INFLUENCE OF CONSTANT ELECTRIC FIELD ON DEHALOGENATION CAPACITY OF THE STRAIN XANTHOBACTER AUTOTROPHICUS GJ 10

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Abstract

Chemical industries produce large amounts of short-chain halogenated aliphatic hydrocarbons, which are used as organic solvents, paints, pesticides, herbicides and intermediates for the synthesis of other organic compounds. Significant quantities of 1,2-dichloroethane (1,2-DCA) have been produced over recent years. Due to toxicity and carcinogenity of this chemical a vast scientific interest has focused on its removal from polluted water. The effect of constant electric field for 1,2-dichloroethane degradation by Xanthobacter autotrophicus GJ 10 was investigated. Processes of dehalogenation were carried out with initial substrate concentrations of 0,12, 0,14 and 0,2 g/l 1,2-DCA in the presence and absence of constant electric field. The anode potential was maintained constant by a potentiostat of value 0,1 V vs. quinhydrone electrode. When no electric field was applied the biodegradation was impeded. It stopped before one chlorine atom in the substrate molecule was released completely. In the case of electric field application the dechlorination process is completed. The concentration of chloride ions in the medium reaches its stoichiometric values. The results shows that the stimulating effect of constant electric field is due to biophysical or biochemical influence of the field applied to bacteria but not because of electrochemical processes.

Key words: *1,2-dichloroethane, biodegradation, Xanthobacter autotrophicus GJ 10, constant electric field*

1. INTRODUCTION

Since the beginning of the last century, halogenated hydrocarbons have been extensively applied in industry and agriculture. Decades after the start of their widespread use, evidence started to accumulate that some of these xenobiotic halogenated compounds are highly toxic. Technologies to eliminate them from waste streams have been actively researched.

Chlorinated organic pollutants can cause potential risk to humans and ecosystems (Yoon et al. 2014). 1,2-Dichloroethane (1,2-DCA) is one of the most common chlorinated industrial products, and is introduced into the environment through its use as a chemical intermediate in the synthesis of a number of chlorinated hydrocarbons. In the past 1,2-DCA has been used as a grain fumigant. It is also one of the intermediates of degradation of hexachloroethane and is a troublesome contaminant, because it can remain for several decades in a contaminated area without being degraded (Song and Carraway. 2005, Van der Zaan et al. 2009). High concentrations of 1,2-DCA are found at many contaminated sites and available treatment processes are not efficient for destroying it (Henderson et al. 2008). 1,2-Dichloroethane has been found in ambient and residential air samples, as well as in groundwater, surface water, drinking water and soils. The predominant use of 1,2-DCA is in the synthesis of polyvinyl chloride pipes, but it is also widely used as a solvent to remove lead from gasoline or extract oil from pesticides and pharmaceuticals (Gwinn et al. 2011). The contamination by 1,2-DCA is frequently found in surface and ground water near locations where vinyl products are manufactured. Due to its high volatility, 1,2-DCA can be released to the air during its manufacture and transport. Currently, more than 17.5 million tons 1,2-DCA are produced annually in the United States, Western Europe and Japan (Field and Sierra-Alvarez, 2004). Furthermore, the U.S. Environmental Protection Agency and the International Agency for Cancer Research have classified 1,2-DCA as a probable human carcinogen (Williams et al., 2001).

Technologies for the removal of 1,2-DCA have attracted a great deal of interest because of its persistent toxicity, potential bioaccumulation, and toxic effects on the environment. At present, there

are a number of proven remediation methods for contaminated sites. The primary technologies are chemical reduction, radiation, sol–gel catalysis, soil vapor extraction, soil washing, thermal treatment, phytoremediation, bioremediation, microwaving and bioventing (Yi Shi et al. 2012). These methods are effective for the removal of volatile organic compounds, but involve high financial or time costs that often make the remedial projects unfeasible. Thus, these methods are usually unsuitable for large contaminated sites.

Approaches to degrade 1,2-DCA have been investigated intensively (Pham et al. 2009, Field et al. 2004). Several articles have dealt with strains capable of hydrolytic dehalogenation of halohaliphatic compounds. Together with physical and chemical methods, biodegradation of 1,2-DCA using microorganisms deserves attention as it offers environment-friendly and low cost remediation (De Wildeman and Verstraete, 2003; Marzorati et al., 2006). The microorganisms utilize 1,2-DCA as a growth substrate, sole carbon and energy source (Janssen et al., 1985; Klecka et al., 1998). 1,2-DCA can be microbially degraded both under aerobic and anaerobic conditions. In the presence of oxygen, 1,2-DCA can completely be oxidized to CO_2, H_2O and Cl^- by different bacterial species, including *Xanthobacter autotrophicus GJ10* (Janssen et al., 1985) and *Ancylobacter aquaticus AD25* (van den Wijngaard et al., 1992). Microorganisms can transform 1,2-DCA rapidly to non-toxic end products via different pathways (Fig. 1) (Hage and Hartmans. 1999).

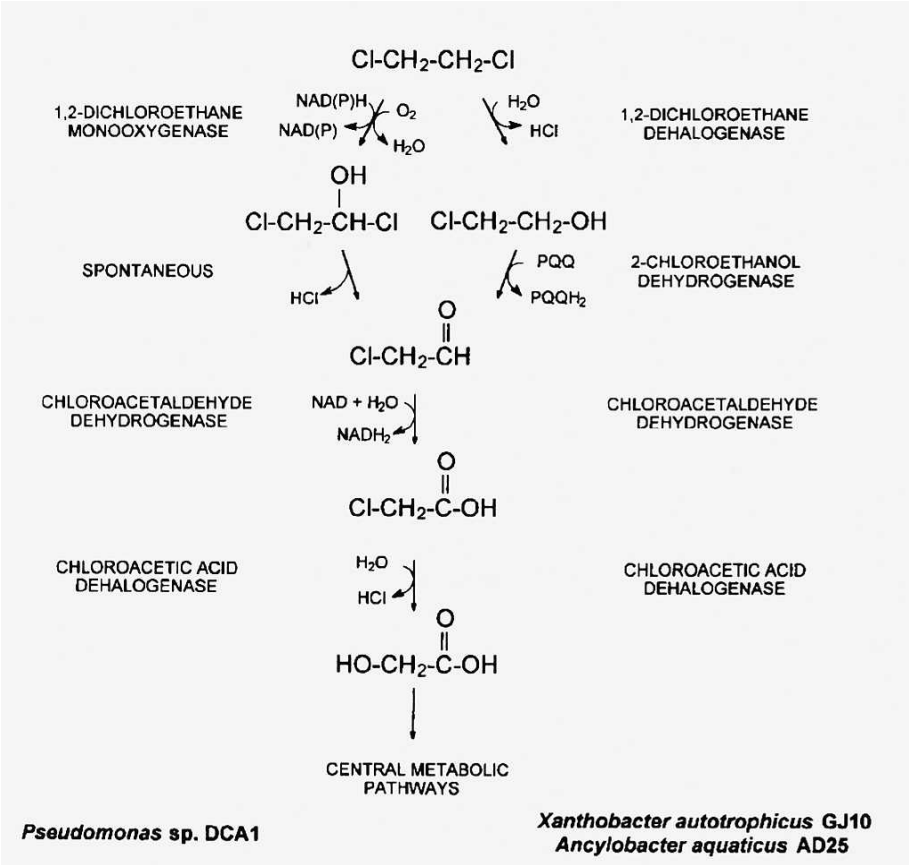

Fig. 1. Pathways of 1,2-DCA degradation by *Pseudomonas sp. DCA1*, *Xanthobacter autotrophicus GJ 10* and *Ancylobacter aquaticus AD 25*

The initial reaction is catalyzed by a haloalkane dehalogenase which converts 1,2-dichloroethane to 2-chloroethanol. Subsequent degradation involves a series of two sequential oxidations catalyzed by alcohol and aldehyde dehydrogenases to yield chloroacetate, which is finally converted to glycolate by the action of a haloacetate dehalogenase. Based on results of extensive biochemical as well as genetic

analysis of the enzymes involved, the authors speculate that evolution of organisms with the ability to grow on 1,2-dichloroethane requires a number of steps (Janssen et al., 1995). While two of the enzymes, namely the alcohol dehydrogenase and the chloroacetate dehalogenase appear to be common in nature, the haloalkane dehalogenase and chloroacetaldehyde dehydrogenase appear to be specifically adapted for the degradation of xenobiotic substrates.

The biodegradation of 1,2-DCA in the absence of oxygen has also been observed with nitrate as alternative electron acceptor (Gerritse et al., 1999). Recently, this process has been described in more detail by Dinglasan-Panlilio et al. 2006. The biotransformation of 1,2-dichloroethane under anaerobic conditions has been reported also by Belay and Daniels,1987 and Egli et al.,1987. They have described the biotransformation of 1,2-dichloroethane to ethene by pure cultures of sulfate reducing or methanogenic bacteria.

In our previous research (Vasileva et al. 2016) we have investigated the influence of constant electric field on dehalogenation capacity of the strain *Xanthobacter autotrophicus GJ 10* by low initial concentration of 1,2-DCA equal to 0.08 g/l. It is interesting to check the effect of electric field on 1,2-DCA biodegradation at higher concentrations where inhibition effects are expected.

In the present paper we have studied an aerobic biodegradation of 1,2-DCA by bacterial strain *Xanthobacter autotrophicus GJ 10.* It was used three different initial concentration of substrate and was investigated the influence of constant electric field on dehalogenation capacity of the bacteria.

2. MATERIALS AND METHODS

2.1. Bacterial strain, media and experimental conditions

Xanthobacter autotrophicus GJ10 was obtained from the Department of Biochemistry, University of Groningen, (The Netherlands) through the National Bank for Industrial Microorganisms and Cell Cultures, Bulgaria (NBIMCC). The strain was stored on agar with 0,08 g/l 1,2–DCE as a carbon source. The strain was grown in mineral medium (MMY) containing (per liter): 5.37g of Na_2HPO_4 .$12H_2O$, 1.36 g of KH_2PO_4, 0.5g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4.7H_2O$, and 0.015 g of $CaCl_2.2H_2O$, supplemented with 1 ml of trace element solution containing (per liter): 2.5 mg $CaCl_2.2H_2O$, 1 mg $FeSO_4.7H_2O$, 0.05 mg $ZnSO_4.7H_2O$, 0.05 mg H_3BO_4, 0.02 mg $CoCl_2.6H_2O$, 0.015 mg $Na_2MO_4.2H_2O$ and 0.02 mg $NiCl_2.6H_2O$. The medium pH was adjusted to 7.2 before being autoclaved. The vitamin solution was substituted by addition of 0.25 g/l yeast extract. The culture was grown in 500-ml glass flasks, which contained 200 ml of medium.

The experiments have been carried out under batch conditions in a 0,5 l Bioflo fermentor (New Brunswick Scientific, Edison, NJ) at 30ºC and agitation speed of 150 rpm. Processes of dehalogenation have been carried out with different initial substrate concentrations of 1,2-DCA in the presence and absence of constant electric field.

The study was focused on biodegradation at constant anode potential. This paper describes the experiments in presence and absence of electric field with three higher initial concentrations of substrate. Processes of dehalogenation were carried out with concentrations of 0,12, 0,14 and 0,2 g/l 1,2-DCA.

For experiments with electric field was used the following experimental installation (Fig.2).

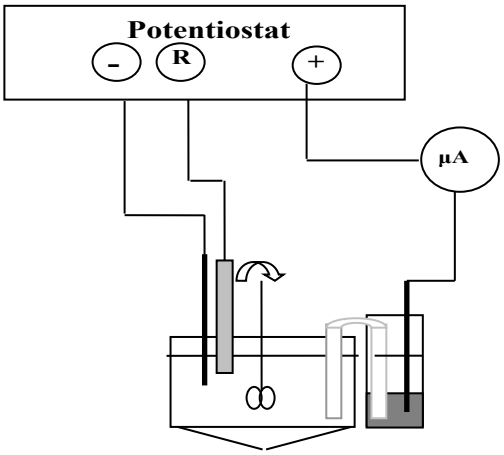

Fig. 2. Experimental installation with constant electric field

When applying electric field, one of the stainless steel baffles of the fermentor was used as a cathode (with surface of 13 cm^2). The cathode potential was maintained constant by a potentiostat at the value 0,1 V vs. quinhydrone electrode at 30 ^{0}C. At this temperature, the reference potential was +0,7029 V, the operating voltage being + 0,6029 V.

The anode was attached to the system by an agar salt bridge, outside the fermentor, in order to avoid undesired anodic processes in the broth.

2.2. Analytical methods

Samples from the broth have been taken periodically and analyzed for biomass and chloride ions. The growth of the biomass was monitored by the optical density of the broth (OD_{500}) vs. medium sample without bacteria using a spectrophotometer (VWR UV-1600 PC). Dry cell concentrations were calculated from the optical density, using a calibration curve.

Chlorides were analyzed by the colorimetric method of Bergman and Sanik 1957. 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). The concentrations of chloride ions were calculated from the optical density, using a calibration curve.

3. RESULTS AND DISCUSSION

The experimental results as time evolution of biomass and chloride release are presented in Fig. 3-8.

Curves of biomass growth with 0,12 g/l 1,2-DCA are shown in Fig.3.

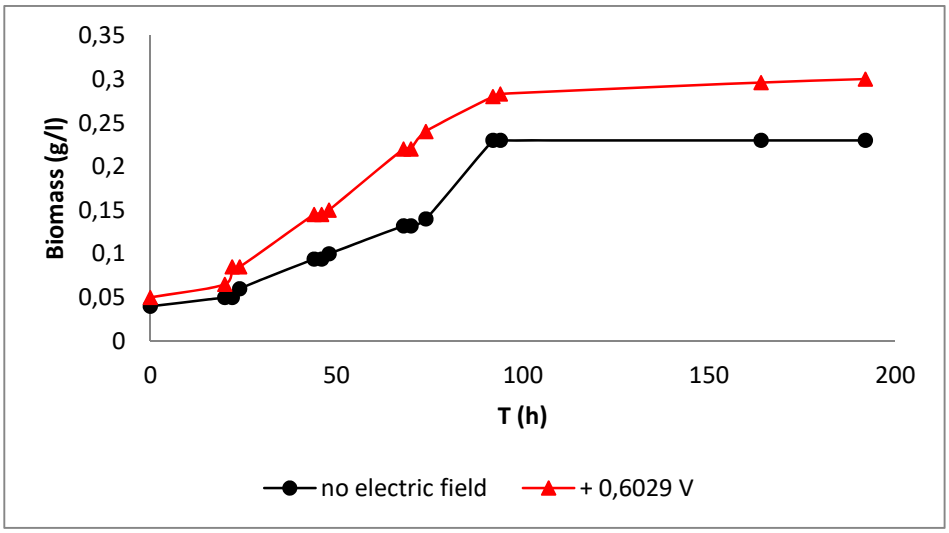

Fig. 3. Biomass growth with 0,12 g/l initial 1,2-DCA concentration

Concerning results without electric field, one short lag-phase and long exponential phase were observed. Biodegradation during exponential phase of bacterial growth took about 80 hours. The curve for microbial growth with electric field had the same profile as it is in the case of the control experiment. The difference was higher concentration of microbial cells during the whole process.

The chloride profiles with absence and presence of electric field with initial 0,12 g/l 1,2-DCA concentration are presented in Fig. 4.

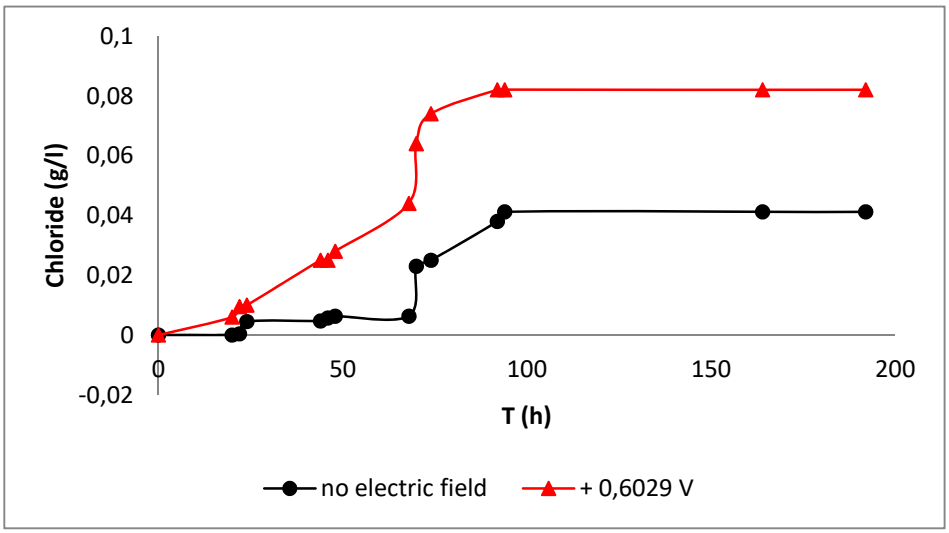

Fig. 4. Chloride profiles with 0,12 g/l initial 1,2-DCA concentration

In the absence of electric field, long constant chloride concentration was registered at the beginning with almost no chloride release for about 70 hours and fast biodegradation process after that. The chloride concentration reached 0,041 g/l, which was a half of stoichiometric one. It implies that only one chlorine atom in the substrate molecule was released completely.

When electric field was applied, the resulting curve showed short initial period of 20 hours, followed by fast chloride release during the exponential phase of bacterial growth until reaching chloride

concentration 0,082 g/l. This value, being comparable to stoichiometric one (0,086 g/l), is an evidence that the biodegradation of 1,2-DCA was completed, evidently due to the stimulating effect of the constant electric field.

Curves of biomass growth with 0,14 g/l 1,2-DCA are shown in Fig.5.

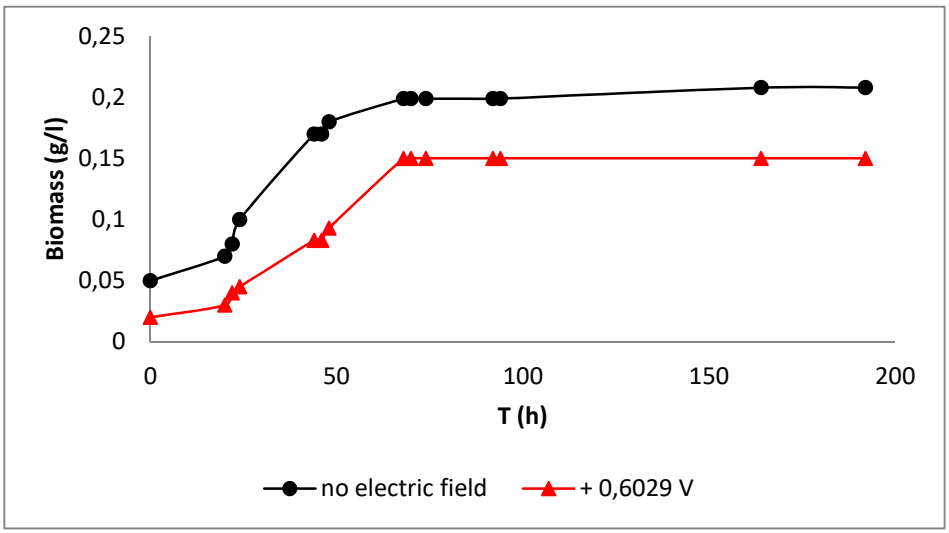

Fig. 5. Biomass growth with 0,14 g/l initial 1,2-DCA concentration

As seen, similar profiles were observed, too. Both graphs showed similar exponential phase of biomass growth of about 50 hours. However, in presence of electric field the amount of accumulated biomass was lower. Nevertheless, the biodegradation of 1,2-DCA was complete under electric field.

Fig. 6 shows the influence of electric field on chloride profiles at the same initial concentration (0,14 g/l).

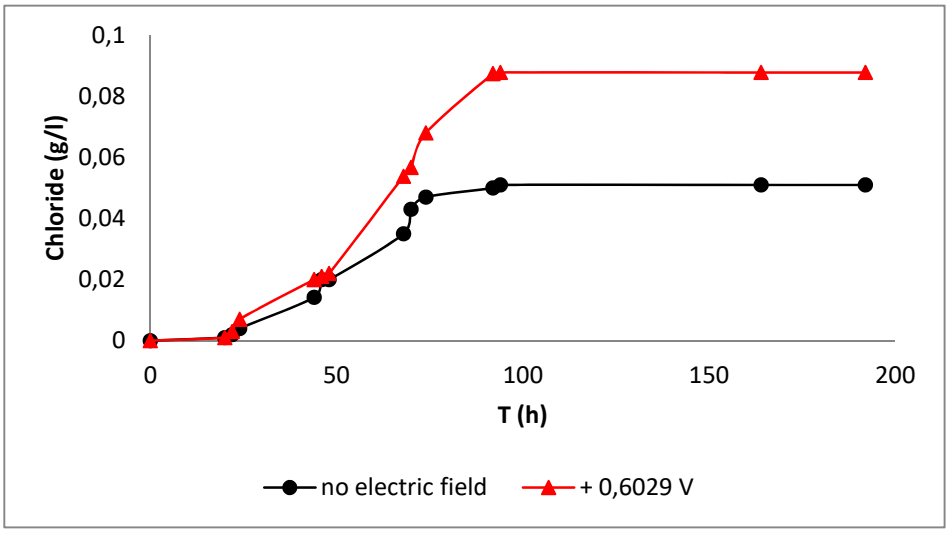

Fig. 6. Chloride profiles with 0,14 g/l initial 1,2-DCA concentration

At these conditions the biodegradation began after 20 hours. Fast chloride release was observed during the exponential phase of bacterial growth. Comparing the graphs of Figures 5 and 6 is visible complete biodegradation with less quantity of bacterial cells was achieved. With no electric field chloride concentration reached 0,051 g/l, while when applying electric field the final chloride concentration became 0,088 g/l, near to stoichiometric 0,1 g/l. Analogously to the previous case, only one chlorine atom from the substrate molecule was released in the process without electric field.

The curves of biomass growth at significantly higher initial concentration 0,2 g/l 1,2-DCA are shown in Fig.7.

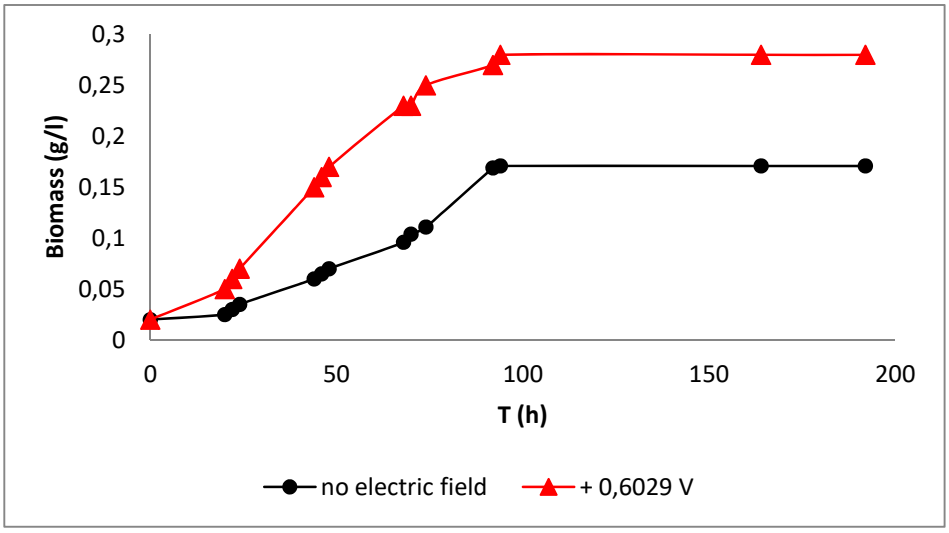

Fig. 7. Biomass growth with 0,2 g/l initial 1,2-DCA concentration

Small biomass accumulation was observed in the absence of electric field. Lag-phase was short and exponential phase continued to 100th hour. With electric field, the microbial culture was completely developed for the same period of time. However, the amount of accumulated biomass was twice as much higher.

The chloride profiles with and without electric field at 0,2 g/l initial 1,2-DCA concentration are presented in Fig. 8.

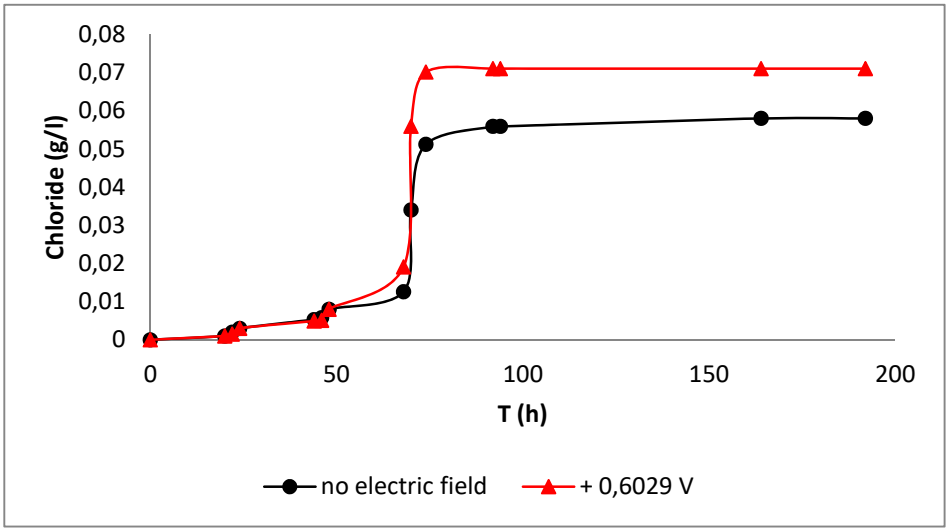

Fig. 8. Chloride profiles with 0.2 g/l initial 1,2-DCA concentration

The chloride profiles for both experiments have similar trend. Chloride release was observed after 50th hour. During a short period of only 25 hours (from 50th to 75th) a half of the chlorine ions were released completely. The final amount of chlorides (0,058 g/l and 0,071 g/l without and with electric field respectively) was much lower than stoichiometric value 0,143 g/l. Even in the case of electric field stimulation only one of the chlorine atoms is released. These results might be due to accumulation of intermediates (e.g. 2-chloroethanol and chloroacetaldehyde) acting as inhibitors of growth and substrate biodegradation.

The electrical current profile for 0,14 g/l 1,2-DCA is shown in Fig.9

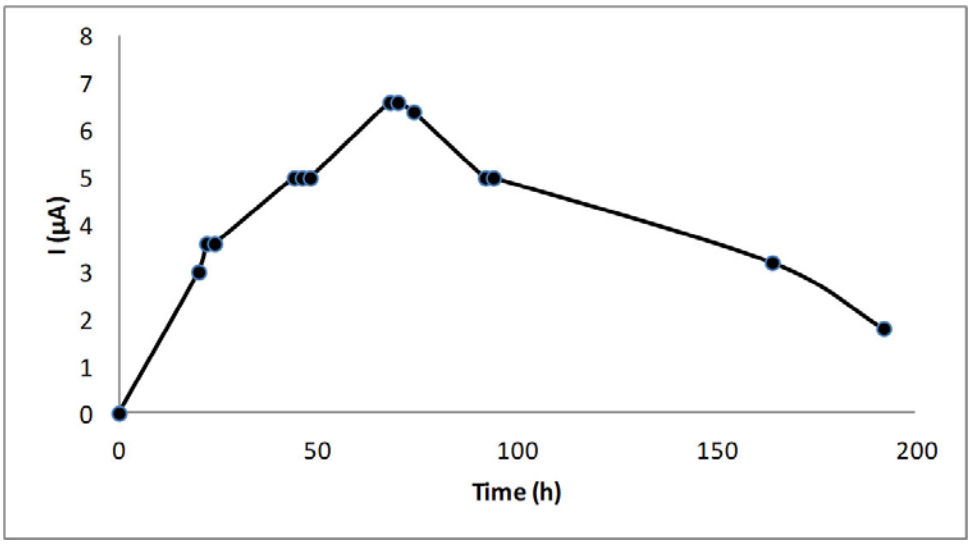

Fig. 9. Current profile with 0,14 g/l 1,2-DCA

A comparison of the chloride concentrations and the monitored values of the electric current, although very small (within 1 and 8 µA) showed that a peak in the electric current corresponded to the almost complete chlorine release and the attainment of stationary phase for biomass growth. Afterwards the current dropped. The increase of electric current corresponded to the continuous release of chlorine and to the microbial growth. Processes with initial substrate concentrations 0,12 g/l and 0,2 g/l were characterized with current values smaller than 6 µA, which is three order of magnitude less than the stoichiometric ones, according to the Faraday's law. These results showed that influence of constant electric field was not because of electrochemical processes but due to biophysical or biochemical processes affecting the enzyme activity of microbial cells and stimulated by the constant electric field.

4. CONCLUSIONS

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

- In the absence of electric field, at all substrate concentrations only one chlorine atom from 1,2–DCE molecule is released. It is because of the inhibition by the intermediates, 2-chloroethanol and chloro-acetaldehyde.
- The constant electric field leads to complete degradation and the concentration of chloride ions in the medium reaches stoichiometric values at low and moderate concentrations.
- Best results are obtained with bio-electrochemical potentiostatic mode at cathode potential 0,1 V vs. quinhydrone electrode.
- It is established that very low currents, less than 8 µA, are measured at potentiostatic mode. That is why the stimulating effect of constant electric field is not due to electrochemical factors but to biophysical or biochemical processes.

ACKNOWLEDGEMENTS

This work was supported under project E02/16/12.12.2014. The authors would like to thank the National Science Fund, Ministry of Education and Science of the Republic of Bulgaria.

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