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Alternating electric fields combined with activated carbon for disinfection of Gram negative and Gram positive bacteria in fluidized bed electrode system

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ABSTRACT

Strong electric fields for disinfection of wastewaters have been employed already for several decades. An innovative approach combining low strength (7 V/cm) alternating electric fields with a granular activated carbon fluidized bed electrode (FBE) for disinfection was presented recently. For disinfection performance of FBE several pure microbial cultures were tested: *Bacillus subtilis*, *Bacillus subtilis* subsp. *subtilis*, *Enterococcus faecalis* as representatives from Gram positive bacteria and *Erwinia carotovora*, *Pseudomonas luteola*, *Pseudomonas fluorescens* and *Escherichia coli* YMc10 as representatives from Gram negative bacteria. The alternating electric field amplitude and shape were kept constant. Only the effect of alternating electric field frequency on disinfection performance was investigated. From the bacteria tested, the Gram negative strains were more susceptible and the Gram positive microorganisms were more resistant to FBE disinfection. The collected data indicate that the efficiency of disinfection is frequency and strain dependent. During 6 h of disinfection, the decrease above 2 Log units was achieved with *P. luteola* and *E. coli* at 10 kHz and at dual frequency shift keying (FSK) modulated signal with frequencies of 10 kHz and 140 kHz. FBE technology appears to offer a new way for selective bacterial disinfection, however further optimizations are needed on treatment duration, and energy input, to improve effectiveness.

1. Introduction

The effluents of wastewater treatment plants (WWTP) often have concentrations of pathogenic microorganisms that can affect the quality of receiving water bodies (Rizzo et al., 2013). Disinfection treatment of WWTP effluent is therefore often required, and chemical and/or electrochemical treatment techniques (such as chlorination, ozonation) are commonly applied (Reinthaler et al., 2003). However, potential carcinogenic substances are produced (e.g. chlorite, bromate) when

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chlorination or ozonation are employed for disinfection (Kraft et al., 1999). Alternatively, UV is nowadays more often used to treat effluents of WWTP. One disadvantage for the application of this method is that water streams usually contain large amount of suspended solids and organics (Carey and Migliaccio, 2009; Rieger et al., 2004), which reduces UV treatment effectiveness due to light dissipation (Loge et al., 1999) and shielding of pathogens from irradiation by particles and inclusion in aggregates (Emerick et al., 1999). A more effective disinfection technique for wastewater effluent is UV combined with ultrasound since both microbial cells and particle aggregates are destroyed (Neis and Blume, 2003; Paleologou et al., 2007).

In addition to traditional microbial pathogens there is an increasing concern about the presence of microorganisms resistant to antibiotics and chemical disinfectants in these treated WWTP effluents (Li et al., 2009; Novo et al., 2013; Reinthaler et al., 2003). These resistant microorganisms are known to cross transfer (horizontally) the resistance genes to natural microorganisms and this is becoming a new emerging environmental and health concern (Rieder et al., 2008). Therefore, there is an urge for novel non-chemical methods that are able to disinfect turbid solutions containing pathogenic microorganisms.

There are many different types of microorganisms in wastewater, and the bacterial composition can be divided into two groups: Gram positive (Gram (+)) and Gram negative (Gram (−)) bacteria. These two groups of bacteria differ mainly in structure and organo-chemical composition of their cell envelope and cell membrane. It is important to understand these differences between both types of bacteria, because it partly determines the disinfection mechanism by which they can be eliminated from water. For instance, Gram (+) bacteria are more susceptible to antibiotics and chemical treatment than to physical treatment methods (Denyer and Maillard, 2002; Qin et al., 1994). Gram (−) bacteria however, are more susceptible to physical disinfection methods (such as ultrasound, photocatalytic, pulsed electric field treatments) than to chemical treatment (Drakopoulou et al., 2009; Foster et al., 2011; García et al., 2007; Rincón and Pulgarin, 2005).

The application of alternating electric fields for influencing cell biology was in the 80/90s a readopted topic for cell research (Chang, 1989; Lagunas-Solar et al., 2005; Valle et al., 2007; Zheng and Chang, 1991). The specific sensitivity of biological cells towards alternating electric fields (AC fields) at certain frequencies is since then being exploited for various purposes such as cell growth, cell killing, diagnostics, sensing devices, healing or gene transfer purposes (Amarjargal et al., 2013; Chang, 1989; Funk and Monsees, 2006). When cells are exposed to AC fields, the polarization of the cell membrane and its components takes place which may further lead to phenomena such as rotation, cell membrane permeability and osmotic imbalance. These effects depend on the frequency and amplitude of the applied electric field, and on the morphology, shape and viability state of cells (Barnes, 2007; Markx and Davey, 1999). In general when AC fields are used for disinfection, the amplitude of electric field should be several to hundreds kV/cm and it is employed in short pulses (ms−μs). The other interesting aspect of an AC field is that it can consist of different signals and contain multiple or modulated frequencies, that possibly lead to an increased disinfection effects (Kotnik et al., 1998; Qin et al., 1994). Low amplitudes (few V/cm) AC fields are reported to suspend cells growth (Giladi et al., 2008).

Recently, we have demonstrated the proof of principle of the granular active carbon (GAC) fluidized bed electrode (FBE) technology for disinfection at only 6 V/cm. *Escherichia coli* YMc10 disinfection of 2.72 Log CFU decay within 6 h with a 140 kHz AC field was achieved. Furthermore, we showed that the FBE disinfection efficiency is dependent on the frequency of the applied electric field. No disinfection was achieved with AC field without GAC, whereas GAC without AC field resulted in 0.5 Log *E.coli* CFU decrease (Racyte et al., 2011).

Activated carbon is well known in the water treatment field. It is used for adsorption of organics, suspended solids and can reduce the concentration of microorganisms (Hijnen et al., 2010). Activated carbon is also used for electrochemical applications in liquids because of it's high surface area and electrical conductivity. A high and a rough surface area facilitate biofilm formation on the activated carbon. Upon adhesion on certain active carbon types (positively charged, acidic), bacteria might lose their viability (van der Mei et al., 2008).

In this work, further studies for extension of proof-of-principle for FBE disinfection were carried out on various bacteria, as representatives of various microbial species occurring in wastewaters and nature. Representatives of Gram (+) (*Bacillus subtilis*, *B. subtilis* subsp. *subtilis*, *Enterococcus faecalis*) and Gram (−) (*Erwinia carotovora*, *Pseudomonas luteola*, *Pseudomonas fluorescens*, *E. coli* YMc10) bacteria were exposed to FBE disinfection at different frequencies with the aim to establish the susceptibility for bacteria disinfection of different types. Four Gram (−) and three Gram (+) bacteria were exposed to a continuous sine wave and to a dual frequency shift keyed (FSK) AC field signals in the FBE system for 6 h. Eight different (combinations of) frequencies were applied based on the optimal frequency peak for disinfection of *E. coli* YMc10 obtained in a previous study (Racyte et al., 2011).

2. Material and methods

2.1. *Granular activated carbon employed for disinfection*

The granulated activated carbon (GAC) Norit RX 3 Extra (Norit B.V. Amersfoort, the Netherlands) was employed in FBE disinfection as suspended bed material. The GAC used had a BET area of 1400 m^2/g, pHpzc 7.77 and particle size of 3 mm. The GAC was prepared for experiments as earlier described by Racyte et al. (2011). The 22 g of GAC were wetted, washed, autoclaved, washed again and stored at 4 °C. One day prior to an experiment the GAC was rinsed and submerged to 30 mL of an electrolyte. The electrolyte for all bacteria was 1:5 diluted tryptic soy broth (1/5 TSB) and in *E. coli* case 1:4 diluted lysogeny broth (1/4 LB) as described previously (Racyte et al., 2011). On the day of the experiment, the electrolyte was removed, the GAC was rinsed with 20 mL reaction medium (Table 1) and subsequently 40 mL of reaction medium were added for disinfection performance investigation in the FBE.