

Model water disinfection with electrolysis using Ti_nO_{2n-1} containing ceramic electrodes*

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ABSTRACT

Water treatment with electrolysis was performed in a specially made electrolytic cell containing Ti_nO_{2n-1} ceramic anode and *Escherichia coli* was used as a model organism for disinfection tests. The results showed that even relatively low energy input (0.5–2.5 kWh·m⁻³, depending on water conductivity) in water samples with chloride ions concentration below 50 mg·l⁻¹, Ti_nO_{2n-1} ceramic electrodes generated active chlorine in the range of 0.4–3.5 mg Cl₂·l⁻¹, which is the level of chlorine used for water disinfection. The results also confirmed that disinfection effect is a result of generation of oxidant species from chlorine rather than effect of electricity per se, or formation of radicals in water. At chloride ion concentration about 7 mg·l⁻¹ *E. coli* is not culturable, not able to divide and

not respiring. Results showed that increase of the current above 0.02 A at chloride ion concentration of 7 mg·l⁻¹ was sufficient to inactivate both culturable and viable but nonculturable (VBNC) *E. coli*. Notably, the ability of bacteria to divide (DVC positive) was lost more rapidly than their ability to respire. Kinetics of disinfection was studied in water sample which was treated with 0.02 A at chloride ion concentration of 7 mg·l⁻¹. After about 15 minutes of exposure no culturable or able-to-divide *E. coli* were detected in the sample. Using the Ti_nO_{2n-1} electrode in the electrolysis process with the presence of chloride ions, in concentration range which is common in raw waters, one can create a level of active chlorine that kills more than 99% of *E. coli* within 15 minutes. A practically applicable simple model for prediction of disinfection efficacy with electrolytic cell has been proposed.

INTRODUCTION

Despite of well developed drinking water disinfection technologies there is still a need of new approaches of water disinfection, which would be effective, safe, easy to perform and less labour intensive. Numerous methods like solar disinfection (McLoughlin et al. 2004), ultrasonication (Jyoti and Pandit 2001; Scherba et al. 1991) and hydrodynamic cavitation (Jyoti and Pandit 2004; Mezule et al. 2010) are based on induction of physiological injury to destroy microorganisms and have shown to have a potential for application in disinfection of drinking water.

Nowadays electrochemical disinfection has gained increasing attention as an alternative for conventional drinking water treatment, because it is regarded as environmentally friendly, amendable to automation, inexpensive, easily operated and is known to inactivate a

wide variety of microorganisms from bacteria to viruses and algae (Bergmann and Koparal 2005; Choon and Jun 2007; Dreesa et al. 2003; Jeong et al. 2007, 2009; Kerwick et al. 2005; Shi et al. 2005). If compared with chlorination (the use of gaseous chlorine or concentrated hypochlorite solution), no addition of chemicals is necessary, because the main disinfecting agents are produced from the naturally occurring ions found in water itself (Kraft et al. 1999a; Kwang et al. 2006; Oliveira et al. 2007; Palmas et al. 2007). Unlike the other non-reagent disinfection methods it still employs disinfection with chlorine – a reliable and well known technique. During electrochemical disinfection oxidants are produced from electrolysis of water molecules and some dissolved chemical compounds (O, OH, H₂O₂, O₃, MnO²⁻, S₂O₈), which are normally present in a drinking water (Bashir et al. 2009; Bejankiwar et al. 2005; Reimanis et al. 2009, 2010). Thus no addition of reagents is

*Presented at the Second International Environmental Best Practices Conference, 14-18 September 2009, Krakow, Poland

required. In the presence of chloride ions after being exposed to electrolysis compounds with high disinfection potential, namely, hypochlorous acid and hypochlorites (HClO , ClO^-) (Bergmann and Koparal 2005; Cheng and Kelsall 2007; Kerwick et al. 2005; Kraft et al. 1999b; Oliveira et al. 2007; Shi et al. 2005; Zaggout and Ghalwa 2008) are formed. These species have residual disinfection effect; therefore their presence is particularly useful for drinking water as they secure water quality in long water distribution networks.

The efficiency of electrolytic process is affected by many factors including the type of electrodes. At the moment electrodes containing Pt, Ti/Sb-doped SnO_2 , IrO_2 , Pt-Ir, RuO_2 , MnO_2 , Ti/boron-doped diamond (BDD), as well as graphite are widely used (Bejan et al. 2005; Chen et al. 2005; Kraft et al. 1999; Leite et al. 2003; Waterston et al. 2006). Electrodes made of titanium oxide with the common formula $\text{Ti}_n\text{O}_{2n-1}$, where n is a number from 4 to 10, are considered as materials of a high potential. Changing the ratio of titanium and oxygen, as well as the manufacturing conditions, one can substantially change the properties of the material and its applications. $\text{Ti}_n\text{O}_{2n-1}$ has a high resistance to corrosion, which is higher than that of pure titanium and titanium dioxide (rutile, anatase). It has also low absorption coefficient and good mechanical strength. In contrast to the anatase and rutile which act as insulating materials $\text{Ti}_n\text{O}_{2n-1}$ shows semiconductivity, and even half-metallic properties. Titanium oxide-based ceramic electrodes have not yet been used in low concentration chloride ion solutions; however, they appear to be potentially applicable (Reimanis et al. 2009, 2010).

Until now the studies on electrochemical disinfection were limited to analyses on culturability of bacteria (Feng et al. 2004; Watts et al. 2008) whereas the other metabolic states, in which pathogenic bacteria may occur in drinking water, have not been investigated. Studies have shown that when subjected to stresses such as disinfection, microorganisms can enter "active but nonculturable (ABNC) state" (Kell et al. 1998), sometimes referred to as "viable but nonculturable" (VBNC) (Oliver 2005), when cells show no potential to divide, they cannot be grown to detectable levels *in vitro* on traditionally used agars, but certain vitality assays show some activity of bacteria (Kell et al. 1998). Lisle et al. (1999) have shown that when *E. coli* is subjected to chlorination, first of all the plate counts decrease, then substrate responsiveness (no ability to divide) and finally parameters other than cell multiplication are lost (Lisle et al. 1999). Thus, when estimating the effect of disinfection, methods involving *in situ* cell viability identification could be a good choice.

The aim of this study was to estimate the effect of electrolysis with $\text{Ti}_n\text{O}_{2n-1}$ containing ceramic anode on viability of bacteria in drinking water. The anode was synthesized in the Riga Technical University, Riga Biomaterials Innovation and Development Centre (Pavlova et al. 2008). The experiments were carried out in laboratory scale using *Escherichia coli* as a model organism for disinfection experiments. The viability of *E. coli* was assessed

by cultivation, substrate responsiveness (direct viable count combined with fluorescent *in situ* hybridization (DVC-FISH)) and respiratory activity.

The influence of the sulphate ion and buffer solution on the disinfection process and the distributed chlorine variation after electrolysis were investigated. Also the influence on the disinfection process exerted by the electrolysis current intensity and duration time as well as changes in the chlorine extracted during electrolysis were investigated.

MATERIAL AND METHODS

Bacterial strains and culture conditions

Escherichia coli ATCC 25922 grown on R2A agar (Scharlau, Spain) was inoculated into prefiltered, liquid Luria-Bertrani (LB) media (tryptone $10\text{g}\cdot\text{l}^{-1}$, yeast extract $5\text{g}\cdot\text{l}^{-1}$, NaCl $10\text{g}\cdot\text{l}^{-1}$) and incubated with constant shaking (150rpm) overnight at 37°C .

Sample preparation

Overnight culture of *E. coli* was centrifuged at $6,000\text{rpm}$ (2500g) for 2min. Then the pellet was washed twice with sterile phosphate buffered saline (7mM Na_2HPO_4 , 3mM NaH_2PO_4 , 130mM NaCl, pH 7.2) and resuspended in sterile distilled water. In order to determine the number of cells in suspension, a small volume of suspension ($0.1\text{--}1.0\mu\text{l}$) was filtered on 25mm diameter $0.2\mu\text{m}$ pore-size filters (Anodisc; Whatman plc) and fixed with 3-4% formaldehyde for 15 minutes, washed with sterile distilled water, air-dried and stained with $10\mu\text{g}\cdot\text{ml}^{-1}$ DAPI (4',6-diamidino-2-phenylindole, Merck) for 5 minutes. Cell numbers were determined by epifluorescence microscopy by counting 20 random fields of view (Ex: $545\pm 30\text{nm}$; Em: $610\pm 75\text{nm}$, dichromatic mirror 565nm , Leica DM, LB). Then the known concentration of cells was added to 0.5 litre of sterile synthetic *E. coli*-free water with different concentrations of SO_4^{2-} or Cl^- and pH ~ 7 (pH ~ 7 was maintained by adding 0.039M NaH_2PO_4 and 0.061M Na_2HPO_4). Maximum concentration of chloride ions ($250\text{mg}\cdot\text{l}^{-1}$) was the allowed limit for drinking water according with EU standarts (Anonymous 1998). All samples were analyzed in triplicates.

Experimental procedure

Water treatment with electrolysis was performed in a specially made electrolytic cell (Figure 1).

Electrolytic cell contained 0.5 litre solution and ceramic anode from pure $\text{Ti}_n\text{O}_{2n-1}$ (Pavlova et al. 2008) with an area 12.1cm^2 and a cathode made of stainless steel (AISI 304), with a total surface area of 18cm^2 . The cathode consisted of two identical plates placed in parallel on either side of the anode, 5mm away from it. Power to the electrochemical reactor was supplied by HQ Power, PS5005 (0-50V DC, 0-5A direct current rectifier). Electrolysis process was carried out at current intensity within the range $0.01\text{--}0.10\text{A}$, temperature $23\pm 2^\circ\text{C}$ and pH 7, with intensive stirring during 15min.

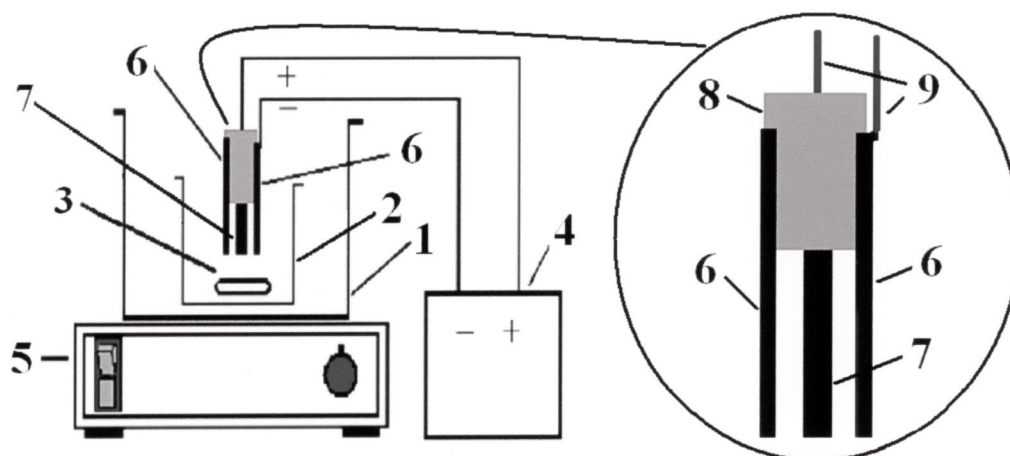


Figure 1. Experimental scheme for electrolysis: 1 – thermostat, 2 – bowl with EL solutions, 3 – magnet, 4 – direct current source, 5 – magnetic stirrer, 6 – stainless steel cathode, 7 – Ti_nO_{2n-1} ceramic rod anode, 8 – epoxide resin, 9 - wire for power supply.

All control samples were left untreated. The amount of released chlorine in the water was determined according to the standard titration method (Anonymous 1985).

After the treatment approximately 100ml of sample were collected in sterile bottles and brought for further processing. To cease the disinfection reaction enough of sodium thiosulphate was added.

***E. coli* cultivation**

Cultivable *E. coli* from both treated and control samples were determined by the plate count technique. 10-fold dilutions were inoculated onto TBX medium (Oxoid Ltd, UK) and incubated for 24 hours at 37°C. Typical blue/green colonies were counted and results expressed as CFU per milliliter. All samples were analyzed in triplicates.

Estimation of respiration activity

Samples were stained with CTC (5-cyano-2,3-ditolyl tetrazolium chloride, Fluka, BioChemika) in accordance with a modification of the procedure described by Rodriguez et al. (1992). In brief, LB media and CTC with a final concentration of 4mM were added to the samples. The mixture was continuously stirred for 2 hours in the dark at the room temperature of about 20°C. Then incubation samples were filtered, fixed and stained with $10\mu\text{g}\cdot\text{ml}^{-1}$ DAPI. Actively respiring and non-respiring cell numbers were determined with epifluorescence microscope (Leica DMLB) equipped with a 50W power supply, mercury lamp, filter sets for DAPI (Ex: $545\pm 30\text{nm}$; Em: $610\pm 75\text{nm}$) and for fluorescent formazan crystals (Ex: 340/380nm; Em: $>425\text{nm}$ or the same channel as for DAPI in order to avoid the counting of any extracellular fluorescing units), and the camera (Cool SNAP

Pro, Media Cybernetics, Inc., USA). For image processing Image Pro Plus 4.5.1. Software (Media Cybernetic Inc., Silver Spring, MD) was used. The enumeration of bacteria was done with direct microscopic counting of 20 random fields of view for each sample. Both respiring and non-respiring bacteria were enumerated in each field of view.

DVC-FISH procedure

Cell substrate responsiveness (potential to divide) was determined by modified DVC method by Kogure et al. (1979) and combined with FISH. In brief, samples were mixed with Tryptone Soya broth (Oxoid Ltd., UK) and $10\mu\text{g}\cdot\text{ml}^{-1}$ of Nalidixic acid and incubated for 6h at 30°C. Then the samples were washed by centrifugation (2min, 2500g) to remove excess culture media, fixed with formaldehyde (final concentration 3-4%) for at least 20 minutes. After fixation the samples were filtered through 25mm diameter $0.2\mu\text{m}$ pore size filter. After washing with sterile distilled water, filters were removed from filtering device and air-dried. Then fluorescent in situ hybridization was performed as described by Mezule et al. (2010).

Statistical analysis

Disinfection kinetics was modelled with Chick-Watson relationship (Dar 2007):

$$-dN/dt = k'Nt^mC^n, \quad (1)$$

where, $-dN/dt$ – rate of decrease in microbial density, k – rate constant, N – microbial density present at time t , t – time, m – empirical constant (2 for *E. coli*), C – concentration of disinfectant, n – coefficient of dilution.

For variable concentrations of the disinfectant, the disinfection efficiency is of the following form (Dar 2007):

$$CT = C^n t_p, \quad (2)$$

where, CT – constant of disinfection efficiency, C – concentration of disinfectant, n – coefficient of dilution, t_p – time required to produce a constant percent kill or die-off. This approach has evolved a “CT” (concentration multiplied by contact time) regulation to ensure a certain percentage of kills of *E. coli*. The percentage of kills are expressed in terms of log removals.

RESULTS AND DISCUSSION

Although *Escherichia coli*, compared to other waterborne pathogens such as *Cryptosporidium*, is not particularly resistant to disinfection, this bacterium is still used as an indicator of hygienic quality of drinking water, therefore it is of interest for water industry. During the last decade VBNC forms of bacteria have received attention as they are suspected as a “hidden source” of infection in drinking water (Juhna et al. 2007; Kalmbach et al. 1997). To identify both cultivable and VBNC bacteria, fluorescent *in situ* hybridization has been selected and combined with DVC in order to differentiate between substrate

responsive and substrate non-responsive cells (Goss et al. 1965).

In order to investigate whether other chemical compounds (e.g. reactive oxygen species) than chlorine are producing disinfection effect during expose to electricity the changes of viability of *E. coli* were determined by addition of different concentrations of sulphate ions (SO_4^{2-}) and buffer ions to distilled water. All samples were treated for 15 minutes with low current ($I=0.1A$, $pH 7\pm 0.2$, $t^\circ=23\pm 2^\circ C$) except the control which was untreated. Results showed that the effect of sulphate ions and reactive oxygen species on viability of *E. coli* is minor (data not shown). A minor decrease in cultivability and potential for dividing were observed only in samples without sulphate ions or in samples with low sulphate ion concentration. The latter phenomenon should be investigated further.

To test the formation of active chlorine from chloride ions, KCl salt in concentrations similar to those in drinking water was added to water. The solution was electrolyzed using permanent process parameters ($pH 7\pm 0.2$, $t^\circ=25^\circ C$, $t=15min$) with intensive stirring.

The intensity of generation of disinfecting substances and herewith the efficiency of electrolysis process in the water supply systems can vary, if the concentration of chloride ions in solution and parameters of electric current change. By changing concentration of chloride ions in solution and intensity of the electric current, a constant concentration of disinfecting substances in solution can be provided.

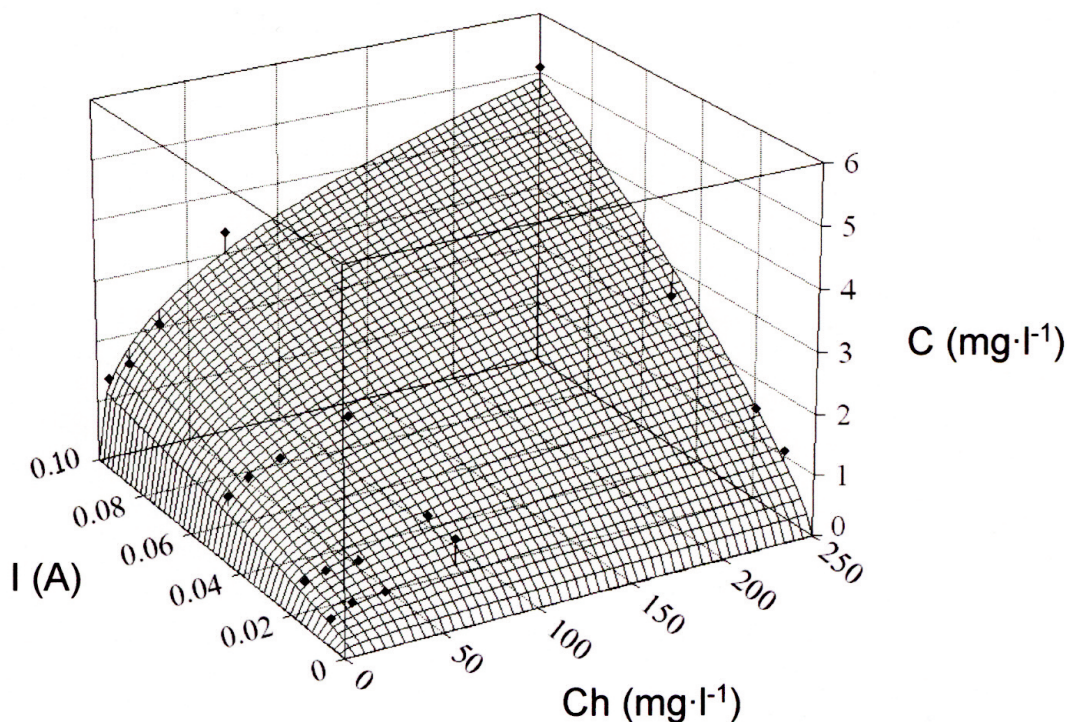


Figure 2. The concentration of chlorine (C) released during electrolysis as a function of concentration of chloride ions (Ch) and electric current intensity (I). Electrolysis was carried out in solution without buffer.

As it can be seen from Figure 2, during electrolysis the amount of released Cl_2 increases if the concentration of chloride ions in solution and the intensity of electric current are increased.

To observe the formation of chlorine during electrolysis, samples with different KCl concentrations in solution were treated (Figure 2). Results show that residual chlorine concentration increases with the increase of chloride ion concentration in solution. In typical surface waters concentration of chloride ions is less than $10\text{mg}\cdot\text{l}^{-1}$, however, in areas subjected to seawater intrusion, the chlorine levels can be much higher. Chlorine can be corrosive to steel pipes at levels of $50\text{mg}\cdot\text{l}^{-1}$, whereas at levels above $250\text{mg}\cdot\text{l}^{-1}$ it causes an objectionable salty taste. From Figure 2 it can be concluded that at even relatively low energy input ($0.5\text{--}2.5\text{kWh}\cdot\text{m}^{-3}$, depending on water conductivity) in water samples containing less than $50\text{mg}\cdot\text{l}^{-1}$ of chloride ions, $\text{Ti}_n\text{O}_{2n-1}$ ceramic electrodes generated active chlorine in the range of $0.5\text{--}3.5\text{mg}\cdot\text{l}^{-1}$, which is the level of chlorine used for drinking water disinfection.

An important factor in electrolysis process is the current intensity, responsible for generation of active chlorine from chloride ions present in the water. If the current intensity is increased, more active chlorine is released on the anode (Figure 2); this intensifies the oxidation processes and increases the disinfection effect in electrolysed solution (Figure 3).

The data obtained (Figure 2) were processed with Systat Software Inc. Table Curve 3D version 4.0. The equation system as two arguments function (3) of x and y was obtained. Obtained information allows to forecast the concentration of Cl_2 if the parameters of electrolysis are changed:

$$C = 1/(-0.15501 + 0.054037 (\ln I)^2 + 2.9544 \ln(\text{Ch})/\text{Ch}) \quad (3)$$

where I – current intensity (A); C – generated active chlorine concentration ($\text{mg}\cdot\text{l}^{-1}$) Ch – chloride ion concentration ($\text{mg}\cdot\text{l}^{-1}$).

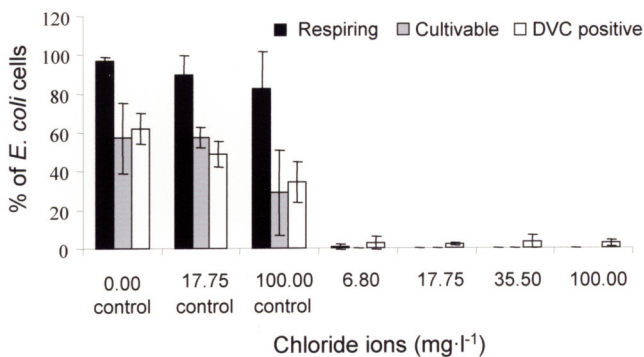


Figure 3. Chloride ion concentration (Ch) influence on disinfection efficiency of *E. coli* (%). Results are presented as percent of metabolically active (respiring), cultivable, and DVC positive (with a potential for dividing) *Escherichia coli* cells. Standard deviation represents the dispersion of the results of three separate experiments. 100% represents the total population. For control samples treatment was not used.

The results also confirmed that disinfection effect is a result of generation of oxidant species from chlorine rather than effect of electricity, i.e. formation of radicals from the water. Effect of chloride ion concentration on disinfection efficiency of *E. coli* is presented in Figure 3. Results showed that at level of about $7\text{mg}\cdot\text{l}^{-1}$ *E. coli* is not culturable and not VNBC (respiring and able to divide). This concentration is within the range of chloride ion concentration in pristine surface waters and ground waters.

Effect of current intensity was studied by exposing *E. coli* for 15 minutes at chloride ion concentration of $6.8\text{mg}\cdot\text{l}^{-1}$ (Figure 4). Results showed that increase of current above 0.02A was sufficient to inactivate both cultivable and VBNC *E. coli*. Notably, the cell ability to divide (DVC positive) was lost more rapidly than the ability to respire.

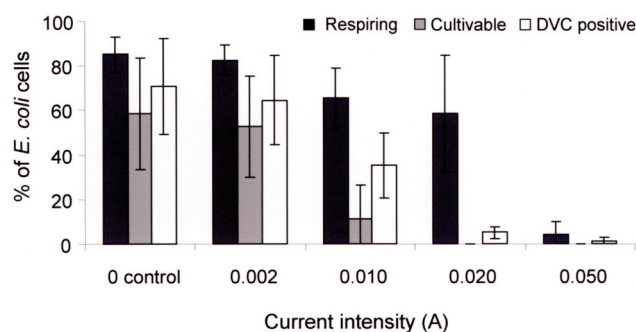


Figure 4. Current intensity (I) influence on disinfection efficiency of *E. coli* (%). Results are presented as percent of metabolically active (respiring), cultivable, and DVC positive (with a potential for dividing) *Escherichia coli* cells. Standard deviation represents the dispersion of the results of three separate experiments. 100% represents the total population. For control samples no treatment was used.

Kinetics of disinfection was studied in water samples which were treated with 0.02A at chloride ion concentration of $6.8\text{mg}\cdot\text{l}^{-1}$ (Figure 5). Previous experiments (Figure 2) show that approximate level of active chlorine would be in around $0.4\text{mg}\cdot\text{l}^{-1}$. After the sample was exposed to this concentration, *E. coli* concentration decreased rapidly following exponential decay rate (viz. first-order rate constant). The rate of decrease was similar for both cultivable and able-to-divide (DVC-FISH) *E. coli*. After about 15 minutes of exposure no cultivable or able-to-divide *E. coli* were detected in the sample. However, respiration ability of *E. coli* decreased with different trend: more rapidly at the beginning and nearly stopped after 3 minutes of the test. This phenomenon should be investigated further.

To develop the mathematical equation which could be used for calculation of disinfection efficiency with

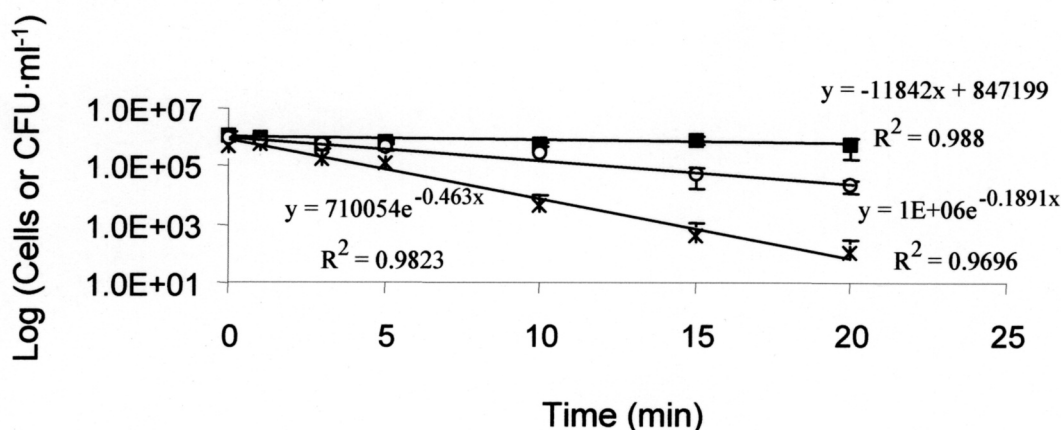


Figure 5. The effect of treatment time on disinfection efficiency (concentrations of chloride ions $6.8\text{mg}\cdot\text{l}^{-1}$, current intensity 0.02A , pH 7 ± 0.2 , $t=23\pm 2^\circ\text{C}$) of different viability states of *E. coli*: respiration activity (■), ability to divide (○) and cultivation (×). Standard deviation represents the dispersion of the results of three separate experiments.

electrolysis using $\text{Ti}_n\text{O}_{2n-1}$ electrodes the disinfection kinetics was fitted to Chick-Watson model ($R^2=0.98$). By combining the obtained equation with the equation (3) the following model was obtained:

$$N=N_0 \cdot e^{((1/(0.155-0.054 \cdot \ln(A) \cdot \ln(A)-2.954 \cdot \ln(C)/C)) \cdot 0.42 \cdot t)} \quad (4)$$

where N – *E. coli* concentration after disinfection (able-to-divide cells·ml⁻¹), N_0 – initial *E. coli* concentration after disinfection (able-to-divide cells·ml⁻¹), C – chloride ion concentration in water sample (mg·l⁻¹), A – current intensity (A), t – contact time (min).

For example according to this model (4) the concentration of (able-to-divide) *E. coli* in water with chloride concentration of $10\text{mg}\cdot\text{l}^{-1}$, current intensity of 0.05A and exposition time of 10 minutes will be 2 cells·ml⁻¹ if the initial concentration was 1000 cells·ml⁻¹.

CONCLUSIONS

1. Using a $\text{Ti}_n\text{O}_{2n-1}$ electrode in electrolysis process ($I \geq 0.5\text{A}$) in the presence of chloride ions at a concentration range $7\text{--}250\text{mg}\cdot\text{l}^{-1}$ (which is common in raw waters) the level of active chlorine can be created that kills more than 99% of *E. coli* within 15 minutes.
2. The mathematical equation which can be used for calculation of disinfection efficiency with electrolysis using $\text{Ti}_n\text{O}_{2n-1}$ electrodes has been fitted to Chick-Watson disinfection kinetics model and it enables prediction of disinfection efficacy of *Escherichia coli* with electrolysis process.

ACKNOWLEDGEMENTS

This work has been partly supported by the European regional development fund within the project “Development of innovative water procession technology using nanostructured ceramics”, No. 2010/0257/2DP/2.1.1.1.0/10/APIA/VIAA/012.

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