problem. Sixty-five classes of such chemical compounds are considered hazardous, and among them, 114 organic compounds have been designated by the U.S. Environmental Protection Agency (EPA) as priority pollutants. The presence of these compounds appears to be attributable largely to inadequate disposal techniques, which have caused contamination of water and soil [1]. The persistence of industrial chemicals may be a necessary requirement for compounds designed specifically for technical use, or of compounds, like insecticides directed towards specific targets. In general, however, persistence of man-made chemicals in the natural environment is undesirable. Therefore studies on degradation including both biotic and abiotic processes occupy a central position in environmental hazard assessments. Substances which persist in the environment for extended periods of time in all of the environments examined have been termed “recalcitrant” [2]. For reasons which will become clear, the term “recalcitrant” is relative rather than absolute. Nonetheless, it should perhaps be pointed out that recalcitrant compounds may not be synthetic necessarily. Natural substances like antibiotics or halogenated metabolites produced by marine algae and animals may also be persistent. Their biodegradability, however, is unknown and indeed has been seldom investigated.

The transformation and complete degradation of readily degraded compounds to carbon dioxide (mineralization) have been extensively investigated, and standardized test procedures have been developed [2]. The situation for less readily degraded compounds is, however, considerably more complicated. Low degradation rates make the application of conventional procedures using, for example, measurements of growth rates, oxygen consumption, evolution of carbon dioxide or decrease in the concentration of dissolved organic carbon, impractical or even impossible. Alternative methods must therefore be sought. In numerous cases, biological treatment can eliminate hazardous compounds by biotransforming them into innocuous forms, degrading them by mineralization to carbon dioxide and water, or anaerobically decomposing them to carbon dioxide and methane. Many compounds are not removed efficiently by existing biological treatment techniques, either because they are metabolized very slowly, or because they are resistant to microbial attack under prevailing environmental conditions.

1,2-Dibromoethane (1,2-DBE) is an organic compound which is only slightly biodegraded in water and persists in the environment. Although 1,2-DBE was prohibited by the U.S. Environmental Protection Agency for use as a soil fumigant in 1983, it is still produced in large quantities, mainly for use as a scavenger in motor fuel antiknock formulations containing lead alkyls [3]. 1,2-Dibromoethane is an extremely toxic chemical owing to its cancerogenic potential [4]. It is one of the most effective and widely used pesticidal soil fumigants [5]. Many years after its last known application as a soil fumigant, residual 1,2-DBE is still found at remarkably high concentrations in soil because it strongly interacts with the soil matrix. 1,2-DBE can slowly leach from such contaminated soils to groundwater over exceedingly long periods, and because of its slow chemical conversion in aqueous milieu, it is a continuous source of contamination of water supplies [6]. A large number of the pesticides (such as 1,2-DBE and other halogenated aliphatic compounds) and industrial solvents found in groundwater, are widely regarded as susceptible to anaerobic reductive dehalogenation but in most cases are thought to be resistant to aerobic oxidation by microorganisms [7, 8, 9]. Bower and McCarty [10] and Belay and Daniels [11] found that 1,2-DBE and other short-chain (one- and two-carbon) halogenated aliphatic compounds could be "transformed" (biotransformed rather than mineralized) under anaerobic conditions at low concentrations. Pignatello [9, 12] and Steinberg et al. [6] carried out several studies of 1,2-DBE degradation in contaminated soils. Pignatello [12] used 1,2-DBE concentrations in soil slurries of 6 to 8 mg/l and obtained stoichiometric release of CO_2 and bromide ions under aerobic conditions.

The metabolism of 1,2-dibromoethane has been extensively studied [13-20]. The metabolic pathway of 1,2 –Dibromoethane degradation is shown in Figure 1.

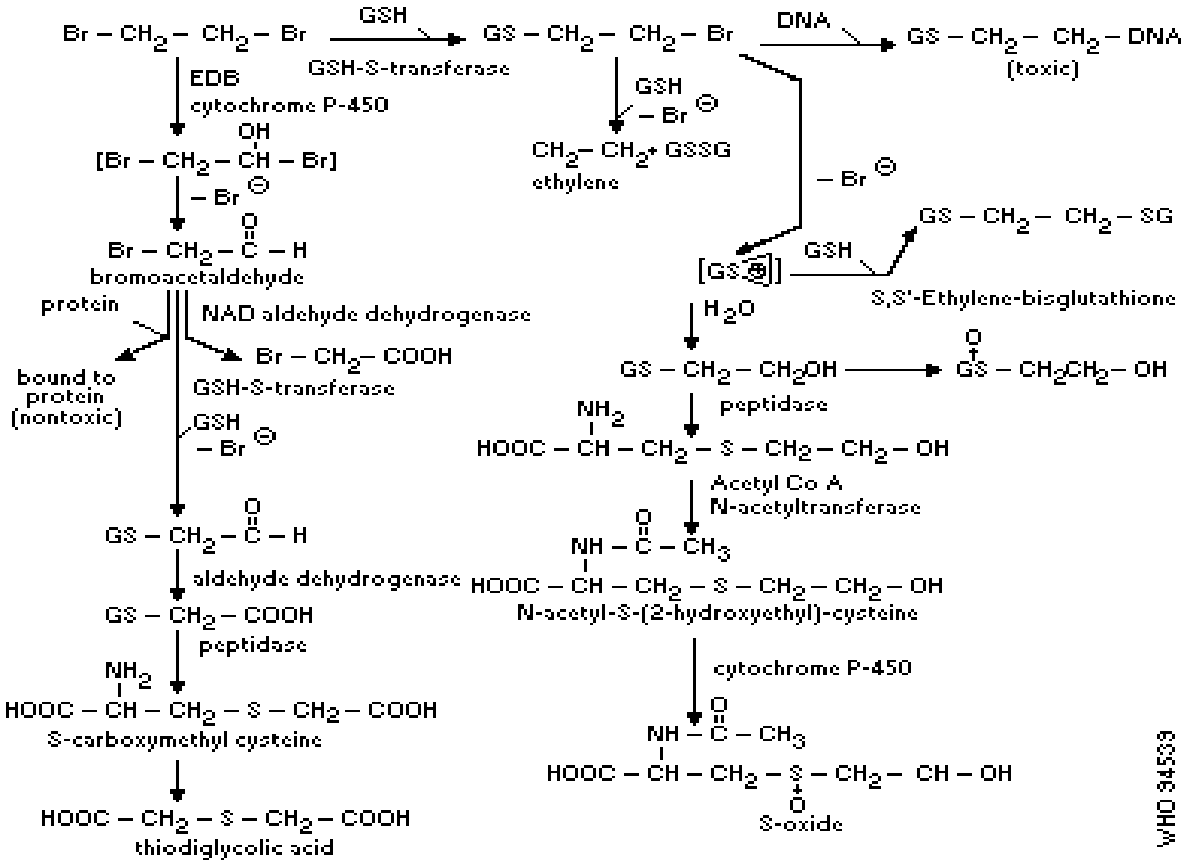

Fig. 1. Metabolism of 1,2-dibromoethane

An important consideration is the choice of microorganism that degrades 1,2-DBE. If a synthetic chemical is biodegradable in a reasonable time frame, then that compound is unlikely to pose a threat to the public health. Nevertheless, while microorganisms can adapt to remove many toxic substances, the great variety of synthetic chemicals used today may disrupt the balance of the carbon cycle [21]. In the future it will be necessary to develop microbial systems that can change this trend.

The evaluation of microorganisms indicates that use of properly selected populations of microbes, and the maintenance of environmental conditions most conducive to their metabolism, can be an important means of improving biological treatment of organic wastes [1]. The microorganisms have potential advantages when the removal of anthropogenic compounds is the goal. A broadened perspective into what constitutes biological treatment opens promising new areas of research and application.

In studies with pure cultures van der Ploeg et al. [22] and Janssen et al. [4] found that 1,2-DBE and 2-bromoethanol were toxic to the bacteria *Xanthobacter autotrophicus* GJ10 and *Ancylobacter aquaticus.* They concluded that the toxicity was due to the lack of a bromoacetaldehyde dehydrogenase and consequent accumulation of highly reactive bromoacetaldehyde. The purpose of this investigation was to find a bacterial culture (single or mixed species) capable of mineralizing 1,2-DBE at concentrations compatible with the treatment of groundwater and chemical industry wastewaters.

Microbes that produce dehalogenases are widely distributed in nature, apparently being evolved to degrade naturally occurring halogenated compounds in order either to exploit them as a carbon source for growth or as a means of protection against the toxicity of these compounds [23]. The bacterial strain *Bradyrhizobium japonicum USDA110* is capable to biodegrade 1,2 – Dibromoethane. Sfetsas et al. [24] investigated the dehalogenase (DHA) from *Bradyrhizobium japonicum USDA110* (BjDHA). The results showed that the BjDHA displays wide substrate specificity towards haloalkanes and particular high activity towards 1,2-DBE. The enzyme has a different catalytic triad topology compared to the *Xanthobacter* haloalkane dehalogenase and is more similar to the *Rhodococcus* enzyme. In addition, consistent with its broad specificity, the BjDHA has a substantially larger and more polar active site cavity compared to the *Xanthobacter* and *Rhodococcus* enzymes and as a consequence, BjDHA is able to dehalogenate longer and polar compounds. These properties make this enzyme very promising bioremediation tool for environmental applications.

In our previous research [25] we have investigated the dehalogenation capacity of strain *Klebsiella oxytoca NBIMCC 8391* for biodegradation of 1,2-DBE. Full degradation was observed at low and moderate initial concentrations equal up to 0,1 g/l.

The main goal of this study is to investigate biodegradation of 1,2-dibromoethane with *Bradyrhizobium japonicum 273* cells. The biodegradation passes through very toxic intermediates e.g. bromoethanol and bromo-acetaldehyde and that is why the complete biodegradation is not observed at higher substrate concentrations. In this paper we compare an aerobic biodegradation in shaking flasks with various initial concentrations of substrate: 0,05 g/l; 0,1 g/l; 0,2 g/l and 0,3 g\l 1,2-DBE.

2. MATERIALS AND METHODS

2.1 Bacterial strain, media and experimental conditions

Bradyrhizobium japonicum 273 was obtained from the National Bank for Industrial Microorganisms and Cell Cultures, Bulgaria (NBIMCC). The strain was grown in mineral medium (MMY) containing (per liter): 1 g of yeast extract, 0,2 g of NaCl, 0,2 g of $MgSO_4$, 0,5 g of K_2HPO_4, 10 g of Glucose, 1 g of Tap water. The medium pH was adjusted to 7.2 before being autoclaved.

The experiments have been carried out in flasks of 250 ml in a rotary shaker at 28^0C and agitation speed of 100 rpm. In each flask the quantity of cultivated inoculums is 30 ml. Processes of dehalogenation have been carried out with different initial substrate concentrations of 1,2-DBE: 0,05 g/l; 0,1 g/l; 0,2 g/l and 0,3 g/l.

2.2 Analytical Methods

Samples from the broth were taken periodically and analyzed for biomass, bromoethanol and bromide ions. The biomass growth was monitored by the optical density of the broth. The light absorption was measured at wavelength 600 nm using a spectrophotometer (VWR UV-1600 PC).

The quantity of bromoethanol was monitored by the optical density of the broth at wavelength 216 nm using a spectrophotometer (VWR UV-1600 PC). The concentrations of bromoethanol were calculated from the optical density, using a calibration curve with coefficient R^2=0,9901.

Bromides 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 $(NH_4)Fe(SO_4).12H_2O$ in 100 ml of 6 M HNO_3) 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 ions were calculated from the optical density, using a calibration curve with coefficient R^2=0,9996

3. RESULTS AND DISCUSSION

The experimental results as time evolution of biomass, bromoethanol and bromide release are presented in Fig. 2-5.

The experimental results with 0,05 g/l initial 1,2-DBE concentration are shown in Fig.2

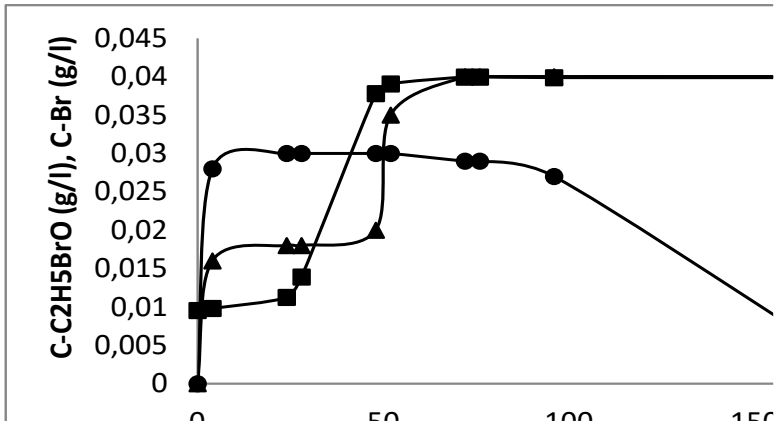

Fig. 2. Curves of biomass growth, concentration of bromoethanol and bromide profile with 0,05 g/l initial 1,2-DBE concentration

The curve of biomass growth shows a clear lag-phase which continues to 24th hour. After that fast biomass growth until 50th hour follows. It helps to accelerate biodegradation of substrate and reaches to the stationary phase of bacterial growth. On the same figure the curves of bromoethanol concentration and of bromide profile are presented. Fast increase of bromoethanol quantity until reaching its stoichiometric concentration 0,033 g/l was observed. During a period from the start and the 50th hour only a half of bromide ions were released completely. After 50th hour a decrease of bromoethanol concentration with the corresponding increase of concentration of bromide ions was observed. At 168th hour complete degradation of bromoethanol is obtained with final accumulation of bromide ions with a concentration being comparable to the stoichiometric one (0,0425 g/l).

The experimental results with 0,1 g/l initial 1,2-DBE concentration are shown in Fig.3.

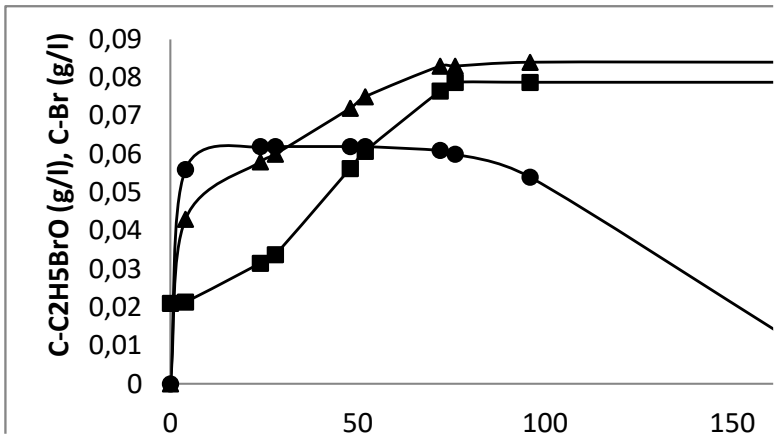

Fig. 3. Curves of biomass growth, concentration of bromoethanol and bromide profile with 0,1 g/l initial 1,2-DBE concentration

The curve of biomass growth shows a very short and not well expressed lag-phase. The exponential phase of bacterial growth continues until 76th hour which is longer than in the previous experiment. Similarly to the previous experiment, the results for bromoethanol and bromide ions concentration show that in the beginning fast increase of bromoethanol concentration took place. In this experiment the concentration of bromoethanol also reaches its stoichiometric value: 0,066 g/l. Dehalogenation is more active during the exponential phase of bacterial growth. After 76th hour decrease of bromoethanol concentration was observed until 168th hour, when it was completely removed. In contrast to the previous experiment bromide concentration increases slightly and after 76th hour reach stoichiometric concentration of 0,0851 g/l.

The experimental results with 0,2 g/l initial 1,2-DBE concentration are shown in Fig.4.

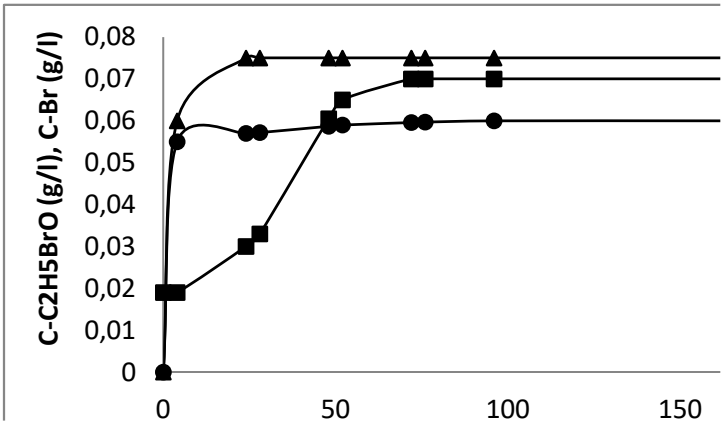

Fig. 4. Curves of biomass growth, concentration of bromoethanol and bromide profile with 0,2 g/l initial 1,2-DBE concentration

This substrate concentration is already much higher. The curve of biomass growth shows that the biomass concentration increases until 72th hour and corresponds to the substrate biodegradation. The concentration of bromoethanol at the end of the process is 0,05 g/l compared to the maximum value of 0,133 g/l. Bromide ions release slightly but in this experiment they didn't reach its final stoichiometric concentration, 0,170 g/l. It was observed that in the end of dehalogenation only 0,075 g/l ions were released. This amount is approximately a half of the stoichiometric one. The results show that

bromoethanol acts as inhibitor of growth and substrate biodegradation. With this initial substrate concentration 0,2 g/l 1,2-DBE only one of the bromide atoms is released.

The experimental results with 0,3 g/l initial 1,2-DBE concentration are shown in Fig.5.

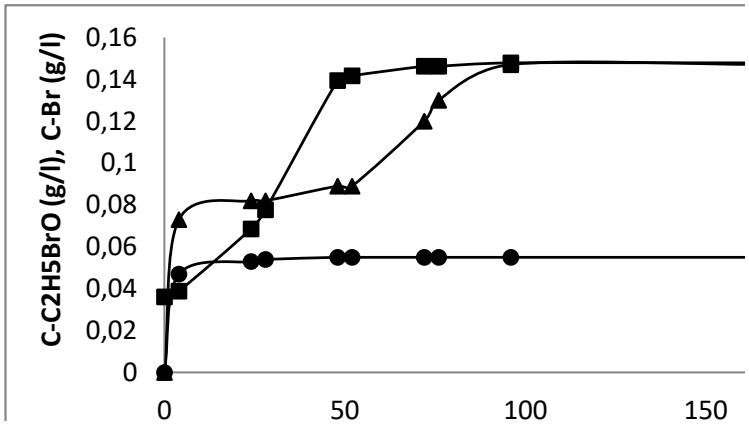

Fig. 5. Curves of biomass growth, concentration of bromoethanol and bromide profile with 0,3 g/l initial 1,2-DBE concentration

The concentration of 0,3 g/l 1,2-DBE is the highest one for our experiments. The curve of biomass growth is similar to the ones of the previous experiment. Biomass quantity increase to 72th hour after that a stationary phase of bacterial growth follows. On the same figure curves of bromoethanol concentration and of bromide profile are presented, too. It is visible that until 50th hour stagnation in accumulation of bromoethanol and bromide ions is observed. Biodegradation is inhibited at this initial substrate concentration. The concentration of bromoethanol is 0,055 g/l compared with stoichiometric one 0,199 g/l. After 50th hour the process of bromide ions release continued, but partially only, as it was at the previous substrate concentration 0,2 g/l. The final concentration of bromide was 0,147 g/l which is approximately a half of stoichiometric 0,255 g/l and corresponds to complete release of only one bromine atom from the 1,2-DBE molecule.

4. CONCLUSIONS

On the basis of the presented experimental results we have come to the following conclusions:

1. Bacterial strain *Bradyrhizobium japonicum 273* biodegrades successfuly 1,2-DBE.
2. At low initial substrate concentrations up to 0,1 g/l full dehalogenation is observed corresponding to stoichiometric values of released bromide ions.
3. At high initial substrate concentrations (0,2 g/l and 0,3 g/l) the intermediate bromoethanol inhibits dehalogenation. In these cases bromoethanol is not fully converted, and the process doesn't reach full degradation of 1,2-DBE.

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