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Combining fluidized activated carbon with weak alternating electric fields for disinfection

Justina Racyte [a,b,*], *Jalal-Al-Din Sharabati* [a,c], *Astrid H. Paulitsch-Fuchs* [a], *Doekle R. Yntema* [a], *Mateo J.J. Mayer* [d], *Harry Bruning* [b], *Huub H.M. Rijnaarts* [b]

[a] *Wetsus, Centre of Excellence for Sustainable Water Technology, Agora 1, P.O. Box 1113, 8900 CC Leeuwarden, The Netherlands*
[b] *Sub-Department of Environmental Technology, Wageningen University, Bornse Weilanden 9, 6708 WG Wageningen, The Netherlands*
[c] *Faculty of Chemistry, University Duisburg-Essen, Universitätsstraße 2, 45141 Essen, Germany*
[d] *EasyMeasure B.V., Breestraat 22, 3811 BJ Amersfoort, The Netherlands*

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ABSTRACT

This study presents fluidized bed electrodes as a new device for disinfection. In the fluidized bed electrodes system, granular activated carbon particles were suspended, and an alternating radio frequency electric field was applied over the suspended bed. Proof-of-principle studies with the luminescent non-pathogenic bacterium *Escherichia coli* YMc10 demonstrated that disinfection with fluidized bed electrodes requires both the presence of granular activated carbon particles and the application of radio frequency electric field. Disinfection was investigated at various frequencies in range from 80 to 200 kHz at electric field strength of 6 ± 0.5 V/cm during 6 h. The largest decrease of *E. coli* viable cell concentration in the liquid (from 10^8 to 10^6 CFU/mL) was obtained at an optimum frequency of 140 kHz. Possible mechanisms of this electromediated disinfection are discussed in the manuscript. The results are promising for development of a new disinfection process with fluidized bed electrodes.

1. Introduction

The quality of drinking water and treated domestic and industrial wastewater is worldwide an issue of concern [1]. Increased requirements of water reuse ask for effective disinfection methods to assure public health, health of cattle and plants in agricultural production systems [2], and to prevent damage to natural ecosystems [3,4]. The oldest and most reliable chemical water treatment methods such as chlorination and ozonation are being widely applied for water disinfection [5,6]. Although effective, these methods require post treatment, mainly due to formation of by-products occurring in the treated water stream [7]. Photolytic methods employing UV radiation are proven to be very effective for killing pathogens; the major issue with these methods is the rather high capital and maintenance costs [8]. Electrical and electrochemical methods for disinfection are widely investigated because they produce effluents less harmful for biological consumers than chemical treatment methods, and they are cheaper than UV treatment [9,10]. Electrochemical disinfection methods treat water streams by electrically produced active species such as radicals [11,12]. The use of solely electric fields for disinfection needs high electric field densities up to 100 kV/cm [13].

Microorganisms have a dielectric nature meaning that they can be polarized in an electric field [14]. Electromagnetic fields are reported to affect several microbial life phenomena such as: microbial growth [15,16], cell fusion (PEF) [17–19],

* *Corresponding author at:* Wetsus, Centre of Excellence for Sustainable Water Technology, Agora 1, P.O. Box 1113, 8900 CC Leeuwarden, The Netherlands. Fax: +31 582 843001.
E-mail address: Justina.Racyte@wetsus.nl (J. Racyte).
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voltage gated channels [20]; and can even cause cell death [16,21]. All mentioned effects employ direct current (DC) or a low frequency electromagnetic field (LF-EMF). The main problem of these DC or LF-EMF methods is the high power consumption and degradation of electrode materials. Therefore, the treated liquid has to be without ions, particles or gas bubbles to avoid electrical short-circuiting or fouling between electrodes spaced on a short distance [22].

Optionally, alternating current (AC) treatment can be used [23,24]. To reach high disinfection efficiency with AC, either strong electric fields ranging from 3 to 100 kV/cm, or high frequencies (60–1000 kHz) are required [13,25]. For wastewater that generally has a low electrical resistance, the application of high voltages results in high currents that contribute to high power consumption. The large power required has restrained the large scale application of AC disinfection [26].

To reduce electric field strength, carbon particles can be added in-between two electrodes of an electrical cell, which results in a so called three-dimensional electrode [5,27–29]. The carbon morphology allows bacteria to adhere [30,31], which may play a role in the inactivation of bacteria. Preliminary experiments indicated that a low amplitude radio frequency electric field (RF-AC) combined with granular activated carbon (GAC) can result in disinfection. In the present study a "fluidized bed electrode" (FBE) was constructed that consisted of a RF-AC applied to a stirred GAC particle suspension. The FBE was operated in batch mode. The proof-of-principle for disinfection by using the FBE system is presented and key experimental factors that determine the disinfection performance were identified. For disinfection experiments non-pathogenic and bioluminescent *Escherichia coli* YMc10 were used as test microorganisms, which are suitable as a representative for enteric pathogens (*E. coli* O157:H7) which can cause waterborne diseases [32]. The use of non-pathogenic *E. coli* YMc10 made experiments possible under normal laboratory conditions. In this paper mechanisms that may play a role in FBE disinfection process are distinguished and discussed.

2. Experimental

2.1. Granular activated carbon

Commercially available GAC NORIT RX 3 EXTRA (Norit BV, The Netherlands) was used. This GAC is extruded, steam activated and acid washed. Prior to the experiments 140 g of GAC was fluidized in a beaker with 1 L Milli-Q water (18.2 MΩ·cm −25 °C, 0.22 μm, Millipore Biocel SAS 67120, France) and wetted (4 h stirring). After this the GAC particles were washed with Milli-Q water and autoclaved at 0.1 MPa, 121 °C for 30 min to avoid contamination of the microbial culture by other bacteria. Subsequently, GAC particles were washed with Milli-Q water again and left standing for 24 h to remove remaining air from GAC pores [33]. Before the experiment the particles were submerged for 24 h in 1/4 LB medium (section 2.3) to saturate the GAC with electrolyte. For the FBE disinfection experiment 400 mL of the prepared medium (1/4 LB media containing ampicillin) and 140 g of pretreated GAC (GAC_{pr}) were used.

2.2. GAC physical characteristics

Activated carbon was analyzed before (GAC_r) and after the pretreatment (GAC_{pr}). Point of zero charge (pH_{pzc}), surface area (BET) and macropore content were determined.

2.2.1. pH point of zero charge determination

The pH_{pzc} was determined by an immersion technique using NaCl as the electrolyte [34]. The 0.05 M NaCl solution was degassed by stirring in N_2 environment for 48 h. Two grams of pretreated and untreated GAC were mixed with 100 mL of decarbonized 0.05 M NaCl and stirred mechanically in N_2 environment at constant room temperature for 24 h (both GACs in triplicate), so that the carbon charges reach a balance (zero charge). After 24 h, the GAC was filtered from the liquid with a 0.45 μm hydrophobic syringe filter and pH_{pzc} was determined with a pH electrode (Liquisys M CPM 253, Endress + Hauser, The Netherlands). The used GAC is brittle, therefore the pH_{pzc} of powder activated carbon NORIT RX 3 EXTRA (PAC) was determined as well. PAC for pH_{pzc} measurement was made by grinding the GAC in a ball mill grinder (PM100, Retsch, Germany) and the above described procedure was applied for determination of pH_{pzc} for PAC.

2.2.2. BET determination

The GAC pore structure properties were determined using nitrogen adsorption on GAC surfaces. GAC was dried and degassed in a N_2 environment for 24 h at 350 °C (VacPrep 061 LB, Micrometrics, Germany). Nitrogen adsorption isotherms were measured (Tristar 3000, Micromeritics, USA) to obtain total BET surface area (m^2/g). The macropore area ($A_{macropore}$) was measured using mercury intrusion porosimetry (Autopore II 9220 porosimeter, Micromeritics, USA).

2.3. Bacteria – Escherichia coli YMc10

Non-pathogenic, genetically modified, bioluminescent bacteria *E. coli* YMc10 (Belgian Coordinated Collections of Microorganisms, Belgium) served as target microorganism for FBE disinfection experiments [35]. This non-pathogenic strain carries a plasmid (pJE202) that contains *Vibrio fischeri* genes on a vector: luxR, luxI, luxC, luxD, luxA, luxB, luxE, luxG specifying the luminescence enzymes and encoding regulatory functions for bioluminescence [36]. It possesses an ampicillin resistance on the same vector to prevent the multiplication in an environment without ampicillin and cross-transfer of the vector to the other microorganisms.

Lysogeny broth (LB) medium was prepared in Milli-Q to cultivate *E. coli* YMc10 (NaCl 10 g/L; BactoTM Tryptone 10 g/L; BBLTM Yeast extract 5 g/L; Ampicillin 0.1 g/L; pH 7). The prepared medium was then autoclaved for 25 min at 0.1 MPa, 121 °C to sterilize the media. A part of the autoclaved medium (100 mL) was inoculated with 1 mL of *E. coli* YMc10 stock solution and incubated for 18 h at 25 °C. After incubation, 90 mL of the bacterial suspension were centrifuged at 3273 g for 15 min. The supernatant was discarded and the pellet was resuspended in 400 mL diluted (1:4) LB medium (1/4 LB medium) which was used in the FBE disinfection experiments. The LB dilution ratio was determined experimentally (data not shown), as the minimum amount of substrate concentration

to maintain a stable culture and achieve active luminescence but inhibit exponential growth of the bacteria. The 1/4 LB medium concentration is comparable to a lightly polluted wastewater concentration [37].

2.4. Experimental set-up

The fluidized bed electrode (FBE) set-up (Fig. 1(C)) consisted of a 1 L beaker glass, 2 stainless steel plate electrodes (35.6 cm^2) and a magnetic stirrer (Heidolph instruments D91126, Germany). A custom made high power, high bandwidth amplifier (f_{max} = 600 kHz, I_{max} = 5 A, V_{maxpp} = 30 V) connected to a function generator (TG 2000 DDS Thurlby-Thandar instruments, UK) provided an alternating voltage, that was connected to the two stainless steel electrodes. The 400 mL of liquid medium to be disinfected was placed in the beaker into which the electrodes were immersed and the GAC particles were suspended. The system was stirred at 650 rpm, which resulted in a fluidized bed with turbulent character. Power was turned on at t = 0 min and 4 mL samples were taken every 30 min for luminescence analysis and every 60 min for viable cell colony forming unit (CFU) analysis. The duration of an experiment was 360 min. Both temperature and pH were measured with a pH electrode and recorded with a data logger (RSG30 Endress + Hauser, Naarden, The Netherlands). Stirring led to a minor pH increase, likely due to release of remaining oxygen complexes from the GAC pores. During operation, pH was maintained at values between 7 and 7.5, by feeding 1 M HCl solution. Temperature was controlled at a value of 23 ± 2 °C.

2.5. Microbiological analysis

To determine the concentration of *E. coli* YMc10 in the samples from the FBE experiments a fast screening was performed with a spectrophotometer measuring the luminescence (1420 Multilabel Counter Victor3; Perkin Elmer, USA). Therefore 200 μL of each sample was pipetted into a well of a 96 well black optical bottom plate (MicroWell, Nunc, Denmark). The intensity of luminescence as photon emissions from a sample in counts per second (CPS) was measured by a extra high scale luminescence detection method. However, luminescence intensity is not only dependent on the amount of bacteria, but also on factors such as bacteria quorum, autoinducers and Lux gene regulators [38]. Bacterial concentrations corresponding to a luminescence level below 10 CPS cannot be detected. Therefore parallel to luminescence measurements, samples were plated on LB agar to confirm that the change in CPS value corresponded to the viable and cultivable *E.coli* concentration – CFU value. The relation between CPS values and CFU values was experimentally determined to be $\Delta CFC = \frac{1}{b}(\Delta log(CPS))$ with r^2 ranging from 0.68 to 0.92. The measured CPS values do not always correspond with the relation; therefore consequently samples were plated to determine the number of viable and cultivable *E. coli* in CFU/mL. The drop technique [39] was used to determine the CFU/mL. Instead of plating different dilutions, three drops of 10 μL of the same dilution were plated (by allowing the drops to run down the agar surface) on the same petri dish as triplicate. The plates were incubated for 18 h at 37 °C.

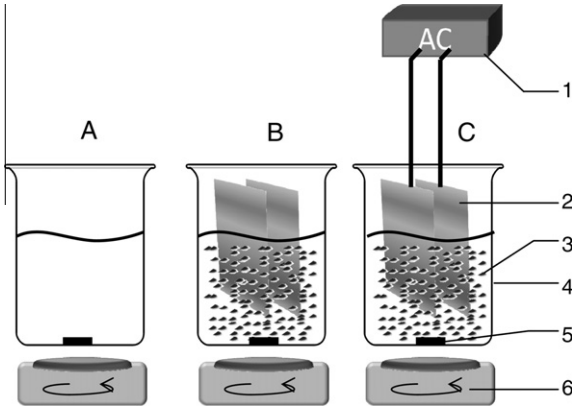

Fig. 1 – The FBE disinfection research set-up. (1) Alternating power source (2) Stainless steel electrode plates. (3) Granulated activated carbon (GAC). (4) 1 L beaker glass. (5) Magnetic stirring bar. (6) Magnetic stirrer. Beaker A: blank 7 (Table 1) (includes 1/4 LB media and bacteria, and excludes electrodes, GAC, applied RF-AC electric field); Beaker B: blank 6 (Table 1) (includes 1/4 LB media, bacteria, electrodes, GAC, and excludes RF-AC electric field); Beaker C: disinfection sample 0 (Table 1 (includes all experimental factors: 1/4 LB media, electrodes, GAC, RF-AC electric field, bacteria).

2.6. Testing the influence of experimental factors on bacterial survival and inactivation in FBE system

A set of blanks was designed (Table 1) to test the relevance of each experimental factor involved in FBE disinfection process. During each experiment different combinations of experimental factors were investigated and the disinfection was measured by the changes in luminescence intensity level. Experimental factors are the parts of the FBE reactor system: Electrodes, RF-AC electric field (100 kHz; 6 ± 0.5 V/cm) and GAC; and the liquid composition: LB medium and bacteria (*E.coli* YMc10). Samples were compared using a hypothesis t-test (compared means with $\alpha = 0.05$). Before the hypothesis tests, samples were verified to be normally distributed.

2.7. The effect of RF-AC on FBE disinfection

The relation between AC frequency and FBE disinfection efficiency was investigated. Duplicate experiments were performed at a various frequencies in a radio frequency range from 80 to 200 kHz, with a step size of 20 kHz. The voltage applied was 6 ± 0.5 V/cm. The reaction media containing 1/4 LB media had a conductivity of 5.2 ± 0.05 mS/cm and the average current measured was 163 ± 5 mA/cm^2 electrode. For each experiment conditions such as temperature, pH, GAC amount, stirring intensity by magnetic stirrer, RF-AC amplitude and media composition were kept constant. A control without AC electric field (Table 1 blank 6; Fig. 1(B)) was running parallel to the disinfection sample (Table 1 disinfection sample (0); Fig. 1(C)).

Samples were taken every hour and plated on agar for CFU counts. The obtained data was statistically analyzed (t-test) to compare the two independent runs from the same frequency (duplicates). Statistically equal samples were averaged. The

Table 1 – Experimental scheme of different experimental factors (LB medium, Electrode plates, GAC, RF-AC, *E.coli* YMc10) investigated for their influence on the performance of FBE disinfection. "+" experimental factor is present during experiment, "–" experimental factor is absent during experiment.

	Disinfection sample (0)	Blank 1	Blank 2	Blank 3	Blank 4	Blank 5	Blank 6	Blank 7
1/4 LB medium	+	+	–	–	+	+	+	+
Electrodes[a]	+	–	+	–	+	+	+	–
Granulated activated carbon (GAC)	+	+	+	+	–	–	+	–
Radio frequency alternating el. field (RF-AC 100 kHz; 6 ± 0.5 V/cm)[b]	+	–	+	–	–	+	–	–
E.coli YMc10 (bacteria)	+	+	+	+	+	+	+	+

[a] Stainless steel electrodes are present but no RF-AC is applied Fig. 1(B).
[b] Stainless steel electrodes are present and RF-AC is applied Fig. 1(C).

normal distribution of the samples was verified using STATDISK 10.4.0 [40].

3. Results and discussion

3.1. GAC properties

The properties of pretreated (autoclaved) carbon (GAC_{pr}) and carbon before pretreatment (GAC_r) are compared in Table 2. Analyzed carbons have moderately high surface areas (BET) [41]. The pretreatment and milling does not influence the change in BET area (Table 2).

The macropore area represents a small percentage of total surface area (Table 2). This small macropore area is not in favor for bacterial adhesion. The size of macropores is defined as larger than 50 nm. The *E. coli* size is in the range of 1 μm, therefore they can only adhere at the external surface and in the larger macropores, and not in meso or micro pores [42].

The pH_{pzc} values (Table 2) indicate that GAC_r is basic, but basicity was diminished after pretreatment (GAC_{pr}). One of the pretreatment steps consisted of autoclaving at a pressure of 0.1 MPa and a temperature of 121 °C. These autoclaving conditions may oxidize the GAC surface slightly [42]. Through oxidation of the GAC, the increase of the amount of surface oxygen complexes influences the amphiphilic character of the solid surface and the pH_{pzc} [43]. The pH_{pzc} together with amphiphilic properties of bacteria and solid surfaces are parameters that determine the extent of bacterial adhesion on the surfaces [44]. *E. coli* pH_{pzc} is in range 2.1 and 4.3 [45,46]. Therefore *E. coli* adhere better on hydrophobic than on hydrophilic surfaces [47]. The increased hydrophilic properties of GAC after pretreatment comparing to GAC_r are expected to decrease the bacterial adhesion. The pretreatment was applied for the sterilization of the materials prior to the experiments, but apparently it also changes GAC properties so that *E. coli* YMc10 adhere in lesser extent on GAC.

3.2. Luminescence measured in a set of blanks

In Figs. 2–4, the luminescence intensity change in counts per second (CPS) of the luminescent *E. coli* YMc10 was used to quantify the disinfection during 360 min: the disinfection sample (Table 1) and different blanks (Table 1) were compared; disinfection sample is presented in all the (Figs. 2–4) to show the relation between sample and the blanks. In case an electric field was applied, the field was 100 kHz; 6 ± 0.5 V/cm.

For blanks 2 and 3 no nutrients were added, (Fig. 2) and an exponential decrease of the CPS value was observed with no difference between RF-AC electric field applied (blank 2) or in absence of an RF-AC electric field (blank 3). The decrease of the CPS value in these blanks is much faster (from 10^6 CPS to 10^1 CPS in 30 min) than for the disinfection sample (from 10^6 CPS to 10^1 CPS in 180 min). The fast decrease of the CPS value is caused by physiological stress due to nutrient limitation and unfavorable osmotic conditions [48]. This indicates the necessity to add salt to maintain the isotonic pressure necessary for an intact cell membrane and nutrients to keep bacteria luminescence active.

For the blanks 4, 5 and 7 that did not contain GAC (Fig. 1A), no decay in the CPS value could be observed in comparison to the disinfection sample (0) (Fig. 3).

For the solution with GAC a decrease in *E. coli* YMc10 luminescence value (CPS) was observed in both cases (Fig. 4): with the applied RF-AC electric field and without the RF-AC electric field (Table 1, blank 6 and blank 1).

In the presence of an RF-AC electric field and after 150 min the CPS values decreased from 10^6 to 10^1, whereas in the absence of an RF-AC electric field CPS values decreased from

Table 2 – GAC physical characteristics (Carbon type, surface area (BET), macropore area ($A_{macropore}$) percentage in total BET area, pH point of zero charge (pH_{pzc}) of GAC and PAC before pretreatment (GAC_r; PAC_r); and GAC and PAC pretreated (GAC_{pr}; PAC_{pr})). Values are given with ± standard deviation.

Activated carbon RX 3 EXTRA type	BET area, m^2/g	$A_{macropore}$, in% of BET	pH_{pzc}
Granulated activated carbon before pretreatment (GAC_r)	1407 (±48)	<1	8.45 (±0.10)
Granulated activated carbon pretreated (GAC_{pr})	1428 (±89)	<1	7.77 (±0.17)
Powder activated carbon before pretreatment (PAC_r)	1424 (±92)	<10	8.80 (±0.21)
Powder activated carbon pretreated (PAC_{pr})	1417 (±90)	<10	7.54 (±0.23)

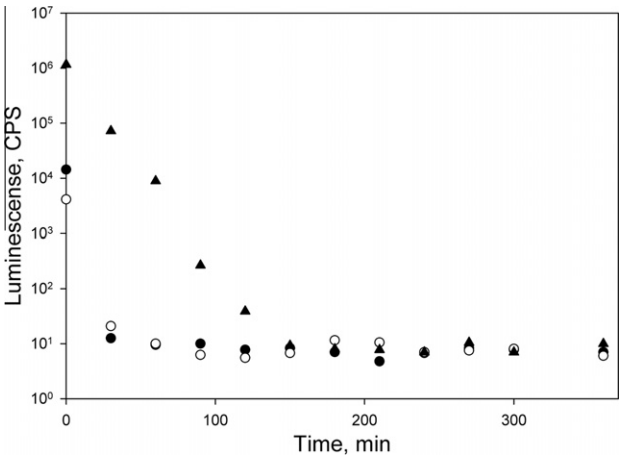

Fig. 2 – The effect of nutrients absence on luminescence intensity. Comparison of disinfection sample 0 ▲ (1/4 LB media, electrodes, GAC, 100 kHz; 6 ± 0.5 V/cm RF-AC electric field, bacteria), Blank 2 ● (no LB media), Blank 3 ○ (no LB media, no electrodes, no RF-AC electric field).

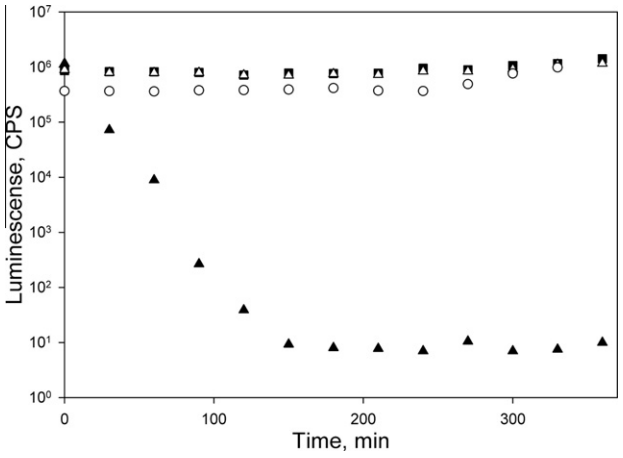

Fig. 3 – The effect of GAC on luminescence intensity. Comparison of disinfection sample 0 ▲ (1/4 LB media, electrodes, GAC, 100 kHz; 6 ± 0.5 V/cm RF-AC electric field, bacteria), Blank 4 ■ (no GAC, no RF-AC electric field), Blank 5 Δ (no GAC), Blank 7 ○ (no electrodes, no GAC, no RF-AC electric field).

10^6 to 10^4 (Fig. 4). The difference in decline of CPS intensity between with and without applied RF-AC electric field demonstrates that the RF-AC electric field combined with GAC disinfects the fluid. However, the presence of GAC alone also leads to the decrease of the CPS values over time. Moreover, CPS measurement was found to be influenced by GAC powder by light scattering [49]. Powder of activated carbon (PAC) is produced whilst fluidizing the reactor, as was confirmed by microscopy studies of fluid samples (data not shown). The GAC powder and the bacteria are likely to form GAC-bacteria aggregates. These aggregates and the presence of GAC powder interfered with the luminescence measurement (CPS), which leads to the observed decrease in the CPS intensity in the absence of RF-AC fields. The CPS monitoring is a valuable and

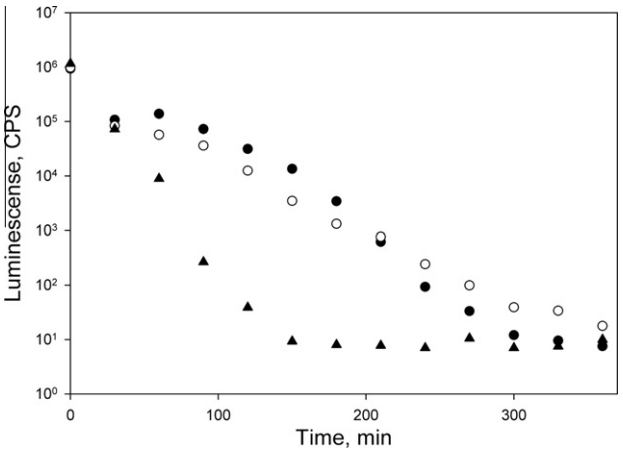

Fig. 4 – The effect of RF-AC on luminescence intensity. Comparison of disinfection sample 0 ▲ (1/4 LB media, electrodes, GAC, 100 kHz; 6 ± 0.5 V/cm RF-AC electric field, bacteria), Blank 1 ○ (no electrodes, no RF-AC electric field), Blank 6 ● (no RF-AC electric field).

handy method for FBE parameter screening, but viable cell concentration determination by plating (CFU) is needed to accurately quantify disinfection as was done in the experiments discussed below. Thus, by using bioluminescent *E. coli* YMc10, we were able to show that disinfection with the FBE is dependent on the combination of RF-AC and GAC particles.

3.3. *The effect of RF-AC on disinfection*

The previous experiments were performed at a constant 100 kHz RF-AC. In this experiment, the effect of various frequencies (in range 80–200 kHz) RF-AC on the FBE disinfection was investigated. The effect of frequency was compared to a control (Table 1, blank 6) without applied electric field. Preliminary experiments (data not shown) showed that luminescence intensity decrease of *E. coli* YMc10 is frequency dependent. The decrease in *E. coli* YMc10 viability was subsequently confirmed with the CFU method as disinfection in time (top to bottom in Fig. 5). The largest decrease of *E. coli* YMc10 concentration was detected at a frequency of 140 kHz. A similar effect of electric field frequency on red blood cell fusion has been reported by Chang [50] at 0.5–5 kV/cm in the range of 80 kHz to 1000 kHz, however without the use of carbon particles. The time required for *E. coli* YMc10 concentration (CFU/mL) to decrease is 1 to 5 h in the FBE. This is longer than disinfection with DC in range 3–110 kV [13,17] or low frequency electric field at 16–60 Hz with electric current 125–370 mA/cm^2 electrode [23,51]. Nevertheless the FBE system operates at a low electric field strength which enables the use of a conductive solution that contains particles or other impurities next to bacteria. This is not the case for DC methods [13,17,22].

The disinfection efficiency depends on the applied frequency (Fig. 5). The highest efficiency within 5 h time was reached at 140 kHz within the frequency range tested at an average power consumption of 99 ± 1 W per 1 L of disinfected solution. To confirm if the disinfection sample is significantly

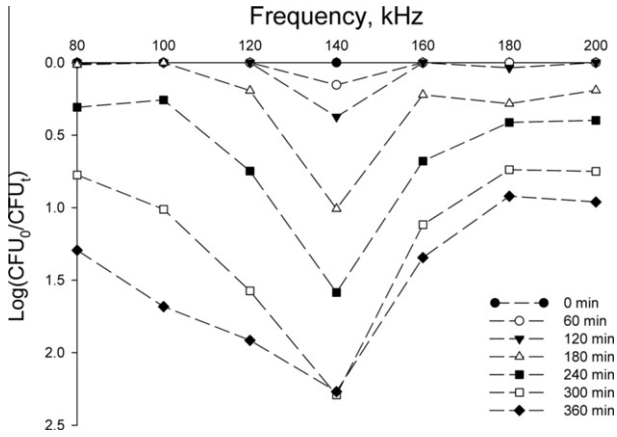

Fig. 5 – Disinfection with FBE system at 6 ± 0.5 V/cm and 163 ± 5 mA/cm^2 electrode at different frequencies. *E. coli* YMc10 viable cell concentration (log(CFU_0/CFU_t)) within 360 min (series). *Dashed lines connect the same time points from different frequencies.

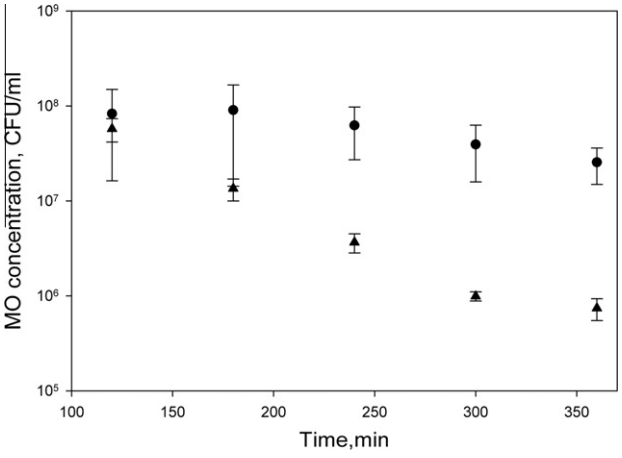

Fig. 6 – Comparison of the effect of GAC (Control 6 ●) and the GAC combined with RF-AC (disinfection sample (0) ▲) at 140 kHz. Error bars indicate standard deviations.

different from a control (without applied RF-AC), the standard deviations of control and the disinfection sample were calculated (Fig. 6). Bacterial concentration (CFU/mL) in the control remains constant during 360 min. In the disinfection sample the bacterial concentration (CFU/mL) exponentially decrease after 120 min of FBE disinfection. The FBE disinfection performance at 140 kHz differs significantly ($p = 0.05$) between disinfection sample and control after 120 min of treatment.

The variation of *E. coli* YMc10 concentration in the control (Fig. 6) can be explained by the following two phenomena: adhesion of *E. coli* YMc10 on GAC particles and/or the effect of the weak magnetic field from the stirrer on the *E.coli* [52]. When bacteria adhere on GAC they might loose the viability by membrane disruption [30]. Bacteria have cell structures that make adherence on rough surfaces possible [53]. To completely adhere *E. coli* on GAC particles at least 3 days of shaking with GAC at 100 rpm is necessary [46]. In our case without an applied RF-AC electric field, 0.5 log CFU/mL (Fig. 6) of expected adhesion was observed. The low adhesion level can be explained with the relatively short experimental time (360 min) and the turbulent character of the fluidized bed. The weak magnetic field from the magnetic stirrer could have a synergistic effect with the alternating current and cause the decrease of *E. coli* YMc10 concentration in the control and in the sample. Nevertheless in this study the magnetic field is not considered as experimental factor as all the blanks and samples were stirred with the magnetic stirrer. Therefore the effect of magnetic field is not investigated in this study. The measured decrease of viable *E. coli* YMc10 concentration by CFU counts and photometric measurement (in CPS) shows the actual disinfection rate due to the applied conditions.

4. General discussion on possible electrical disinfection mechanisms

Electric fields can lead to induced currents in microorganisms. Different frequencies can lead to phenomena such as: surface polarization [54,55]; increased cell membrane permeability and change in shape [50]. At the resonance frequency for each microorganism the polarization effect will be maximal. Due to the polarization effect on the outer cell membrane, a potential difference can build up between the inside of the cell membrane and the outer wall similar to a diode. By this effect cells can be damaged through the blockage of the voltage gated channels [56,57]. The effect of the resonance frequency on cell wall permeability has been established in cell fusion by induced increase in membrane potential [50]. An increased membrane potential could also cause membrane disruption followed by leakage of cellular content [16].

Cells are found to rotate in alternating electrical fields [54] possibly causing mechanical cell fatigue [58]. Cell rotation characteristics are dependent on a lot of factors such as: cell aging, viability etc. [59,60]. For the pathogenic *E. coli* K12 rotation torque arises at the frequency between 100 and 500 kHz [61], which is consistent with the frequency range applied in our study.

In RF-AC electric fields a time dependent effect of cavitation [62] can cause changes in cell shape [50]. Moreover it induces physical disintegration of microbial cells and e.g. *E. coli* cells are highly susceptible to it [63].

Furthermore, the magnetic field from the magnetic stirrer may play a role in FBE disinfection. Studies have shown that weak magnetic fields in combination with electric fields could disturb the biochemical equilibrium in microbial cells e.g. by the formation of radical species [64–66]. The weak magnetic fields, in combination with alternating electric fields influence the dissociation probability of Ca, Mg, Zn ion-proteins from their carrier DNA strands in *E.coli* cells [52].

The cause of microorganism death in most above mentioned cases is affected by pH, temperature, radical formation and conductivity of the disinfected medium [19,64,67]. Granulated activated carbon seems to serve as fluidized conductive component with a high surface area that enhances these cell-electric field interactions.

5. Conclusions

This study presents the first data on the disinfection with a fluidized bed electrode (FBE) system using an alternating radio frequency field (RF-AC) combined with a granulated activated carbon (GAC) suspension. This opens the way to evolve the FBE to a technology in addition to other disinfection systems such as chemical treatment and UV light application. The advantage of the FBE system is its simplicity, which leads to a robust and low maintenance technology, not requiring addition of chemicals. The FBE could be added to a water treatment chain as a tertiary step. It was found that GAC was the main experimental factor allowing FBE disinfection to take place at the low electric field strength (6 ± 0.5 V/cm). Furthermore, the disinfection performance in the FBE was found to be dependent on the frequency. The best disinfection rate, with a decrease of *E. coli* concentration from 10^8 to 10^6 CFU/mL, was obtained at 140 kHz. Further research is needed to establish whether this is a unique frequency or whether other optima exist under different circumstances. Additionally, it is required to gain insights in the exact mechanisms underlying the disinfection process. This will be of great importance in developing FBE to a technology that can be applied in practice.

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REFERENCES

[1] WHO/UNICEF. Progress on sanitation and drinking-water: WHO/UNICEF; 2010.
[2] EPA US. Guidelines for water reuse. Washington, DC; EPA/625/R-04/108; 2004.
[3] Howard G, Charles K, Pond K, Brookshaw A, Hossain R, Bartram J. Securing 2020 vision for 2030: climate change and ensuring resilience in water and sanitation services. J Water Clim Change 2010;01(1):1–15.
[4] Richter BD, Mathews R, Harrison DL, Wigington R. Ecologically sustainable water management: managing river flows for ecological integrity. Ecol Appl 2003;13(1):206–24.
[5] Ferreira BK. Three-dimensional electrodes for the removal of metals from dilute solutions: a review. Miner Process Extr Metall Rev 2008;29:330–71.
[6] Kim BR, Anderson JE, Mueller SA, Gaines WA, Kendall AM. Literature review-efficacy of various disinfectants against Legionella in water systems. Water Res 2002;36(18):4433–44.
[7] Tyrrell SA, Rippey SR, Watkins WD. Inactivation of bacterial and viral indicators in secondary sewage effluents, using chlorine and ozone. Water Res 1995;29(11):2483–90.
[8] Andreozzi R, Caprio V, Insola A, Marotta R. Advanced oxidation processes (AOP) for water purification and recovery. Catal Today 1999;53(1):51–9.
[9] Azbar N, Yonar T, Kestioglu K. Comparison of various advanced oxidation processes and chemical treatment methods for COD and color removal from a polyester and acetate fiber dyeing effluent. Chemosphere 2004;55(1):35–43.
[10] Melemeni M, Stamatakis D, Xekoukoulotakis NP, Mantzavinos D, Kalogerakis N. Disinfection of municipal wastewater by TiO_2phtocatalysis with UV-A visible and solar irradiation and BDD electrolysis. In: Proceedings of the 9th International Conference "Protection and Restoration of the Environment" (PRE9). Greece: Global Nest Journal: 2009.
[11] Feng C, Suzuki K, Zhao S, Sugiura N, Shimada S, Maekawa T. Water disinfection by electrochemical treatment. Bioresour Technol 2004;94(1):21–5.
[12] Sato M, Ohgiyama T, Clements JS. Formation of chemical species and their effects on microorganisms using a pulsed high-voltage discharge in water. IEEE Trans Industry Appl 1996:106–12.
[13] Mazurek B, Lubicki P, Staroniewicz Z. Effect of short HV pulses on bacteria and fungi. IEEE Trans Dielectr Electr Insul 1995:418–25.
[14] Barnes FS. Interaction of DC and ELF electric fields with biological materials and systems. In: Barnes FS, Greenebaum B, editor. Handbook of biological effects of electromagnetic fields: bioengineering and biophysical aspects of electromagnetic fields. 3rd ed. CRC/Taylor & Francis; 2007. p. 115–52.
[15] Aaron RK, Boyan BD, Ciombor DM, Schwartz Z, Simon BJ. Stimulation of growth factor synthesis by electric and electromagnetic fields. Clin Orthopaed Rel Res 2004;419:30–7.
[16] Palaniappan S, Sastry SK, Richter ER. Effects of electricity on microorganisms: a review. J Food Process Pres 1990;14(5):393–414.
[17] Narsetti R, Curry RD, McDonald KF, Clevenger TE, Nichols LM. Microbial inactivation in water using pulsed electric field and magnetic pulse compressor technology. IEEE Trans Plasma Sci 2006:1386–93.
[18] Zimmermann U, Neil GA. Electromanipulation of cells. CRC press; 1996.
[19] Jeyamkondan S, Jayas DS, Holley RA. Pulsed electric field processing of foods: a review. J Food Prot 1999;62:1088–96.
[20] Chiabrera A, Bianco B, Moggia E, Kaufman JJ. Zeeman–Stark modeling of the RF EMF interaction with ligand binding. Bioelectromagnetics 2000;21:312–24.
[21] Matsunaga T, Nakasono S, Kitajima Y, Horiguchi K. Electrochemical disinfection of bacteria in drinking water using activated carbon fibers. Biotechnol Bioeng 1994;43:429–33.
[22] Lian M, Islam N, Wu J. AC electrothermal manipulation of conductive fluids and particles for lab-chip applications. IET Nanobiotechnol 2007;1(3):36–43.
[23] Birbir M, HacIoglu H, Birbir Y, Altug G. Inactivation of *Escherichia coli* by alternative electric current in rivers discharged into sea. J Electrostat 2009;67(4):640–5.
[24] Tracy RLJ. Lethal effect of alternating current on yeast cells. J Bacteriol 1932;24(6):423–38.
[25] Tekle E, Astumian RD, Chock PB. Electroporation by using bipolar oscillating electric field: an improved method for DNA transfection of NIH 3T3 cells. Proc Natl Acad Sci USA: PubMed 1991:4230–4.

[26] Geveke DJ. Non-thermal processing by radio frequency electric fields. In: Sun D-W, editor. Emerging technologies for food processing. Elsevier Ltd: 2005. p. 307–22.
[27] Mayer MJJ, Metz SJ, Klijn G-J. Device and method for disinfection and/or purification of a fluid. NL patent 033021 2010.
[28] Wang B, Kong W, Ma H. Electrochemical treatment of paper mill wastewater using three-dimensional electrodes with Ti/Co/SnO2-Sb2O5 anode. J Hazard Mater 2007;146(1-2):295–301.
[29] Xiong Y, Strunk PJ, Xia H, Zhu X, Karlsson HT. Treatment of dye wastewater containing acid orange II using a cell with three-phase three-dimensional electrode. Water Res 2001;35(17):4226–30.
[30] van der Mei HC, Atema-Smit J, Jager D, Langworthy DE, Collias DI, Mitchell MD, et al. Influence of adhesion to activated carbon particles on the viability of waterborne pathogenic bacteria under flow. Biotechnol Bioeng 2008;100:810–3.
[31] Yamamoto O, Nakakoshi K, Sasamoto T, Nakagawa H, Miura K. Adsorption and growth inhibition of bacteria on carbon materials containing zinc oxide. Carbon 2001;39(11):1643–51.
[32] Carlson S. Mikrobiologie des Wassers. In: Höll K, ed. Wasser: nutzung im kreislauf, hygiene, Analyse und Bewertung. 8th ed. Walter de Gruyter; 2002. p. 285–389.
[33] Corapcioglu MO, Huang CP. The surface acidity and characterization of some commercial activated carbons. Carbon 1987;25(4):569–78.
[34] Fiol N, Villaescusa I. Determination of sorbent point zero charge: usefulness in sorption studies. Environ Chem Lett 2009;7(1):79–84.
[35] Steinberg SM, Poziomek EJ, Engelmann WH, Rogers KR. A review of environmental applications of bioluminescence measurements. Chemosphere 1995;30(11):2155–97.
[36] Engebrecht J, Nealson K, Silverman M. Bacterial bioluminescence: Isolation and genetic analysis of functions from Vibrio fischeri. Cell 1983;32(3):773–81.
[37] Mavrov V, Fähnrich A, Chmiel H. Treatment of low-contaminated waste water from the food industry to produce water of drinking quality for reuse. Desalination 1997;113(2-3):197–203.
[38] Boyer M, Wisniewski-Dyé F. Cell–cell signalling in bacteria: not simply a matter of quorum. FEMS Microbiol Ecol 2009;70:1–19.
[39] Miles AA, Misra SS, Irwin JO. The estimation of the bactericidal power of the blood. Int J Hyg Environ Health 1938;38:732–49.
[40] Triola MF. Elementary statistics: with multimedia study guide: Pearson Education, Limited; 2007.
[41] Gryglewicz G, Machnikowski J, Lorenc-Grabowska E, Lota G, Frackowiak E. Effect of pore size distribution of coal-based activated carbons on double layer capacitance. Electrochim Acta 2005;50(5):1197–206.
[42] Bandosz TJ. Activated carbon surfaces in environmental remediation: Elsevier; 2006.
[43] Marsh H, Rodriguez-Reinoso F. Activated carbon: Elsevier; 2006.
[44] Rijnaarts HHM, Norde W, Lyklema J, Zehnder AJB. The isoelectric point of bacteria as an indicator for the presence of cell surface polymers that inhibit adhesion. Colloids Surf B 1995;4(4):191–7.
[45] Lytle DA, Rice EW, Johnson CH, Fox KR. Electrophoretic mobilities of *Escherichia coli* O157:H7 and wild-type *Escherichia coli* strains. Appl Environ Microbiol 1999;65(7):3222–5.
[46] Moreno-Castilla C, Bautista-Toledo I, Ferro-Garcķa MA, Rivera-Utrilla J. Influence of support surface properties on activity of bacteria immobilised on activated carbons for water denitrification. Carbon 2003;41(9):1743–9.
[47] Ong Y-L, Razatos A, Georgiou G, Sharma MM. Adhesion Forces between E. coli Bacteria and Biomaterial Surfaces. Langmuir 1999;15(8):2719–25.
[48] Prescott LM, Harley JP, Klein DA. Microbiology. 5th ed.: McGraw-Hill; 2002.
[49] Masschelein W, Rice RG. Ultraviolet light in water and wastewater sanitation: Lewis Publishers; 2002.
[50] Chang DC. Cell poration and cell fusion using an oscillating electric field. Biophys J 1989;56(4):641–52.
[51] Park J-C, Lee MS, Lee DH, Park BJ, Han D-W, Uzawa M, et al. Inactivation of bacteria in seawater by low-amperage electric current. Appl Environ Microbiol 2003;69(4):2405–8.
[52] Binhi VN, Alipov YD, Belyaev IY. Effect of static magnetic field on E. coli cells and individual rotations of ion–protein complexes. Bioelectromagnetics 2001;22(2):79–86.
[53] Coelhoso I, Boaventura R, Rodrigues A. Biofilm reactors: An experimental and modeling study of wastewater denitrification in fluidized-bed reactors of activated carbon particles. Biotechnol Bioeng 1992;40:625–33.
[54] Markx GH, Davey CL. The dielectric properties of biological cells at radiofrequencies: applications in biotechnology. Enzyme Microb Technol 1999;25(3–5):161–71.
[55] Pethig R, Kell DB. The passive electrical properties of biological systems: their significance in physiology, biophysics and biotechnology. Phys Med Biol 1987;32(8):933.
[56] Beurrier C, Bioulac B, Audin J, Hammond C. High-frequency stimulation produces a transient blockade of voltage-gated currents in subthalamic neurons. J Neurophysiol 2001;85(4):1351–6.
[57] Panagopoulos DJ, Karabarbounis A, Margaritis LH. Mechanism for action of electromagnetic fields on cells. Biochem Biophys Res Commun 2002;298(1):95–102.
[58] Pohl S, Herbert A. Natural cellular electrical resonances. Int J Quantum Chem 1982;22:399–409.
[59] Holzapfel C, Vienken J, Zimmermann U. Rotation of cells in an alternating electric field theory and experimental proof. J Membr Biol 1982;67(1):13–26.
[60] Mischel M, Pohl HA. Cellular spin resonance. Theory and experiment. J Biol Phys 1983;11(3):98–102.
[61] Berg HC, Turner L. Torque generated by the flagellar motor of *Escherichia coli*. Biophys J 1993;65(5):2201–16.
[62] Gogate PR. Cavitation: an auxiliary technique in wastewater treatment schemes. Adv Environ Res 2002;6(3):335–58.
[63] Foladori P, Laura B, Gianni A, Giuliano Z. Effects of sonication on bacteria viability in wastewater treatment plants evaluated by flow cytometry-Fecal indicators, wastewater and activated sludge. Water Res 2007;41(1):235–43.
[64] Funk RHW, Monsees TK. Effects of electromagnetic fields on cells: physiological and therapeutical approaches and molecular mechanisms of interaction. Cells Tissues Organs 2006;182(2):59–78.
[65] Grissom CB. Magnetic field effects in biology: a survey of possible mechanisms with emphasis on radical-pair recombination. Chem Rev 1995;95(1):3–24.
[66] Sheppard AR, Swicord ML, Balzano Q. Quantitative evaluations of mechanisms of radiofrequency interactions with biological molecules and processes. Health Phys 2008;95(4):365–96.
[67] Murphy JC, Kaden DA, Warren J, Sivak A. Power frequency electric and magnetic fields: A review of genetic toxicology. Mutat Res-Rev Gen 1993;296(3):221–40.