DEHALOGENATION CAPACITY OF THE STRAIN *KLEBSIELLA OXYTOCA*

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Abstract

The dehalogenation capacity of Klebsiella oxytoca is demonstrated on 1,2-dichloroethane and 1,2-dibromoethane as substrates and sole carbon sources. Full degradation was observed at low and moderate initial concentrations. The chlorine is released in two steps of substrate biodegradation. It is observed that the cells retain chloride ions during their exponential phase of growth. About biodegradation of 1,2-dibromoethane, bromide ion release and reach their stoichiometric value when the concentration of microbial cells in the broth is the highest.

Key words: *1,2-dichloroethane (DCE), 1,2-dibromoethane (DBE), dehalogenation, biodegradation*

1. INTRODUCTION

Halogenated compounds are extremely important and diverse class of environmental by adverse chemicals. Short-chain halogenated aliphatic compounds are manufactured in bulk quantities by the chemical industry. As a result of these applications, and due to spillage or improper disposal, these compounds have entered the environment and have caused contamination of soil and groundwater (Topalova et al. 2003). Some of these compounds are very toxic and cause enormous problems to human health and to the environment (Cook et al. 2004). 1, 2- Dichloroethane (DCE) has been produced in larger quantities than any other chlorinated hydrocarbouns (Janssen et al. 1985). This compound is mainly used as precursor for polyvinilchloride production and as solvent. DCE enters in the environment through atmospheric emissions, waste effluents to waterways and land disposal of liquid and solid wastes. DCE exerts strong cytotoxic and cancerogenic effect, and it is classified as probable human carcinogen in group II.

Microbiological research on the biodegradation of halogenated compounds is mainly focused on the physiological processes responsible for their mineralisation and on the enzymes involved in cleavage of the carbon–halogen bond. The characterization of dehalogenating organisms is important for their industrial application. Their physiological properties and substrate range will determine the process conditions that should be used and the range of transformations that can be obtained in practical treatment systems (Janssen et al. 2001). In general, degradation can be based on one of four processes: use of a halogenated compound as a carbon source and oxidisable substrate with either oxygen or nitrate as an electron acceptor; fermentative metabolism, in which a dehalogenated intermediate serves as electron acceptor, the use of a halogenated compound as an electron acceptor with liberation of halide; and co-metabolic transformation and halide release linked to any metabolic process. The last process arises through the lack of specificity of an enzyme with another physiological function. Janssen et al. 2001 discuss important recent findings in the field of microbial dehalogenation. Much work has been carried out on the identification and characterisation of dehalogenating enzymes, both from aerobic and anaerobic organisms Figure 1. Several biochemical and biophysical studies on new dehalogenases have been performed, and this has led to considerable insight into their evolution, diversity and catalytic mechanisms.

Many different organisms have been found that are capable of using halogenated compounds as a growth substrate. Relatively new examples of compounds that are known to be degradable by pure bacterial cultures include 1, 3-dichloropropylene and 1, 2-dibromoethane (DBE). In both cases, initial dehalogenation starts with a reaction catalysed by a hydrolytic haloalkane dehalogenase (Poelarends et al. 1999, Poelarends et al. 2000). The haloalkane dehalogenases that degrade these compounds are homologous to the classical enzyme from *Xanthobacter autotrophicus* (Verschueren et al. 1993). Even though sequence similarities are low, the overall structures are very similar and the catalytic residues are conserved (Newman et al. 1999]. Another example of a recently studied hydrolytic dehalogenase is

LinB from *Sphingomonas paucimobilis* (Marek et al. 2000); this enzyme is involved in the conversion of tetrachlorocyclohexadiene, an intermediate in the γ-hexachlorocyclohexane degradation. Pathway is demonstrated on Figure 1 as follows.

(a) Dehalogenation of 1, 2-dibromoethane by a haloalkane dehalogenase (DhaAf) (Poelarends et al. 2000).

(b) Dechlorination of chloroacetate by a group II dehalogenase (L-DEX or DhlB) (Hill-Ke et al.199, Li-YF et al. 1998, Ridder et al. 1999].

(c) Reduction of tetrachlorohydroquinone during pentachlorophenol metabolism by tetrachloro-hydroquinone dehalogenase (TCD) (Anandarajah et al. 2000).

(d) Initial dehalogenation steps during the metabolism of lindane. Two dehydrohalogenations are followed by two hydrolytic steps (Marek et al. 2000, Nagata et al. 1997 Trantirek et al.2001)

(e) Reduction of trichloroethene by a reductive dehalogenase (TRD) (Magnuson et al. 2000).

(f) The activity of orthochlorophenol reductive dehalogenase (CRD) (Van de Pas et al.1999, Smidt et al. 2000).

The most appropriate way to treat DCE is the microbial one, particularly at low concentrations. Special attention has been paid to *Xanthobacter autotrophicus GJ10* strain. There is also oxidative pathway for DCE biodegradation by *Pseudomonas* strain, like the described in Inguva et al. 1999. Comparison of these two metabolic pathways is shown in Eq. 1a and Eq. 1b(Mileva 2009). The difference consists in the first enzyme converting the substrate directly into 2-chloroethanol or 2-chloroacetaldehyde. Pathway (1a) is the hydrolytic one, proposed in (Janssen 1985); pathway (1b) is the oxidative one, proposed in (Inguva et al. 1999). The enzymes involved in these metabolic pathways are denoted as follows:

“1” - haloalkane dehalogenase;

“2” - 2-chloroethanol dehydrogenase;

“3” - chloroaldehyde dehydrogenase;

“4” - haloacetate dehalogenase;

“5” 1; 2-dichloroethane monooxygenase.

The oxidative pathway (1b) does not require the enzymes haloalkane dehalogenase and 2-ethanol dehydrogenase because the formation of 2-chloroacealdehyde is a spontaneous process due to keto-enol tautomerism.

$$\text{(1a)} \qquad \begin{aligned} &CH_2Cl - CH_2Cl \xrightarrow{1} CH_2Cl - CH_2OH \xrightarrow{2} CH_2Cl - CHO \xrightarrow{3} \\ &CH_2Cl - COOH \xrightarrow{4} CH_2OH - COO^- \end{aligned}$$

$$\text{(1b)} \qquad \begin{aligned} &CH_2Cl - CH_2Cl \xrightarrow{5} CH_2Cl - CH(OH) - Cl \longrightarrow CH_2Cl - CHO \xrightarrow{3} \\ &CH_2Cl - COOH \xrightarrow{4} CH_2OH - COO^-. \end{aligned}$$

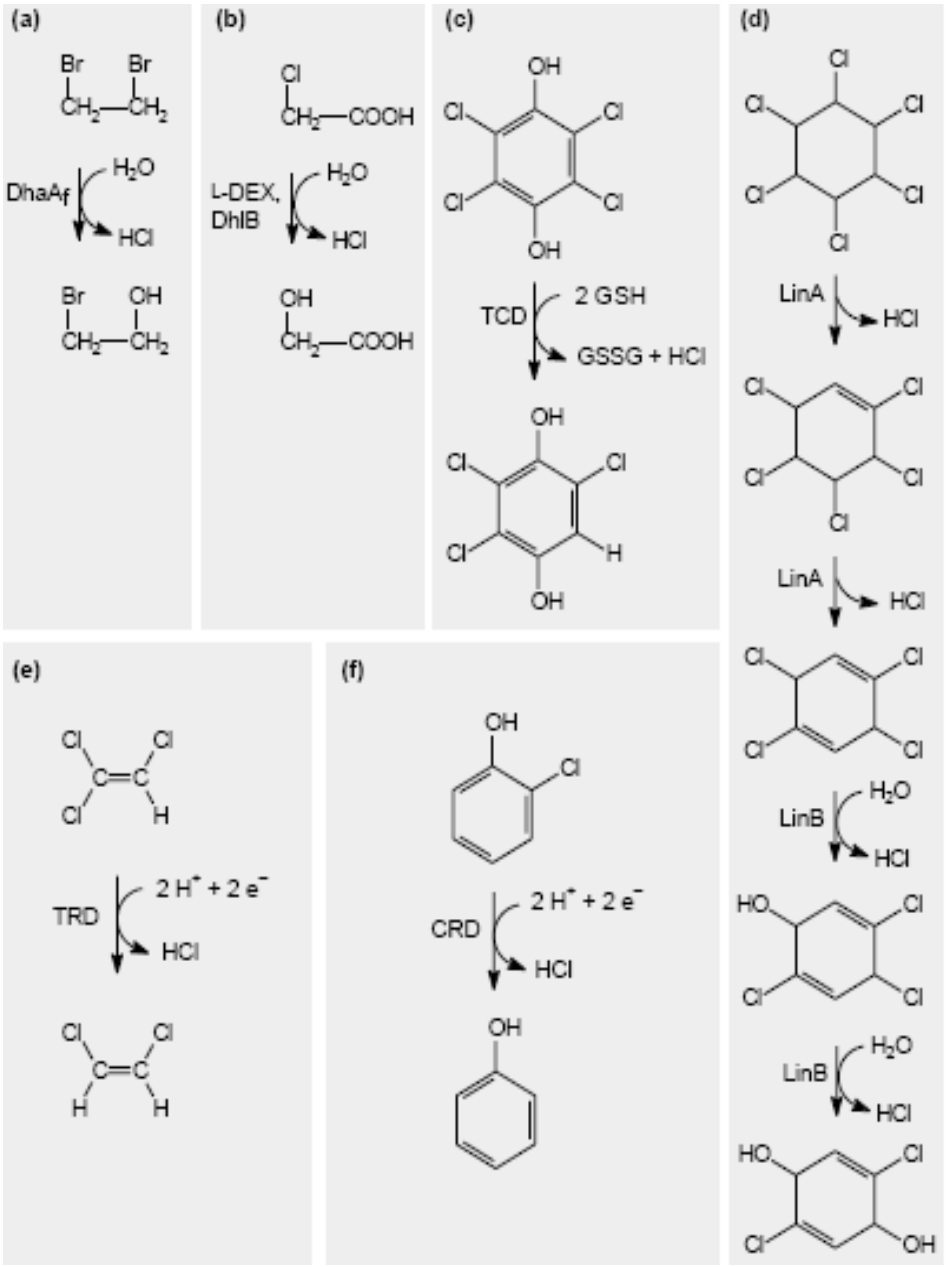


Figure 1. Dehalogenation reactions in bacterial cultures.

There are some other strains from the genera *Xanthobacter, Pseudomonas and An-cylobacter* with a capability to degrade DCE, like *X. autotrophicus GJ11, A. aquaticus AD20, AD25, AD27 and Pseudomonas sp. RB8* (Nardi-Dei et al. 1999).

In regard to biodegradation of DBE by the strain *Xanthobacter autotrophicus GJ 10* is observed strong substrate inhibition. Results are shown on Figure 2. Only slight part from DBE was utilized at 24 hour. In this time biomass reaches his peak and then quickly drops down.

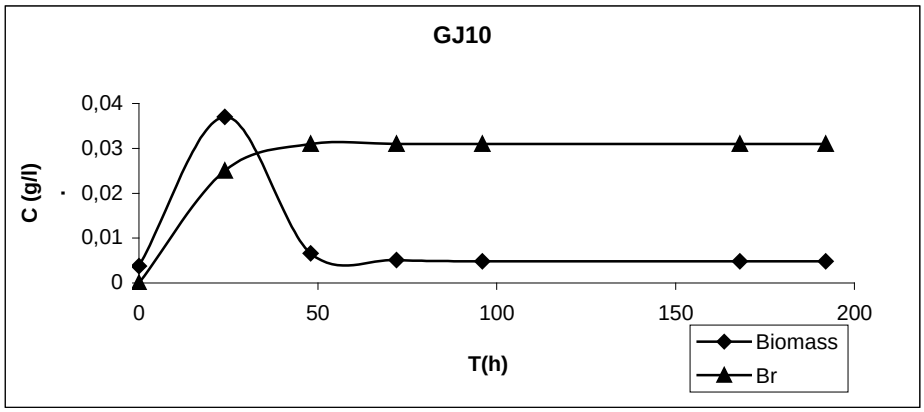


Figure 2. Dehalogenating activity and cell growth of *Xanthobacter autotrophicus GJ* 10 by initial concentration of DBE=0, 05 g/l

1,2-Dibromoethane is a synthetic organic chemical and is one of the most effective and widely used pesticides soil fumigants. Reduction in the use of DBE for agricultural applications in the late 1980s, owing to its cancer-causing potential and its detection in groundwater supplies, has reduced human exposure to this extremely toxic xenobiotic. However, it is still produced in large amounts for use as a lead scavenger in some countries; as a fumigant for stored grain; as a solvent for resins, gums, and waxes; and as an intermediate in the synthesis of dyes and pharmaceuticals (Alexeeff et al. 1990). For a better understanding of the fate and persistence of DBE in the environment and for the development of bioremediation techniques for the cleanup of polluted locations, it is important to study the physiology and ecology of bacteria that degrade this toxic compound (S. Keuning et al. 1985).

The strain *Klebsiella oxytoca* is recommended as a "novelty" in dehalogenation of chlorinated compounds, namely tetrachlorinated phenols Nagata et al. 1997. In the present paper we demonstrate the dehalogenating potential of this strain on 1, 2-dibromoethane.

2. BACTERIAL STRAIN, MEDIA AND CULTIVATION CONDITIONS

The strain Klebsiella oxytoca NBIMCC 8391 was isolated from activated sludge in the wastewater treatment plant in Luckoil Neftochim refnery in Burgas, Bulgaria (Mileva 2009). The strain is Gram-negative. The identification was made by DNA-analysis using the PCR technique in GBF, Braunschweig, Germany. This identification was confirmed in the National Bank for Industrial Microorganisms and Cell Cultures by biochemical tests. The strain was grown on liquid medium MMV containing (g/l): Meat extract 10, Peptone 10, NaCl 5. 1, 2-Dibromoethane was used as sole substrate. Initial amounts were added to the medium to correspond to concentrations 0,05 g/l, 0,08 g/l, 0,1 g/l, 0,2 g/l, 0,5 g/l.

3. ANALYTICAL METHODS

Biomass concentration was assayed by the optical density at 550 nm and its dry weight was calculated by standard calibration curve.

Bromides were determined photometrically by the method of Bergman and Sanik [11]. A sample of centrifuged incubation mixture (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 6M HNO_3) and 3 ml saturated solution of 1.5g $Hg(SCN)_2$ in 500 ml of 98% ethanol. The mixture was shaken and after 10 min filtered through 0.2 μm filter. The adsorption was measured at wavelength 460 nm.

4. RESULTS AND DISCUSSION

Batch mode experiments were carried out under aerobic conditions using sealed shaking asks in a rotary shaker (220 rpm) at 30^0 C. Samples of 2 ml volume were taken at appropriate periods of time. The results are shown on Figures 3-7.

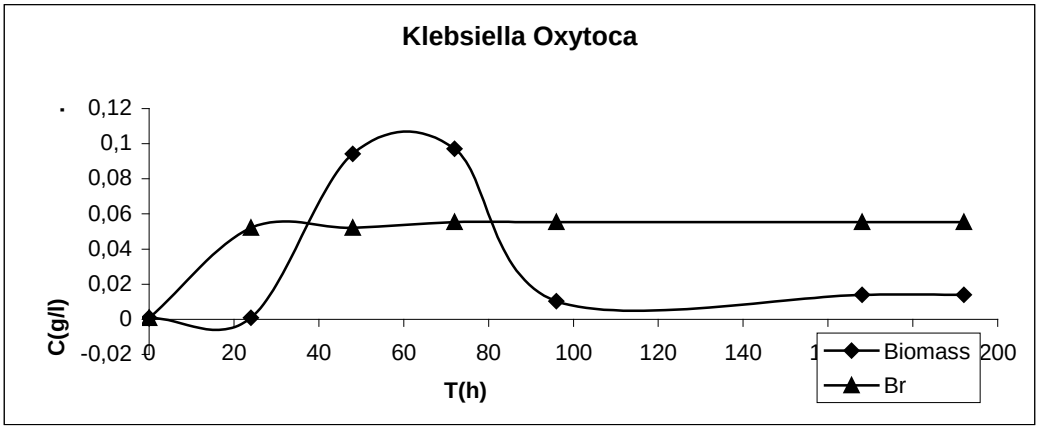


Figure 3. Dehalogenating activity and cell growth of *Klebsiella Oxytoca* by initial concentration of DBE=0, 05 g/l

The period of bromide retention coincides with the microbial exponential growth. Review of the kinetic curves shows that in this period of time (25h-100 h), DBE is completely degraded and the concentration of bromide ions in the medium reaches stoichiometric value of 0,0425 g/l..

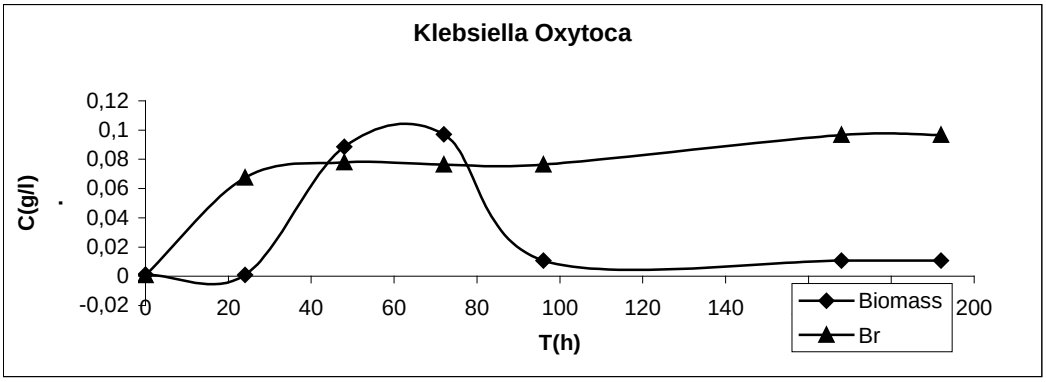


Figure 4. Dehalogenating activity and cell growth of *Klebsiella Oxytoca* by initial concentration of DBE=0, 08 g/l

The experimental results on the kinetics in Fig. 4 show that at activing and increasing biomass keeps the biodegradation. The first bromide atom in the DBE molecule is released in the lag-phase of microbial growth and bromide ions reaches stoichiometric value of 0,0681 g/l.

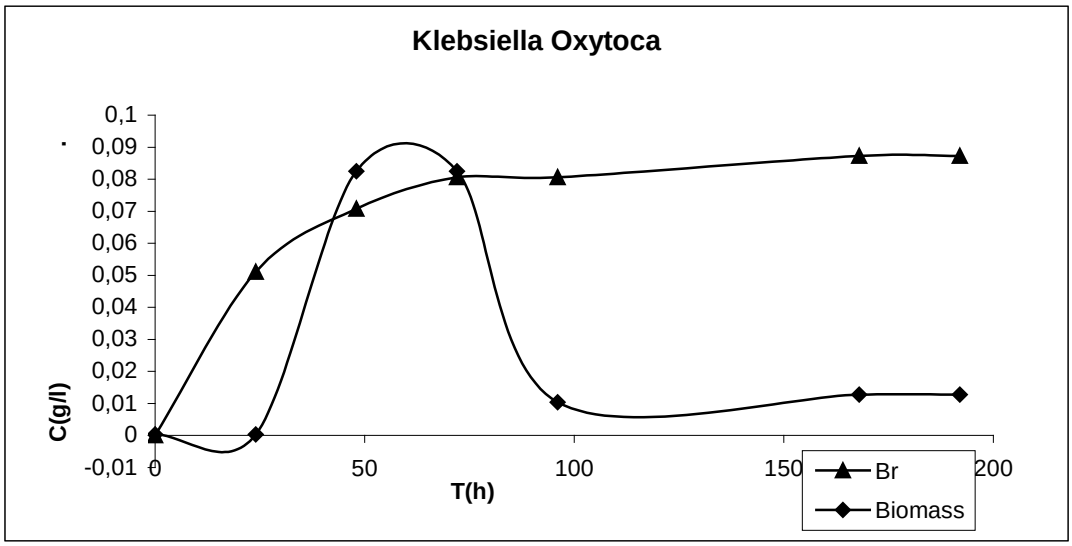


Figure 5. Dehalogenating activity and cell growth of *Klebsiella Oxytoca* by initial concentration of DBE=0, 1 g/l

By initianal concentration of 0,1 g/l, the results are similar regarding to another processes. DBE is degraded and bromides reaches stoichiometric value of 0,0851 g/l.

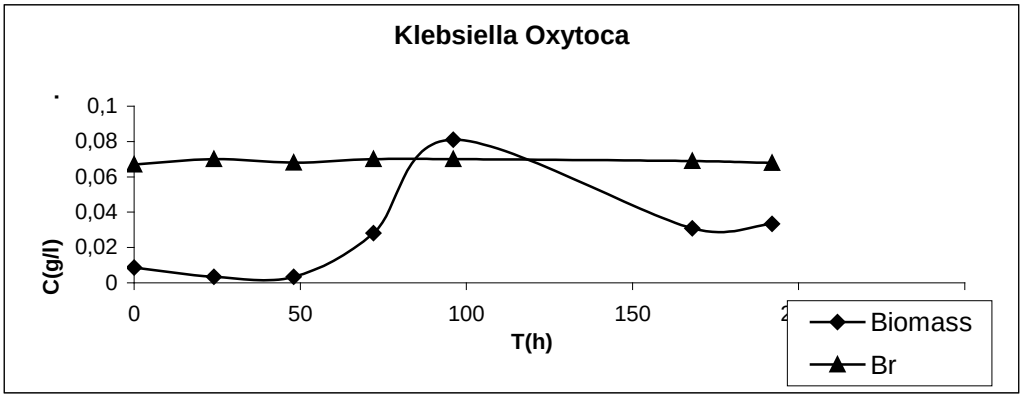


Figure 6. Dehalogenating activity and cell growth of *Klebsiella Oxytoca* by initial concentration of DBE=0, 5 g/l

By this initial concentration of 0,5 g/l DBE, kinetic curves show slightly results. The substrate inhibition is strong and the biodegradation is getting more complicated. The cells are with long lag-phase and bromides didn't released.

CONCLUSION

1. Full degradation of DCE and DBE was observed at low and moderate initial concentrations.
2. The chlorine is released in two steps of substrate biodegradation. It is observed that the cells retain chloride ions during their exponential phase of growth.
3. The bromide ion release and reach their stoichiometric value when the concentration of microbial cells in the broth is the highest.

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