Alternating field activated carbon fluidized bed electrode for water disinfection

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Alternating field activated carbon fluidized bed electrode for water disinfection

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For my parents and grandparents
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Chapter 1

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General introduction
1.1. Water scarcity in global perspective

Society is founded on water: we need water for drinking, food production, cleaning, sanitation, energy production, commercial and industrial applications. The water use distribution per sector on a global scale is shown in Figure 1.1 (adapted from Cisneros et al.(2008)).

About 70% of the earth’s surface is covered with water. However, from this amount only 0.3% is easily accessible for humans, and only 0.06% is of drinking water quality (Ahuja 2009, Stikker 1998). Most of the drinking water is located in the northern hemisphere, leaving the areas close to the equator and other localized regions in a risk of scarcity for drinking water (Fung et al. 2011). The other two world-wide problems related to water are due to i) extensive urbanization (expanding agriculture and industry); and ii) a lack of access to safe drinking water and proper sanitation in developing countries. The lack of sanitation facilities is one of the major causes of 2 million children death each year (Ahuja 2009).

United Nations released the document “World Development Millennium Goals” in which Goal number 7 stimulates to ensure environmental sustainability; with one of the
targets to improve access of populations to safe drinking water and basic sanitation (United Nations 2013).

The access to safe drinking water improved since the introduction of these millennium goals in 2001, but still 10 % of the world’s population was without access to safe drinking water in 2010. Moreover, 605 million people will still lack access to safe drinking water and 2.5 billion people (36 % of world population) will lack access to improved sanitation facilities in 2015 (United Nations 2013). Furthermore, climate change and intensive urbanization are of the increasing concern (Cisneros et al. 2008). Most of the world’s fresh water is used by agriculture in developing countries and shared between agriculture and industry in developed countries (Figure 1.1). Potential drinking water sources – rivers and lakes (such as: Yangtze in China; Sarno, Danube in Europe, King River in Australia and 40-50 % of U.S. rivers and lakes) are microbiologically and chemically polluted. For instance, Asian rivers have on average 3 times more pathogens from feces than the worlds average (Ahuja 2009), and some local watersheds are even much more polluted.

According to a current scenario, by 2030 half of the world’s population will experience drinking water stress (Smakhtin et al. 2004). There are already many places in the world where water demand and consumption (due to population growth) are exceeding the regional water resources availability (Kummu et al. 2010). From Figure 1.2 it can be observed that there are regions (such as Asia and Africa) in which part of the population is already experiencing extreme water shortage (dark read filling) (Kummu et al. 2010). The world average water availability was 8.4 m³ per capita per year, according the data in 2006. However, variations between continents and sub-continents are very high e.g. in the Middle East and North Africa it is 1.4 m³ per capita per year, in Australia and Oceania is 53.3 m³ per capita per year and in Europe 10.7 m³ per capita per year (Cisneros et al. 2008).
The expression per capita per year per country/region does not always represent the real water availability situation, e.g. in Australia and Oceania high water availability is indicated, however inland Australia has very arid areas (such as Great Victoria and Great Sandy deserts). This indicates that in many water scarce regions of the world vast and appropriate measures should be taken to reduce current and future risks of water scarcity. “As a society, we no longer have the luxury of using water only one time; however, we have the responsibility of maintaining and ensuring water quality” (Levine and Asano...
2004). Among one of the most important measures it is the reclamation of used water and purification to appropriate level needed for reuse.

Reclaimed water reuse is being practiced in developed and developing countries. For developed countries, it is mainly driven by economical and legal reasons. For example with urban water management implementation in the cities when centralized and decentralized water approaches are combined it can reduce external water demand up to 50 % (Agudelo-Vera et al. 2011). In developing countries untreated wastewater is often used in households for lower quality applications such as irrigation to recycle the nutrients to the land for (urban) agriculture (Jiménez et al. 2012). In both cases wastewater is considered as a resource, however, due to missing legislative basis, the water reuse concept is still difficult to realize in practice (Cisneros et al. 2008, Jefferson et al. 2004).

Considering water reuse, care should be taken for pathogenic microorganisms (Pandit et al. 2012b) to prevent waterborne diseases and viruses spread through water. Here, disinfection is a very important process that can be implemented in various places in the water cycle, and which will need to be extended in the future.

1.2. Disinfection principles and headlines

1.2.1. The historic perspective in the approach to pathogenic organisms

Disinfection has been mentioned already 2000 years BC in ancient Sanskrit, where was prescribed to expose water to sunlight, boil it and dip a hot piece of copper into it seven times and then filter it through charcoal (Faust and Aly 1983, Rajeshwar and Ibanez 1997). In 300 BC in Roman Empire drinking water was supplied by an aqueduct system and wastewater was collected separately in a central sewer that discharged to the river Tiber. However, it took almost 1500 years before the first central sewer was build in the U.K. and another few hundred years till it was also build in France. In the mean time, waterborne pandemics such as Typhus (e.g. in 1 month killed 18 000 people in Granada in 15th century) and Cholera (e.g. during few months killed 20 000 people in Paris in mid-19th century) were devastating Europe, India, Russia, and U.S. It reached its apogee in 1850
with 3 deaths per 100 people (Kaufman 2009). The rate of waterborne pandemic diseases were increasing due to doubling population in urban areas and untreated sewage use for agriculture, discharge to the rivers that were used as drinking water source and shallow drinking water wells. Back in those times, people believed in the theory of spontaneous generation of diseases (Cooper 2001). In mid-19th century, John Snow discovered the link between diseases and polluted food or water consumption. The relation between waterborne and airborne microorganisms and diseases was established in 1870s in the works of Louis Pasteur (Sykes 1958). However, water disinfection to reduce outbreaks of diseases has been introduced only since 1900; and shortly after that continuous disinfection of drinking water by chlorine was established (Pandit et al. 2012b, Rajeshwar and Ibanez 1997). Nowadays, in urbanized areas part of drinking water and in some regions also wastewater is disinfected (Davis 2010).

The distinction between disinfection and sterilization should be mentioned. **Sterilization** is referring to total removal/inactivation of living organisms present, whereas **disinfection** refers to the removal/inactivation of organisms to a safe level (Sykes 1958).

The specific amount of infectious organisms (viruses, bacteria and their spores, protozoa and their cysts, worms and their eggs) in water can cause waterborne diseases. The pathogens can be spread via drinking water or other sources such as environmental, recreational, contaminated foods and industrial run-offs (Figure 1.4). However, the cause of waterborne infection is dependent on the virulence of the pathogens and immunity of the host (Mara and Horan 2003). Vulnerable groups of the population are young, elderly, sick and immune-deficient people (Pandit et al. 2012b). The large scale introduction of sewers and use of water treatment techniques since beginning of 20th century, such as filtration and disinfection, reduced the waterborne outbreaks in developed areas. Exceptions are rare cases due to failure of treatment systems or mankind mistakes, or when untreated sewage is discharged to receiving waters that are used as potable water source (e.g. 20 000 people got infected in Delhi in 1950s and in Shanghai in 1980s) (Chen et al. 2006). In developing countries, however, waterborne diseases and infections remain
the most common causes of deaths and contribute to 80% of all sicknesses (Pandit et al. 2012b).

Knowledge of the environmental and health effects caused by wastewater discharges gained through the years made the shift from conventional treatment techniques to advanced techniques for effective removal of micro-pollutants and pathogens, that otherwise might contribute to long term hazards (Gogate 2002).

1.2.2. Disinfection in water cycle perspective

The disinfection of water can take place in any stage of the water lifecycle (Figure 1.3). The lifecycle of water begins from its source. The treatment level depends on the source properties and intended use (Figure 1.4) (Levy et al. 2011). Water after it has been used; can be either discharged or recycled. In many situations, water that is supplied to the drinking water infrastructure needs to be treated to prevent introduction of harmful microorganisms. Also, wastewater and in some cases wastewater treatment plant effluents have to be treated before being discharged into lakes or streams as self-purification can only take place to a system-dependent extent (Davis 2010). Recycling of water is a very important issue in the perspective of water scarcity. Regulations for reuse of wastewater were introduced in California state in 1918 (California Code regulations Title 22) (Cornel and Meda 2008) and large scale reuse began in 1970s (Strathmann et al. 2011). It took almost two decades, when in 1989 the WHO released guidelines for the quality of wastewater (including wastewater treatment effluents) for use in agriculture and aquaculture. These guidelines were adopted by the U.S. EPA in their water reuse manual of 1992. In all these documents one of the main criteria to be monitored is the microbiological water quality, leading to disinfection of wastewater as one of the important steps in the water reclamation-and-reuse cycle (Exall et al. 2004). Many possible cycles are possible for water reuse (Figure 1.3) and disinfection can have a different function in each of them.
Here, distinction should be made between direct and indirect water reuse. **Direct** water reuse refers to treated or untreated wastewater use straight from the facility (e.g. treatment plant, industry effluent); **indirect** water reuse refers to wastewater use after it has been discharged to receiving waters that are again used as a water source (Exall et al. 2004). Direct water reuse is mainly implemented in industry due to financial and legislative reasons. For direct wastewater reuse in irrigation, secondary treatment combined with disinfection is the lowest level of treatment recommended, but often not required (Levy et al. 2011). Water reuse is practiced in water scarce areas such as parts of Middle East, Australia and southwest U.S. In Europe most projects for wastewater reuse are in southern countries such as Cyprus, Spain, Italy and France (Cornel and Meda 2008, Exall et al. 2004). Otherwise, water reuse is mainly indirect, where the treated water is discharged to receiving waters that subsequently are used as source for production of drinking water. In general, there is a lack of information on direct wastewater reuse scale in Europe, probably due to the lack of European Union legislations that prohibit or stimulate reuse of wastewater. The only juridical document in the European Union is the Urban Wastewater Directive (91/271/EEC), which states “treated wastewater should be reused wherever appropriate”. When implementing wastewater reuse in a country level, authorities rely on WHO guidelines and California guidelines (Title 22) (Cornel and Meda 2008). Direct non-potable water reuse is mainly applied for industrial process water, cooling water and agricultural irrigation (Shannon et al. 2008). Unfortunately, direct reuse of untreated wastewater is also practiced worldwide for food crop irrigation. This practice accounts for 10 % of the food crop irrigation worldwide and has a growing tendency. In Vietnam even 80 % of food crops are irrigated with untreated wastewater (Cornel and Meda 2008). Possibly, appropriate disinfection also for these situations will play a very important role for improving public health and food safety in the future.
Water disinfection should not introduce new problems. Therefore, the following criteria for disinfection are to be considered (EPA 2004), it:

- should reduce the concentration of pathogenic organisms below required level;
- should be safe;
- should not increase water toxicity due to disinfection by products;
- should be reliable and cost effective;
- should not result in risks to human health or the environment due to the transport, storage, or handling of disinfection chemicals or byproducts;
- should not affect the receiving environment (such as: land, surface water or groundwater).

Prior to the treatment of effluent the required quality of water has to be determined to identify what level of treatment is required. Potential water reuse areas that are distinguished are: potable, recreational, environmental, agricultural and industrial (Figure 1.4) (EPA 2004).

Figure 1.4. Potential water use areas such as potable, recreational, environmental, industrial, agricultural.
From the area of application different quality criteria for water treatment applies; for instance potable water has to be treated to a higher extent, compared to the water to be used for e.g. irrigation of non-food crops (Figure 1.5) (EPA 2004).

Disinfection standards depending from the water application:

- Potable: More strict regulations
- Agricultural for food (crops/livestock)
- Recreational
- Urban irrigation
- Industrial
- Environmental
- Agricultural for non-food (crops/livestock): Less strict regulations

*Figure 1.5. Schematic representation of water quality requirements for different application areas, generated from information found in the literature (EPA 2004).*

**1.2.3 Potable water versus wastewater disinfection**

Potable water has to be treated to a higher quality level than the water to be used for other purposes. Furthermore, potable water after disinfection often needs to have some amount of residual disinfectant upon feeding into distribution networks due to possible recontamination during transportation (Davis 2010).

Wastewater has to be treated to such a level that discharge does not harm humans, the environment, sustains the good state of receiving water bodies and does not introduce recalcitrant anthropogenic effects. In the cases where wastewaters or wastewater treatment effluents have to be disinfected, the target is to reduce the pathogenic organism concentration to meet the limits for discharge in specific areas. If chlorine or other chemical disinfectants (peracetic acid, ozone (Collivignarelli et al. 2000))
are used for wastewater disinfection decontamination/dechlorination has to be performed as sequential step in order remove remaining chemicals and to prevent formation of harmful by-products (Davis 2010). The most common disinfectant for wastewater is chlorine and for dechlorination sulfur dioxide (SO₂), sodium sulfite (Na₂SO₃), bisulfite (NaHSO₃), and metabisulfite (Na₂S₂O₅) are used (Tchobanoglous et al. 2003). During effluent dechlorination the salinity level increases in water and dissolved oxygen is consumed requiring additional water aeration before discharge (Lazarova et al. 1999).

1.3. Types of pathogens, caused infections and susceptibility to disinfection

There is a wide variety of organisms that cause different diseases and infections. In wastewater four main classes of pathogens can be distinguished (bacteria, viruses, protozoa and worms) that are responsible for causing the thread for human and cattle health (Table 1.1).

<table>
<thead>
<tr>
<th>Pathogen class and size</th>
<th>Caused disease/symptoms</th>
<th>Association to source</th>
<th>Treatment susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria (0.5 to 10 µm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella sp.</em></td>
<td>Typhoid fever, dysentery, diarrhea;</td>
<td>Insufficiently treated potable water; some cases through infected host; unsanitary sewage disposal and poor hygiene; contaminated food.</td>
<td>Bacteria type specific. Vegetative bacteria are easily removed. Bacterial spores are more recalcitrant. Sufficient removal can be achieved with filtration.</td>
</tr>
<tr>
<td><em>Schigella sp.</em></td>
<td>Dysentery, diarrhea, tenesmus, fever;</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>Cholera, diarrhea, vomiting ; Gastroenteritis, enteric fever.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Fecal coliforms</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viruses (0.01-0.1 µm)</td>
<td>Muscular paralysis,</td>
<td>Insufficient treated potable water; poor</td>
<td>More resistant than bacteria and poor</td>
</tr>
<tr>
<td><em>Enterovirus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1.1. Pathogen groups and their potential hazards and origin (information adapted from (Burch and Thomas 1998, Pandit et al. 2012b, Rajeshwar and Ibanez 1997)).
Due to the organisms variety in types (Table 1.1) and structures, the organisms also possess different resistances to disinfection. Therefore, it is important to understand the main differences between the organisms and the disinfection point of action.

- **Bacteria.** One group of organisms that play an important role in water and airborne infection spread are bacteria. Bacteria are prokaryotic, unicellular microorganisms. Based on their membrane properties, bacteria are divided into two types: Gram (-) and Gram (+) bacteria (Figure 1.6, a and b). The difference in their membrane structure makes them differently susceptible to disinfection treatment. The Gram (-) bacterial membrane consists of three morphologically defined layers (Figure 1.6): the cytoplasmic (inner) membrane that regulates the passage of metabolites in and out of the cytoplasm; the thin (2-7 nm) peptidoglycan layer that provides mechanical resistance; and the outer membrane consisting of lipids, polysaccharides and proteins, providing an interface between the cell and the environment (Molloy et al. 2000). Gram (-) bacteria are more susceptible to physical treatments such as ultrasonication and pulsed electric field due to the complexity of the outer membrane (Drakopoulou et al. 2009, Foster et al. 2011, García
et al. 2007, Rincón and Pulgarin 2005). Gram (+) bacteria membrane consists of 2 morphologically defined layers: the cytoplasmic membrane (inner) and a thick (20-80 nm) peptidoglycan layer (Figure 1.6). A number of Gram (+) bacteria additionally can form endospores that give them the ability to survive in a non-vegetative state, until transferred to a favorable environment reviving into the normal vegetative state. Gram (+) bacteria are more vulnerable to chemical treatments such as antibiotics (mostly attacking or blocking the peptidoglycan layer formation) and peroxides (oxidizing organic molecules in the cell membrane or cytoplasm). These chemicals have to surpass the porous peptidoglycan layer and one single (cytoplasmatic) membrane as barrier (Denyer and Maillard 2002, McDonnell and Russell 1999, Qin et al. 1994).

Figure 1.6. (a) Gram (-) and (b) Gram (+) bacteria structural representation (Denyer and Maillard 2002). Copyright ©, John Wiley and Son.

- **Viruses.** This important group of pathogens is quite different from other groups of microorganisms. Viruses are the simplest and smallest in size (0.01-0.3 µm) microorganism, most types consist of a nucleic acid and a protein “head”. Viruses don’t have the complex machinery for reproduction and therefore the spread and multiplication of viruses is dependent on a host organism. All viruses are very specific regarding the invaded hosts and their caused diseases (Mason and Tiehm 2001). As parasites, viruses
invade host cells and after the infected cell dies from lysis, the replicated viruses are released and able to infect other cells (Heritage 2003).

- **Protozoa.** Numerous waterborne diseases originate from parasitic protozoa, unicellular, eukaryotic organisms. The size of protozoa is in range of 10-20 µm and they can be found in natural soils and waters, and wastewaters. They play an important role in biological wastewater treatment (Mason and Tiehm 2001). A protozoa cell is able to switch to a resting state e.g. to form a cyst/(oo)cyte, which has a very resistant cell wall. Not all of these oocysts and cysts are viable and will cause diseases (Tchobanoglous et al. 2003). Protozoan cysts (*Giardia lamblia* and *Cryptosporidium parvum*) are very resistant to chlorination and are found in almost all surface waters around the globe. Some parasitic protozoa originate from feces of infected humans and animals (Horan 2003, Prescott et al. 2002).

- **Helminths** or worms, is another class of microorganisms that can cause infections. Worms can be divided in three major groups: Nematodes (round-worms), Platyhelminthes (flat-worms), and Annelida (segmented-worms), with nematodes as the biggest group. Some types of worms are parasitic, but most of the worms are harmless to people and cattle (Tchobanoglous et al. 2003). Worms are multicellular microorganisms that have complex reproductive systems and life-cycles. They need intermediate hosts for larval/egg stage and definite hosts for growth into an adult stage. Worm eggs can survive in water or soil for a few months and it is longer than other pathogens. Helminth larvae are transmitted through direct consumption of contaminated water or foods (Chen et al. 2006).
1.4. Extent of disinfection

Due to the variety of pathogens, different disinfection mechanisms are to be considered. One can distinguish between “cidal” (bactericidal, fungicidal) disinfection referring to the process of killing the target organism and “static” (bacteriostatic, fungistatic) disinfection, referring to the hindering of growth and multiplication of target organism (Sykes 1958). The generalized disinfection pathways (both for ‘-cidal’ and ‘-static’) are shown in (Figure 1.7) and can be described in the following sequence (McDonnell and Russell 1999, Rajeshwar and Ibanez 1997, Sykes 1958):

Firstly (I), the disinfectant interacts with the cell surface and this can operate in three possible ways: i) adsorption of disinfectant on the cell membrane suspending the function of the membrane; ii) direct damage/destruction of the cell wall causing the direct leakage of the cytoplasm; iii) increase of cell permeation (lethal damage) and further penetration for the site of action (Figure 1.7 marked as (I)).

Secondly (II), after having overcome the first barrier (cell envelope structure), the disinfectants take action on targeted sites in the cytoplasm or at the cell membrane (often due to internal active radical reactions damaging the cell organelle infrastructure). This suspends growth or cell division, and other life functions of the cell. More specifically, this leads to inhibition of cell homeostasis, modification of nucleic acids (DNA, RNA), inhibition of protein synthesis, and intervening integrity (aggregation, coagulation), activity or even full destruction of structures of proteins including enzymes (Figure 1.7 marked as (II)). This generally results in inhibition of electron transport or ATP synthesis, and eventually in cell death.

Different factors are influencing the severity of disinfection such as activity and life stage of the microorganisms, concentrations of microorganisms as well as disinfecting agents, contact time, temperature, pH, organic and inorganic matter concentration in the treated media.
Resistance to disinfection can be intrinsic or acquired by mutations. Acquired resistance exists mainly to chemical disinfectants or metal salts (such as mercury) (McDonnell and Russell 1999). The other factor decreasing disinfection efficiency is biofilm formation. It occurs when microorganisms attach to the surfaces and form a slimy layer called biofilm. The vegetative states will differ in different layers of the biofilm resulting in different susceptibility to disinfection (McDonnell and Russell 1999).

Figure 1.7. General scheme of disinfection pathways adapted from (Li et al. 2008, McDonnell and Russell 1999, Rajeshwar and Ibanez 1997).
1.5. State-of-art technologies for disinfection

State-of-art technologies for disinfection are chlorination, ozonation or ultraviolet radiation. These are commercially proven technologies applied on large scale.

1.5.1. Chlorination

Chlorine disinfection is one of the first chemical disinfection methods introduced, in the early 20th century, in water preparation plants in England and U.S. following by worldwide application until present (Pandit et al. 2012b). Chlorine is one of the most aggressive disinfectants, that introduces negative effects in concentrations as low as 2\times10^{-3} \text{ mg/L} (EPA 2004). Chlorine dissolves in water and forms so called “free chlorine” in the form of either hydrochlorous acid (HOCl) or hypochlorite ion (OCl\(^-\)) that react with organics. The free chlorine reactions are dependent on the pH which affects the disinfection efficiency. In case ammonia is present, chloramines are formed that also have disinfection ability (Davis 2010, Rajeshwar and Ibanez 1997).

Another option for disinfection is chlorine dioxide (ClO\(_2\)), a highly explosive gas, which reacts savagely with organics. It is a better deactivator of viruses than chlorine, but in contrast to chlorine, the level of Giardia cysts removal is too low to reach water treatment standards (Rajeshwar and Ibanez 1997).

Disinfection pathways from Cl\(_2\) and ClO\(_2\) are different. The Cl\(_2\) molecule inhibits membrane transport and ATP synthesis reactions and suspends the growth by damaging nucleic acids, therefore it is also effective against viruses. With respect to protein synthesis inhibition Cl\(_2\) has a relatively low activity, and ClO\(_2\) is a much stronger disinfectant in that aspect, and therefore often chosen for organisms that need to be targeted on this process (McDonnell and Russell 1999).

The advantage of disinfection with chlorine is that a residual concentration can be applied to assure a remaining bactericidal effect in water distribution.
networks. This brings also a drawback, as microorganisms can develop resistance to chlorine compounds through DNA mutations because of low concentrations of residual chlorine present in distribution systems. Furthermore, microorganisms included in microbial agglomerates (flocks/ biofilms) will possess higher resistance to chlorine (McDonnell and Russell 1999, Ridgway and Olson 1982). Chlorine can be produced at site trough electrochemical reactions to avoid accidents during transportation and handling (Davis 2010).

Water chlorination with Cl₂ or ClO₂ generates disinfection byproducts (DBPs) in the presence of organics; these byproducts are in part halogenated hydrocarbons that are hazardous for humans and the environment (Weinberg et al. 2002). Besides DBPs toxicity, the chlorine reaction with organics reduces the available chlorine for disinfection and requires an increase in the chlorine dose.

1.5.2. Ozonation

This disinfection technique employs the powerful oxidant ozone (O₃). It can be applied for disinfection and persistent pollutant decomposition. Ozone is a natural substance that is present in our atmosphere; it can be also produced from air, or oxygen gas in several ways. Corona discharge and ultraviolet light (UV) and cold plasma electrolytic discharge are commonly used technologies resulting in ozone production from oxygen molecule dissociation (Rajeshwar and Ibanez 1997). The first O₃ generator was made in mid-19th century in Berlin and it was mainly used for drinking water disinfection (Gottschalk et al. 2009, Rajeshwar and Ibanez 1997). Most ozone installations are in Japan, namely 400 – 600 installations, and 150 - 200 installations in the rest of the world (Paraskeva and Graham 2002). Three mechanisms are involved in ozone disinfection (Gehr et al. 2003): i) direct reaction of dissolved molecular ozone with organic cell components, which has a low reaction rate (Lazarova et al. 1999); ii) radical production in water (such as hydroxyl (OH·) and hydro-peroxide (HO₂·)), which subsequently react quickly and damages
lipids, proteins and nucleic acids (McDonnell and Russell 1999); iii) protein coagulation and oxidation of nucleic acids (Paraskeva and Graham 2002). According to the data of Liberti and Notarnicola (1999) in line with Collivignarelli et al. (2000) installation and operation costs of ozonation facilities are higher than chlorination and is close to UV disinfection facilities. Ozonation has technical advantages over chlorination such as lower residual chemicals in treated water, higher oxidizing power and faster disinfection than with chlorine, three times lower DBP production rate (DBPs are produced only if bromides, and/or aldehydes are present in water), easier and safer handling and storage, less pH sensitivity, and no development of resistant pathogens to ozone. Another advantage of ozonation is the increase of the concentration of dissolved oxygen in the water, which is beneficial when disinfected water to be discharged into surface waters. Ozonation improves the physico-chemical properties (such as color, odor, taste) of water (Paraskeva and Graham 2002); ozone has a low solubility in water, and aggressive nature of ozone leads to corrosion of materials (Rajeshwar and Ibanez 1997). A drawback of ozone installations is the complexity and energy intensity at high voltage (5-20 kV); and ozone has to be produced on-site as it is unstable and fast dissociates into oxygen (Liberti and Notarnicola 1999, Paraskeva and Graham 2002).

1.5.3. Ultraviolet irradiation

Ultraviolet irradiation (UV) for disinfection was introduced already in 1900, but due to unstable operation and lack of reliability it was originally competed-out by chlorination (Rajeshwar and Ibanez 1997). The advantage of UV disinfection is, that it does not produce DBPs at the low irradiation doses that are generally used for disinfection. Since 1950 – 1970s when analytics advanced and DBPs were detected and their harmful effects on life were identified (Richardson 2005). The disinfection with UV was since then reintroduced for water disinfection, replacing gradually most of chlorination and ozonation facilities (Chen et al. 2006, Weinberg et al.
The other advantage of UV disinfection is the effective deactivation of Cryptosporidium and Giardia compared to chemical disinfectants (Hijnen et al. 2006). In Europe UV was widely employed for drinking water disinfection since 1980s counting more than 2000 installations in 2006 (Chen et al. 2006). UV disinfection of water generally employs low pressure mercury vapor lamps with UV emission in the region of 254 nm (Masschelein and Rice 2002).

The disinfection mechanism of UV light consists of two components: the main component is the UV light emitted in the range of 240-290 nm is damaging the organism’s nucleic acids (DNR, RNR) through light adsorption leading to denaturation and replication function termination (Oppenländer 2003); the successive component is radical production such as OH\(^+\), O\(_2\)\(^+\), HO\(_2\)\(^+\), that destroy cells by damaging the outer membrane, proteins and nucleic acids (McDonnell and Russell 1999). To increase UV radical production rate, it can be combined with chemicals such as hydrogen peroxide (H\(_2\)O\(_2\)), ozone or a photocatalysts (TiO\(_2\)) (Dalrymple et al. 2010). The UV dose required for 3 – 5 Log fecal coliform removal in water is 30 – 45 mJ/cm\(^2\) (Lazarova et al. 1999). UV penetration depth in water is limited and is decreasing with turbidity resulting in decreased disinfection efficiency at larger distances from the light source (Masschelein and Rice 2002). When turbid water is treated with high contents of organic matter, the UV dose required for disinfection will increase up to 200 mJ/cm\(^2\) (Liberti and Notarnicola 1999), therefore often prior UV disinfection dissolved organic matter and turbidity are removed by filtration and activated carbon sorption (Chen et al. 2006). During UV disinfection of water with high natural organic mater and nitrate the formation of DBPs is possible and will increase with the increased irradiation doses (Buchanan et al. 2006).

The effect of UV disinfection has been found to be organism dependent and decreases in the following order: E.coli pure culture > Bacillus sp. pure culture > indigenous total coliforms (Lazarova et al. 1999). In case insufficient treatment time and UV light dose is applied, some microorganisms can photo-reactivate, and a water source can get re-contaminated. Furthermore, reactivation, absence of a
pursued disinfection effect due to residual disinfectant, reduced penetration in turbid solutions and lamp scaling effects are limitations of UV disinfection (Davis 2010, Liberti and Notarnicola 1999).
<table>
<thead>
<tr>
<th>Disinfection Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Areas of use</th>
<th>Remarks</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorination</td>
<td>- residual bactericidal action to avoid recontamination;</td>
<td>- Disinfection Byproducts (DBP) production;</td>
<td>Potable water, wastewater, recreational artificial waters, cooling water systems, as cleaning detergent.</td>
<td>Being slowly replaced by other disinfection methods due to aggressive nature and toxicity.</td>
<td>(Davis 2010, Lazarova et al. 1999, Levy et al. 2011, Rajeshwar and Ibanez 1997, Shannon et al. 2008).</td>
</tr>
<tr>
<td></td>
<td>- effective against bacteria and viruses;</td>
<td>- high concentrations required if organics are present in water resulting in DBPs production;</td>
<td></td>
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<tr>
<td></td>
<td>- low cost.</td>
<td>- has unpleasant smell and taste;</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>- poor inactivation of spores, cysts and some viruses at standard doses;</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>- after wastewater disinfection, dechlorination is required to remove remaining chlorite resulting in higher disinfection costs up to 30%;</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>- efficiency is pH and temperature dependent;</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>- bacteria and viruses can develop resistance.</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ozonation</td>
<td>- no residual chemicals; - short contact times;</td>
<td>- high capital and maintenance costs;</td>
<td>Used for potable water and secondary treated wastewater disinfection</td>
<td></td>
<td>(Collivignarelli et al. 2000, Lazarova et al. 1999, Liberti and Notarnicola 1999,</td>
</tr>
<tr>
<td></td>
<td>- limited Disinfection Byproduct formation (DBP) compared to chlorine;</td>
<td>- DBPs production (bromates, aldehydes);</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- increases dissolved oxygen level;</td>
<td>- efficiency is effected negatively by high COD/BOD/TOC;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- improves water physico-chemical properties (odor, taste, color);</td>
<td>- aggressive resulting in corrosion;</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>- operation is less dangerous than chlorine;</td>
<td>- does not have residual bactericidal effect;</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>- has to be produced on-site (complex</td>
<td></td>
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</tr>
</tbody>
</table>
| Ultraviolet irradiation | - Disinfection Byproduct (DBP) formation possibility is low;  
|                        | - effectively inactivates *Giardia lamblia* and *Cryptosporidium parvum*;  
|                        | - effective against most of the studied viruses;  
|                        | - efficiency independent from pH and temperature. | - efficiency is negatively affected by turbidity, suspended solids, color, organic matter;  
|                        | - for protozoan cysts and viruses disinfection requires high doses up to 200 mJ/cm²;  
|                        | - lamp scaling and aging reduces efficiency;  
|                        | - requires intensive maintenance;  
|                        | - high capital and maintenance costs;  
|                        | - no residual bactericidal effects;  
|                        | - bacteria and viruses can develop resistance;  
|                        | - photoreactivation and recontamination possibility. | Drinking water disinfection, wastewater disinfection. |
1.6. Other methods for water disinfection

Since 2000 years BC the first means to clean water from pathogens were: silver, charcoal and filtration and these methods are still simple, easy and useful ways for water disinfection (Rajeshwar and Ibanez 1997).

1.6.1. Silver. In 350 BC Aristotle advised to Alexander the Great to transport the drinking water in silver cans and boil it before use. The first scientific proof of silver use was in 1869 when Ravelin discovered that a concentration as low as \(9 \times 10^{-9} \text{ M}\) was effective in killing fresh-water algae (Ellis and Luscombe 1994). Generally pure silver is less active for disinfection than silver compounds such as silver acetate, silver nitrate, as well as silver sulphadiazine (Ellis and Luscombe 1994). Silver ions have been described to target at bacterial proteins, membranes and nucleic acids (McDonnell and Russell 1999). Nowadays silver is mainly used in medicine and not used for water disinfection anymore, however there are new applications being explored such as biofouling reduction (Heidarpour et al. 2010, McDonnell and Russell 1999).

1.6.2. Membranes. Use of membranes has long lasting history. First membranes of natural origin were discovered in 18\textsuperscript{th} century (Cecen and Aktas 2011). For disinfection ultra-filtration and microfiltration membranes are in some cases used to remove pathogenic microorganisms (Lazarova et al. 1999). The main disadvantage of membranes is biofouling reducing filtration efficiency and membrane lifetime, high energy costs for application to large water quantities. Therefore, activated carbon adsorption is used before filtration to remove organics and biological and non-biological particles, to increase the lifetime of the membrane units and to reduce the level of fouling (Tsujimoto et al. 1998).

1.6.3. Activated carbon application for filtration is a quite recent development which can be regarded as an optimized form of charcoal filtration. In ancient times (already in 3500
BC in Egypt) charcoal was used in water and air purification, and later also for medical treatment (Cecen and Aktas 2011). First written sources of charcoal use for disinfection purposes were recorded in ancient Sanskrit in 200 BC, where it was recommended to use charcoal for filtration of water that was stored in copper vessels (Rajeshwar and Ibanez 1997). In the 15th century sailors noticed, that after wooden vessels were blackened with fire, the water stored would stay fresh much longer. It took until the 18th century to identify the mechanisms of charcoal adsorption. In 1862, activated charcoal was used for potable water treatment for the first time and since then subsequently introduced in a large scale. In 1909 the first activated carbon factory was opened that produced activated carbon powder, two years later also a Dutch activated carbon company (Norit) was founded. In the early years, activated carbon was primarily used for potable water and in the food and chemistry industries to decolorize solutions (Cecen and Aktas 2011). First applications of tertiary wastewater treatment using activated carbon are reported since 1970 (Cecen and Aktas 2011). The wastewater was post-treated for reuse in industry or to meet surface water discharge limits (Strathmann et al. 2011).

Nowadays, activated carbon is used in the water treatment chain: i) to reduce COD (Chemical Oxygen Demand, i.e. a measure for organic compound concentration). Removed COD also reduces organic substrate and microorganisms growth (due to substrate exhaustion in water), in this way is also minimized membrane fouling extending membrane lifetime (Monod 1949); ii) as a filter for suspended solids in specific size ranges, including bio-particles such as worm eggs (such as helminthes) and protozoa cysts. The helminthes eggs and protozoa cysts can be reasonably retained in activated carbon filters. In contrast, activated carbon was found to be less efficient in removing bacteria and bacterial spores, and is totally not effective against viruses (Hijnen et al. 2010). Activated carbon, as all other filters, is susceptible to non-biological and especially biofouling. Bacteria get easily attached to carbon due to rough, porous surface, facilitating the shelter for bacteria to attach (Busscher et al. 2008, Moreno-Castilla et al. 2003). However, the sterilizing effect of activated carbon is limited because attached bacteria are viable and may even grow inside activated carbon filters forming biofilms. Those biofilms in water
stream can detach as flocs in this way contaminating treated water stream (Ying and Ping 2006).

To easier retain the activated carbon adsorbent in filter systems, often granular activated carbon (GAC) is used. For water treatment applications, activated carbon has to possess high mechanical strength, to prevent erosion/disintegration during operation. During treatment a certain degree surface disintegration is generally occurring and the released fragments can be transferred to the treated water stream. Washed off activated carbon powder may contain attached pathogens which will be transferred to treated water stream that might get contaminated (Geldreich 1996, Hijnem et al. 2010). Due to recontamination risk from carbon filters, successive disinfection or membrane filtration is used (Davis 2010).

1.6.4. **Thermal disinfection** is one of the traditional methods to kill pathogens in drinking water on household scale. By heating above 80 °C for at least 20 minutes most of pathogens and their spores can be deactivated. However, thermal pasteurization is less efficient against viruses (Burch and Thomas 1998). In industry it can be used in a form of distillation, however it is not widely applied because it is energy intensive (~1 kWh/L) (Johanstone 2001). Thermal disinfection can as well be used as emergency disinfection for potable water, required during big scale disasters such as floods, tornadoes, fail in potable water distribution or treatment system (Davis 2010). Thermal/steam induced (120 °C) and high pressure sterilization is a common method used in microbial and medical laboratories (Siwach and Singh 2007).

1.6.5. **Photo-catalytic (TiO₂) disinfection.** Heterogeneous photocatalysis can also be employed for disinfection, firstly reported by Matsunaga in 1985, and since then TiO₂ was applied for various parasites inactivation (Matsunaga et al. 1985, Rajeshwar and Ibanez 1997). As catalyst combined with light, TiO₂ is used for disinfection because it enhances OH⁻ radical production. This method is effective against bacteria and viruses, but deactivation of cysts and bacterial spores was not reported (Rajeshwar and Ibanez 1997).
In the dark, TiO$_2$ is not toxic for cells resulting in no disinfection effect. The disinfection rate depends on the light intensity and concentration of TiO$_2$. The general disinfection susceptibility for TiO$_2$ photocatalytic disinfection is decreasing in the order: Gram (+) bacteria > Gram (-) bacteria > viruses. This method can also be applied to inactivate antibiotic resistant microorganisms (Tsai et al. 2010). Furthermore, due to its simple performance and low concentrations of TiO$_2$ required for disinfection and no specific equipment required, it can be used in distant locations, where electrical power is not available. The disadvantage of the method, however, is the price of TiO$_2$, and exhaustion of catalyst (Rajeshwar and Ibanez 1997). Another limitation of the method is pH dependency for disinfection efficiency (McCullagh et al. 2007).

1.6.6. Peroxogens (Hydrogen peroxide, per-acetic acid)

**Hydrogen peroxide (H$_2$O$_2$)** is a well-known disinfectant which is available in the market at concentrations from 0.3 – 90 %. It is environmentally friendly as it decomposes to water and oxygen. It is active against bacteria, protozoa and viruses. For a sporicidal effect, higher concentrations and longer contact times are required. Gram (-) bacteria possess higher resistivity to this chemical than Gram (+) bacteria. It’s main disinfection action is due to radical production, that attack lipids, proteins and nucleic acids (McDonnell and Russell 1999).

**Peracetic acid (PAA)** is unstable organic per-acid (CH$_3$COOOH). It is effective in acidic conditions due to release of active oxygen. However, it has quite some limitations most importantly the microbial re-growth due to acetic acid as a carbon source, and the increase in organic content of treated water, initiating growth of other bacteria in the system. Applicability depends highly on water quality and is not particularly effective against viruses and protozoa, and has very high costs such as 2.6 € per m$^3$ of treated water (Liberti and Notarnicola 1999). An advantage comparing to ozonation and chlorination, is its activity in highly organic loaded water. It’s disinfection effect is similar to peroxide, but the mechanisms are not well known (McDonnell and Russell 1999). Toxic
or carcinogenic effects were not thoroughly investigated, due to little data available (Collivignarelli et al. 2000).

1.6.7. Ultrasonic disinfection. Ultrasonic (US) technology was firstly mentioned in the beginning of 20\textsuperscript{th} century and was described as high frequency sound waves resulting in chemical effects (Mason et al. 2005). Upon first applications, it was used for emulsification and surface cleaning. In recent years US technology is applied in food industry processes (such as homogenization, flavor extraction, filtration, freezing, crystallization of fats and sugars, alteration of enzyme activity, equipment sterilization, bactericidal action) (Mason et al. 2005) as well for water decontamination it has been widely researched (Gogate 2002).

The mechanism of US disinfection depends on the amount of power applied. In cases that the applied US power in the liquid is low, micro-bubbles are created that oscillate and induce liquid jets around them (micro-streaming). Upon US application at a high power, the micro-bubbles oscillate in a transient mode resulting in their collapse or implosion, called cavitation. This creates high mechanical friction and locally high temperature and pressure gradients, which can induce chemical reactions in the fluid (Mason et al. 2008). The US source can be either mechanical (piezoelectric transducer) that will create the sound waves by mechanical movement; or hydrodynamic (liquid whistle) in which liquid is pushed mechanically through an orifice from which it expands into the reaction chamber, where the pressure waves are produced (Mason et al. 2005). US produced mechanically can be applied at different frequencies; for disinfection, however mainly frequencies in the range of 20 to 80 kHz are employed. Different frequencies, generally result in different disinfection efficiencies (Antoniadis et al. 2007).

US is mainly used for bacterial decontamination, removal of viruses is energy intensive, time consuming and has limited efficiency (Chrysikopoulos et al. 2013, Su et al. 2010). Limited disinfection efficiency of US on viruses is related to the mechanism of US disinfection; due to liquid jets and forces induced during cavitation it ruptures mechanically the cell membrane. Local temperature increase, pressure waves and
chemical reactions are playing a secondary role in microorganism inactivation. It is also thought that US increases cell membrane permeability (Mason et al. 2003).

The ultrasound disinfection efficiency depends on various factors such as acoustic power (force), frequency, media composition, liquid temperature, treatment time (Jyoti and Pandit 2003, Joyce et al. 2003). Ultrasonic efficiency for disinfection can be enhanced by addition of solid particles (Ince and Belen 2001, Tuziuti et al. 2005). Particles will enhance the disinfection due to additional friction between particles resulting in faster cell membrane rupture (Murphy et al. 1993, Salter and Smith 1984). Furthermore, ultrasound is used for pretreatment of fluids before disinfection, as it can well disintegrate particles, microorganism consortia and flocks, in this way increasing transparency of the fluids to be treated and making bacteria more vulnerable for disinfection (Blume and Neis 2004). The main US drawbacks are: that it is electrical energy intensive; it is less effective for specific microorganisms viruses, bacterial spores and protozoan cysts and it’s efficiency is decreasing with decreasing microorganisms concentration (Joyce et al. 2003). Furthermore, US disinfection efficiency is decreasing with the increasing distance from the US source (Mason et al. 2005). Some bacteria are not affected by ultrasound such as Pseudomonas spp. and in general it is more efficient towards Gram (-) bacteria than Gram (+) bacteria, and for anaerobic bacteria than aerobic bacteria (Mason et al. 2005). Considerably good bacterial disinfection can be achieved with high power ultrasound taking into account the factors mentioned above (Hua and Thompson 2000, Salleh-Mack and Roberts 2007). US is efficient against protozoa, plankton and algae destruction, however is working poor for viruses removal (Mason and Tiehm 2001).
1.6.8. Electric fields for disinfection

Pulsed electric field (PEF) for disinfection. In 1970s it was discovered that high pulses of electric field can cause pore formation in cell membranes, which is referred to as electroporation (Neumann and Rosenheck 1972). When the short pulses (in the range 1-40 µs) of high electric field strengths (10-100 kV/cm) are applied in the cell suspension, pores in the cell membrane are formed that are either irreversible, or reversible i.e. can be repaired by the cell (Sugar and Neumann 1984). This high field strength can be achieved in micro-channels (Sugar et al. 1987). When the cell is in the liquid between the electrodes, the current will flow through the cell, which will induce pore formation in the cell membrane and with time, the size of pore can increase eventually causing leakage of the cytoplasm and cell death (Kekez et al. 1996).

PEF is mainly used for liquid food products pasteurization as due to very short pulses, the disinfected product is not exposed to substantial heating. Low temperature pasteurization is beneficial for maintaining freshness, smell, taste and look of the food products (Wouters and Smelt 1997). Furthermore, there were few attempts to use PEF for hospital wastewater disinfection, however the high costs of the method remains the main limitation (Rieder et al. 2008). PEF is not possible in the presence of air bubbles or high conductivity (saline liquids), which need to be removed prior treatment (Johnstone 2001). Main advantages of PEF are, that cells are not able to develop resistance to PEF (Rieder et al. 2008) and the absence of electrochemical reactions (electrode corrosion and byproduct formation) due to the short duration of the pulses (Johnstone 2001).

Direct current electric fields (DC field) are static electric fields with no amplitude variation in time. When negatively charged cells are exposed to the DC field, the cells will move against the direction of electric field from negative electrode to the positive one. At neutral pH, most of the cells generally have a negative surface charge (Voldman 2006). However, using a DC field for disinfection has several disadvantages due to electrochemical reactions: disintegration of electrode materials (Giladi et al. 2010) and scaling (Lian et al. 2007). DC fields are widely used for electrochemical reactions in water
(Li et al. 2002) and cell electrophoresis (cells movement in the electric field (Pitta and Berg 1995)). DC field is used for on site generation of chlorine (Li et al. 2002). A DC field causes cell damage at a higher rate compared to an AC field (Voldman 2006).

**Alternating current electric field (AC field)** is a dynamic electric field with amplitude variation in time. An AC field does not (at high frequency) or hardly result in disintegration of the electrode material and in less chemical reactions (Lian et al. 2007).

Generally in AC field, cells are being polarized and a cell dipole is formed, that is oriented opposite to the electrode charges (Voldman 2006). Cells will get aligned perpendicularly to the field lines accompanied by the elongation of the cell minimizing the exposure to the electric field (Funk et al. 2009).

AC fields can be applied at a large range of frequencies (Figure 1.8.) and signal shapes, therefore a wide variety of physiological effects on cells exist (Barnes 2007, Markx and Davey 1999, Voldman 2006).

![Frequency range of alternating current electric field (AC field) applications. The arrow indicates the frequency range applied in the present work.](image-url)

**Figure 1.8. Frequency range of alternating current electric field (AC field) applications. The arrow indicates the frequency range applied in the present work.**
1.7. Limitations at specific conditions of above discussed disinfection methods

Despite vast application and research performed on state-of-the-art disinfection methods, none of them is universally applicable, and all have various negative aspects. Most of the disinfection techniques in general can reach at least 3 Log reduction of microorganisms, but for achieving higher disinfection efficiencies high input (chemicals, energy, time) is required (Collivignarelli et al. 2000).

The disinfection techniques can be grouped according their specific properties and associated effects. If we consider Ozonation and Chlorination, the addition of chemicals can be regarded as non-sustainable. Furthermore, the techniques involve costs due to purchase and storage of chemicals and risk of DBPs formation and increased toxicity of water.

When water with high turbidity and high concentrations of dissolved organics is to be treated, Ultraviolet irradiation, Ultrasonic disinfection and PEF will experience decreased penetration depth and fouling. Mentioned effects will result in decreased disinfection efficiency.

Water rich in organics will require higher consumption of disinfectant chemicals as well as higher energy input and longer exposure times for physical methods. High organic and salt content will also increase water conductivity causing problems e.g. electrode disintegration for PEF.

Due to complexity of the treatment methods especially Ultraviolet irradiation, Ozonation and PEF installations will require advanced equipment, complex installation and intensive maintenance; therefore those methods will result in high capital and maintenance costs.

1.8. Emerging and combined disinfection technologies

Alternatively an improved performance can be achieved by emerging disinfection methods, and by combined applications. Among this group we discuss especially the electric techniques and US. Techniques that have not been proven on a large scale, but are
currently being investigated can be called emerging disinfection techniques. For emerging
techniques extensive experimental data and pilot applications are yet missing to provide
serious alternatives to the state-of-the-art techniques. Different techniques have different
limitations and none of them, as stand alone process, can guarantee water of sufficient
quality, without extensive contact times, and high concentration of chemicals. Different
techniques can be combined in parallel or simultaneously providing enhanced treatment
effects (Pandit et al. 2012a). Some examples are: US and UV (prior to UV treatment, US
disintegrates flocks and particles mechanically and UV disinfection efficiency is enhanced);
sequential application of activated carbon and membrane filtration (activated carbon
adsorbs organics and acts as a filter for particles and protozoan cysts and reduce the
substrate for microorganisms and thus the membrane fouling). A novel combination of
techniques we propose here is activated carbon combined with an AC field in a fluidized bed.

1.9. Fluidized bed electrodes of granular activated carbon with an AC field

We introduce a new combination technology that is based on two known disinfection
mechanisms, the AC field and activated carbon. We expect a synergistic effect for two
reasons. First, activated carbon can induce a stress factor on microorganisms due to COD
adsorption that might enhance vulnerability for the AC field. Second, the activated carbon
particles can act as a fluidized bed electrode that locally increases the electric field
strength i.e. amplitude of applied AC field.

1.9.1. Effects of AC fields on cell physiology

Cells possess electric properties, as was noticed already in 1930s, when
electrophoresis with blood cells was introduced by A. Tiselius (Chawla 2003). Cells are
composed of different molecules such as amino acids, peptides, proteins and
polysaccharides, which have ionisable groups and in electrolyte solution will exist as
electrically charged species (Markx and Davey 1999, Wilson and Walker 2010). Electric fields in mammalian cells are proved to affect main function performance such as protein synthesis, ion pumping activity, cell cycle and division. The field can be employed for modification of cell surface chemical events that would influence the vital functions such as hormone binding, antibodies and neurotransmitters activity. The above mentioned effects are used in medicine for diagnostic and healing purposes (Funk et al. 2009).

However, mammalian cells are different from microorganisms by their structure and rigidity. Bacteria and mammalian cells have a cytoplasmic membrane, but contrary to mammalian cells, bacterial membranes consist of one or more layered structures (transmembrane, outer membrane Figure 1.6). The ability to form spores and the outer membrane envelope in bacteria contribute to the resistance to chemical and physico-chemical treatments and environmental factors. Furthermore, bacteria and viruses are smaller in size than mammalian cells. Viruses on the other hand have only nucleic acid and a protein head and might not have a membrane at all (Palmer et al. 2012). Due to the outer cell membrane and smaller size, microorganisms are affected by electric fields to a lesser extent than the larger mammalian cells, and higher electric field strengths are required, or longer exposure times are needed. Furthermore, due to the different cell envelope structures of microorganisms, they have different responses to electric fields (Funk et al. 2009). For instance Gram (-) bacteria have different outer cell wall structure than Gram (+) bacteria (Figure 1.6 a and b) and therefore, have different susceptibility to electric fields. In general Gram (-) bacteria are more sensitive to electric fields due to the complex outer cell membrane structure that consists of large molecules such as phospholipids, polysaccharides, proteins, porins and other natural polymers (Figure 1.6) (Hülsheger et al. 1983). Those large molecules have electrical charge, and can respond to electrical signals by structural changes. The conductivity and permittivity of the outer cell membrane will depend on the ion concentration in the surrounding medium and applied electric field properties (Markx and Davey 1999). The cytoplasmic cell membrane is, a non-conductive barrier for external DC and low frequency AC fields. With increasing electric field frequency, the cytoplasmic cell membrane conductivity will increase, modifying the
membrane from polarized (charge storage) to conductive (current flow) (Markx and Davey 1999).

The electric field effects on cells can be achieved in two ways, either as direct damage due to polarization of the cell membrane causing electroporation and cytoplasm leakage (at high field strengths 1 – 100 kV/cm) or as indirect effects due to function inhibition (at low field strengths few V/cm) (Voldman 2006). Here the electric field frequency plays a crucial role.

1.9.2. Effects of AC fields on cells at specific frequencies

Low frequency AC fields can cause dielectrophoresis (polarized particles movement towards higher field intensity). Low frequency fields (DC to 300 Hz), also called extremely low frequency (ELF) fields (Figure 1.8) have largest effect on cells due to their coupling to cells indigenous electrical activity (Barnes 2007). However, at higher electric field strengths the ELF frequencies can result in electrolysis, as electrolysis can occur up till 1 kHz frequency (Tracy 1932).

Attempts to apply AC field of 50-60 Hz frequency for disinfection were reported in 1930 by Tracy (Tracy 1932). However, the effects obtained are assigned to a thermal disinfection due to ohmic heating (Palaniappan et al. 1990). For disinfection of sea water a 50 – 60 Hz frequency AC field was employed and electrochemical reactions (i.e. electrochemical chlorine evolution) were responsible for 4-5 Log disinfection. The disinfection performance achieved was of the same range as with DC, only a lower level of chlorine production was measured (Park et al. 2003).

AC fields in medium radio frequency range can result in suspended cell division (Giladi et al. 2010) and in the frequency range from 10 kHz to 10 MHz cell membrane permeability will increase with a peak at 100 kHz (Caubet et al. 2004, Chang 1989). Ion and molecule transport through the membrane will be enhanced already at low AC field strengths (10-30 V/cm) (Xie et al. 1997). Enhanced antibiotic uptake by E.coli at 10 MHz due to this property was reported (Caubet et al. 2004). Amarjargal et al. (2012) found a
higher disinfection rate at 100 kHz compared to 1 Mhz with ceramic tourmaline nanoparticles.

At frequencies of 10 MHz to some GHz (higher range radio frequency and microwave radiation (Figure 1.8)), heating and ionization of molecules inside the cell will occur.

The effects of ELF and microwave frequency AC fields are quite well investigated, however the data on effects taking place in medium radio frequency range are quite limited (Barnes 2007). In the medium radio frequency (RF) range (10 kHz to 10 MHz – Figure 1.8) the electric field mainly acts on the cell membranes (Barnes 2007, Voldman 2006) and the effects will depend on AC field frequency, cell physiological state and electrolyte composition (Markx and Davey 1999).

The effects of AC fields on cells are frequency specific and can be complex leading to a wide variety of physiological changes including cell death (disinfection), which can occur even at low AC field strengths when applied at adequate frequencies.

1.9.3. Physico-chemical properties of activated carbon relevant to disinfection

Activated carbon can affect microorganisms in two ways: negatively through a change of environment by chemical adsorption of organics (i.e. decrease in available substrate concentration) which is stressful for microorganisms, and positively through adsorption of bacteria providing a “protective environment” from harsh environment effects (e.g. disinfection).

Activated carbon is good adsorbent of organic matter, especially non-polar molecules (Marsh and Rodriguez-Reinoso 2006). Organics adsorption on activated carbon (so depletion in the liquid) can introduce stress on planktonic bacteria (Nachin et al. 2005). When cells are transferred from mineral rich media to mineral depleted media, they become more vulnerable for other chemical or physical stress factors due to outer membrane damage (Desnues et al. 2003, Zaske et al. 1980). Therefore, organics depletion stress can make pathogens more vulnerable for disinfectants. On the other hand, bacteria
that are inside/ adhered onto activated carbon or other particles will be well sheltered from disinfectants, reducing the efficiency of disinfection (Emerick et al. 1999).

The surface of activated carbon is complex and contains several types of surface groups (such as oxygen, nitrogen, hydrogen, hydroxyl, carboxyl, phenol, ethers) depending on the production method. The surface groups determine the specific adsorptive behavior (Al-Degs et al. 2000, Marsh and Rodriguez-Reinoso 2006). Furthermore, activated carbon is a hydrophobic material and in water it will have either a negative or a positive charge depending on media pH and point of zero charge of the activated carbon (Moreno-Castilla et al. 2003). These properties will influence interactions of the activated carbon and the bacteria, such as attraction or repulsion forces (Busscher et al. 2008, Rijnaarts et al. 1995).

Optionally, to facilitate pathogen inactivation, modified, positively charged activated carbons can be used, and upon attachment pathogens would loose their viability (van der Mei et al. 2008). Acidic properties, surface charge and other specific properties (ash content, density, hardness, pH, particle and pore size distribution, surface area) can be controlled by choosing the right source material and manufacturing process (Kinoshita 1988, Marsh and Rodriguez-Reinoso 2006). In general activated carbons are considered as relatively low cost materials (Chandrasekaran et al. 2008).

1.9.4. Fluidized bed electrodes

A fluidized bed electrodes system is obtained when particles are fluidized in between two electrodes over which an electric field is applied. Fluidized bed electrodes (FBE) systems were introduced in 1970s in order to recover dissolved metals from a fluid stream employing a DC field (Ferreira 2008). The main idea of fluidized bed electrodes employing a DC field is the increase of electrode surface, to achieve high volumetric electrochemical reaction rates. In addition, along the reactor a well-mixed suspension with uniform temperature, concentration and pH distribution is achieved due to particles that are being carried in the reactor along with the fluid (Werther 2000). Highest efficiencies were achieved with graphite fluidized particles (Hampson and McNeil 1983). These
systems also have several limitations, such as clogging with particles, spatial distribution of potentials in the bed and high power consumption. Charge can be transferred in a FBE in two ways: the first way is due to conductive charging by direct transfer of electrons that occurs either by particles forming a chain between the electrodes and by elastic collisions with other particles that are floating and not touching the electrode (Ferreira 2008); the second way is due to capacitive charging by diffuse double layers that are formed by ions in the electrolyte solution (Zhao et al. 2012). The highest electric field strength will occur at the interface between solid surfaces and the liquid. In the case of a GAC and water fluidized bed, voltage gradients will occur due to the low resistivity of the GAC (with the conductivity in the range of $S/cm$) compared to high resistivity of water (with the conductivity in the range of $mS/cm - \mu S/cm$). The good electrical conductivity of GAC is resulted by layered structure and high surface area of steam activated carbons (Kinoshita 1988).

In a FBE system, potential differences will occur through the bed, depending on the different packing density. Lower fluidization speed will result in a higher electric current strength; and a higher fluidization speed due to lower packing density will result in a lower electric current strength (Bareau and Coeuret 1979). Only FBE systems for DC application were reported so far and employed mainly for electrochemical reactions. However, this system has not been employed for disinfection and has not been investigated with the application of a medium radio frequency range AC field ($10^5$ Hz – Figure 1.8). Another advantage of a fluidized bed may be an enhanced bacteria adhesion to GAC surfaces because of high mass transfer rates (Busscher et al. 2006, van der Mei et al. 2008).
1.10. Research focus: knowledge gaps and research objectives

The challenge of this thesis research is the development of a GAC – FBE system with AC field for disinfection.

The knowledge gaps related to GAC-FBE systems

- The operation of a FBE system with AC field for disinfection has not been reported.
- Preliminary findings indicate disinfection effects but there is no systematic proof-of-principle that disinfection is a result of the combination of AC field and GAC.
- It is not known whether the FBE disinfection method is able to remove all types of microorganisms efficiently.
- Effects of GAC on microorganisms are known, however they are not characterized and quantified for FBE conditions.
- There is a lot of information on physical chemical properties of GAC, however GAC properties responsible for disinfection in FBE combined with AC field are not identified.
- It is not known whether other stress factors than interaction with GAC, such as stress caused by ultrasound, can make microorganisms vulnerable for AC field disinfection.
- Next to the proof-of-principle, understanding the mechanism of disinfection is required to develop the technology towards full scale application and public acceptance.
The research objectives

- To give a proof-of-principle of the disinfection with radio frequency electric fields combined with GAC;
- To quantify the effect of the different components on FBE disinfection performance;
- To determine the effect of radio frequency electric fields combined with or without GAC on bacteria survival;
- To determine applicability of FBE disinfection for different types of microorganisms;
- To determine disinfection effects in systems with ultrasound and alternating field;
- To identify the mechanism of disinfection occurring in FBE.

1.11. Outline of the thesis

In the second chapter, the proof-of-principle is provided and the relevant components for FBE disinfection are described. In the third chapter, the proof-of-principle is extended by testing FBE disinfection on different types of bacteria (Gram (-) and Gram (+)). In the fourth chapter, bacteria metabolic activity and survival in presence of GAC and different microbiological media are investigated as having an additional effect sustaining the disinfection in FBE. In the chapter five, the effect of different GAC types is tested for the disinfection performance in FBE, and properties of GAC are related to disinfection performance. In the chapter six, FBE is combined with ultrasonication in order to increase the disinfection and energy efficiency. Finally, in chapter seven, general conclusions are presented and various results obtained throughout the thesis are discussed with their implications for the disinfection mechanisms, future research directions and practical applications.
1.12. References


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Combining fluidized activated carbon with weak alternating electric fields for disinfection

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 hours. 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.

2.1. Introduction

The quality of drinking water and treated domestic and industrial wastewater is worldwide an issue of concern (WHO/UNICEF 2010). Increased requirements of water reuse ask for effective disinfection methods to assure public health, health of cattle and plants in agricultural production systems (EPA 2004), and to prevent damage to natural ecosystems (Howard et al. 2010, Richter et al. 2003). The oldest and most reliable chemical water treatment methods such as chlorination and ozonation are being widely applied for water disinfection (Ferreira 2008, Kim et al. 2002). Although effective, these methods require post treatment, mainly due to formation of by-products occurring in the treated water stream (Tyrrell et al. 1995). 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 (Andreonzi et al. 1999). 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 (Azbar et al. 2004, Melemeni et al. 2009). Electrochemical disinfection methods treat water streams by electrically produced active species such as radicals (Feng et al. 2004, Sato et al. 1996). The use of solely electric fields for disinfection needs high electric field densities up to 100 kV/cm (Mazurek et al. 1995).

Microorganisms have a dielectric nature meaning that they can be polarized in an electric field (Barnes 2007). Electromagnetic fields are reported to affect several microbial life phenomena such as: microbial growth (Aaron et al. 2004, Palaniappan et al. 1990), cell fusion (PEF) (Jeyamkondan et al. 1999, Narsetti et al. 2006, Zimmermann and Neil 1996), voltage gated channels (Chiabrera et al. 2000); and can even cause cell death (Matsunaga et al. 1994, Palaniappan et al. 1990). 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 (Lian et al. 2007).

Optionally, alternating current (AC) treatment can be used (Birbir et al. 2009, Tracy 1932). 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 (Mazurek et al. 1995, Tekle et al. 1991). 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 (Geveke 2005).

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 (Ferreira 2008, Mayer et al. 2010, Wang et al. 2007, Xiong et al. 2001). The carbon morphology allows bacteria to adhere (Yamamoto et al. 2001, van der Mei et al. 2008), 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 (Carlson 2002). 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.
2.2. Experimental

2.2.1. 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 0.2 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 (Corapcioglu and Huang 1987). Before the experiment the particles were submerged for 24 h in ¼ LB medium (section 2.3) to saturate the GAC with electrolyte. For the FBE disinfection experiment 400 mL of the prepared medium (¼ LB media containing ampicillin) and 140 g of pretreated GAC (GAC_{pr}) were used.

2.2.2. GAC physical characteristics

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

2.2.2.1. pH point of zero charge determination. The pH_{pzc} was determined by an immersion technique using NaCl as the electrolyte (Fiol and Villaescusa 2009). The 0.05 M NaCl solution was degassed by stirring in N₂ 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 N₂ 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 $\text{pH}_{\text{pzc}}$ of powder activated carbon NORIT RX 3 EXTRA (PAC) was determined as well. PAC for $\text{pH}_{\text{pzc}}$ 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 $\text{pH}_{\text{pzc}}$ 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 $\text{N}_2$ environment for 24 hours at 350°C (VacPrep 061 LB, Micrometrics, Germany). Nitrogen adsorption isotherms were measured (Tristar 3000, Micromeritics, U.S.) to obtain total specific surface area BET ($\text{m}^2/\text{g}$). The macropore area ($\text{A}_{\text{macropore}}$) was measured using mercury intrusion porosimetry (Autopore II 9220 porosimeter, Micromeritics, U.S.).

2.2.3. 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 (Steinberg et al. 1995). This non-pathogenic strain carries a plasmid (pJE202) that contains *V.fischeri* genes on a vector: luxR, luxI, luxC, luxD, luxA, luxB, luxE, luxG specifying the luminescence enzymes and encoding regulatory functions for bioluminescence (Engebrecht et al. 1983). 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 (¼ 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 ¼ LB medium concentration is comparable to a lightly polluted wastewater concentration (Mavrov et al. 1997).

### 2.2.4. Experimental set-up

The fluidized bed electrode (FBE) set-up (Figure 2.1 (C)) consisted of a 1 L glass beaker, 2 stainless steel plate electrodes (35.6 cm²) 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, U.K.) 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 revolutions per minute (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 22±2.5°C.
Figure 2.1. 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 2.1) (includes ¼ LB media and bacteria, and excludes electrodes, GAC, applied RF-AC electric field); Beaker B: blank 6 (Table 2.1) (includes ¼ LB media, bacteria, electrodes, GAC, and excludes RF-AC electric field); Beaker C: disinfection sample 0 (Table 2.1) (includes all experimental factors: ¼ LB media, electrodes, GAC, RF-AC electric field, bacteria).

2.2.5. 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, U.S.). 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 (Boyer and Wisniewski-Dyé 2009). 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

$$\Delta CFU = \frac{1}{b} (\Delta \log CPS)$$

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 (Miles et al. 1938) 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 hours at 37 °C.

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

A set of blanks was designed (Table 2.1) 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.
Table 2.1. 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.

<table>
<thead>
<tr>
<th>Disinfection sample (0)</th>
<th>Blank 1</th>
<th>Blank 2</th>
<th>Blank 3</th>
<th>Blank 4</th>
<th>Blank 5</th>
<th>Blank 6</th>
<th>Blank 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>¾ LB medium</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Electrodes(^a)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Granulated activated carbon (GAC)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Radio frequency alternating el. field (^b)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>(RF-AC 100 kHz; 6 ± 0.5 V/cm) (^b)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>E.coli YMc10 (bacteria)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^a\) Stainless steel electrodes are present but no RF-AC is applied Figure 2.1 (B).
\(^b\) Stainless steel electrodes are present and RF-AC is applied Figure 2.1 (C).

2.2.7. The effect of RF-AC on FBE disinfection

The relation between AC frequency and FBE disinfection efficiency was investigated. Duplicate experiments were performed at 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 ¾ 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 2.1, blank 6; Figure 2.1 (B)) was running parallel to the disinfection sample (Table 2.1 disinfection sample (0); Figure 2.1 (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 normal distribution of the samples was verified using STATDISK 10.4.0 (Triola 2007).
2.3. Results and discussion

2.3.1. GAC properties

The properties of pretreated (autoclaved) carbon (GAC$_{pr}$) and carbon before pretreatment (GAC$_{r}$) are compared in Table 2.2. Analyzed carbons have moderately high specific surface areas (BET) (Gryglewicz et al. 2005). The pretreatment and milling does not influence the change in BET area (Table 2.2).

The macropore area represents a small percentage of total surface area (Table 2.2). 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 (Bandosz 2006).

The pH$_{pzc}$ values (Table 2.2) 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 (Bandosz 2006). 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$_{pzc}$ (Marsh and Rodriguez-Reinoso 2006). The pH$_{pzc}$ together with amphiphilic properties of bacteria and solid surfaces are parameters that determine the extent of bacterial adhesion on the surfaces (Rijnaarts et al. 1995). E. coli pH$_{pzc}$ is in range 2.1 and 4.3 (Lytle et al. 1999, Moreno-Castilla et al. 2003). Therefore, E. coli adhere better on hydrophobic than on hydrophilic surfaces (Ong et al. 1999). 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 YM10 adhere in lesser extent on GAC.
Table 2.2. GAC physical characteristics (Carbon type, specific 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.

<table>
<thead>
<tr>
<th>Activated carbon Rx 3 EXTRA type</th>
<th>BET area, m²/g</th>
<th>A_{macropore}, in % of BET</th>
<th>pH_{pzc}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulated activated carbon before pretreatment (GAC_r)</td>
<td>1407 (± 48)</td>
<td>&lt;1</td>
<td>8.45 (± 0.10)</td>
</tr>
<tr>
<td>Granulated activated carbon pretreated (GAC_{pr})</td>
<td>1428 (± 89)</td>
<td>&lt;1</td>
<td>7.77 (± 0.17)</td>
</tr>
<tr>
<td>Powder activated carbon before pretreatment (PAC_r)</td>
<td>1424 (± 92)</td>
<td>&lt;10</td>
<td>8.80 (± 0.21)</td>
</tr>
<tr>
<td>Powder activated carbon pretreated (PAC_{pr})</td>
<td>1417 (± 90)</td>
<td>&lt;10</td>
<td>7.54 (± 0.23)</td>
</tr>
</tbody>
</table>

2.3.2. Luminescence measured in a set of blanks

In Figures 2.2-2.4, 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 2.1) and different blanks (Table 2.1) were compared; disinfection sample is presented in all the Figures (2.2 – 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, (Figure 2.2) 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 (Prescott et al. 2002). 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.

Figure 2.2. The effect of nutrients absence on the change in luminescence intensity. Comparison of disinfection sample 0 ▲ (¼ 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).

For the blanks 4, 5 and 7 that did not contain GAC (Figure 2.1 A), no decay in the CPS value could be observed in comparison to the disinfection sample (0) (Figure 2.3).

Figure 2.3. The effect of GAC on the change in luminescence intensity. Comparison of disinfection sample 0 ▲ (¼ 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).
For the solution with GAC a decrease in *E. coli* YMc10 luminescence value (CPS) was observed in both cases (Figure 2.4): with the applied RF-AC electric field and without the RF-AC electric field (Table 2.1, blank 6 and blank 1).

**Figure 2.4. The effect of RF-AC on the change in luminescence intensity. Comparison of disinfection sample 0 ▲ (¼ 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).**

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 $10^6$ to $10^4$ (Figure 2.4). 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 (Masschelein and Rice 2002). 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 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.

### 2.3.3. 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 2.1, 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 Figure 2.5). 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 (1989) 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 hours in the FBE. This is longer than disinfection with DC in range 3-110 kV (Mazurek et al. 1995, Narsetti et al. 2006) or low frequency electric field at 16-60 Hz with electric current 125-370 mA/cm² electrode (Birbir et al. 2009, Park et al. 2003). 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 (Lian et al. 2007, Mazurek et al. 1995, Narsetti et al. 2006).
Figure 2.5. Disinfection with FBE system at 6 ± 0.5 V/cm and 163 ± 5 mA/cm² electrode at different frequencies. E. coli YMc10 viable cell concentration (log(CFU₀/CFUₜ)) within 360 min (series). Dashed lines connect the same time points from different frequencies.

The disinfection efficiency depends on the applied frequency (Figure 2.5). The highest efficiency within 5 hour time was reached at 140 kHz within the frequency range tested at an average power consumption of 1.5 kWh per 1 L of disinfected solution. To confirm if the disinfection sample is significantly different from a control (without applied RF-AC), the standard deviations of control and the disinfection sample were calculated (Figure 2.6). 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.
Figure 2.6. 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.

The variation of *E. coli* YMc10 concentration in the control (Figure 2.6) 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* (Binhi et al. 2001). When bacteria adhere on GAC they might lose the viability by membrane disruption (van der Mei et al. 2008). Bacteria have cell structures that make adherence on rough surfaces possible (Coelhoso et al. 1992). To completely adhere *E. coli* on GAC particles at least 3 days of shaking with GAC at 100 rpm is necessary (Moreno-Castilla et al. 2003). In our case without an applied RF-AC electric field, 0.5 log CFU/mL (Figure 2.6) 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.
2.4. 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 (Markx and Davey 1999, Pethig and Kell 1987); increased cell membrane permeability and change in shape (Chang 1989). 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 (Beurrier et al. 2001, Panagopoulos et al. 2002). The effect of the resonance frequency on cell wall permeability has been established in cell fusion by induced increase in membrane potential (Chang 1989). An increased membrane potential could also cause membrane disruption followed by leakage of cellular content (Palaniappan et al. 1990).

Cells are found to rotate in alternating electrical fields (Markx and Davey 1999) possibly causing mechanical cell fatigue (Pohl 1982). Cell rotation characteristics are dependent on a lot of factors such as: cell aging, viability etc. (Holzapfel et al. 1982, Mischel and Pohl 1983). For the pathogenic E. coli K12 rotation torque arises at the frequency between 100 and 500 kHz (Berg and Turner 1993), which is consistent with the frequency range applied in our study.

In RF-AC electric fields a time dependent effect of cavitation (Gogate 2002) can cause changes in cell shape (Chang 1989). Moreover it induces physical disintegration of microbial cells and e.g. E. coli cells are highly susceptible to it (Foladori et al. 2007).

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 (Funk and Monsees 2006, Grissom 1995, Sheppard et al. 2008). 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 (Binhi et al. 2001).

The cause of microorganism death in most above mentioned cases is affected by pH, temperature, radical formation and conductivity of the disinfected medium (Funk and Monsees 2006, Jeyamkondan et al. 1999, Murphy et al. 1993). Granulated activated carbon seems to serve as fluidized conductive component with a high surface area that enhances these cell-electric field interactions.

2.5. 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 an 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.
Acknowledgements

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Chapter 3

Alternating electric fields combined with activated carbon for disinfection of Gram negative and Gram positive bacteria in Fluidized Bed Electrode system

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 six hours 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.

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3.1. 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 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. 2012, 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 cell 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. E. coli YMc10 disinfection of 2.7 Log CFU decay within 6 hours 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 its 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, Bacillus subtilis subsp. subtilis, Enterococcus faecalis) and Gram (-) (Erwinia carotovora, Pseudomonas luteola, Pseudomonas fluorescens, Escherichia 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 hours. 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).

3.2. Material and Methods

3.2.1. Granular activated carbon employed for disinfection

The granulated activated carbon (GAC) Norit RX 3 Extra (Norit, The Netherlands) was employed in FBE disinfection as suspended bed material. The GAC used, had a BET area of 1400 m$^2$/g, pH$_{pzc}$ 7.8 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 3.1) and subsequently 40 mL of reaction medium were added for disinfection performance investigation in the FBE.

GAC in FBE is considered as a component that facilitates the electric field strength and bacteria adhesion. In this study the disinfecting effect occurring due to AC field was the primary objective. Therefore, GAC was pretreated with organics and mineral rich electrolyte to avoid environmental stress factors introduction on the microbial cultures, such as pH variation, starvation and isotonic imbalance; as these stress factors would obscure the effect of disinfection by the AC electric field. Other stresses occurring in real wastewater situations are expected to increase the disinfection rate achieved with FBE.

### 3.2.2. Microorganisms and cultivation

Different bacteria were used as model microorganisms for wastewater effluents and selected for disinfection investigation in FBE. Specific bacteria were chosen as representatives for their specific characteristics: *E. coli* and *E. faecalis* as fecal contamination indicative bacteria (Foladori et al. 2007). *E. corotovora* is a plant pathogen commonly found in the soil together with *Pseudomonas* and *Bacillus* (Glick 2010, Thomas et al. 2011). *Pseudomonas* genus was chosen from the group of Gram negative bacteria as flock forming species and it has been previously shown that it is resistant to some physical and chemical disinfection methods (Li et al. 2009). *Bacillus* is a representative of endospore forming bacteria. All bacteria used are rod shaped except *E. faecalis* which is coccioid shaped.

Two species from the same genus *Pseudomonas* (*luteola* and *fluorescens*) and two species from another genus *Bacillus* (*subtilis* and *subtilis* subsp. *subtilis*) were used to test the disinfection on the genus and investigate whether the FBE disinfection effect is strain specific or genus specific. All bacteria were obtained from DSMZ (Leibniz–Institute,
German Collection of Microorganisms and Cell Cultures, Germany) except *E. coli* YMc10 which was obtained from Coordinated Collections of Microorganisms, Belgium.

All bacteria used were non–pathogenic strains. Pure microbial cultures were used for disinfection performance quantification and to get insights in the FBE disinfection process. Microbial cultures were grown at optimal growth conditions. Bacteria were harvested in an end exponential, beginning stationary phase for reproducible results. *E. coli* was cultivated in Lysogeny broth (LB) media as described previously (Racyte et al. 2011). As *E. coli* was genetically modified to have a luminescence transcription, all media and GAC that were used for *E. coli*, were supplemented with 0.1 g/L ampicillin (Sigma-Aldrich, Inc.). The other bacteria were cultivated in 30 g of tryptic soy broth (TSB) obtained from Fluka (Sigma Aldrich, Inc.), which was dissolved in 1 L of Milli-Q water (18.2 MΩ•cm - 25 ºC, 0.22 µm, Millipore Biocel SAS 67120, France) and was autoclaved for 25 min at 0.2 MPa, 121 ºC. The bacteria were grown in the sterile growth medium overnight. Before the start of an experiment the cells were harvested by centrifugation and removal of the supernatant, followed by re-suspension of the bacteria to reach the concentration of ~10^8 bacteria per mL medium and transferred to the FBE disinfection beaker. The original media concentrations were diluted with the ratio given in Table 3.1, further referred to as reactor media. The dilution ratios for reactor media for each bacterium were experimentally established (data not shown) to obtain a stable microbial culture (where growth was equal to decay) in GAC suspensions at ambient temperature for at least 6 hours (disinfection experiment duration). Due to the dilution the salt concentration is lowered in TSB and LB (for *E. coli*), therefore addition of NaCl (VWR international) is required to maintain the normal isotonic pressure (Table 3.1). To avoid contamination of microbial cultures, all procedures were performed in sterile conditions.
Table 3.1. Bacteria and their corresponding media concentrations used as FBE reactor media. The growth media were diluted to enable stable microbial cultures at ambient conditions for 6 hours.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Reactor medium</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> YMc10</td>
<td>1:15 LB +NaCl 5 g/L</td>
</tr>
<tr>
<td><em>Pseudomonas luteola</em></td>
<td>1:15 TSB + NaCl 0.25 g/L</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>1:13 TSB + NaCl 0.25 g/L</td>
</tr>
<tr>
<td><em>Erwinia carotovora</em></td>
<td>1:25 TSB</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> subsp. subtilis</td>
<td>1:20 TSB + NaCl 0.28 g/L</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>1:20 TSB + NaCl 0.28 g/L</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>1:10 TSB</td>
</tr>
</tbody>
</table>

3.2.3. Experimental set-up

Experiments were performed with 8 glass beakers of 100 mL (Duran, Germany) in parallel, each filled with 22 g of GAC and 40 mL bacterial suspension. The duration of experiments was 6 hours, based on the method applied in our previous study (Racyte et al. 2011). In four beakers, the electric field was applied for 6 h continuously using two square stainless steel plate electrodes (13 cm$^2$) per beaker with a distance of 1.9 cm between the electrodes (Figure 3.1). Beakers that were exposed to electricity are denoted as ‘sample’ or FBE reactor cell with closed electrical circuit and applied electricity. The other four beakers were treated the same way, however no electric field was applied, these are further denoted as ‘control’.

A magnetic stirrer (240 rpm) with multiple plates (Variomag telesystem, H+P Labortechniek AG, Germany) was used for mechanical agitation of the GAC suspension (Figure 3.1). The temperature inside the FBE reactor cells was controlled at 22.5± 2.5 °C in an ice bath. Nd-Fe-B magnets (type S-08-30-N Supermagnete, Germany) coated with rubber were used as stirring magnets to lift the GAC particles to a well mixed suspension (Teflon coated stirring magnets did not accomplish this and therefore not used).
The alternating electric field (AC field) was supplied by a custom made amplifier, capable of high power output at frequencies up to 500 kHz, and a frequency generator (TG2000- 20 MHz, Thurlby Thandar Instruments, U.K.).

Figure 3.1. Experimental set-up. An AC field was applied with different wave forms: a) continuous sinus wave, and b) FSK modulated dual sinus wave.

The FBE disinfection experiments were performed with AC field frequencies ranging from 10 kHz to 285 kHz, with a step size of 60-80 kHz. The frequencies were chosen based on disinfection optima obtained with *E. coli* in a previous work (Racyte et al. 2011). These signals were applied as a single continuous frequency and as frequency modulated signals by dual frequency shift keying (FSK) further denoted as [frequency 1]& [frequency 2] dual FSK i.e. continuously switched between two frequencies every 50 ms. The set of AC field frequencies applied in the FBE disinfection experiments are given in Table 3.2. The applied AC electric field was 7 V$_{\text{RMS}}$/cm and the average current density measured was $325 \pm 5$ mA/cm$^2$ electrode. Impedance analysis (Impedance analyzer HP4194A (Hewlett-Packard, California, U.S.)) showed that impedance of FBE system was frequency independent. Each microorganism was exposed to each frequency in duplicate or triplicate independent experiments.
Table 3.2. AC field continuous sinus wave and dual FSK modulated frequencies tested for FBE disinfection performance.

<table>
<thead>
<tr>
<th>AC field frequencies employed for disinfection, (±2 kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 kHz</td>
</tr>
<tr>
<td>80 kHz</td>
</tr>
<tr>
<td>140 kHz</td>
</tr>
<tr>
<td>200 kHz</td>
</tr>
<tr>
<td>10 kHz &amp; 80 kHz dual FSK</td>
</tr>
<tr>
<td>10 kHz &amp; 140 kHz dual FSK</td>
</tr>
<tr>
<td>80 kHz &amp; 140 kHz dual FSK</td>
</tr>
<tr>
<td>280 kHz &amp; 285 kHz dual FSK</td>
</tr>
</tbody>
</table>

3.2.4. Microbiological analysis

To quantify the disinfection performance in the FBE system, samples were collected every 90 minutes and stored at 4 °C till further analysis. Each sample was analyzed the same day. The concentration of bacteria in each sample was determined by plating the diluted aliquot on agar plates. The dilution series was performed in $\frac{1}{5}$ TSB and for *E. coli* in $\frac{1}{4}$ LB (containing ampicillin). Subsequently $10^{-3}$-$10^{-6}$ dilutions were plated on TSB agar or LB agar (for *E. coli*) plates employing the three drop technique (Racyte et al. 2011). Three drops of 10 µL of the same diluted sample were pipetted (allowing the drops to run down the agar surface) on the same petri dish as triplicate. The agar plates were incubated in the incubator for 18 h for almost all bacteria and for 40 h for *Pseudomonas* and *E. faecalis*. The cultivation temperature was selected for each bacterium according to the specifications (*E. faecalis, Bacillus, Pseudomonas* at 30 °C; *E. coli, E. corotovora* at 27 °C). Colony forming units (CFU) were quantified after 18 h or 40 h and bacteria concentration was recalculated to colony forming units per mL (CFU/mL).
3.2.5. Microbiological analysis of bacteria immobilized inside GAC particles

Random GAC particles were taken out with sterile tweezers one by one from the sample beaker and from the control beaker (~30 GAC particles at each sampling time from each beaker). Each GAC particle was rinsed briefly in polyphosphate buffered saline (PBS) to remove remaining disinfection solution. PBS was prepared by dissolving 9.55 g of 10x Dulbecco’s Powder (Applichem, Germany) in 1 L of Milli-Q water. The rinsed GAC particle was subsequently transferred to a 2.5 mL PBS solution. Then the PBS solution was mechanically shaken at 2000 rpm (MultiReax Heidolph, Germany) for 2 hours in ambient conditions to detach bacteria from the GAC surface. After 2 hours agitation the GAC particles were removed from the PBS solution and the aliquots of PBS solution containing detached bacteria were serially diluted and plated on agar plates; plates were incubated and the amount of CFU/mL was determined.

One particle from each bacteria suspension was taken for scanning electron microscope (SEM) visualization (before rinsing with PBS). The GAC particles were fixated by suspending GAC particles in 1 mL of 2.5 % glutaraldehyde solution (Sigma Aldrich, Germany) in 0.1 M PBS and left for 24 hours in 4 ºC. Afterwards, particles were rinsed three times (for 15 min) in PBS. Then the particles were dehydrated by using a six-step ethanol/water gradient (30 % – 100 %) and left overnight at room temperature to dry. Subsequently, samples were kept in a desiccator until analysis with SEM. Prior SEM analysis, samples were coated with gold and examined with SEM – JEOL JSM-6480LV (Acceleration voltage 6 kV, HV-mode, SEI detector).

3.2.6. Data processing

Each set of microorganisms was exposed to its designated frequency in duplicate or triplicate independent experiments to test the FBE disinfection reproducibility. Each sample was plated in triplicate. Disinfection data from independent experiments of the same frequency were statistically compared with a t-test, outliers were rejected and
statistically equal samples were averaged. The normal distribution of the data was verified using STATDISK 10.4.0. (Triola 2007) for the 95 % confidence interval ($\alpha=0.05$). The bacteria concentration CFU/mL was normalized to its initial concentration and the logarithmic value was calculated $\log \left( \frac{\text{CFU}_t}{\text{CFU}_0} \right)$ for disinfection experiments. In case there was no bacteria concentration variation between time data points (in experiment – bacteria immobilized inside GAC particles), the time average of the difference between sample and blank was determined ($\Delta \log \text{CFU}$). Standard deviations of the Log values were calculated from the triplicates using a Taylor series expansion.

To obtain the disinfection data due to electricity the disinfection without AC field applied (control) was subtracted (in Log scale) from the total disinfection obtained with the AC field applied (sample). A larger decay of sample compared to control is expressed with a negative sign (-Log), whereas a larger decay in control compared to sample is expressed with a positive sign (+Log). For data comparisons the disinfection due to the electric field was denoted negligible if the decay of bacteria in the control was equal or higher than in the sample.

To compare, the disinfection rate at all frequencies of all bacteria, the un-weighted average and disinfection range over all frequencies applied were calculated for each bacterial species. The un-weighed average was calculated by taking an absolute value of the disinfection average for each bacteria at all tested frequencies and is further denoted as “FBE sensitivity”. The disinfection range, further denoted as “frequency specificity” is the difference between highest and the lowest disinfection value achieved for each bacteria at all investigated frequencies after 360 minutes of disinfection.
3.3. Results

3.3.1. Disinfection of different bacteria at different frequencies

The effect of different AC field frequencies and FSK electric field frequencies (Table 3.2) on the disinfection of 3 Gram (+) and 4 Gram (-) bacteria in the FBE system was investigated.

It was previously shown (Racyte et al. 2011) that *E. coli* was sensitive at specific frequencies to disinfection in the FBE system. In the present study disinfection of *E. coli* in FBE system was tested at the same 3 frequencies and another 5 different frequencies. The same 8 frequencies were applied on 6 other types of bacteria.

Figure 3.2. *E. coli* YMc10 disinfection performance with the FBE system at 7 ± 0.5 V\(_{\text{RMS}}\)/cm and 325 ± 5 mA/cm\(^2\) electrode at different conditions: x - at ambient conditions with no treatment; ○ - with application of AC field at 10 kHz & 140 kHz without GAC; ■ - with GAC treatment; ▼-with FBE treatment at 10 kHz & 140 kHz. Error bars indicate standard deviations (α=0.05).

Figure 3.2 shows the change in *E.coli* concentration over time when exposed to different conditions: *E.coli* in reactor electrolyte, exposed to AC field alone, exposed to
GAC alone and exposed to FBE disinfection (combination of AC field and GAC). From the data obtained (Figure 3.2) in reactor electrolyte without GAC, E.coli shows a slight growth (0.3 Log CFU increase) in the initial state, and does not further increase and thus remains stable over 6 hours. Under the exposure to AC field the growth is still observed (0.2 Log CFU increase), but slightly lower compared to the conditions without AC field, and then remains stable. The exposure to GAC alone results in disinfection (0.4-0.6 Log CFU E.coli decay). The exposure to FBE (combination of AC field and GAC) at double modulated frequency of 10 kHz & 140 kHz results in E.coli 3.3 Log CFU disinfection. Thus, when the GAC suspension is exposed to an AC field at specific frequency, the disinfection is enhanced with 2.5 Log CFU decrease.

Furthermore, as GAC disinfection (control) was found to be constant, therefore only enhancement of disinfection due to AC field is further taken into account. The summary of AC field effects in GAC suspensions of E.coli at 8 different frequencies is summarized in Figure 3.3.

Figure 3.3. E. coli YMc10 disinfection performance with the FBE system at 7 ± 0.5 V_RMS/cm and 325 ± 5 mA/cm² electrode at different continuous and FSK modulated AC field signals. E. coli YMc10 viable cell concentration (Log(CFU_t/CFU_0)) over time (within 180 min and 360 min). Error bars indicate standard deviations (α=0.05). Data is corrected for decay in control without electricity.
Figure 3.3 shows that *E. coli* was sensitive for frequency of 140 kHz as published earlier (Racyte et al. 2011). In current study similar disinfection results were obtained with 2.2 Log *E. coli* reduction at 140 kHz. Moreover, it was discovered that the disinfection also occurs at a low AC field frequency of 10 kHz (1.8 Log CFU decrease) and at dual FSK modulated frequencies of 10 kHz & 140 kHz (2.5 Log CFU decrease).

Figure 3.4. *Bacteria disinfection performance with the FBE system at 7 ± 0.5 V_{RMS}/cm and 325 ± 5 mA/cm² electrode at different continuous and FSK modulated signal AC field. Gram (-) bacteria (Pseudomonas fluorescens, Erwinia carotovora, Pseudomonas luteola) and Gram (+) bacteria (Enterococcus faecalis, Bacillus subtilis, Bacillus subtilis subsp. subtilis) viable cell concentration (Log(CFU₉₀/CFU₀)) over time (within 180 min and 360 min). Error bars indicate standard deviations (α=0.05). Data is corrected for decay in control without electricity.
*P. luteola* and *E. corotovora*, were disinfected in the FBE system, only to a lower extend compared to *E. coli*. The highest disinfection achieved was a 2 Log reduction during 6 h treatment time for *P. luteola* and 1.5 Log reduction during 6 h treatment time for *E. corotovora* for both bacteria at 80 kHz & 140 kHz dual FSK frequencies (Figure 3.4).

*P. fluorescens* is the only bacterium, from Gram (-) bacteria investigated, which did not show any disinfection. From three Gram (+) bacteria tested for disinfection in FBE, only *Bacillus subtilis* subsp. *subtilis* was disinfected at 80 kHz and at 10 kHz & 140 kHz AC field frequencies and 0.5-1 Log CFU decay was achieved.

For comparison of the total disinfection performance between all bacteria, data at all frequencies were summarized in the Table 3.3, in terms of i) un-weighted average value for all frequencies tested indicating frequency unspecific FBE sensitivity and ii) the range of variation, indicating the frequency specificity in disinfection.

*Table 3.3. The overall FBE disinfection performance comparison for all bacteria at all frequencies: the average of Log CFU decrease at all frequencies (FBE sensitivity) and the difference between highest and lowest Log CFU decrease (Frequency specificity).*

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>FBE sensitivity</th>
<th>Frequency specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> YMc10</td>
<td>1.2</td>
<td>2.3</td>
</tr>
<tr>
<td><em>Pseudomonas luteola</em></td>
<td>1.3</td>
<td>0.97</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>0.16</td>
<td>n.d.*</td>
</tr>
<tr>
<td><em>Erwinia carotovora</em></td>
<td>0.5</td>
<td>1.3</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> subsp. <em>subtilis</em></td>
<td>0.3</td>
<td>0.67</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>0.05</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*n.d. is not determined due to the occurring growth in the control; therefore the number is left out.

Data analysis in Table 3.3 quantitatively expresses the sensitivity of different bacteria for FBE disinfection. On the average the disinfection of *E. coli* is moderately
effective at all frequencies investigated with a FBE sensitivity of 1.2 Log, and larger effectiveness can be obtained at specific frequencies with a frequency specificity of 2.3 Log. The same pattern but at lower disinfection levels was found for *E. carotovora* that has a FBE sensitivity of 0.5 Log and a frequency specificity of 1.3 Log. The only Gram (+) bacterium *B. subtilis* subsp. *subtilis* which was to some extent sensitive for disinfection, shows a FBE sensitivity value of 0.3 Log and a frequency specificity of 0.7 Log: these bacteria do not show high overall sensitivity for FBE disinfection, however there is higher disinfection level obtained at specific frequencies. *P. luteola* has high FBE sensitivity of 1.3 Log, although the frequency specificity is 0.97 Log meaning that *P. luteola* is less sensitive for specific frequencies but it is more susceptible for overall FBE disinfection. The rest of the bacteria investigated have negligible disinfection rates, i.e. less than 0.5 Log.

### 3.3.2. Effect of the AC field on bacteria immobilized inside the GAC particles

One of the essential components in the FBE disinfection system are the GAC particles. Therefore, during experiments particles were collected, fixated and visualized with a Scanning Electron Microscope (SEM).
Figure 3.5. SEM pictures from GAC particles and attached bacteria from the control beakers. Gram (-) bacteria: A - Pseudomonas fluorescens, B - Erwinia carotovora, C - Pseudomonas luteola; and Gram (+) bacteria: D - Enterococcus faecalis, E - Bacillus subtilis, F - Bacillus subtilis subsp. subtilis. There were no bacteria found on GAC particles taken from the sample beakers (data not shown).

In the SEM micrographs of GAC particles taken from the control beakers (Figure 3.5) the presence of adhered bacterial cells can be observed. The cell exteriors seem intact and bacteria are present in groups or micro-colonies. However, SEM photos indicated a difference in bacterial quantity attached on GAC particles taken from sample and control beakers (on GAC taken from sample beakers there were no bacteria observed with SEM, not shown). Experimentally the amount of each species adhered to GAC particles in the
sample (with electric field) and in the control (no electric field) beakers during the 6 h disinfection experiment was determined. The amount of bacteria adhered on GAC particles in the sample and in the control was different; however there was no concentration variation during the time in the same beaker. The difference in bacteria concentration immobilized inside the GAC particles between the sample and the control GACs was determined as $\Delta \log (\text{CFU})$. The $\Delta \log (\text{CFU})$ (equals the Log amount of cells recovered from the sample GAC subtracted from the Log amount of cells recovered from the control GAC) was plotted versus disinfection data $\log (\text{CFU}_t/\text{CFU}_0)$ at the same frequency for each bacterium (Figure 3.6). A value of $\Delta \log (\text{CFU})=0$ indicates equal recovered cell amounts (for GAC sample and GAC control), i.e. cells were not killed while adhered on the GAC surface in sample where the electric field was applied. A value of $\Delta \log (\text{CFU})$ with a significant number indicates that cells were killed when immobilized on the GAC and upon electric field application.

Figure 3.6. Relation of disinfection performance versus bacteria immobilization inside GAC particles. The disinfection data in $\log (\text{CFU}_t/\text{CFU}_0)$ at the same frequency for each bacterium is plotted versus the difference of bacteria immobilized inside GAC (from sample and control) $\Delta \log (\text{CFU})$ for each bacterium at the same frequency.
Figure 3.6 shows that there is a relation between disinfection data \( \log(CFU_t/CFU_0) \) (bacteria death) and \( \Delta \log(CFU) \) (difference of bacteria immobilized inside sample GACs and control GACs).

The highest \( \Delta \log(CFU) \) value is obtained for \( E. coli \) (1.2 Log) as well as the highest disinfection value of 2.5 Log (Figure 3.3). The second best disinfection value in the FBE is achieved for \( P. luteola \) with a \( \Delta \log(CFU) \) value of 0.8 and a disinfection value of 2 Log. For the rest of the bacteria the \( \Delta \log(CFU) \) values lay between 0.5 and 0.

### 3.4. Discussion

The studied Gram (-) bacteria are sensitive to FBE disinfection with the exception of \( P. fluorescens \). Due to different disinfection rates achieved for \( P. luteola \) and \( P. fluorescens \); and for \( B. subtilis \) and \( B. subtilis \) subsp. \( subtilis \), it can be concluded that FBE disinfection is frequency specific and strain specific.

Amarjargal et al. (2012) reported \( E. coli \) disinfection in media with ceramic tourmaline nanoparticles with AC fields in frequency range of 0.1 to 1 MHz. They observed a higher disinfection rate at a frequency of 100 kHz than at 1 MHz, which corresponds with our findings for the bacteria being sensitive in the 100 kHz frequency range.

Our data shows an enhanced \( E. coli \) disinfection effect in the FBE system by applying AC field at dual FSK frequencies (10 kHz & 140 kHz) compared to single frequencies of 140 kHz and 10 kHz. A similar observation was published by Qin et al. (1994) who investigated the disinfection rate with a pulsed electric field system at 10-35 kV/cm. They concluded that for disinfection bipolar pulses were more effective than the single pulses. This added effect might occur, according Barnes (2007), because cells are rather recalcitrant to a constant signal, but more vulnerable to a varying signal. Frequencies in the range from 10 kHz to 1 MHz and at low field strengths (1-4 V/cm) can result in the vibration of ions and molecules at the exterior of cells as well as inside the cell (Xie et al. 1997). This does not cause adverse effects on the cells immediately, but can
however suspend cell division and increase cell fatigue and wear-out (Giladi et al. 2008). The suspended growth can be observed in Figure 3.2, when compared *E. coli* concentration change in time without treatment and with applied AC field. The difference in concentration is small (0.1 Log CFU) therefore, actually it can not be distinguished whether it is an experimental error or suspended growth due to exposure to AC field.

Bacterial membranes become more permeable in electric fields above 100 kHz (Chang 1989). Bacterial membrane permeability increase is used for instance to enhance antibiotics uptake by Gram (-) bacteria in a radio frequency field (Giladi et al. 2008). Another cause of disinfection might be due to the continuous sinusoidal AC field and FSK-modulated AC fields that can affect the trans-membrane voltage. This might explain why some applied frequencies have more effect than others (Kotnik et al. 1998). Challis et al. (2005) reported non uniform electric fields at radio frequencies (in range of 0.1-1 Mhz). This introduces voltage drops and adverse effects causing non-linear effects on the cell membrane that might result in a short circuiting of a cell membrane resistivity.

Furthermore, the effects due to GAC have to be considered as well, because bacteria can attach to GAC as also observed in the SEM pictures, contributing to bacteria removal (Hijnen et al. 2010). The difference of attached bacteria on GAC sample and control (Figure 3.6) implies that cells sensitive to disinfection, are also less attached to GAC. The data confirm that higher disinfection of planktonic bacteria was paralleled by an increased cell removal (killing) at the GAC surface. Therefore, the crucial effect of GAC in an AC field has to be considered. Voltage gradients will be present on the GAC surface due to differences in GAC and liquid resistances, facilitating unfavorable conditions for the attached bacteria that can be enhanced by surface properties of GAC resulting in reduced viability of attached bacteria (van der Mei et al. 2008). Furthermore, the strength of the electric field will be locally increased in between GAC particles (Ferreira 2008). In this way, bacteria can get electrocuted in the surroundings of GAC particles.

In the results of our present study, not all Gram (-) bacteria did show a similar sensitivity for specific frequencies during FBE disinfection, and disinfection achieved was lower than the one obtained for *E. coli* (Figures 3.3 and 3.4). This might be due to the
applied frequencies (10, 80, 140, 200 kHz), which were chosen with respect to the optima obtained in a previous study with *E. coli* YMc10 (Racyte et al. 2011). Non occurrence of disinfection with *P. fluorescens* might be due to the presence of cell surface polymers (extracellular polymers) that also contribute to flock forming nature known for these bacterial strains. The capsule may cover cell membranes and make these cells more resistant to environmental effects (Yao et al. 2012, Li et al. 2009, Xavier 2011) and thus also disinfection in FBE.

Interestingly, the investigated Gram (-) bacteria (*E. coli*, *P. luteola*, *E. corotovora*) showed higher disinfection susceptibility at the same energy input and different frequencies in the FBE system at the applied conditions compared to the Gram (+) bacteria. It appears that the sensitivity for specific frequencies also exists for some organisms of Gram (+) type (such as *Bacillus subtilis* subsp. *subtilis* in this study), even though the disinfection performs at low rates.

The difference between Gram bacteria types is mainly the cell exterior architecture such as presence/absence of peptidoglycan cell wall matrix, other polymeric structures, outer membrane. Therefore, the disinfection mechanism in the FBE system possibly has a physical origin, similar to pulsed electric field and ultrasonication (García et al. 2007, Neis and Blume 2003) rather than a chemical origin (such as antibiotics and peroxides).

A rough energy calculation shows that for a liter of artificial wastewater disinfection containing gram negative bacteria at least 2.2 kWh/L was required, which is too high to be competitive with other disinfection technologies. The presented setup was used for the proof-of-principle study and to test bacteria susceptibility for AC fields and frequency specificity. It is likely that there are possibilities for improvements with respect to energy use and disinfection duration. Further research is required to enhance disinfection rates before the FBE technology can be developed to a stand alone disinfection technique or a selective disinfection technique for specific applications.
3.5. Conclusions

FBE disinfection is a novel technology oriented at post-treatment of specific wastewater streams.

- The described disinfection is frequency dependent for the studied bacteria (*P. luteola, P. fluorescens, E. corotovora, E. coli, B. subtilis, B. subtilis subsp. subtilis, E. faecalis*). *E. coli* is most susceptible to disinfection at specific frequencies and with a 2.5 Log concentration decay in (live cell numbers), however *P. luteola* is the most susceptible for FBE disinfection with a 1.3 Log concentration decay in (live cell numbers) at all frequencies investigated.

- Different bacterial species show different disinfection rates. The bacteria investigated that were exposed to the optimum frequency for *E. coli* showed different disinfection rates. This might be due to differences in cell exterior architecture of bacteria creating a variable sensitivity to electric field frequencies.

- In general Gram (-) bacteria were more susceptible for disinfection at the applied frequencies than Gram (+) bacteria. Gram (+) bacteria were not considerably affected by disinfection in FBE at tested frequencies, and this is an indication that the disinfection mechanism is of a physical nature rather than due to the chemical effects.

- The dual frequency modulated (FSK) AC field works better for the disinfection in FBE than a continuous frequency.

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3.6. References


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Chapter 4

Effect of granular activated carbon and substrate availability on survival of Escherichia coli

Abstract

This paper describes the effects of granular activated carbon (GAC) on the metabolic activity and survival of a pure culture Escherichia coli YMc10. The change in E.coli concentration and metabolic activity was studied with 5 media differing in organic, mineral and salt content and exposed to 5 different GAC concentrations between 23 and 350 g GAC /L. The changes in E. coli concentration and metabolic activity were related to changes in the media such as COD (organic concentration), osmotic balance (salt concentration) and buffer capacity.

Our results show that E. coli YMc10 is sensitive to changes in organic concentrations. The adsorption of organics on GAC is proportional to the GAC concentration present in the suspensions. Severe pH variations were observed in phosphate buffered saline when exposed to GAC suspensions. E. coli suspended in 350 g/L of GAC in media with low organics and low salt concentrations resulted in 3.5 Log decay after 6 hours exposure. The presence of GAC alone does not result in disinfection in rich media, but establishes unfavorable conditions which can enhance adverse effects on microorganism survival in water treatment systems.

4.1. Introduction

Recent outbreaks of infections originating from pathogenic waterborne bacteria rise a concern of efficiency and reliability of conventional water and wastewater disinfection technologies. Disinfection is a key process for the supply of safe water (WHO 2011). There is a vast variety of disinfection systems that are capable to disinfect water (Pandit et al. 2012). The most efficient and broadly used disinfection systems either have high operational and maintenance costs (such as UV and ozone systems) or are cheaper but are hazardous (such as chlorination, ozonation) and lead to the formation of carcinogenic halogenated compounds (Lazarova et al. 1999).

Innovative approaches are needed to decrease eco-toxicological effects originating from water treatment, and to be able to provide safe water. The Fluidized Bed Electrode (FBE) was introduced as an alternative for state of the art disinfection techniques (Racyte et al. 2011). The FBE system consists of a granular activated carbon (GAC) suspension, over which an alternating radio frequency electric field is applied using stainless steel electrodes (Racyte et al. 2011). GAC particles in suspension function as a three-dimensional electrode, allowing the device to operate at low voltages (Racyte et al. 2011).

The first disinfection studies with the FBE system have used *Escherichia coli* YMc10 as target organism (Racyte et al. 2011). These studies showed a 2.7 Log reduction in microbial concentration after 6 hours of treatment at a frequency of 140 kHz. The study showed that the presence of both GAC particles and electric fields is necessary for disinfection. However, the exact death inducing mechanisms underlying this technology at the cellular level are not yet known. It is probable that GAC as conductive component enhances cell-electrical field interactions in FBE (Ferreira 2008).

GAC is widely used in water and wastewater treatment systems as a filter for organic matter and micro-pollutants (Hijnen et al. 2010, Karanfil et al. 1999). It has a relatively low cost, possesses a high surface area, and a broad range of surface functional groups that are responsible for its adsorption capacity. These surface characteristics in
GAC filters favor microorganism adhesion on GAC acting as pathogen barrier, thus functioning as a disinfection method (Moreno-Castilla et al. 2003, Weber et al. 1978). On the other hand, when nutrients and organics are present in the treated water, GAC can facilitate biofilm growth, which introduces the risk of water recontamination by pathogens regrowth in distribution networks (Hijnen et al. 2010). Therefore, insight into the effect of GAC on metabolic activity and survival of bacteria is needed.

In our previous studies, GAC was considered as a physical component in the FBE disinfection system with AC field (Racyte et al. 2011). However, physico-chemical effects of GAC on bacteria disinfection have not been studied so far under FBE conditions.

The metabolic activity and survival of microorganisms are affected by environmental factors such as chemical composition of the media (e.g. osmotic pressure, mineral composition, pH) and physical factors (e.g. temperature, pressure, physical properties of surfaces) (Monod 1949, Prescott et al. 2002). The metabolic activity of cells can be measured efficiently using bioluminescent bacteria, and survival of bacteria can be measured by optical density. Bacteria that possess a bioluminescent gene, will express a certain bioluminescence activity (light) depending on their metabolic activity (Boyer and Wisniewski-Dyé 2009, Ritchie et al. 2003). The expression of the bioluminescent phenotype requires energy. This expression depends on the energy status of the microbial cell (Ritchie et al. 2003, Unge et al. 1999). Therefore, luminescence is a useful tool for the indication of bacterial state.

This paper describes the metabolic activity and survival of bioluminescent E. coli YMc10 at various GAC suspension conditions, such as GAC concentration and chemical concentrations (e.g. organics, mineral availability and osmotic conditions). By identifying the conditions that provide a minimal survival of E.coli, indirect disinfection can be facilitated in a treatment system, with disinfection as a crucial step in water treatment chain to provide society with safe and pathogen-free water.
4.2. Materials and methods

4.2.1. Media

Various media (tap water, pure and diluted phosphate buffered saline (PBS), pure and diluted lysogeny broth) were used for the batch experiments, and are described below.

Tap water was autoclaved for 25 min at 121 °C, 0.2 MPa.

Phosphate buffered saline (PBS) is commonly used in biological research. It is water based buffer solution containing salts with phosphate groups that sustain constant pH and isotonic conditions. PBS was prepared by dissolving 9.55 g of 10x PBS Dulbecco’s Powder (Applichem, Germany) in 1 L of Milli-Q water (18.2 MΩ•cm 25 °C, 0.22 µm, Millipore Biocel SAS 67120, France) and was autoclaved for 25 min. \( \frac{1}{4} \) PBS solution was made by diluting PBS in autoclaved tap water with a ratio of 1:4.

Lysogeny broth (LB) is a complex growth medium consisting of organics (such as: amino acids, peptides, proteins) and “microbial nutrients” in the form of minerals and trace elements (such as: nitrogenous compounds, sulfur, trace nutrients, vitamin complex). LB medium was prepared by dissolving 10 g/L NaCl (GPR Rectapur, VWR, Netherlands); 10 g/L BactoTM Tryptone (BD, U.S.) and 5 g/L BBLTM Yeast extract (BD, U.S.), and autoclaving for 25 min. \( \frac{1}{4} \) LB solution was made by diluting LB in autoclaved tap water with a ratio 1:4.

In experiments with bacteria, 0.116 g/L Ampicillin (Sigma-Aldrich, Inc.) was added.

Five media that varied in concentrations of organics, minerals and salts were used (Table 4.1) to investigate the different conditions imposed on *E. coli* in the presence and absence of GAC.
Table 4.1. General profile of mineral medium, osmotic pressure and buffer capacity of the investigated 5 media.

<table>
<thead>
<tr>
<th></th>
<th>LB</th>
<th>½ LB</th>
<th>PBS</th>
<th>¼ PBS</th>
<th>Tap water</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mineral media</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(organics &amp; nutrients)</td>
<td>Normal</td>
<td>Low</td>
<td>None*</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td><strong>Osmotic pressure</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(salts)</td>
<td>Normal</td>
<td>Low</td>
<td>Normal</td>
<td>Low</td>
<td>None</td>
</tr>
<tr>
<td><strong>Buffer capacity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pH)</td>
<td>Normal</td>
<td>Low</td>
<td>Normal</td>
<td>Low</td>
<td>None</td>
</tr>
</tbody>
</table>

*PBS contains only phosphorus and no organics which is not enough to induce growth, therefore it is defined as “none” in mineral medium category.

4.2.2. Microorganism

The microorganism in the experiments was a genetically modified, bioluminescent, non-pathogenic *Escherichia coli* strain YMc10 (BCCM Belgian Coordinated Collections of Microorganisms, Belgium). This strain contains a plasmid with *Vibrio fischeri lux*ICDABEG operon, *luxR* genes and its promoters, along with an ampicillin selective marker (Engebrecht et al. 1983, Racyte et al. 2011). Bioluminescence is an indicator of the energy status of the cell (Unge et al. 1999), and can thus be used as an indicator of cell health and metabolic activity. Ampicillin resistance allows easy cultivation and preventing contamination by other microorganisms. In addition, ampicillin is required for the expression of the bioluminescent phenotype, because resistance to ampicillin and bioluminescence are on the same plasmid.

*E. coli* was cultivated in LB medium supplemented with ampicillin (0.1 g/L), and incubated at 27 ºC on a shaker at 120 rpm (Labnet 311DS, U.S.) for 18 ± 2 h. The cultivation temperature of 27 ºC provided an active luminescence gene (supplier
specifications). All experiments and analyses performed with *E.coli* pure culture were carried under sterile conditions.

### 4.2.3. Granular Activated Carbon

The granular (3 mm size) activated carbon (GAC) used in all experiments was NORIT RX3 EXTRA (Norit, The Netherlands). To quantify the adsorption of organics and salts and effect on *E.coli* 5 GAC concentrations were chosen: 23 g/L; 44 g/L; 88 g/L; 175 g/L and 350 g/L. The highest concentration (350 g/L) was previously successfully employed for disinfection in fluidized bed electrodes (FBE) (Racyte et al. 2011). Before its use, GAC was weighted, wetted, washed and autoclaved as described by Racyte et al. (2011) to remove industrial impurities and for sterilization. After this treatment, GAC possesses a specific surface area (BET) of 1400±50 m²/g and pH of zero charge (pHₚzc) of 7.8 (Racyte et al. 2011).

### 4.2.4. Experimental set-up

#### 4.2.4.1. Chemical adsorption of media components on GAC.

*a) Isotherms of organics adsorption on GAC.* The data for organics adsorption isotherms of LB to GAC was obtained using Chemical Oxygen Demand (COD) quantification. The experiments were carried out until an equilibrium concentration was reached, which occurred at day 6 (raw data is supplemented in appendix – Figure A.4.2).

The measurement of COD with cuvette tests requires the absence of Cl⁻ to obtain an accurate result. Therefore, LB without NaCl was used during these experiments which also change the ionic strength of the media. At the ionic strength applied salting out effects are small, therefore it was not expected to affect the adsorption performance.

Weighed and pretreated GAC particles (23 - 350 g/L) were immersed overnight in 100 mL of LB medium without NaCl. Prior to the experiments, GAC particles were rinsed and subsequently submerged in 100 mL of fresh medium. Continuous stirring with a
multiple plate magnetic stirrer at 300 rpm (Variomag telesystem, H+P Labortechniek AG, Germany) at ambient temperature was used to simulate the mechanical agitation conditions of the FBE system (Racyte et al. 2011). Experimental conditions are shown in supplementary material (appendix- Figure A.4.1). Samples (2 mL) for isotherm construction were collected every day, filtered with disposable syringe filters and stored at 4 ºC in closed centrifuge tubes until COD analysis.

For the calculation of the Freundlich isotherms, the COD measurements from day 6, when equilibrium was reached, were used to calculate the equilibrium concentration of solutions exposed to each GAC concentration and to adjust it to the Freundlich Model (Bautista-Toledo et al. 2008).

\[(x/m) = K_f \cdot C_e^{(1/n)}\]  

where \(x/m\) is the mass of adsorbate adsorbed per unit of adsorbent (in \(\text{mg}_{\text{COD adsorbate}}/\text{g adsorbent}\)). The mass of adsorbate is determined from the difference between initial \((C_0)\) and equilibrium \((C_e)\) concentration (in \(\text{mg}_{\text{COD}}/\text{L}\)); \(K_f\) is the Freundlich capacity factor (in \(\text{mg}_{\text{COD adsorbate}}/\text{g adsorbent})(\text{L/}\text{mg}_{\text{COD}})^{(1/n)}\)); and \(1/n\) is the Freundlich intensity parameter.

**b) Adsorption of organics in LB on GAC during 6 h.** A corresponding experiment to simulate FBE experimental conditions (6 hours) was performed to determine organics adsorption to different concentrations of GAC in suspensions during 6 hours. Samples (2 mL) for COD analysis were collected every two hours, filtered with disposable syringe filters and stored at 4 ºC in closed centrifuge tubes until COD analysis.

The adsorption data obtained during 6 h experiment was used to design the incubation experiment (4.2.4.2.). The final organics concentration at 6 h with each GAC concentration was used to calculate the concentrations for the simulated inoculation media concentrations.
c) Adsorption of salts in PBS on GAC. Corresponding experiments to adsorption of organics were performed for adsorption of salts. Instead of LB, PBS was used here to test salt (NaCl, KCl, Na$_2$HPO$_4$, KH$_2$PO$_4$) adsorption on GAC. Adsorbed salts in PBS would modify buffering and osmolarity properties that might have an effect on microbial culture. Adsorption was quantified by IC and ICP measurements. Samples (2 mL) were collected every two hours, filtered with disposable syringe filters and stored at 4 ºC in closed centrifuge tubes until IC and ICP analysis.

4.2.4.2. Effect of mineral medium concentration on the metabolic activity of *E. coli* (without GAC). Twelve solutions with 6 different organic concentrations and two different NaCl concentrations representing LB (10 g/L NaCl) and ¼ LB (2.5 g/L NaCl) were used to investigate the effect of mineral medium (LB) concentration change on the metabolic activity and survival change of *E. coli*.

*E. coli* was cultivated overnight in LB media, after 18 h cells were harvested by centrifugation for 10 minutes at 1300 g (Beckman coulter Allegra X-12R centrifuge) in 50 mL sterile centrifuge tubes (Cs500, VWR, Netherlands). Supernatant was removed and the pellets were resuspended to different media without intermediate washing step to sterile Erlenmeyer flasks and closed with air permeable stoppers. The flasks were incubated at ambient conditions for 18 - 24 hours on the shaker. During the incubation, the metabolic activity (luminescence) and concentration of *E.coli* (optical density) were determined at 0, 2, 6, 18 and 24 hours to investigate the change over time. Additionally for SEM analysis, samples (2 mL) were filtrated with PTFE filters and the filters were fixated for SEM analysis.

4.2.4.3. Metabolic activity and survival of *E. coli* in different media (without GAC). *E. coli* was cultivated overnight, cells were harvested and resuspended to different media (Table 4.1) without intermediate washing step. Subsequently, after shaking for 1 minute, samples were taken for optical density and luminescence measurement (t=0). This measurement was repeated after 2 hours incubation at ambient conditions on the shaker.
4.2.4.4. Effect of different media and different GAC concentrations on \textit{E.coli} survival. Weighed and pretreated GAC particles (23 - 350 g/L) were immersed overnight in 300 mL to the various media (Table 4.1) to allow the saturation of GAC with medium (Racyte et al. 2011). Prior to the experiment, GAC particles were rinsed and subsequently submerged in 400 mL of fresh medium.

\textit{E. coli} was cultivated overnight and cells were harvested and resuspended to the GAC suspensions to obtain an initial microbial concentration of about \(10^8\) colony forming units per mL (CFU/mL). During the experiment all glass beakers were continuously stirred with a multiple plate magnetic stirrer at 300 rpm at ambient conditions. The pH was monitored every 5 minutes and stabilized at 7.25 ± 0.25 by the addition of either 1 M HCl or 1 M NaOH (GPR Rectapur, VWR, Netherlands).

Experiments were performed for 6 hours, samples (2 mL) were collected every two hours. Samples were kept at 4°C until further analysis of the microbial concentration.

4.2.5. Analyses

4.2.5.1. Chemical analyses. For COD determination, collected samples were filtered twice with Cronus PTFE hydrophobic syringe filters with a nominal pore size of 0.2 µm (Milllex-LG; Millipore, U.S.) to remove GAC powder, as remaining GAC powder would interfere with the analysis. COD was determined with a cuvette test LCK 514 COD (Hach Lange, Germany) with the measuring range between 100 and 2000 mg/L. The anion concentration was measured with an IC (metrohm 761 Compact IC – metrosep Organic acids 6.1005.200). The cation concentration was measured with an ICP-OES (PekinElmer, Optima 5300DV).

4.2.5.2. Microbial analysis and plating. For optical density and luminescence determination 200 µL of each sample was pipetted into optiplates and was measured in a Multilabel Counter (1420 Multilabel Counter Victor 3; Perkin Elmer, U.S.). For optical
density analysis 96 well transparent optiplates and for luminescence analysis 96 well black optiplates were used (Thermo Scientific, Germany). The optical density was measured as UV adsorption at a wavelength of 680 nm (\(\text{OD}_{680}\)). The intensity of luminescence was quantified as photon emissions in counts per second (CPS).

For experiments with GAC, plating on agar was used for \textit{E.coli} survival (microbial concentration) determination. Samples were serially diluted in the same reactor medium except for experiments with autoclaved tap water, where PBS was used as dilution medium to avoid extended osmotic imbalance for microbial culture. All dilutions were plated on solidified LB agar petri dishes to determine \textit{E.coli} concentration change in viable colony forming units (CFU). The three drop technique (Miles et al. 1938) was used for plating. Three drops of 10 µL of the same diluted sample were pipetted (allowing the drops to run down the agar surface) on the same petri dish as triplicate. Subsequently, petri dishes were placed in the incubator (Snijders scientific, The Netherlands) at 27°C overnight. \textit{E. coli} colonies were quantified after 18 hours of incubation as CFU/mL.

\textbf{4.2.5.3. Microscopic analyses.} For SEM analysis, GAC particles and filters were fixated in a 2.5 % glutaraldehyde (Sigma Aldrich, Germany) solution for 24 hours at 4 ºC. Afterwards, particles and filters were rinsed three times (15 min each) with PBS. Dehydration of the samples was performed using a six-step ethanol/water gradient (30 % - 100 %). Subsequently, samples were kept in a desiccator until analysis with SEM. For SEM analysis, samples were coated with gold and examined with a JEOL JSM-6480LV SEM microscope (Acceleration voltage 6 kV, HV-mode, SEI detector).

\textbf{4.2.6. Data processing}

The obtained data was statistically analyzed (T-test with 95 % confidence interval) to compare the data from triplicate analyses (OD, luminescence, CFU/mL) of two or three independent experiments. Statistically equal samples were averaged. The normal distribution of the samples was verified using STATDISK 10.4.0 (Triola 2007). The
logarithmic value of relative change in microbial concentration was calculated 
\[ \log(\text{CFU}_t/\text{CFU}_0) \], where \( \text{CFU}_t \) is microbial concentration at a time \( t \) and \( \text{CFU}_0 \) is initial microbial concentration. A negative value indicates a decrease in microbial concentration compared to initial concentration, whereas a positive value indicates growth.

4.3. Results

4.3.1. Chemical adsorption of media components on GAC

4.3.1.1. Isotherms of organics adsorption on GAC. Organic compounds have an affinity to adsorb on GAC. The change in organic composition in LB medium was quantified by COD. LB medium without NaCl has a COD value of 18 g/L. The equilibrium of organic compound adsorption on GAC was reached at day six.

The COD data were fitted to the Freundlich adsorption model (Figure 4.1) and resulted in a Freundlich capacity factor \( (K_f) \) of \( 10.6 \pm 0.1 \, (\text{mg}_{\text{COD}}/\text{g}_{\text{GAC}})(\text{L/mg}_{\text{COD}})^{1/n} \), and a Freundlich intensity parameter \( (1/n) \) of \( 0.351 \pm 0.009 \, (R^2 = 0.946) \). The Freundlich isotherm (Figure 4.1) shows that the GAC used has a low capacity for adsorption of the organic compounds in the LB medium (low \( K_f \)). Nonetheless, the low value of \( 1/n \) indicates, that there is a strong bond between GAC and the adsorbed organics compounds.

![Figure 4.1. Freundlich isotherm for adsorption of organics compounds on GAC (dashed line) and experimental data (filled dots). Error bars denote standard deviation of the measurements.](image)
4.3.1.2. Adsorption of organics on GAC in LB medium. The low capacity for removing organic compounds is noticeable during the 6 hours batch experiments with GAC concentrations lower than 88 g/L GAC (Figure 4.2). However, when the GAC concentration is higher, the adsorption of organic compounds increases. The initial COD concentration decreased with 62 % in a 175 g/L GAC suspension and with 87 % in a 350 g/L GAC suspension.

![Graph showing COD concentration of LB media with different GAC concentrations](image)

Figure 4.2. COD concentration of LB media (without NaCl) in suspensions with different concentrations of GAC (23, 44, 88, 175, 350 g/L) after 6 hours of incubation. Vertical bars denote standard deviation values for measurements (α=0.05).

LB medium exposed to 350 g/L of GAC for 6 hours had a remaining concentration of organic compounds of 2.27 gCOD/L or 13 % from the initial organic concentration.

4.3.1.3. Adsorption of salts on GAC in PBS medium. IC and ICP analysis showed minor ions adsorption in PBS (< 10 % adsorbed from initial concentration) except for phosphate ion in 175 g/L and 350 g/L GAC suspensions. In 175 g/L GAC suspension, 14 % of phosphate was adsorbed and in 350 g/L GAC suspension 16 % of phosphate was adsorbed.
4.3.2. Effect of mineral medium concentration on the metabolic activity of *E. coli*

Organic substances in LB medium are adsorbed on GAC as shown in Figure 4.2. To test whether organics depletion due to adsorption on GAC causes starvation of *E. coli*, simulated incubation solutions with adapted concentration of mineral media (bacto tryptone and yeast extract) and salts were prepared and used for the incubation of *E. coli* (Table 4.2).

Organic concentration was calculated from the ratio of organics in LB (15 g/L bacto tryptone and Yeast extract) to COD (18 g/L COD). LB concentration equals to incubation 1 and ¼ LB concentration would fall in between incubations 9 and 10 (Table 4.2).

*Table 4.2. Composition of incubation media used to determine microbial state of *E. coli***

<table>
<thead>
<tr>
<th>Incubation Number</th>
<th>LB media components, g/L</th>
<th>Incubation Number</th>
<th>LB media components, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tryptone</td>
<td>Yeast extract</td>
<td>NaCl</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>5.0</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>6.7</td>
<td>3.3</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>3.3</td>
<td>1.7</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>1.3</td>
<td>0.7</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>0.7</td>
<td>0.3</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>0.3</td>
<td>0.2</td>
<td>10</td>
</tr>
</tbody>
</table>

Metabolic activity and survival of *E. coli* were investigated in 12 different incubation solutions (Table 4.2) that could be encountered in solution with depleted organics due to adsorption on GAC. *E. coli* was incubated in 12 different solutions with 6 different concentrations of the two main sources of organic components of LB medium (bacto-tryptone and yeast extract) to simulate organics depletion effect. The organic composition of incubation solutions was chosen to match the organic composition of LB media exposed to the different GAC concentrations for 6 hours (Figure 4.2). Here, a decrease in
luminescence is related to a decrease in metabolic activity and a decrease in survival (OD$_{680}$) is related to lysis (cell death).

Different osmotic conditions were applied for solutions 1 to 6 (10 g/L NaCl concentration – LB media) and for solutions 7 to 12 (2.5 g/L NaCl concentration ¼ LB). The used composition of diluted mineral media and NaCl concentration are given in Table 4.2. The effect of these 12 incubation conditions on the *E. coli* metabolic activity (luminescence) and concentration (OD$_{680}$) is shown in Figure 4.3.

![Figure 4.3](image)

*Figure 4.3. E. coli metabolic activity (Luminescence, cps) and concentration (OD$_{680}$) for microbial suspensions in LB media with different concentrations of organics, minerals and salts (Table 4.2). Vertical error bars denote standard deviation ($\alpha=0.05$) of duplicate experiments (duplicated only selection of incubations: 1, 3, 6, 7, 10, 12).*
The luminescence which represents metabolic activity (Figure 4.3) is stable in all 12 incubation suspensions for 4 hours, after which it was decreasing. The decrease was 1 Log higher in suspensions with low isotonic conditions and low organic concentrations (incubations 10-12) compared to normal isotonic conditions and low organic concentrations (incubations 4-6).

The OD<sub>680</sub> data show (Figure 4.3), that microbial concentration was increasing with time indicating microbial growth. Growth was observed in all incubated suspensions except 6 and 12. However, slightly higher level of growth was observed in isotonic suspensions with high mineral and organic concentration (incubations 1-3) compared to suspensions with low isotonic conditions (incubations 7-9). The growth was not observed in suspensions with lowest organic and mineral concentration (incubations 6 and 12 (Table 4.2)).

4.3.3. Metabolic activity and survival of *E. coli* in different media

To relate *E.coli* metabolic activity (luminescence) and survival (microbial concentration) to different conditions, *E.coli* was incubated in 5 different media (Table 4.1). The luminescence values show over 90 % decrease in organics and nutrients low media (autoclaved tap water, ¼ PBS and PBS) and 50-80 % decrease for 1/4 LB and LB (Figure 4.4). The survival (OD<sub>680</sub>) of *E.coli* on the other hand, after 2 hours of incubation in all media gives less than 20 % decrease compared to the initial concentration. The decrease in OD<sub>680</sub> values is minimal for 1/4 LB and LB suspensions. The highest decrease in luminescence (90-95 %) and OD<sub>680</sub>(15-20 %) is observed for ¼ PBS and PBS suspensions.
Figure 4.4. Change in metabolic activity (luminescence decrease) and microbial concentration ($\text{OD}_{680}$ decrease) in 5 media (tap water, ¼ PBS, PBS, ¼ LB, LB) after 2 hours of incubation.

4.3.4. Effect of different media and different GAC concentrations on *E.coli* survival

*E.coli* was exposed to different concentrations of GAC (23-350 g/L) in various media (Table 4.1). Experiments showed that an increase in GAC concentration resulted in a microbial survival decrease in all tested suspensions (Figure 4.5). Little or no effect at low GAC concentrations was observed (23-44 g/L), whereas higher GAC concentrations showed a higher decrease in microbial survival with a maximum decrease of 3.5 Log in PBS suspension with a GAC concentration of 350 g GAC/L after 6 hours. In LB and ¼ LB with the GAC concentration of 175 g/L showed growth (although small). In LB suspension with GAC concentration of 350 g/L the slight growth of *E.coli* was observed, whereas in ¼ LB suspension *E.coli* survival was affected resulting in a slight concentration decay (up to 0.5 Log) after 6 hours.
Figure 4.5. Relative E.coli concentration (Log (CFU_t/CFU_0)) in time at different GAC concentrations (0, 23, 44, 88, 175, 360 g/L) in different media (Table 4.1). Error bars are the standard deviation of triplicate analysis in duplicate experiments (α=0.05).

The GAC used has a tendency to alkalinize the media, as the media without GAC have a pH between 7 and 7.5, therefore to the media with GAC, it was needed acid
addition (for LB base addition) to maintain a stable pH. The alkalization is a consequence of GAC surface charge characteristics (Racyte et al. 2011), therefore the pH was monitored and adjusted throughout the experiments. The level of pH change is related to the amount of acid (base) added (Table 4.3).

Table 4.3. Amount (mL) of added acid (base) to adjust the pH of the media in suspensions with different GAC concentrations (g/L).

<table>
<thead>
<tr>
<th>Media</th>
<th>Concentration of GAC suspensions</th>
<th>23 g/L</th>
<th>44 g/L</th>
<th>88 g/L</th>
<th>175 g/L</th>
<th>350 g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB*</td>
<td></td>
<td>3.6</td>
<td>3.4</td>
<td>3.6</td>
<td>3</td>
<td>3.7</td>
</tr>
<tr>
<td>¼ LB</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>PBS</td>
<td></td>
<td>0</td>
<td>0.5</td>
<td>0.9</td>
<td>4.4</td>
<td>7</td>
</tr>
<tr>
<td>¼ PBS</td>
<td></td>
<td>1.7</td>
<td>3.2</td>
<td>4.4</td>
<td>8.8</td>
<td>10.7</td>
</tr>
<tr>
<td>Autoclaved H₂O</td>
<td></td>
<td>0.9</td>
<td>1.4</td>
<td>2.1</td>
<td>3.9</td>
<td>5.6</td>
</tr>
</tbody>
</table>

*In this case NaOH was added because the medium was too acidic.

The amount of acid (base), which was required to adjust the pH in low concentration (23-88 g/L) GAC suspensions shows that ¼ PBS and autoclaved tap water required highest amount of acid to adjust pH (1-4.5 mL) meaning that it had the weakest buffer capacity. PBS and ¼ LB only lost their buffer capacities in suspensions with high GAC concentrations (175, 350 g/L). ¼ LB medium had the highest buffer capacity from tested media that required only 1 mL acid addition during 6 hours.
4.3.5. SEM analyses of GAC particles and filtrated incubation suspensions

SEM pictures of different experiments are compared in Figure 4.6.

Figure 4.6. SEM images of GAC and microbial cells on filters from different experiments. GAC particle from 23 g/L GAC in ¼ LB suspension at 3.5 h (A) and 6 h (B); filtrated microbial suspensions of Table 4.2 incubation 6 (C) and incubation 12 (D).

SEM images for cells exposed for 3.5 h to 23 g/L GAC in ¼ LB suspension, show healthy bacteria with a normal size, a defined rod shape and an even surface (Figure 4.6 A). However, after 6 h contact (Figure 4.6 B) the cells are smaller and not voluminous. Cells suspended in solutions with 10 g/L of NaCl (Table 4.2; incubation 6) have a normal size and shape (Figure 4.6 C), whereas the cells suspended in solutions with 2.5 g/L of NaCl (Table 4.2; incubation 12) are smaller and show more damage (Figure 4.6 D). Similar trend
was observed in other SEM images provided in supplementary material (appendix – Figures A.4.3 and A.4.4).

4.4. Discussion

Mechanisms related to bacterial stability in mechanically agitated GAC suspensions were investigated. The adsorption of chemical compounds and pH shifts in GAC suspensions could cause indirect disinfection, due to stress for bacteria such as starvation. Adsorption properties of GAC were investigated in 5 media, representing different conditions with respect to organics, nutrients, salts and pH stability, and were related to change in *E.coli* metabolic activity and survival.

4.4.1. Chemical adsorption of media components on GAC

The COD measurements of LB exposed to different concentrations of GAC showed that GAC has a low capacity for adsorbing the organics from LB medium, given the low value of Freundlich capacity factor \( (K_f) \). The organics in LB medium is mainly composed of amino acids. However, when amino acids are adsorbed on GAC (in LB and ¼ LB), the total amount of available organic nutrients in solution is reduced (Lin et al. 2010).

GAC also adsorbs ions to some extent. IC and ICP measurements showed that the change during 6 hours in the concentration of ions in all media analyzed was not high. However, in PBS and ¼ PBS phosphate was more extensively adsorbed on GAC, which resulted in a loss of pH buffering capacity. The buffering capacity loss in ¼ PBS occurred sooner and at lower GAC concentrations (required amount of chemicals addition to control the pH - Table 4.3) compared to PBS.
4.4.2. Organics limitation and osmotic stress

A microbial culture of *E.coli* was harvested in its exponential growth phase in rich medium, and transferred to adverse conditions, e.g. buffer solution or diluted mineral media or tap water. In general, microbial cultures can adapt to adverse conditions when given the time to adapt. Vital et al. (2008) grew microbial cultures for 3 days in carbon limited minimal medium, prior to their experiments. Their research showed that *E. coli* can grow at low organic concentration (2 – 8 mg/L of dissolved organic carbon with a conductivity of 0.57 – 1.13 mS/cm) such as river waters (Vital et al. 2008). The transfer of bacteria from a medium with high organics and minerals concentrations to medium with low concentrations of organics and minerals has adverse effects on cells such as decrease in metabolic rate (Egli 2010), shrinkage (Nyström 2004) and damage of cell envelopes, rendering cells more susceptible to other stresses (Zaske et al. 1980). A lack of organic compounds (growth substrate) is known to trigger several stress factors in *E. coli* (Sezonov et al. 2007). Bacteria can adapt to stresses through activation or suppression of gene expression helping them to survive (Costanzo and Ades 2006), however this adaptation in genome requires time.

In our case, the mineral medium concentration required to keep the microbial culture stable was around 0.5 g/L (organics and mineral medium concentration) with 2.5 g/L NaCl (conductivity 4.5 mS/cm). Therefore, our incubations used had two orders of magnitude higher concentration of minerals and organics compared to Vital et al. (2008) with conductivity in the same range. Despite the constant bacterial concentration (Figure 4.3) at the lowest organics and nutrient conditions for 24 hours (Table 4.2 - incubations 10, 11, 12), the metabolic state of *E.coli* decreased in time at lower mineral media concentrations as measured by luminescense. The metabolic activity is one of the cell functions which indicate the status of the cell. In our experiments, metabolic activity was expressed through luminescence. Luminescence is regulated by complex cell mechanisms such as autoinducer concentration and quorum sensing. The metabolic activity and thus
luminescence is affected by abiotic factors such as pH, temperature and medium composition (Boyer and Wisniewski-Dyé 2009).

Similar luminescence and $\text{OD}_{680}$ data obtained for different isotonic conditions related to LB and $\frac{1}{4}$ LB (10 and 2.5 g/L NaCl), indicate that the salt concentration of the medium in this range has no influence on the survival of *E.coli*. However, the limited amount of organics and minerals did affect the metabolic activity of *E.coli*, and resulted in *E.coli* metabolic activity decrease in time. Low isotonic conditions at low organics and minerals concentrations seem to have an effect on cell mechanical strength, given that, *E.coli* from incubation 12 (2.5 g/L NaCl), show more damage in the SEM pictures than in incubation 6 (10 g/L NaCl) in Figure 4.6 C and D, also the difference in microbial membrane damage was observed between other suspensions 7 and 11 (appendix – Figure A.4.4)

The luminescence data showed that bacteria were less metabolically active in media with low concentrations of organics and minerals (autoclaved tap water, $\frac{1}{4}$ PBS and PBS) with the lowest metabolic activity in PBS. Although PBS is a balanced salt solution that provides isotonic conditions, it lacks organic and mineral sources, resulting in the low metabolic activity and suspended growth. In contrast, autoclaved tap water and $\frac{1}{4}$ PBS may contain residual minerals, originating from the autoclaved tap water (Ravva and Korn 2007), because PBS was prepared in Milli-Q water, whereas PBS and LB were diluted with autoclaved tap water.

### 4.4.3. Influence of different media and different GAC concentrations on *E.coli* survival

The nature of GAC can also have adverse effects on cells, as positively charged activated carbons reduce cell viability (van der Mei et al. 2008) and this effect can be enhanced in combination with other stresses, e.g. limited organics and nutrients.

Furthermore, the GAC in suspensions had not only an adverse effect on the change in the concentration of the organics causing starvation stress for bacteria, but also severe pH variations were observed. To maintain a stable pH, buffering solutions are used such as
PBS. However, the pH variation was high in PBS suspensions with high GAC concentrations, implying that PBS is not a suitable buffer in combination with GAC. Although *E. coli* can survive in a pH range between 4 and 9, a shift to an acid or alkali environment is stressful for bacteria requiring adaptation time (Padan et al. 2005). Zilberstein et al. (1984) showed that an extracellular pH shift can injure *E. coli* cells. They suggest that cell growth is pH sensitive. A high pH can be more harmful in nutrient and salt poor media, as cells are already under non-favorable conditions which compromise their response to changing environments and stresses (Egli 2010). The pH shifts impose a new stressful factor to microbial cells, as the processes of intracellular pH regulation require additional energy (Zilberstein et al. 1984), in this way reducing the metabolic activity. Decrease in phosphate ion concentration due to adsorption on GAC seems to be enough for buffer capacity disruption in the PBS and ¼ PBS media. Our data indicates that the buffer capacity is considerably changed if GAC is present in PBS suspensions.

The pH measurements of solutions in suspensions with different concentrations of GAC showed that, for suspensions with GAC concentrations higher than 88 g/L, the buffer capacity of each medium except LB was ruptured (Table 4.3). In LB, however, pH stability in GAC suspensions was high due to the amphoteric character of amino acids (zwitterions) and their buffer capacity. Although LB also lost its buffer capacity, this occurred to a much lower extent. Apparently, some amino acids are less adsorbed than phosphate, which in turn results in a smaller loss of buffering capacity.

SEM visualization of *E. coli* attached to GAC particles and on the filters confirms the statement that organics depletion in ¼ LB media exposed to GAC suspensions affected microbial survival after 6 hours resulting in smaller and less voluminous cells. Deformation of the cell membranes was visible earlier for cells incubated in suspensions with lower organics, nutrient and salts concentrations, compared to cells that were incubated at higher concentrations of organics and nutrients and normal isotonic conditions (Figure 4.6 and in appendix- Figure A.4.4).

The experiments and analysis performed in this research confirmed that the healthy condition of microbial cells is affected by depletion of organics and minerals and
osmotic stress, which on their turn are affected by the GAC concentration. This change was enough to impose a stress on the bacterium tested, especially in PBS suspensions with high GAC concentrations.

4.5. Conclusions

GAC causes a decrease in organics concentration and buffer capacity in the five studied media (tap water, LB, ¼ LB, PBS and ¼ PBS). Especially the media that are poor in organics and minerals (PBS, ¼ PBS and tap water) cause stress on *E. coli* and can eventually result in disinfection. Higher GAC concentrations result in a lower survival of *E. coli*.

These new insights reinforce the importance of GAC in FBE systems. GAC alone does not cause disinfection in organics and minerals rich media, but establishes stress conditions for the microbial culture, which, in combination with other stress conditions (e.g. AC field) can enhance disinfection in wastewater treatment plants.

**Acknowledgements**

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4.6. References


4.7. Appendix to chapter 4.

Experimental set-up of experiments with GAC.

![Experimental set-up for organics adsorption on GAC.](image)

Figure A.4.1. Experimental set-up for organics adsorption on GAC.

Experimental data for adsorption isotherm construction.

![Change in COD content of LB media without NaCl exposed to different concentrations of GAC. The GAC amounts were all suspended in 0.4 L of media. Seven days experiment.](image)

Figure A.4.2. Change in COD content of LB media without NaCl exposed to different concentrations of GAC. The GAC amounts were all suspended in 0.4 L of media. Seven days experiment.
**E. coli** state attached on GAC in time.

![Image of E. coli attached on GAC](image1)

*Figure A.4.3. E. coli attached on GAC in ¼ LB during 1 hour (A) and during 3 hours (B) of exposure.*

**Experiment- mineral medium concentration on the metabolic activity of E. coli (4.3.2).**

![Image of microbial suspensions](image2)

*Figure A.4.4. Filtrated microbial suspensions of Table 4.2 incubation 1 (A), incubation 7 (B) and incubation 11 (C).*
Chapter 5
__________________________

Performance of fluidized bed disinfection with different types of granular activated carbon

Abstract
The disinfection of effluents is important to promote the reuse of these water resources and safeguarding human health, especially in water scarce areas worldwide. A proof-of-principle of a method for water disinfection consisting of fluidized bed electrodes (FBE) with Rx 3 Extra granular activated carbon (GAC) and a low strength alternating electric field (AC field) in radio frequency range (80 - 200 kHz) was published. In the study presented here we investigated the mechanistic role of 10 different types GAC in radio frequency FBE disinfection with E.coli YMc10 as the model microorganism. The disinfection performances with only GAC, and GAC combined with an AC field were quantified. Seven GACs showed poor to intermediate and three GACs (Norit RB3 (2.7 Log CFU E.coli decrease), Sorbonorit 3 (2.8 Log CFU E.coli decrease) and Rx 3 Extra (3.4 Log CFU E.coli decrease) showed substantial disinfection. The results suggest a relation between the pH_{pzc} of the GAC and the disinfection performance. Disinfection performance increased with bigger particle size and decreasing conductivity of GAC. We conclude that these physico-chemical and physical properties of GAC are important factors controlling the disinfection performance of these type of FBE systems.

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5.1. Introduction

A major part of the world population is threatened by fresh water scarcity, and therefore, water reuse is becoming increasingly important as a valuable water resource (Kummu et al. 2010, Levine and Asano 2004). In water scarce areas, one of the ways to sustain this resource is treated municipal wastewater reuse (Levy et al. 2011), to be used e.g. for irrigation to meet the demands of agriculture that accounts for 70% of the worlds’ water consumption (Cosgrove et al. 2000). To minimize the risk of diseases originating from pathogenic microorganisms present in wastewater effluent, disinfection as post-treatment should be applied (Mujeriego and Asano 1999). From disease prevention point-of-view, disinfection is the most important process in the treatment chain reclaiming wastewater for reuse (Lazarova et al. 1999, Sawyer 1976, Tansel 2008). The level of disinfection to be achieved depends on source and intended use of the recovered water (EPA 2004, Liberti and Notarnicola 1999).

Even though there are many processes that are used for disinfection of water, none is universally applicable or meets all the requirements of disinfection for different situations (Blatchley et al. 2007, EPA 2004). To ensure that water leaving sewage treatment plants is safe for humans and the environment, there is a need for innovative disinfection methods that meet the requirements for specific reuse situations (Sawyer et al. 1976, Shannon et al. 2008, Snowden-Swan et al. 1998).

Recently, a proof-of-principle study (Racyte et al. 2011), has shown that low strength (6 – 8 V/cm) alternating electric fields (AC field) at radio frequency of 140 kHz combined with a granular activated carbon (GAC) suspension can be applied for disinfection in a fluidized bed electrodes system (FBE). The application of FBE for disinfection has numerous advantages compared to state-of-the art disinfection techniques (e.g. UV radiation, chlorination, ozonation, electrochemical disinfection): the system is robust, easy to operate, does not require addition of chemicals; disinfection of turbid wastewater is possible, and post-treatment is not required. Moreover, the
combination of AC field and GAC does not require a high voltage and is not susceptible to corrosion of electrode materials as opposed to pulsed electric field (PEF) disinfection (Roodenburg et al. 2005). The proof-of-principle study showed a 2.7 Log concentration (CFU/mL) reduction of \textit{E.coli} YMC10 within 6 hours of treatment in AC field – FBE. A blank experiment without AC field (GAC alone) showed 0.5 Log CFU reduction. Disinfection was found to be frequency dependent and could not be achieved without a GAC, demonstrating that GAC is an important component in FBE disinfection. However, the precise disinfection mechanism has not been resolved yet.

This paper investigates the relation between FBE disinfection performance and GAC properties. It can be hypothesized that physico-chemical properties of GAC such as surface charge might influence FBE disinfection through attraction/repulsion between bacteria and GAC (Busscher et al. 2008, Rijnaarts et al. 1995). Microbial cell killing processes may operate differently for adsorbed and suspended planktonic bacteria, and sensitivity to these processes depends on the type of bacterium (Chapter 3 this thesis). Physical and electrical properties such as conductivity and particle size distribution determine voltage gradients on the liquid/GAC interfaces (Ferreira 2008) and these voltage gradients can have an effect on microbial cell viability (Challis 2005). Physico-chemical and physical properties of GAC depend on the source of raw material from which the GAC is produced (for example: coconut, biomass, peat) and preparation procedure, i.e. chemical or thermal activation of the GAC (Marsh and Rodriguez-Reinoso 2006).

The objective of this research is to determine the effect of commercially available GACs on disinfection of a pure culture \textit{E.coli} YMC10 in FBE, and to relate different GAC properties (chemical and physical) to the disinfection performance. Furthermore, the contribution of GAC without AC field to the overall disinfection was identified.
5.2. Materials and methods

5.2.1. Granular activated carbons tested for disinfection

Ten different GACs (Norit, The Netherlands) were selected to be examined for disinfection performance. Industrial GAC names were changed with abbreviations for simplicity: A – RX1.5 EXTRA; B – R1.5AA; C – Norit RB3; D – Sorbonorit 3; E – Norit PK 3-5; K – Norit C GRAN; L – Norit RO 3515; M – Norit GAC 830 PLUS; N – Norit GCN 830 PLUS; Rx – Norit RX3 EXTRA (In appendix- figure A.5.1 are depicted tested GACs).

All GACs except GAC K were thermally activated by steam. Activation by steam enhances GAC electrical conductivity. GAC K was chemically activated (by phosphoric acid). Chemical activation enhances very open (macro/meso) pore structure (i.e. high adsorption capacity) and specific surface chemistry, but not electrical conductivity. GACs A, B, C, D, L, and Rx are produced from the same raw material (biomass and a binder) and are physically pressed into a cylindrical, uniform shape (extruded). GAC A, Rx, M, N and L are acid washed activated carbons, which results in a high purity level e.g. have very low concentrations of metals. GACs A, B and Rx are a modification of the same GAC. GACs A and Rx are the same and differ only in particle size.

5.2.2. Physical GAC analysis

5.2.2.1. Surface area and porosity of GAC. N₂ adsorption isotherms were measured (Tristar 3000, Micromeritics, U.S.) to obtain a specific surface area (BET), micropore and total pore volume. All experiments were performed twice in triplicate. Before measuring N₂ adsorption isotherms, the samples were dried and degassed in an N₂ environment for 24 h at 300 °C (VacPrep 061 LB, Micromeritics, Germany).

5.2.2.2. Electrical conductivity of GAC. The electrical conductivity of GACs was measured with an LCR meter (4338B Milliohmometer, Agilent, U.S.). The distance and conductivity were measured between two points of a granule, clamping the granule between two clamps; 10-30 samples were measured and averaged.
5.2.3. Physico-chemical analysis of GAC

5.2.3.1. Point of zero charge of GAC. The point of zero charge (pH$_{pzc}$) of treated and untreated activated carbons was determined using two methods in parallel. The 1$^{st}$ method used was an immersion method (Fiol and Villaescusa 2009) and was adapted as following: instead of KNO$_3$, 0.05 M NaCl was used as background electrolyte, which was degassed for 24 h in N$_2$ environment prior to the experiment. In 50 mL of NaCl, 20 g/L of GAC was suspended. GAC was mixed with NaCl (120 rpm) with stirrer in N$_2$ environment at ambient temperature for 48 hours. After 48 h GAC was filtered twice from the liquid with a 0.45 μm hydrophobic PTFE syringe filters, and the pH$_{pzc}$ of filtered NaCl was determined with a pH electrode (Liquisys M CPM 253, Endress+Hauser, The Netherlands).

To validate the pH$_{pzc}$ data obtained with the 1$^{st}$ method, mass titration was used as 2$^{nd}$ method (Wang and Lu 1998). This method was adapted as following: instead of NaNO$_3$, 0.05 M NaCl was used as background electrolyte at 3 different pH values (pH = 3, 6, 11). For each initial pH, four containers were filled with 50 mL of degassed NaCl solution and different concentrations of activated carbons (6 g/L, 12 g/L, 20 g/L, 30 g/L). The suspensions were mixed for 48 h, and subsequently, the GAC was filtered twice and the pH$_{pzc}$ was determined in all 12 suspensions. Data of both methods corresponded with each other, and for matter of simplicity, the immersion method (1$^{st}$ method) was chosen for pH$_{pzc}$ determination of all GACs.

5.2.3.2. Chemical composition of activated carbons. Scanning electron microscope (SEM JEOL JSM-6480LV) combined with energy dispersive X-ray (EDX) spectroscopy was used to determine the chemical composition of activated carbons. Data on raw material and ash content were obtained from supplier.
5.2.3. GAC preparation for disinfection experiments

For disinfection investigation in the FBE system, 50 mL of each GAC was suspended with the same GAC volume. From 10 investigated GACs concentrations in suspensions are given in Table 5.1. The GACs were pre-treated as described earlier (Racyte et al. 2011): stocks of each GAC type were wetted in Milli-Q water (18.2 MΩ·cm – 25°C, 0.22 µm, Millipore Biocel SAS 67120, France), sterilized (121°C, 0.2 MPa for 25 min), rinsed with Milli-Q water and stored at 4°C until further use for experiments and analyses and is denoted as “pre-treated GAC”.

Table 5.1. GAC concentrations used for disinfection experiments in FBE (GAC volume/liquid volume was 50 mL/40ml).

<table>
<thead>
<tr>
<th>GAC</th>
<th>Concentration, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A RX 1.5 EXTRA</td>
<td>460</td>
</tr>
<tr>
<td>B R 1.5 AA</td>
<td>425</td>
</tr>
<tr>
<td>C Norit RB3</td>
<td>560</td>
</tr>
<tr>
<td>D Sorbonorit 3</td>
<td>475</td>
</tr>
<tr>
<td>E Norit PK 3 – 5</td>
<td>300</td>
</tr>
<tr>
<td>K Norit C GRAN</td>
<td>370</td>
</tr>
<tr>
<td>L Norit RO 3515</td>
<td>745</td>
</tr>
<tr>
<td>M Norit GAC 830 plus</td>
<td>710</td>
</tr>
<tr>
<td>N Norit GCN 830 plus</td>
<td>650</td>
</tr>
<tr>
<td>Rx Norit RX3 Extra</td>
<td>550</td>
</tr>
</tbody>
</table>

5.2.4. Reactor medium

Lysogenum Broth (LB) medium was prepared by dissolving 10 g/L NaCl (VWR, The Netherlands), 10 g/L BactoTM Tryptone (BD, U.S.) and 5 g/L BBLTM Yeast extract (BD, U.S.) in Milli-Q water. The pH was adjusted to 7.0 ± 0.2 by adding 1 M HCl or 1 M NaOH (VWR, The Netherlands) and this medium was sterilized at 121°C for 20 min. This medium was used to cultivate bacteria. Previously, the minimum concentration of substrate required to keep the bacterial culture stable (when growth equals decay) for the duration of an
experiment (360 min) was established with GAC Rx 3 Extra (Racyte et al. 2011). Each GAC however, adsorbed organics and salts to a different extent. Therefore, for each GAC, the organics and salt concentration of the LB medium was adjusted so that the organic concentration and conductivity were in the same range for each GAC at the end of an experimental run (Data is shown in appendix –Table A.5.2).

5.2.5. Bacterium

A genetically modified, non-pathogenic, bioluminescent bacteria *Escherichia coli* YMc10 (*E.coli*) obtained from the Belgian Coordinated Collections of Microorganisms (Belgium) served as a target microorganism in the disinfection experiments. *E.coli* was chosen because it is an indicative microorganism for the enteric pathogen contamination that causes waterborne diseases (Foladori et al. 2007). For experiments with *E.coli*, 0.116 g/L of ampicillin (Sigma-Aldrich, Inc.) was added to each medium and GAC suspension, because it is required for the expression of the bioluminescent character of *E.coli*. Additionally, presence of ampicillin avoids contamination of the pure culture with other microorganisms that are not resistant to ampicillin (Racyte et al. 2011). Bacterial culture for every experiment was obtained by inoculating 100 mL of autoclaved LB medium with 1 mL of the liquid stock culture (~$10^8$ CFU/mL) and incubating for 18 ± 2h at 27 °C with constant shaking. All procedures with pure culture *E.coli* were carried out under sterile conditions.

5.2.6. Experimental set up

The fluidized bed electrode set up was adapted from (Racyte et al. 2011). Experiments were performed with 8 glass beakers of 100 mL (Duran, Germany) in parallel, each filled with the same volume of GAC (Table 5.1) and 40 mL bacterial suspension. In four beakers, an alternating electric field of 140 kHz (AC field) was applied for 360 min continuously using two square stainless steel plate electrodes (13 cm$^2$) per beaker with a
distance of 19 mm between the electrodes (Figure 5.1). Beakers with GAC that were exposed to the AC field were denoted as “total disinfection”, which indicates disinfection due to a combination of GAC and AC field. The other four beakers were treated the same way, however no electric AC field was applied. These were denoted as “control” which indicates disinfection due to GAC alone.

Temperature and pH were monitored with a combined temperature/pH electrode. Temperature was controlled at a value of 22.5 ±2.5 °C by cooling in an ice bath, and pH was maintained at a value between 7.0 and 7.5 by adding either 1 M HCl or 1 M NaOH solution. Magnetic stirrer (240 rpm) with multiple plates (Variomag telesystem, H+P Labortechniek AG, Germany) was used for stirring the GAC suspension (Figure 5.1). Nd-Fe-B magnets (type S-08-30-N Supermagnet, Germany) coated with rubber were used as stirring magnets to lift the GAC particles to a well-mixed suspension. The AC field of 140 kHz was supplied by a custom made, class B amplifier, capable of high power output at frequencies up to 500 kHz, and a frequency generator (TG2000- 20 MHz, Thurlby Thandar Instruments, U.K.). An oscilloscope was used to monitor the frequency and wave shape of the signal and to record voltage and the current.

Figure 5.1. Experimental set-up.
Prior to the disinfection experiments the GAC was rinsed with Milli-Q water and left overnight in 40 mL of 1:4 diluted LB (¼ LB) solution to saturate GAC with electrolyte. On the day of experiment, overnight medium was rinsed away with the reactor medium (specific for each GAC). Then, each beaker was filled with 40 mL of reactor medium with bacteria added to a concentration of $10^8$ colony forming units per mL (CFU/mL), electrodes were immersed into these beakers, and the GAC particles were suspended by stirring at 250 rpm. For each experiment, conditions such as media composition, stirring intensity, and GAC volume were the same.

5.2.7. Microbiological analysis

To quantify the *E. coli* concentration change in the FBE during disinfection experiments, 0.5 mL samples were collected every 90 minutes and stored at 4 °C till further analysis. Each sample was analyzed the same day. The concentration of bacteria in each sample was determined by plating the diluted aliquot on agar plates. The dilution series was performed in ¼ LB with ampicillin. Subsequently $10^{-1}$-$10^{-6}$ dilutions were plated on LB agar plates employing the three drop technique (Miles et al. 1938). Three drops of 10 µL of the same diluted sample were pipetted (allowing the drops to run down the agar surface) on the same petri dish as triplicate. The agar plates were incubated in the incubator for 18 h, at 27°C. CFU were quantified after 18 h and bacteria concentration was recalculated to CFU per mL.

5.2.8. Data processing

Each GAC was tested for disinfection in duplicate or triplicate independent experiments. Each liquid medium sample was analyzed in triplicate. Disinfection data from independent experiments with the same GAC were statistically compared with a t-test, outliers were rejected, and statistically equal samples were averaged. The normal distribution of the data was verified using STATDISK 10.4.0. (Triola 2007) for a 95 %
confidence interval (α=0.05). The bacteria concentration at each time point CFU_t/mL was related to its initial concentration, yielding the relative concentration CFU_t/CFU_0 and the logarithmic value was calculated, i.e. Log (CFU_t/CFU_0).

To determine the effect of disinfection due to the AC field in FBE, the data of control (GAC without AC field) was subtracted (in Log scale) from the total disinfection data (AC field with GAC). Decay in E.coli concentration was expressed with a negative sign (-Log). If decay in the control was higher than the total disinfection, the data was expressed as (+Log) representing growth.

To evaluate and compare the disinfection performance of different GACs, the evaluation criteria as proposed by Madge and Jensen (2002) were used (Table 5.2).

Table 5.2. Disinfection evaluation criteria as proposed by Madge and Jensen (2002).

<table>
<thead>
<tr>
<th>Log reduction of E.coli concentration, (CFU/mL)</th>
<th>Disinfection evaluation criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 Log</td>
<td>Poor</td>
</tr>
<tr>
<td>1-2 Log</td>
<td>Intermediate</td>
</tr>
<tr>
<td>2-3 Log</td>
<td>Good</td>
</tr>
<tr>
<td>&gt; 3 Log</td>
<td>Very good</td>
</tr>
</tbody>
</table>

5.3. Results and Discussion

5.3.1. AC field and time effect on disinfection performance in FBE

To analyze the effect of the AC field on the disinfection in FBE, from the Log CFU reduction of the total disinfection (combined GAC and AC field) the Log reduction obtained from the control (GAC only) was subtracted. Here the FBE disinfection corrected for the GAC effect (control) is discussed.

Three from ten GACs tested showed considerable disinfection (E.coli CFU decay) in FBE (Figure 5.2): Rx (2.3 Log CFU decay), C (1.9 Log CFU decay) and D (1.4 Log CFU decay).
The disinfection performance of these was similar during the first 90 minutes (~0.5 Log CFU decay), however the next 90 minutes GAC Rx resulted in 1.5 Log CFU decay, whereas GAC D showed less than 1 Log CFU decay. The GAC Rx and D disinfection performance after the first 180 min slowed down and within the next 180 minutes resulted in additional 0.5-0.8 Log CFU decay. Bacterial decay for GAC C was constant (~0.5 Log CFU /90min) throughout the experiment.

The other GACs employed for FBE disinfection did not enhance substantially the AC field disinfection, however, they showed a different performance in time. The first group of GACs (A, B, E – Figure 5.2 A) did show some disinfection during treatment and the bacterial concentration was exponentially decreasing for GAC A (0.3 Log CFU decay) and GAC B (0.8 Log CFU decay). GAC E resulted in 0.3 Log CFU decay during the first 90 minutes, and during the remaining disinfection time (270 minutes), the bacterial concentration was constant. The second group of GACs (K, L, M, N – Figure 5.2 B) resulted in negligible disinfection, because during 360 min of FBE disinfection treatment the bacterial concentration variation was either constant (M, K) or slightly varying (L, N) between 0 and 0.3 Log CFU decay.

The total disinfection effect includes the contribution of both GAC and AC field. However, to distinguish the AC field effect from the GAC effect (Figure 5.2), the GAC effect (control) data were subtracted from the total effect.
Figure 5.2. Disinfection due to the AC field (corrected for control) of E.coli YMc10 in FBE with 10 GAC types. The relative bacterial concentration Log(CFU/CFU₀) in sample beakers is plotted versus treatment time (360 min). In figure (A) disinfection performance in FBE with GACs A, B, C, D, E is depicted. In figure (B) disinfection performance in FBE with GACs K, L, M, N, Rx is depicted. The error bars denote standard deviations from duplicate/triplicate experiments (α=0.05).

AC fields in the frequency range of 100 kHz are reported to increase microbial membrane permeability (Chang 1989). Furthermore, it seems that the presence of specific GACs enhances disinfection effect in FBE.

5.3.2. Physico-chemical and physical properties of GACs

Physico-chemical and physical properties of the 10 GACs were determined and summarized in Table 5.3. No direct relation was observed between the GAC properties (Table 5.3): source of raw material, BET area, pore volume distribution or purity (ash content), on the one hand, and FBE disinfection performance on the other hand (Table 5.4). GAC chemical composition as determined with SEM - EDX showed no relation to disinfection (data shown in the appendix - Table A.5.1).
Table 5.3. GAC Physico-chemical and physical properties. Considerable disinfection in FBE was achieved with grey shaded GACs.

<table>
<thead>
<tr>
<th>GAC /Parameters</th>
<th>Norit RX 1.5 Extra (A)</th>
<th>Norit R 1.5 AA (B)</th>
<th>Norit RB 3 (C)</th>
<th>Sorbo norit 3 (D)</th>
<th>Norit PK 3-5 (E)</th>
<th>Norit C RAN (K)</th>
<th>Norit RO 3515 (L)</th>
<th>Norit GAC 830 (M)</th>
<th>Norit GCN 830 (N)</th>
<th>Norit RX3 Extra (Rx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw material</td>
<td>Binders/ biomass</td>
<td>Binders/biomass</td>
<td>Binders/biomass</td>
<td>Binders/biomass</td>
<td>Peat</td>
<td>Phosphoric acid/biomass</td>
<td>Binders/biomass</td>
<td>Coal</td>
<td>Coconut</td>
<td>Binders/biomass</td>
</tr>
<tr>
<td>BET surface area, m²/g</td>
<td>1815</td>
<td>n.a.</td>
<td>1074</td>
<td>1453</td>
<td>795</td>
<td>1297</td>
<td>1425</td>
<td>958</td>
<td>998</td>
<td>1521</td>
</tr>
<tr>
<td>Micropore volume, cm³/g</td>
<td>0.79</td>
<td>n.a.</td>
<td>0.49</td>
<td>0.61</td>
<td>0.41</td>
<td>0.23</td>
<td>0.42</td>
<td>0.38</td>
<td>0.69</td>
<td>0.66</td>
</tr>
<tr>
<td>Meso and macro pore volume, cm³/g</td>
<td>0.32</td>
<td>n.a.</td>
<td>0.18</td>
<td>0.37</td>
<td>0.04</td>
<td>n.a.</td>
<td>0.91</td>
<td>n.a.</td>
<td>0.09</td>
<td>0.33</td>
</tr>
<tr>
<td>pH_{pzc} (untreated)</td>
<td>9.4</td>
<td>10.5</td>
<td>9.8</td>
<td>10.9</td>
<td>10.8</td>
<td>6.2</td>
<td>3.9</td>
<td>10.3</td>
<td>8.6</td>
<td>9.4</td>
</tr>
<tr>
<td>pH_{pzc} (treated)</td>
<td>6.1</td>
<td>7.5</td>
<td>8.7</td>
<td>8.5</td>
<td>9.2</td>
<td>5.9</td>
<td>2.9</td>
<td>7.0</td>
<td>6.5</td>
<td>7.8</td>
</tr>
<tr>
<td>Ash content, mass-%</td>
<td>3</td>
<td>~5</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Particle size, mm</td>
<td>1.5</td>
<td>1.5</td>
<td>3</td>
<td>3</td>
<td>2.8-5</td>
<td>0.5-1.7</td>
<td>1.5</td>
<td>0.43-2.4</td>
<td>0.6-2.4</td>
<td>3</td>
</tr>
<tr>
<td>Conductivity, S/cm</td>
<td>4.3</td>
<td>2.8</td>
<td>1.6</td>
<td>2.8</td>
<td>2.3</td>
<td>0</td>
<td>6.9</td>
<td>1.2</td>
<td>3.6</td>
<td>1.1</td>
</tr>
</tbody>
</table>
5.3.2.1. **Effect of $p\text{H}_{\text{pzc}}$ on FBE disinfection.** Loss of bacterial viability due to the interaction between GAC and bacteria can occur (Abu – El - Sha’r and Gharaibeh 1999, Hijnen et al. 2010, van der Mei et al. 2008). This might be attributed to the nature and charge of surfaces and electrostatic properties of bacteria (Busscher et al. 2006, Rijnaarts et al. 1995). The surface properties of GAC are a result of both initial raw material and preparation procedure. Mei et al. (2008) reported that due to GAC surface charge, adhered bacteria lose their viability resulting in disinfection. The loss of viability of cells can be enhanced by the use of acidic and positively charged activated carbons. The surface charge is influenced by the solution pH. At a pH below the $p\text{H}_{\text{pzc}}$, the GAC surface has a net positive charge, and at a pH above $p\text{H}_{\text{pzc}}$, the GAC has a net negative charge (Lopez-Ramon et al. 1999).

The disinfection (bacterial decay) in control beakers (without AC field) was quantified for all GAC to determine the different contributions to bacterial decay by interaction between GAC and bacteria only, and by interaction with combined GAC and AC field (Table 5.4). In FBE without electric field (control), the GACs A, C, K resulted in a CFU decrease in the range from 0.5 to 1 Log, and the GACs D, E, M and Rx resulted in a CFU decrease of 1-1.25 Log.

The GACs B, C, D, E, Rx used in the disinfection experiments have a $p\text{H}_{\text{pzc}}$ (after pre-treatment) in the range of 7.5 to 9.2, therefore those GACs would be positively charged in the FBE reactor. GACs D, E, M, Rx resulted in more than 1 Log CFU reduction in FBE due to GAC alone without the AC field, indicating a possible contribution of adhesion to the bacteria decay. The relation of total disinfection versus $p\text{H}_{\text{pzc}}$ shows that GAC with high disinfection have a $p\text{H}_{\text{pzc}}$ range (of 7.8 – 8.7). The graphical relation is given in supplementary material (in appendix - Figure A.5.2)

Several factors may cause a smaller than expected adherence of *E.coli* to positively charged GAC. The positive charge of GAC in the liquid media with organic ions can be neutralized due to a stabilizing layer of adsorbed organic ions surrounding the GAC particle. Moreover, *E.coli* bacteria have a low surface charge resulting in weak interaction
force to the surfaces and low adherence to positively charged GAC (van der Mei et al. 2008). Therefore, it seems that the GACs tested and the *E.coli* interfacial interactions are weak, or the time of experiment (6 h) is short in comparison with of 21 day experiments performed by (Busscher et al. 2006). In addition mechanical agitation in FBE introduces shear forces that can hinder adhesion of bacteria to the GAC surface (Rijnaarts et al. 1993).

Considering the total disinfection due to the combination of the GAC and AC field, disinfection performance is intermediate to very good (Table 5.4) for GACs B, C, D, E, M, Rx that would be positively charged (except GAC M that would be neutral) according to their $pH_{pzc}$ (Table 5.3). Never the less, the GACs that performed relatively well in disinfection with GAC alone, did not consistently lead to enhanced performance in total FBE disinfection and vice versa, supporting only a weak relation between AC field disinfection and positively charged GACs. Furthermore, it seems that other, than $pH_{pzc}$, GAC properties play a more dominant role in the effectiveness of a certain GACs for total disinfection in FBE.
Table 5.4. *E. coli* disinfection performance due to GAC and GAC combined with AC field of 10 GACs tested in FBE for 360 min.

<table>
<thead>
<tr>
<th>GAC type</th>
<th>GAC disinfection (control) Log(CFU&lt;sub&gt;t&lt;/sub&gt;/CFU&lt;sub&gt;0&lt;/sub&gt;) decay</th>
<th>Combined AC field and GAC disinfection (total) Log(CFU&lt;sub&gt;t&lt;/sub&gt;/CFU&lt;sub&gt;0&lt;/sub&gt;) decay</th>
<th>Disinfection evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-0.6 ± 0.17</td>
<td>-0.9</td>
<td>Poor</td>
</tr>
<tr>
<td>B</td>
<td>-0.2 ± 0.14</td>
<td>-1.0</td>
<td>Intermediate</td>
</tr>
<tr>
<td>C</td>
<td>-0.8 ± 0.14</td>
<td>-2.8</td>
<td>Good</td>
</tr>
<tr>
<td>D</td>
<td>-1.2 ± 0.052</td>
<td>-2.7</td>
<td>Good</td>
</tr>
<tr>
<td>E</td>
<td>-1.1 ± 0.14</td>
<td>-1.4</td>
<td>Intermediate</td>
</tr>
<tr>
<td>K</td>
<td>-0.7 ± 0.08</td>
<td>-0.8</td>
<td>Poor</td>
</tr>
<tr>
<td>L</td>
<td>-0.2 ± 0.08</td>
<td>-0.2</td>
<td>Poor</td>
</tr>
<tr>
<td>M</td>
<td>-1.25 ± 0.11</td>
<td>-1.3</td>
<td>Intermediate</td>
</tr>
<tr>
<td>N</td>
<td>-0.4 ± 0.05</td>
<td>-0.7</td>
<td>Poor</td>
</tr>
<tr>
<td>Rx</td>
<td>-1.1 ± 0.1</td>
<td>-3.4</td>
<td>Very good</td>
</tr>
</tbody>
</table>

5.3.2.2. Effect of particle size and conductivity on FBE disinfection. The disinfection performance for GAC alone (control) and GAC with AC field (total disinfection) was compared for particle size. No relation could be found between disinfection with GAC alone and particle size, whereas there seemed to be a relation between FBE disinfection due to the AC field and particle size. Therefore, here the FBE disinfection corrected for the GAC effect (control) is discussed. Three GACs (C, D, Rx) that resulted in considerable disinfection in FBE with AC field were all extruded GACs with particle size of 3 mm. This indicates that a physical/electrical phenomenon may be the cause of disinfection in FBE. To establish if there is a relation between physical parameters and disinfection, the disinfection achieved in FBE due to AC field was plotted versus the electrical conductivity and particle size (Figure 5.3).
Considerable *E.coli* disinfection in FBE was obtained with extruded 3 mm GACs: C, D and Rx due to applied AC field (sample). The disinfection performance (CFU decay) in FBE decreased in the order (Figure 5.2) Rx (2.3 Log) >C (2 Log) >D (1.5 Log). However, their electrical conductivity (Table 5.3) decreased in reverse order D (2.8 S/cm) >C (1.6 S/cm) >Rx (1.1 S/cm). The other three extruded GACs A, B, and L with particle size of 1.5 mm resulted in disinfection performance (CFU decay) in the order B (0.8 Log) >A (0.3 Log) >L (0.005 Log). The electrical conductivity (Table 5.3) of these GACs decreased as well in the reverse order L (6.9 S/cm) >A (4.3 S/cm) >B (2.8 S/cm). Therefore, it seems that the disinfection performance due to AC field in FBE is inversely proportional to electrical conductivity of the GAC material.

The GAC concentration in g/L (Table 5.1) was also compared to FBE disinfection performance. The concentrations of the GACs that were working for disinfection were 550 g/L for Rx, 560 g/L for C, and 475 g/L for D. GACs Rx and C had a similar concentration and also resulted in similar disinfection. GAC D had a lower concentration and also showed a lower disinfection (1.8 Log decay CFU – Figure 5.2). However, no relation was found between disinfection in FBE and GAC concentration for the 1.5 mm size, extruded GACs (A,
B, L). It might be that an optimal GAC/liquid ratio as (C, D, Rx) is needed for FBE disinfection, however particle size and conductivity (Figure 5.3) seem to play major role in the disinfection mechanism.

To confirm the conclusions, bigger than 3 mm extruded GAC should be tested to see whether higher disinfection performance can be achieved. The biggest sized GAC tested in FBE for disinfection was non extruded GAC E. However, it did not result in enhanced disinfection when combined with AC field (0.3 Log decay).

5.3.3. Disinfection in FBE and field strength

The conductivity of GAC, particle size and GAC concentration are important parameters for the field distribution in the GAC fluidized bed (Figure 5.4).

![Figure 5.4. Simplified equivalent scheme, potential (U) and electric field strength (E) distribution in FBE.](image)

The fluidized bed as is used in the FBE experiments can be described as many separate particles moving freely in the medium between two electrodes over which an AC
field is applied. Because GAC particles have several orders of magnitude lower resistance $R_{\text{GAC}}$ (conductivity 1-6 S/cm) than the liquid $R_{\text{liquid}}$ (conductivity of 4.5 mS/cm), there will be a different field strength over GAC and the liquid (Figure 5.4). For a liquid with large particles with a relatively low conductivity the electric field strength between the particles is most likely higher since there is in total less liquid in between, compared to the liquid between highly conductive small GAC particles. In practice this problem is more complex since the structure is not one-dimensional as represented here but 3D: our reasoning here must be regarded as a first approximation.

Several phenomena can take place at the GAC-liquid interface. In FBE mechanical agitation of fluidized GAC particles result in changes in bed expansion followed by a varying field distribution (Bareau and Coeuret 1979). In the paper of Fereira (2008) it is proposed that the electrical field is stronger nearby the GAC particle surface, since micro-chemical effects occur at that location when a voltage is applied across the bed. Moreover, the polarity change (due to applied AC field) may cause local high amplitude fluctuations on the various microbial physiological processes on bacteria which are close to the GAC-liquid surface (Challis 2005). Since we found a relation between the physico-chemical and physical properties of GAC and AC field mediated disinfection in FBE we conclude that the dynamics of micro-scale physico-chemical and physical processes at the GAC-liquid interface play an essential role in FBE disinfection.
5.5. Conclusions

Ten commercially available GACs were compared for *E.coli* YMc 10 disinfection performance in an AC filed FBE. Physico-chemical and physical properties of GACs were investigated, to relate GAC properties to the disinfection efficiency.

- Depending on the type of GAC used in FBE, total disinfection resulted in 0.2 to 3.4 Log CFU reduction of *E.coli*.
- A slightly positive surface charge is in favor for disinfection in FBE.
- A considerable higher disinfection performance was found for extruded GACs with particle size of 3 mm compared to GACs with smaller particle size and not extruded.
- The disinfection performance increased with a decrease in GAC conductivity.
- No relation could be established between other investigated GAC properties (raw material, chemical composition, surface area, pore distribution and purity) investigated and disinfection performance in FBE.

Acknowledgements

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5.6. References


5.7. Appendix to chapter 5.

GAC photos.

Figure A.5.1. Photos of tested GACs (A, B, C, D, E, K, L, M, N, Rx) for disinfection in FBE.

GAC properties.

Figure A.5.2. Relation between disinfection (Log (CFU/CFU₀)) and pHₚzc value. Empty circles is total disinfection. Filled circles: disinfection due to GAC alone.
GAC surface chemical composition SEM-EDX data.

Table A.5.1. Surface chemical composition of GACs (A, B, C, D, E, K, L, M, N, Rx) obtained with SEM EDX.

<table>
<thead>
<tr>
<th>GAC / Chemical composition, (weight %)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>K</th>
<th>L</th>
<th>M</th>
<th>N</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Counts</td>
<td>273126</td>
<td>618140</td>
<td>203811</td>
<td>179735</td>
<td>158419</td>
<td>156701</td>
<td>169807</td>
<td>90827</td>
<td>112579</td>
<td>141741</td>
</tr>
<tr>
<td>Carbon</td>
<td>99.3</td>
<td>87.56</td>
<td>83.3</td>
<td>88.9</td>
<td>85.2</td>
<td>79.62</td>
<td>94.95</td>
<td>92.2</td>
<td>99.15</td>
<td>99.4</td>
</tr>
<tr>
<td>Sodium</td>
<td>n.d.</td>
<td>0.07</td>
<td>0.11</td>
<td>n.d.</td>
<td>0.13</td>
<td>0.58</td>
<td>0.17</td>
<td>0.04</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Magnesium</td>
<td>n.d.</td>
<td>0.36</td>
<td>0.27</td>
<td>0.53</td>
<td>2.00</td>
<td>n.d.</td>
<td>0.09</td>
<td>0.06</td>
<td>0.01</td>
<td>n.d.</td>
</tr>
<tr>
<td>Aluminium</td>
<td>n.d.</td>
<td>0.17</td>
<td>1.05</td>
<td>0.19</td>
<td>0.40</td>
<td>0.01</td>
<td>0.14</td>
<td>0.45</td>
<td>0.10</td>
<td>n.d.</td>
</tr>
<tr>
<td>Silicium</td>
<td>0.46</td>
<td>0.61</td>
<td>1.58</td>
<td>0.27</td>
<td>0.44</td>
<td>0.08</td>
<td>0.36</td>
<td>0.66</td>
<td>0.15</td>
<td>0.18</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>n.d.</td>
<td>0.21</td>
<td>0.03</td>
<td>0.07</td>
<td>0.43</td>
<td>1.09</td>
<td>0.04</td>
<td>0.03</td>
<td>0.09</td>
<td>n.d.</td>
</tr>
<tr>
<td>Sulfur</td>
<td>0.27</td>
<td>0.34</td>
<td>0.31</td>
<td>0.92</td>
<td>0.62</td>
<td>0.04</td>
<td>0.52</td>
<td>0.73</td>
<td>0.10</td>
<td>0.35</td>
</tr>
<tr>
<td>Chlorine</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.02</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.02</td>
<td>n.d.</td>
<td>0.14</td>
<td>0.27</td>
<td>n.d.</td>
</tr>
<tr>
<td>Potassium</td>
<td>n.d.</td>
<td>1.31</td>
<td>0.04</td>
<td>0.61</td>
<td>0.36</td>
<td>0.05</td>
<td>3.27</td>
<td>0.05</td>
<td>0.01</td>
<td>n.d.</td>
</tr>
<tr>
<td>Calcium</td>
<td>n.d.</td>
<td>1.22</td>
<td>1.25</td>
<td>1.10</td>
<td>3.50</td>
<td>0.06</td>
<td>0.36</td>
<td>0.07</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Iron</td>
<td>n.d.</td>
<td>0.43</td>
<td>2.10</td>
<td>0.87</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.55</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Zinc</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.01</td>
<td>0.21</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.03</td>
</tr>
<tr>
<td>Cadmium</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.12</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.05</td>
<td>0.04</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

*n.d. – not detectable
Addition to paragraph 5.2.4 - reaction medium adaptation for different GACs

To avoid the difficult reproducibility of results due to microbial growth, LB media concentrations were adapted according each GAC chemical adsorption data. After adaptation, LB media during disinfection experiments had equivalent availability of organics and minerals for *E.coli* in all GAC suspensions. Furthermore, *E.coli* can grow optimally in the concentration range of 0.5 % to 5 % of NaCl (Abdulkarim et al. 2009, Hrenovic et al. 2009, Snoeyink et al. 1990), therefore NaCl concentration was not adapted and was 2.5 g/L as previously published (Racyte 2011). For the tested GACs (A, B, C, D, E, K, L, M, N, Rx) adsorption of NaCl was minor.

*Table A.5.2. LB media concentrations used with GACs (A, B, C, D, E, K, L, M, N, Rx) for disinfection experiments.*
Ultrasonic disinfection enhanced with alternating electric field and granular activated carbon particles

Abstract

There is a demand for alternatives to chemical disinfection of used waters including wastewater treatment effluents that have low energy, chemical and material consumption, do not form toxic by-products, and are robust and reliable in operation. A potential candidate is ultrasonic (US) disinfection combined with an alternating electric field (AC field) and/or suspended granular activated carbon (GAC) and this was investigated in this study. The disinfection and energy consumption was compared for 7 different combinations of process components (US, GAC and AC field). Combining US and AC field the disinfection achieved was 2.5 Log \( E.coli \) colony forming units reduction with 0.82 kWh/L energy input. A synergistic disinfection effect was observed when combining AC field and US without GAC. However, the lowest energy input of 0.05 kWh/L and highest disinfection of 3.7 Log \( E.coli \) colony forming units reduction was achieved with the combination of US and GAC.

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6.1. Introduction

Increasing exhaustion in fresh water supply and awareness on water scarcity and quality problems, is making reuse of used water, including wastewater treatment effluents, an important issue (Kummu et al. 2010). The conventional wastewater treatment methods are not always suitable to deliver the water of required quality that would be suitable for multipurpose reuse (Brenner 2011). For example, food industry wastewater effluents could be reused for food crop irrigation, but this requires the removal of pathogenic microorganisms (Kretschmer et al. 2002). Conventional methods to remove pathogenic microorganisms rely on the use of biocides (e.g. active chlorine), which may lead to formation of carcinogenic and mutagenic by-products. These by-products (such as halogenated hydrocarbons) are of great public concern for water reuse (Antoniadis et al. 2007, Nissinen et al. 2002). Thus, there is a need for non-chemical disinfection methods. These methods have to be energy efficient, robust, easy maintainable and a safe alternative for chemical disinfection methods (EPA 2004).

Alternating current fluidized bed electrode (FBE) might offer an alternative to existing technologies for effluent disinfection (Racyte et al. 2011). FBE employs an alternating electric field (AC field) and granulated activated carbon (GAC). A robust and low maintenance design, no required addition of chemicals and low investment costs make this method a potentially attractive disinfection technology. Previously, a disinfection of 2.7 Log reduction of E.coli colony forming units (CFU) during 6 hours of disinfection treatment was achieved at energy input of 1.5 kWh/L (Racyte et al. 2011). Therefore, further development is required to decrease the treatment time and energy consumption to make this technology feasible in practice.

Acoustic cavitation created by ultrasonication (US) is another method successfully employed for disinfection (Jyoti and Pandit 2001, Joyce et al. 2003). Cavitation is being described as the formation, irregular oscillation and collapse/implosion of micro-bubbles that create locally high pressure and high temperature zones. US for disinfection mainly
works mechanically due to friction created by micro-bubbles i.e. the liquid jets caused by the sound waves; chemical (radical formation) and heat effects add up to this main disinfection mechanism (Gogate 2007, Lambert et al. 2010, Mason et al. 2003, Piyasena et al. 2003). It is not precisely known which minimal US power is needed for cavitation to occur, as cavitation is dependent from several factors such as liquid media composition, conductivity, viscosity, surface tension, salinity (Borkent et al. 2007, Mohajerani et al. 2010).

When low power US is applied and cavitation is not occurring, microbial cell damage can occur due to micro-streaming (Mason et al. 2003, Tuziuti et al. 2005). Micro-streaming is as well as cavitation, a process of micro-bubble formation, but due to low US power the micro-bubbles constantly oscillate and don’t collapse. Due to the micro-bubble formation and constant oscillation high intensity liquid flows are generated, that cause mechanical stress on cell membranes and leads to microbial cell fatigue (Mason et al. 2003).

The ultrasonic disinfection can be achieved with an energy input of 0.05 kWh/L for zooplankton microorganisms (Mason et al. 2003), however, in general higher energy inputs are required for successful disinfection especially for pathogenic microorganism that have a cell architecture more resilient to the agitation of their local environment (Piyasena et al. 2003). Therefore, ultrasonication is at this stage considered as a more complex and more expensive alternative than chemical disinfection methods (Hulsmans et al. 2010, Lambert et al. 2010, Piyasena et al. 2003). Ultrasonic disinfection is also not yet adequate at low bacterial concentrations and for specific pathogenic microorganisms such as viruses (Joyce et al. 2003, Su et al. 2010).

The ultrasonic disinfection rate is affected by many factors, including the frequency and the power of ultrasound (Jyoti and Pandit 2003, Joyce et al. 2003). There are several ways to improve US disinfection such as: change of reactor geometry, transducer position and combination of different treatment methods. One of the options to improve US disinfection efficiency and to reduce energy input is to enhance it’s intensity through the use of additives. Additives can be divided into two groups: the first, is catalysts and
chemicals such as TiO$_2$, ozone, hydrogen peroxide, chlorine (Dadjour et al. 2005, Gogate 2007, Jyoti and Pandit 2003) and the second, is the inert or reactive solid particles (such as glass beads, activated carbon, alumina, ceramic granules and metallic zinc particles (Ince and Belen 2001, Murphy et al. 1993, Tuziuti et al. 2005). In the case of the first group, US energy weakens microbial cell membranes making it easier permeable, so chemicals can better penetrate to the inside of bacterial cells in this way improving the chemical disinfection yield; catalysts (such as TiO$_2$) add up to radical production rate during sono-chemical cavitation (Dadjour et al. 2005). In the case of second group, solid particle addition introduces liquid/solid interfacial zones enlarging wave magnitudes at interference, that reduce the power threshold to begin the cavitation or micro bubble formation (Gogate 2007). The effect of particles on US enhancement is well known, as glass beads combined with US have been used in microbiology for bacterial cell opening (Murphy et al. 1993, Salter and Smith 1984). However, at a lower power (below the cavitation threshold) disinfection might occur due to micro-streaming (Mason et al. 2003, Miller 1981). Hydrophobic particles act as a surface to increase the friction and liquid jets in the solid/liquid interface and enhance the rate of disinfection (Borkent et al. 2007, Tuziuti et al. 2005).

Another way to improve US disinfection is through combination with other disinfection methods such as advanced oxidation processes (AOP), photolysis (UV), electrochemical disinfection and membrane technologies (Blume and Neis 2004, Jyoti and Pandit 2003, Lambert et al. 2010). US can be used as a pretreatment in a two step disinfection technology to disintegrate flocks and bacteria consortia as well to increase the bacterial cell permeability, or as one step simultaneous treatment to reduce the time of disinfection and to increase the effect of chemicals distribution due to mixing (Blume and Neis 2004, Jyoti and Pandit 2003, Jyoti and Pandit 2001, Mason et al. 2003).

The objective of this study is to prove the principle of disinfection by simultaneous combination of alternating electric field (AC field) and ultrasonication (US) for disinfection enhanced by granulated activated carbon (GAC) particle addition. For investigating the effect on disinfection performance, 7 different combinations of US, AC field and GAC were
tested for disinfection of pure culture non-pathogenic *E.coli* YMc10. The results were compared in terms of disinfection performance and energy input.

6.2. Materials and methods

6.2.1. Microorganisms

In this research non-pathogenic, genetically modified, bioluminescent bacteria *E.coli* YMc10 (Belgian Coordinated Collections of Microorganisms, Belgium) were employed as model microorganism for disinfection performance quantification. *E.coli* YMc10 (*E.coli*) possesses an ampicillin resistance on the same vector as bioluminescence to prevent the multiplication in an environment without ampicillin and cross-transfer of the vector to other microorganisms. The bacteria were cultivated in Lysogeny broth (LB) medium overnight as described previously (Racyte 2011). Before the start of an experiment, the cells were harvested by centrifugation, the supernatant was removed, and the bacteria were re-inoculated to the reactor medium. The reactor medium with GAC suspension was 1:4 diluted LB medium (¼ LB) in which there was no net bacterial growth as mentioned previously (Racyte et al. 2011). However, when GAC particles were not used (US and/ or AC field methods), the ¼ LB medium was too concentrated and induced bacterial growth, therefore the LB medium was 1:15 diluted (1/15 LB) (experimentally determined ratio, Chapter 3 this thesis). The concentration of bacteria before disinfection was ~10^8 cells per milliliter (mL). All media that were used for *E. coli*, were supplemented with 0.1 g/L ampicillin (Sigma-Aldrich, Inc.). All procedures with pure culture *E.coli* were carried out under sterile conditions.

The disinfection reactor is further denoted as “sample” where bacteria were exposed to disinfection treatment (US, AC field). In the control reactor bacteria were exposed to the same conditions as the treated sample (such as medium, GAC, stirring, temperature) only the disinfection treatment was not applied.
6.2.2. Granulated activated carbon

In the experiments commercially available granulated activated carbon (GAC) NORIT RX 3 EXTRA (Norit, The Netherlands) was used as fluidized particles. The GAC used had a BET area of 1400 m$^2$/g, pH$_{pzc}$ 7.8 and particle size of 3 mm.

This GAC is produced by extrusion, and is thermally activated and acid washed. In all experiments with GAC, three different concentrations of GAC particles in the media were employed: 350 g/L, 475 g/L and 550 g/L. These concentrations were chosen, because in previous work (Racyte et al. 2011) 350 g/L concentration was employed for disinfection experiments and 550 g/L was the concentration that would maximally fit to the beaker; the concentration of 475 g/L was chosen as one in between.

Prior to the experiments, the required amount of GAC was weighted, wetted, sterilized, rinsed with ultrapure Milli-Q water (18.2 MΩ•cm at 25 °C, 0.22 µm, Millipore Biocel SAS 67120, France) and stored at 4 °C till further use as explained in detail earlier (Racyte et al. 2011). The GAC was rinsed with Milli-Q water 18 hours prior to an experiment and submerged into 300 mL ¼ LB solution containing ampicillin to saturate GAC with electrolyte. Approximately 2 hours prior to an experiment the GAC particles were rinsed with 200 mL of ¼ LB containing ampicillin, drained and filled with reaction medium 400 mL of ¼ LB supplemented with ampicillin.

6.2.3. Experimental set-up

The disinfection reactor set-up consisted of a 1 L beaker glass and 2 stainless steel electrodes (with 112 cm$^2$ area per electrode at a distance in between of 1.9 cm) (Figure 6.1). The alternating electric field (AC field) was supplied by a custom made high power, class B, amplifier and a frequency generator (TG2000- 20 MHz, Thurlby Thandar Instruments, U.K.). Voltage and current were monitored with a digital oscilloscope (Rigol DS1022C, U.S.). In the experiments with an AC field the applied voltage was kept at 13±1 V($\text{RMS}$). For the ultrasound generation, a Branson sonifier W-250 (P$_\text{max}$=250 W, f= 20 kHz)
was used with a horn sonotrode with tip of 1.3 cm diameter (Figure 6.1). In case US was used, a 20 W ultrasonic power was applied discontinuously in “on-off cycles” with an overall duty cycle of 17 % to prevent substantial heating of reactor suspension (Lambert et al. 2010, Salter and Smith 1984) as well to reduce the energy consumption. The “on cycles” were applied for 10 minutes during every hour of 6 hours experiment ((0 to 10 min), (60 to 70 min), (120 to 130 min), (180 to 190 min), (240 to 250 min), (300 to 310 min)). The AC field was applied continuous during the 6 hour experiment.

The electric energy input of AC field \(E_{AC}\) to the disinfection reactor was calculated with:

\[
E_{AC} = V_{RMS} \cdot I_{RMS} \cdot t_{TR}
\]  

(1)

where \(V_{RMS}\) is voltage applied over the electrodes, \(I_{RMS}\) is the current measured at the electrodes and \(t_{TR}\) is the treatment time. The ultrasonic energy output at the horn was taken from the manufacturer specifications\(^1\).

![Figure 6.1. Schematic representation of the experimental setup.](image)

For the disinfection experiments - suspensions containing 400 mL of medium (\(\frac{1}{4}\) LB medium or \(\frac{1}{15}\) LB medium) supplemented with ampicillin and \textit{E.coli} were employed. The

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temperature inside the reactors was controlled at 22.5 ± 2.5 °C in an ice bath; for suspending the GAC a magnetic stirrer (RET basic IKAMAG®, Germany) with the speed of 250 rpm was employed (Figure 6.1). Nd-Fe-B magnets (type S-08-30-N Supermagnete, Germany) coated with rubber were used as stirring magnets to suspend the GAC particles to a well-mixed suspension (Teflon coated stirring magnets did not accomplish this and therefore not used).

A control without AC field and US application was running in parallel to the disinfection sample. Both temperature and pH were monitored with a pH electrode and recorded with a data logger (RSG30 Endress + Hauser, The Netherlands).

6.2.4. The set of experiments

The combinations investigated for disinfection rate are summarized below. Additionally, experiments 1, 3, 6 and 7 were carried out with three GAC concentrations (350 g/L, 475 g/L and 550 g/L) to be able to evaluate the optimal GAC liquid ratio for the efficient inactivation of bacteria.

1. GAC
2. AC field
3. AC field + GAC (FBE)
4. US
5. US + AC field
6. US + GAC
7. US + GAC + AC field

6.2.5. Microbial analysis and plating

Every hour 2 mL samples were taken, each sample was serially diluted; all dilutions were plated on LB agar petri dishes for determination of concentration change in viable colony forming units (CFU). The three drop technique (Racyte et al. 2011) was used for plating. Three drops of 10 µL of the same diluted sample were pipetted (allowing the
drops to run down the agar surface) on the same petri dish as triplicate. Subsequently, petri dishes were placed in the incubator (Snijders scientific, The Netherlands) at 27 °C for 18 hours. Afterwards, CFU were counted and recalculated to CFU/mL.

6.2.6. Data analysis

The obtained data as CFU/mL was statistically analyzed (t-test) to compare the triplicate analyses of two or three independent experiments. Statistically equal samples were averaged. The normal distribution of the data was verified using STATDISK 10.4.0 (Triola 2007). The bacterial concentration CFU/mL was normalized to its initial concentration and the Log reduction was calculated as Log(CFU_t/CFU_0). Standard deviations of the Log values were calculated from the analysis in triplicate.

To obtain the Log reduction due to AC field and/or US (sample), the Log reduction without AC field and/or US applied (control) was subtracted from the total Log reduction obtained with the AC field and/or US applied. A larger decay of the sample compared to the control is expressed with a negative sign (− Log), whereas a larger decay in control compared to sample is expressed with a positive sign (+ Log).

The synergy factor (SF) of a combined disinfection processes was calculated as following (Mohajerani et al. 2010):

\[
SF = \frac{k_{AB}}{k_A + k_B} \tag{2}
\]

Where SF is the synergy factor; \( k_{AB} \) is the disinfection rate constant of combined disinfection processes; \( k_A \) and \( k_B \) are the disinfection rate constants of individual disinfection processes.

Disinfection turned out to follow 1st order kinetics. The 1st order disinfection rate constant \( k_1 \) was calculated as following:

\[
k_1 = -\frac{d(\text{LN(CFU)})}{dt} \tag{3}
\]
6.3. Results and discussion

6.3.1. Combination of US and AC field without GAC

A 140 kHz alternating electric field (AC field) was applied through the *E. coli* disinfection media (without GAC), and the disinfection achieved was negligible (Figure 6.2 A). It was also measured the disinfection for US alone and a 0.9 Log decay of *E. coli* was achieved (Figure 6.2 B). If US was combined simultaneously with the AC field of 140 kHz (without GAC), a disinfection of 2.5 Log CFU/ml decay was achieved (Figure 6.2 B).

![Figure 6.2](image)

*Figure 6.2. Different treatments (US, AC field, GAC, and their combinations) for *E. coli* YMc10 disinfection. The normalized bacterial concentration Log(CFU/CFU₀) is plotted versus treatment time. In the figure (A) treatments not employing US are depicted. In the figure (B) treatments employing US are depicted. The error bars denote standard deviations from duplicate or triplicate experiments (α=0.05).*

The combination of AC field and US result in a high synergy factor (Table 6.1). US acts on bacterial membranes, increasing the microbial cell membrane permeability and inducing mechanical friction effects (Mason et al. 2003, Piyasena et al. 2003). The AC field as well increases the cell membrane permeability (Chang 1989). Thus microbial membrane
permeability seems to be the limiting factor in US disinfection when it is applied solely, which seems to be overcome when US and AC field were combined.

The occurrence of cavitation was not monitored in our study, however it is not expected at the power level applied. The US power applied in our study was 8.3 W/L, and is at least 10 times lower than is used by others for ultrasonic disinfection (Antoniadis et al. 2007, Ince and Belen 2001, Jyoti and Pandit 2003), and the micro-streaming is likely to be taking place at the power level applied here.

*Table 6.1. Different treatments (US, AC field, GAC, and their combinations) were investigated for *E.coli* YMc10 disinfection. The methods and their combinations applied for disinfection; disinfection efficiency, disinfection reaction rate versus energy consumption and synergy factor for the combined treatments are quantified during the time of the disinfection treatment applied (360 min).*

<table>
<thead>
<tr>
<th>Method applied for disinfection</th>
<th>Disinfection efficiency achieved, Log</th>
<th>Energy consumed, kWh/L</th>
<th>Energy required per Log disinfection, kWh/L per Log</th>
<th>Reaction constant k₁, 1/min</th>
<th>Synergy factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 GAC</td>
<td>-0.47 ± 0.14</td>
<td>0</td>
<td>0</td>
<td>0.0030</td>
<td>-</td>
</tr>
<tr>
<td>2 AC field</td>
<td>-0.13 ± 0.03</td>
<td>0.32</td>
<td>2.56</td>
<td>-0.0012</td>
<td>-</td>
</tr>
<tr>
<td>3 AC field+GAC (FBE)</td>
<td>-2.21 ± 0.27</td>
<td>1.5</td>
<td>0.68</td>
<td>0.017</td>
<td>9.1</td>
</tr>
<tr>
<td>4 US</td>
<td>-0.92 ± 0.16</td>
<td>0.05</td>
<td>0.05</td>
<td>0.0055</td>
<td>-</td>
</tr>
<tr>
<td>5 US+AC field</td>
<td>-2.53 ± 0.32</td>
<td>0.84</td>
<td>0.33</td>
<td>0.017</td>
<td>3.8</td>
</tr>
<tr>
<td>6 US+GAC</td>
<td>-3.74 ± 0.27</td>
<td>0.05</td>
<td>0.01</td>
<td>0.025</td>
<td>2.9</td>
</tr>
<tr>
<td>7 US+GAC+AC field</td>
<td>-3.94 ± 0.44</td>
<td>1.55</td>
<td>0.39</td>
<td>0.025</td>
<td>3.4</td>
</tr>
</tbody>
</table>
6.3.2. Combination of US and GAC versus US, GAC and AC field

If GAC was combined with US, *E.coli* disinfection of 3.7 Log CFU reduction was achieved. The disinfection achieved with GAC alone was 0.5 Log CFU reduction and with US alone was 0.9 Log *E.coli* CFU reduction (Figure 6.2). There was a synergistic effect if GAC was combined with US (Table 6.1). The increase in disinfection when GAC and US are combined could be due to several reasons. The US combined with particles (glass beads) is used in microbiology for bacterial cell opening (Salter and Smith 1984). The US and glass beads method works according the principle that US energy creates mechanical friction between glass beads surfaces and creates interferences among the sound waves. Microorganisms in these regions are killed due to bacterial membrane rupture by a combination of mechanical friction and enhanced cavitation (Murphy et al. 1993). A similar effect of disinfection is expected for the combination of US with GAC. Ince and Belen (2001) reported that the addition of solid particles to a ultrasonic reactor might reduce the energy threshold for cavitations to begin, due to sound wave interference. They tested ceramic, metallic zinc, and activated carbon particles. From these particles, activated carbon particles increased the US disinfection rate the most. The rough surface, sharp edges, uneven shape and presence of pores in GAC seem to contribute to substantial improvement of US disinfection. Tuziuti et al. (2005) reported an increase in US disinfection rate in a liquid with alumina particles compared to a liquid without particles. Furthermore, they found an additional effect when the solution was saturated with air prior ultrasonication. A possible mechanism is that the protective peroxidase system of aerobic bacteria (Desnues et al. 2003), is less capable to function under agitated and high oxygen concentration conditions. In our research the mechanically agitated GAC suspension was also saturated with air.

Another possible mechanism contributing to disinfection could be related to micro air bubbles that are stuck in GAC pores (depicted in the appendix – Figure A.6.1) and are expanding and shrinking with the sound waves (US). This causes local pressure differences
in the media and the GAC pores that could cause fatigue on bacteria vital functions (Alpas et al. 1999, Welch et al. 1993) and eventually kill the bacteria that are trapped in these pores. Thus, it is expected that more GAC in suspension would result in more disinfection. To test this hypothesis, three GAC concentrations (350 g/L, 475 g/L, 550 g/L) were investigated for combined US and GAC disinfection. The difference in disinfection rate however, was found insignificantly dependent on GAC particle concentrations in the range studied (data provided in the appendix - Figures A.6.2 and A.6.3), thus falsifying our hypothesis. To assess all different particle-US related mechanisms contributing to disinfection, and to explain our observations, further detailed investigations are required in the future.

Additionally, when US and GAC were combined with an AC field, it resulted in 3.9 Log E.coli CFU/mL decay. The combined US and GAC (thus without AC field) method however, resulted in 3.7 Log CFU/mL reduction. Therefore, the additional effect of the AC field to disinfection with US and GAC is negligible. It seems that the AC field does not further increase the microbial membrane permeability if GAC is combined with US. Thus, to enhance US disinfection, two different approaches are possible: 1) simultaneous application with AC field or 2) addition of GAC particles.

6.3.3. Disinfection efficiency versus power consumption

Two electrical devices were used, the commercially available US “Branson sonifier”, and a custom made AC power supply for disinfection. The US sonifier was operated at a low power, and during 6 hours treatment a 0.9 Log CFU/mL E.coli reduction was achieved with an energy input of 0.05 kWh/L. If US was combined with GAC for disinfection, a 3.7 Log CFU/mL reduction was achieved at the same energy input. The energy input for US disinfection was a factor five higher than US and GAC combined disinfection, i.e. from 0.05 kWh/L to 0.01 kWh/L for 1 Log CFU/mL E.coli disinfection (Table 6.1).

In case of industrial process water disinfection (Nybom et al. 2012) where contact with GAC might be unwanted (for example in food and chemical industries) US combined with AC field might be an attractive option. Then, in this case, disinfection can be achieved
with US and AC field, thus without the disadvantage of disinfection by-products formation, that are formed if ozonation or chlorination are used for disinfection (Nissinen et al. 2002). The disinfection achieved with low power US and AC field was 2.5 Log CFU/mL reduction at 0.84 kWh/L energy input, and for 1 Log reduction of *E. coli* was required 0.33 kWh/L (Table 6.1).

The highest *E. coli* disinfection performance (3.9 Log CFU/mL reduction) was achieved with a combination of low power US, AC field and GAC. However, this process also required the highest energy input of 1.55 kWh/L (for 1 Log reduction - 0.39 kWh/L (Table 6.1)). Such a high energy input is due to interaction between GAC and the AC field, because GAC is electrically conductive resulting in electrical current flowing through GAC suspension. The energy input of the combination of US, AC field and GAC was 40 times the energy input of the combination of US and GAC. However, the disinfection only slightly increased. Therefore, with the respect to the energy input, the US and GAC combination is the best option of the investigated combinations for disinfection.

In order to decrease energy input in the tested system different measures can be considered such as: variation in AC field pulse shape and duration; the conductivity of fluidized material (replace GAC by other solid particles); replacing US transducers by hydrodynamic cavitation system.

### 6.4. Conclusions

The goal of this research was to provide a proof-of-principle for various combined technologies using US, alternating electric field (AC field) and/or solid particles (GAC), and to compare disinfection rates and the required energy input.

- Combined US and AC field resulted in a disinfection of 2.5 Log CFU/mL. However, each of them (US and AC field) separately resulted in less than 1 Log disinfection. This shows that there is a synergistic effect if US and AC field are combined (synergy factor of 3.8). To our knowledge, this is the first report on this phenomenon. The disinfection achieved with combined US and AC field is similar to the one achieved with
FBE (combined AC field and GAC) 2.2 Log CFU/mL disinfection, however when combining US and AC field there is lower total energy input required.

- The disinfection achieved with combined US and GAC (3.7 Log CFU/mL reduction) was higher than FBE (GAC and AC field combined (2.2 Log CFU/mL reduction)). However, the synergetic effect of FBE was higher (9.1) than that of US and GAC (2.9).

- Combined US and GAC (3.7 Log CFU/mL reduction) and combined US, GAC and AC field (3.9 Log CFU/mL reduction) resulted in a similar disinfection performance. The energy input however, was more than 40 times higher for the combined US, GAC and AC field method. Combined US and GAC showed 1.5 Log CFU/mL more disinfection than FBE (AC field and GAC), indicating that combined US and GAC works better for disinfection than AC field combined with GAC.

Further studies are necessary to investigate the applicability of these techniques combinations for specific applications in the future, and to elucidate the underlying mechanisms in killing the microbial cells in the various individual and combined techniques.

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6.5. References


6.6. Appendix to chapter 6.

GAC surface structure at different SEM magnifications.

![Figure A.6.1. SEM micrographs of GAC Rx 3 particle surface at 500 µm (A) and 5 µm (B) magnification.](image)

Disinfection achieved due to different GAC concentrations:

![Figure A.6.2. Disinfection (Log (CFU<sub>t</sub>/CFU<sub>0</sub>)) achieved with US, GAC and AC field combination for different GAC concentrations 350, 475 and 550 g/L over time.](image)

![Figure A.6.3. Disinfection (Log (CFU<sub>t</sub>/CFU<sub>0</sub>)) achieved with US and GAC combination for different GAC concentrations 350, 475 and 550 g/L over time.](image)
Chapter 7

General discussion and outlook

This thesis describes the proof-of-principle of FBE disinfection for bacteria with radio frequency AC fields and granular activated carbon. Furthermore, we found a synergistic effect when a radio frequency AC field was combined with US for disinfection. In this outlook, the contributions of the key components of the FBE disinfection system are highlighted and possible mechanisms for cell killing/inactivation taking place in the FBE and AC/US systems are briefly described. Furthermore, remaining scientific and technological challenges and possible application areas of this novel technology are discussed.
7.1. Effect of AC field frequency on disinfection

In Chapter 2 the AC field and combination of AC field and GAC for disinfection in FBE were investigated. The results show that there is no noticeable effect on microbial concentration change by application of AC field alone, however it enhanced the disinfection achieved by GAC. The dependence of disinfection on the AC field frequency shows the relevance of the AC field for cell inactivation (Figure 7.1). Disinfection was achieved at the highest rate at 140 kHz, and lower rate at the other tested frequencies in the range (80-200 kHz).

![Figure 7.1. Comparison of different systems disinfection (Log(CFU_t/CFU_0)) performance in time (min).](image)

To determine whether the same optimum frequency in FBE is applicable for all microorganisms or only for a specific type, different Gram type bacterial strains were tested at different single and dual frequencies for disinfection in FBE (Chapter 3). Not all tested bacterial species were susceptible for disinfection in FBE. However, best performance for each strain of tested bacteria was achieved at different AC field frequencies (Table 7.1).
Table 7.1. Optimum frequencies for disinfection in FBE for Gram negative and Gram positive bacteria.

<table>
<thead>
<tr>
<th>Gram negative bacteria</th>
<th>Frequencies, kHz</th>
<th>Gram positive bacteria</th>
<th>Frequencies, kHz</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens</em></td>
<td>280 &amp; 285 (dual)</td>
<td><em>B. subtilis</em></td>
<td>80 &amp; 140 (dual)</td>
</tr>
<tr>
<td><em>P. luteola</em></td>
<td>80 &amp; 140 (dual)</td>
<td><em>B. subtilis</em> subsp. <em>subtilis</em></td>
<td>80 (single)</td>
</tr>
<tr>
<td><em>E. corotovora</em></td>
<td>80 &amp; 140 (dual)</td>
<td><em>E. faecalis</em></td>
<td>280 &amp; 285 (dual)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>10 &amp; 140 (dual)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Gram negative bacteria were more susceptible to FBE induced cell killing compared to Gram positive bacteria (Figure 7.2), indicating a possible physical origin of the disinfection mechanism in FBE (similar to ultrasonication and pulsed electric field), rather than a chemical origin (antibiotics and peroxides).

Figure 7.2. Gram negative (A) and Gram positive (B) bacteria disinfection (Log(CFU<sub>t</sub>/CFU<sub>0</sub>)) in time (min) at optimum frequencies (Table 7.1).
Susceptibility to disinfection in FBE of various strains was different, ranging from 2.5 Log CFU decrease for *E. coli* to 0.2 Log CFU decrease for *B. subtilis*. Susceptibility of the same genera but different strains was not the same, indicating that the disinfection in FBE is strain specific, resulting in a different optimum frequency for bacteria inactivation between the representatives of the same genera (Table 7.1).

A different effect of frequency specificity on microbial strains in FBE was observed. Some strains (e.g. *P. luteola*) were in general sensitive to disinfection at all frequencies tested in FBE (Figure 3.4 and Table 3.3 in Chapter 3) showing low frequency specificity, whereas other strains (e.g. *E. coli*) showed high frequency specificity meaning high disinfection at specific frequencies in FBE (Figure 3.3 and Table 3.3 in Chapter 3).

Disinfection performance in FBE depends on the AC field frequency. For the tested bacteria the highest disinfection was achieved at a frequency which differed for each bacterium. Gram negative bacteria were more susceptible to FBE disinfection and therefore, the inactivation mechanism is probably of physical origin related to cell envelope structure.

### 7.2. Effect of GAC on disinfection

In Chapter 2 we saw that GAC is an important component in FBE, which also causes disinfection without an AC-field (Figure 2.6 Chapter 2). However, disinfection caused by GAC alone is low (~0.5 - 1 Log CFU decrease) compared to disinfection caused by combined AC field and GAC (2.7 Log CFU decrease).

We were working with diluted growth media (Lysogeny broth - LB), which showed to be best to keep pH of the medium and *E. coli* culture stable, with conditions keeping cell growth equal to decay. In Chapter 4 it was observed that the metabolic activity of the microbial culture was reduced in media low in organics and salts. Depleted organics combined with lower isotonic conditions resulted in visible microbial membrane damage.
(SEM pictures C and D in Figure 4.6, Chapter 4). Adsorption effects and pH variation due to GAC influenced microbial culture state, resulting in stress that can eventually lead to disinfection (van der Mei et al. 2008).

We showed in Chapter 3 that for bacteria sensitive to FBE treatment there was a difference in adhesion level on the sample and on the control GAC particles (Figure 3.6 in Chapter 3). This difference in adhesion implies that bacteria sensitive to AC field either got less attached on GAC or were disinfected on the GAC surface.

GAC was found to be an important factor in FBE disinfection. Therefore, different GACs (10 types) were tested for *E. coli* inactivation in FBE and different properties of these GACs were analyzed to be related to disinfection performance. From GACs tested, only three GACs combined with AC field resulted in substantial disinfection in FBE (2.7 - 3.4 Log CFU decrease). A relation was observed between disinfection performance and the following GAC properties: surface charge (pH$_{pzc}$), GAC conductivity and particle size. The conductivity and particle size of GAC might be important parameters because they influence the electric field distribution in the FBE.

Obtained data indicates that interactions between bacteria - GAC - organics contribute to the FBE disinfection. Furthermore, physical and physico-chemical properties of GAC affect FBE disinfection performance.

7.3. Effect of Ultrasound on disinfection

The FBE disinfection seems to be of physical origin (Chapter 6). Combinations with other disinfection methods that are of physical origin might lead to a synergy.

To test this hypothesis, and to increase the rate of FBE disinfection, we combined FBE with a known technique for disinfection, i.e. ultrasound (US) in Chapter 6. Low power
US for disinfection was investigated, and resulted in 0.9 Log CFU *E.coli* concentration decay. Furthermore, the low power US disinfection was enhanced with the AC field, which resulted in 2.5 Log CFU decay. The combination of US and AC fields thus yields in a high synergy factor of 3.8. The enhanced disinfection achieved when US and AC field were combined, may be attributed to enhanced cell membrane permeability. Both techniques seem to enhance cell membrane permeability (Caubet et al. 2004, Chang 1989). Moreover, US alone leads to mechanical cell membrane damage, resulting in disinfection (Mason et al. 2003).

The disinfection was also improved when US was combined with GAC compared to US alone. This is likely caused by enhancement of effects that are known for US, i.e. mechanical friction and cell membrane permeability by the physical stress caused by GAC. A combination of US, GAC and AC field did not further increase the disinfection.

When combining US and AC field the cell membrane permeability may be enhanced by the AC field. In case of combining GAC and US, GAC causes stress and mechanical friction and US causes cell membrane permeability. Also for the combination of GAC and AC field (FBE), the GAC would cause stress and mechanical friction, and the AC field would cause cell membrane permeability.

### 7.4. Possible mechanisms of disinfection in FBE

Most likely, the disinfection mechanism in FBE is complex and is caused by a combination of effects such as enhanced local field strength on/at GAC particles and stressfull conditions caused by GAC on microbial cells. In FBE, bacteria inactivation occurring in the liquid is paralleled by cell killing on GAC particle surface. Possible disinfection mechanisms in the FBE system, are divided into 4 groups according their nature and these are discussed below.
7.4.1. Disinfection mechanisms related to electrolysis

One of the possibilities to disinfect water electrically is to apply low frequency alternating current (AC fields) in the frequency range 16-60 Hz (Birbir et al. 2009, Park et al. 2003). Here, active radicals will act against microorganisms and will result in disinfection (McDonnell and Russell 1999). Electrolysis in the FBE case would produce small quantities of Cl\(_2\) and when there would be a catalytic activity on the carbon surface also other radicals can be produced (e.g. Cl*, O* and OH*). However, in the FBE system, an AC field in medium radio frequency range was chosen to avoid electrolysis and its secondary effects (radical formation, toxic gases generation, corrosion of electrode materials). According Tracy (1932) and Kirson et al. (2004) electrolysis is not occurring with an AC field with frequencies above 1 kHz.

Electrolysis is not possible to occur in the FBE system, due to the applied medium range radio frequency electric field. Therefore, an electrochemical mechanism of disinfection was unlikely and was not investigated in this thesis (excluded based on theoretical basis).

7.4.2. Disinfection mechanisms related to the change of environmental factors

The growth and survival of microorganisms is greatly affected by environmental factors such as chemical composition (e.g. osmotic pressure, COD, pH) and physical effects (e.g. temperature, pressure, physical properties of surfaces). These factors influence microorganisms’ growth and decay (Prescott et al. 2002). A lack of organics and minerals (Figure 2.2 in Chapter 2) or presence of GAC (Figure 2.6 in Chapter 2) was observed to result in decrease of the *E.coli* concentration. Therefore, several mechanisms that can cause bacterial decay apart from electrical effects are distinguished: (i) microorganism
adhesion on GAC (Hijnen et al. 2010, van der Mei et al. 2008); (ii) bacteria disinfection because of GAC mechanical grinding (Salter and Smith 1984); (iii) toxic effects of GAC on microorganisms (Yamamoto et al. 2001); (iv) essential change in COD and osmotic conditions due to chemical adsorption on GAC (Csonka 1989, Monod 1949).

All these environmental effects can take place in FBE, which results in decay of bacteria concentration. These effects however, were limited, as was observed for GAC without AC field (0.5-1 Log CFU E.coli decay).

7.4.3. Hypotheses for disinfection in FBE due to microorganisms and AC field interactions

Changes in the environment make bacteria suspended in the liquid (planktonic bacteria) and on the GAC surface (adhered bacteria) more susceptible to AC field effects. AC field effects that could affect bacteria are:

a) AC field induced mechanical wear of planktonic bacteria
b) AC field induced increase in membrane permeability of planktonic bacteria
c) AC field induced increase of voltage over bacteria adhered to the GAC surface

7.4.3.1 AC field induced mechanical wear (rotation). The polarization of the outer cell membrane makes the cells form an electrical dipole (Markx and Davey 1999, Voldman 2006). This dipole effect causes a cell to rotate in rotating electrical fields, as has been found for yeast cells in the frequency close to 100 kHz (Mischel and Pohl 1983). Rotation only occurs at optimum frequencies, which will vary for different cells (Holzapfel et al. 1982). When electric fields are not rotating but change the direction (the polarity in case of an AC field) in short time intervals, the outer cell membrane will try to ‘follow’ this field (Markx 2008) and can also rotate, or instead of rotating, it will ‘flip’ every time when the field switches the sign (Figure 7.3.). However, cells will be able to ‘follow’ the AC field only
till a certain frequency, above which the time for a cell polarization will become too short and friction too high due to the size of the cell (Markx and Davey 1999).

![Image of AC field induced rotation leading to mechanical wear of microbial cells]

Figure 7.3. AC field induced rotation leading to mechanical wear of microbial cells.

This theory can explain the sensitivity of microbial cells tested in FBE disinfection for the AC field specific frequency. Probably, the AC field in the FBE system exerts specific mechanical movements on cells and thus here the AC field frequency would be an important parameter as these movements are expected to take place at the highest amplitude at optimum AC field frequency. Furthermore, if mechanical movements occur, the rate of them will increase with increasing electric field strength (Markx 2008). In FBE, the electric field strength is expected to be locally increased between GAC particles, as suggested in Chapter 5.

7.4.3.2. AC field induced increase in cell membrane permeability. AC fields can induce an increase in cell membrane permeability at frequencies close to 100 kHz (Chang 1989). Furthermore, as mentioned in the previous paragraph due to polarization effect on the outer cell membrane, a difference of potential can build up between the inside of the cell membrane and the outer wall (Markx and Davey 1999, Voldman 2006). To explain this phenomenon one could think of an equivalent electrical circuit. In this analogy, the voltage gated channels in the membrane act as diodes and the cell membrane as a capacitor (Figure 7.4.). Application of an electric AC field across the cell membrane could cause the function disruption and blockage of the voltage gated channels (Beurrier et al.
which leads to microbial cell damage.

*Figure 7.4. The equivalent circuit of a microbial cell in liquid.*

Enhancement of effects mentioned in 7.4.3.1 and 7.4.3.2 due to surrounding GAC particles in the suspension. Through collisions of GAC particles with electrodes or with other GAC particles electrons may be transferred between GAC particles and between GAC particles and electrodes. This process changes the charge of individual GAC particles. When planktonic bacteria are present in between 2 particles or in between a particle and electrode with different surface charge (and thus potential) they will experience a locally high electric field strength (Figure 7.5.). These locally high electric field strengths may result in disinfection mechanisms similar to those that occur with pulsed electric field (PEF), i.e. an increase in membrane permeability and formation of pores, possibly along with effects mentioned above in paragraphs 7.4.3.1. and 7.4.3.2.

*Figure 7.5. Micro organism in high electric field between two charged GAC particles.*
7.4.3.3. AC field induced increase of voltage over bacteria adhered to the GAC surface. According to Markx and Davey (1999) the outer cell membrane, cytoplasmic membrane and cytoplasm of a planktonic cell have different electrical resistances and permittivity. From the theory proposed there, an equivalent electrical circuit can be generated in which the cell membrane is represented as a resistor and capacitor in parallel, and the conductive cytoplasm (inside of the cell) as a resistor. Additionally, we took into account the liquid surrounding the cell and that could be represented as a resistor and capacitor in series (liquid double layer) (Figure 7.6.) (Silva et al. 2008).

![Figure 7.6.](image)

**Figure 7.6. Equivalent circuit of planktonic microbial cell generated from the theory proposed by Markx and Davey (1999).**

The cell consists of a symmetrical circuit (Figure 7.6.). For such a circuit, there would be no change in voltage strength over parts of the cell. The electrical current through the membrane however, would increase with increasing frequency. Nevertheless, no optimum frequency would be expected in this situation.

In case a microbial cell adherence to a GAC particle, the equivalent circuit may be represented as shown in figure 7.7. Here the electrical circuit is no longer symmetrical,
due to attachment of the cell to the conductive and infinitively large (comparing to the size of microbial cell) GAC particle.

**Figure 7.7. Cell adhered to an infinitely large GAC particle: A) schematic representation and B) equivalent circuit.**

This situation of a cell attached to GAC conductive particle (Figure 7.7.) can be simplified as follows (Figure 7.8.). A capacitor ($C_{\text{liquid DL}}$) and resistor ($R_{\text{DL}}$) connected in series would represent the liquid double layer $R_{\text{liquid DL}}$ and the resistance inside the cell $R_{\text{Cell Inside}}$. The ‘cell inside’ conductivity is assumed to be Ohmic. A capacitor ($C_{\text{CM}}$) and resistor ($R_{\text{CM}}$) connected in parallel represent the impedance over twice the cell membrane (CM).

**Figure 7.8. Simplified equivalent circuit of liquid double layer (DL) and cell membrane (CM).**
This situation (Figure 7.8.) is electrically analogous to a filter of which the double layer capacitor \( (C_{DL}) \) has a high impedance for low frequencies (Figure 7.9. liquid impedance) and a low impedance for high frequencies due to a capacitor and resistor in parallel \( (C_{CM} \text{ and } R_{CM}) \) (Figure 7.9. cell impedance). These filters are connected in series as is shown in figure 7.8, with a resulting voltage between the cell inside and the GAC particle which is shown in figure 7.9. The voltage across the cell has a maximum value at the resonance frequency (designated at a relative frequency of about ‘0’). In the graph (Figure 7.9.) all values are relative.

**Figure 7.9 Frequency dependent behavior of the microbial cell adhered to a GAC particle.** \( X_{axis} \) (relative frequency, \( Y_{primary axis} \) (relative impedance), \( Y_{secondary axis} \) (relative voltage amplitude).

Considering that the total impedance of the simplified circuit (Figure 7.8) is \( Z \), which equals to the sum of the two impedances \((Z_{DL}+Z_{CM})\) of liquid double layer and cell membrane. Where \( Z_{DL} \) is the impedance of the liquid double layer:

\[
Z_{DL} = R_{DL} + \frac{1}{i \cdot \omega \cdot C_{DL}}
\]

and \( Z_{CM} \) is the impedance of the cell membrane:
\[ Z_{CM} = \frac{1}{R_{CM} + \frac{i \cdot \omega \cdot C_{CM}}{1}} \]

Then the peak resonance angular frequency can be expressed as:

\[ \omega = \frac{1}{\sqrt{C_{DL} \cdot C_{CM} \cdot R_{DL} \cdot R_{CM}}} \]

This peak resonance frequency would result in the highest voltage inside the cell \( (V_{\text{inside cell}}) \), which might lead to disruption of the structure and therefore the functioning of the cell, eventually leading to cell death. The actual values are not known, but this demonstrates the principle and might explain why disinfection in FBE occurs at specific frequencies. This effect might be further enhanced by other effects of AC fields on microbial cells such as are described in sections 7.4.3.1 and 7.4.3.2.

**Concluding remarks on hypotheses covering AC field - GAC - bacteria interactions in FBE disinfection**

There are different leads indicating that microbial cells in AC fields can be destroyed by mechanical effects and structure/function inhibition. Due to the low amplitude AC field applied in the FBE system, it seems that this disturbance is not enough to cause disinfection in the absence of the GAC particles. However, to confirm any of the discussed hypotheses in this section more research is required.

### 7.4.4. Hypothesis on disinfection performance versus time in liquid or on GAC surface

Disinfection data analysis indicates that the microbial concentration initially decreases slowly (figures 7.1 and 7.2), followed by an exponential decrease and finally does not substantially decrease anymore is depicted in figure 7.10 (dashed line). In chapter 3 another effect was observed: the bacteria concentration recovered from GAC over time was constant see also figure 7.10 (solid horizontal line). This indicates that the disinfection rate is limited, possibly due to 1) a rate limiting adsorption of bacteria to the GAC, or 2) a rate limiting desorption of bacteria from the GAC.
Theory 1 implies that FBE disinfection occurs mainly for bacteria on the GAC particle surface, and not in the liquid. Initially, a large number of bacteria are adsorbed on the GAC ($C_{\text{GAC}}$). Bacteria on the GAC are inactivated, and replaced by suspended bacteria present in the liquid (planktonic bacteria ($C_{\text{plankt}}$)). Consequently, the concentration of viable planktonic bacteria decreases. Because the concentration of suspended planktonic bacteria decreases, the driving force for adsorption of bacteria from the liquid to the GAC surface also decreases. This results in a disinfection performance decrease, which is finally limited by the adsorption rate of planktonic bacteria to the GAC. This theory could explain why recovered bacteria from GAC had the same concentration in time ($\Delta \text{Log CFU}$ Figure 3.6 in Chapter 3).

Theory 2 implies that FBE disinfection occurs mainly for planktonic bacteria, and not for adsorbed bacteria on GAC surface. Initially, there is an equilibrium situation between adhered bacteria ($C_{\text{GAC}}$) and planktonic bacteria ($C_{\text{plankt}}$). Planktonic bacteria are affected by FBE conditions (e.g. stress due to environment changes, AC field, shear forces), whereas adhered bacteria are protected from the FBE conditions because of attachment to GAC (Emerick et al. 1999). The concentration of planktonic bacteria decreases, which increases the driving force for desorption of bacteria from the GAC surface to the liquid. The desorption rate decreases again with the decrease in adhered bacteria on GAC surface. However, in SEM observations it was difficult to detect bacteria on GAC surface after the experiments, indicating that microbial adhesion on GAC was not extensive.

![Figure 7.10. Variation of bacterial concentration in time in the liquid (dashed line) and on GAC surface (solid line).](image-url)
To better quantify and understand the mechanism of disinfection in FBE and its occurrence either on GAC surface or in the liquid, different fluidized particles in FBE can be tested. The alternative fluidized particles in FBE should be either not facilitating bacterial adhesion, or could be specialized such as positively charged - acidic GACs (upon attachment of bacteria to GAC surface, it would reduce bacteria survival (van der Mei et al. 2008)).

If microbial disinfection takes place mainly in the liquid phase, locally created high field strength in the proximity of two GAC particles could occur, that may kill the microbial cell in between those particles (as shown in Figure 7.5.).

The disinfection in FBE occurs in the liquid, or on the GAC particles, but is most probably a combination of the two. Further research is needed to elucidate the relative contributions of both mechanisms to FBE bacterial disinfection.

7.5. Comparison of energy efficiency versus disinfection performance

The disinfection level achieved in the FBE due to AC field varies from 2.2 Log to 2.5 Log CFU reduction of *E.coli*. Tests with other bacteria did not reach such a high level of disinfection. For energy calculations the contribution of GAC to disinfection was not included (GAC disinfection was subtracted from the total disinfection values). For achieving *E.coli* reduction of 2.2 Log CFU, an energy input of 1.5 kWh/L was required (Chapter 6). In the smaller set-up, a higher disinfection level of *E.coli* was achieved of 2.5 Log CFU (Chapter 3), however, also power consumption increased up to 2.2 kWh/L. This rough energy consumption calculation shows that the system is not yet optimized for energy efficiency, and this part needs further systematic investigation.
The disinfection level achieved when GAC and US were combined (again the disinfecting effect of GAC is subtracted from total disinfection values) was 3.7 Log CFU reduction of *E. coli* with a power consumption of 0.05 kWh/L. When we look to the combination of AC field and US for disinfection a reduction of 2.5 Log CFU *E. coli* was achieved with an energy consumption of 0.84 kWh/L. Furthermore, when FBE and US were combined for disinfection, enhanced disinfection was achieved of 3.9 Log CFU *E. coli* reduction but required an energy input of 1.55 kWh/L. To compare, e.g. boiling 1 liter of water for about 5 minutes requires practically about 1 kWh/L \(^2\). Thus, the combination of AC field and US is an energetically favorable technology compared to a classical microbial inactivation measure. Nevertheless, the energy efficiency of the combination of AC field and US needs to be further improved to become practically relevant.

The disinfection level achieved with the FBE system and variations of it can be classified between “good” and “very good” (Table 5.4 in Chapter 5) when only *E. coli* disinfection is considered. To achieve this level of disinfection 5-6 hours treatment time was required which resulted in high energy input. However, the disinfection in FBE was tested for bacteria only (so not for the other groups of microorganisms); and Gram (+) bacteria were found less susceptible for FBE disinfection compared to Gram (-) bacteria at the applied conditions. Alternatively, here specific applications for FBE could be considered, such as biotechnology exploiting the frequency specificity to maintain a batch pure culture producing high value products, and where high energy input might be acceptable to protect the (costly) pure culture biomass from contamination.

For water disinfection with FBE, optimization of energy input is required to be able to compete with existing water and wastewater disinfection technologies. The optimization of FBE technology towards shorter reaction times and lower energy input is expected to be possible, however, only after the disinfection mechanism will be resolved, which will require more research.

7.6. General conclusions and future perspectives

The fluidized bed electrode disinfection system proved to work for the inactivation of microorganisms with low field strength (5-10 V/cm) at a radio frequency (10^4 – 10^5 Hz) electric field. The key components GAC and the AC field should be present to achieve considerable disinfection. In the absence of the GAC (with only AC field), no disinfection was observed, whereas in the absence of AC field (with only GAC) disinfection is limited. The combination of these components leads to a very high synergy factor (9.1). The disinfection was tested on pure cultures of seven types of bacteria (E. coli, P. luteola, P. fluorescens, E. corotovora, E. faecalis, B. subtilis, B. subtilis subsp. subtilis). The best disinfection was achieved with E.coli i.e. 3.4 Log CFU/mL decrease during 6 hours of treatment time.

The main advantages of the FBE system are:

- It is robust (no electrode corrosion, stable GAC abrasion);
- It does not require addition of chemicals;
- Formation of toxic compounds is unlikely;
- It mainly requires periodic replacement of GAC, and no other maintenance.

The FBE system needs electricity and supply of fresh GAC. The frequency of replacement of the GAC depends on the purity of the water to be treated and the flow rate.

At present, the main drawback of the stand-alone FBE system is the low disinfection rate, resulting in a long disinfection time, and thus high energy input. To be able to enhance the disinfection rate further, the disinfection mechanism must be elucidated, through possible research lines as indicated earlier in this chapter.
This research resulted in important leads in understanding the disinfection mechanism in FBE:

- The disinfection mechanism is likely of physical origin;
- GAC is an essential component and results in bacteria stress and decay, which is enhanced by the AC field;
- Disinfection is frequency specific for every type of bacteria;
- The type, size and conductivity of fluidized material are important.

To further extend the research on FBE disinfection mechanism and application the following research directions could be considered:

Thermo-graphic analysis of the system could give insights on whether there is a heat transfer through particles, caused by an electrical current flowing through these particles. In this way the charge transfer mechanism that takes place in FBE and the extent of joule heating on GAC particles could be clarified.

To investigate the hypothesis of locally increased field strengths that are created by conductive particles causing disinfection, an FBE experiment with non carbon materials with low conductivity could be performed. Materials that could be tested are Ebonex (Ti₄O₇), zeolites and conductive polymers. Particle mixtures could also be investigated, because higher potential differences may be formed between different type of particles.

After the disinfection mechanism has been resolved it is important to investigate the disinfection of different types of pathogenic microorganisms in mixed populations as well disinfection of real water and wastewater samples. Furthermore, the continuous fluidized bed (flow through) reactor needs to be investigated, as a possible up-scaled design instead of the batch fluidized bed reactor as was used in this thesis.

The FBE technology may not be applied as standalone disinfection technology in a full scale, however could be considered as pretreatment step for organics, and suspended solids removal with applied AC field to reduce bacteria concentration before for instance UV or pulsed electric field disinfection.
Furthermore, the FBE technology (due to long retention times required for disinfection) might be applied for industrial process waters and cooling waters, that are being stored in tanks and are applied weekly or monthly. In this way FBE technology would achieve COD reduction and reduce the amount of bacteria (so also biofilm formation) inside these systems.

The combination of AC field and low power US (without GAC) is a second promising disinfection method. The possible applicability of this process (AC field +US) for selective removal of microorganisms in biotechnological applications, e.g. fermentation broths (AC field frequency specificity) should be investigated and optimized.

The elegance of disinfection with low capital and maintenance costs make FBE a potentially attractive technology for disinfection, however scientific and technological challenges, such as elucidating the mechanism, increasing the inactivation rate, and reducing the energy input, remain for the future research.
7.7. References


List of abbreviations

AC field – alternating current electric field
BC – before christ
BET – specific surface area
CFU – colony forming units
COD – chemical oxygen demand
CPS – counts per second
DBPs – disinfection by products
DC field – direct current electric field
DNA – deoxyribonucleic acid
EPA – environmental protection agency
FBE – fluidized bed electrode
FSK – frequency shift keying
GAC – granular activated carbon
Gram (-) – gram negative type bacteria
Gram (+) – gram positive type bacteria
LB – lysogeny broth
LF – low frequency
MO – microorganism
OD – optical density
PAC – powder activated carbon
PBS – polyphosphate buffered saline
PEF – pulsed electric field
pH_{pzc} – pH at point of zero charge
RF – radio frequency
RMS – square root of the mean
RNA – ribonucleic acid
rpm – rounds per minute
SEM – scanning electron microscope
EDX – energy dispersive X-ray spectroscopy
SF – synergy factor
TSB – tryptic soy broth
US – ultrasound
UV – ultraviolet light
WHO – world health organization
WWTP – wastewater treatment plant
English Summary

The scarcity of fresh water is a large concern for human health. Part of the world population (10%) does not have access to safe drinking water and 36% of the population does not have basic sanitation. Most of these people live in water scarce areas, and reuse of water is relevant to provide the required amounts of fresh water. To increase the reuse of water resources, wastewater effluents could be used for e.g. irrigation, and disinfection of these effluents is important to safeguard health of humans and livestock.

State-of-art water disinfection technologies have limitations, such as use of toxic chemicals, a high energy input, formation of disinfection by-products, complex equipment with intensive maintenance and limited disinfection efficiency. Therefore, there is a demand for alternative technologies that have low energy demand, low chemical and material consumption, no formation of toxic by-products, and are robust and simple in operation.

One of possible alternatives is disinfection based on electric fields. Strong electric fields (e.g. typically as pulsed electric fields) for disinfection of waters have been employed already for several decades. However, this technology needs high electric field strength, which is a disadvantage in turbid water with high organic content. This could be avoided through the use of a low strength alternating electric field combined with a fluidized bed electrode (FBE) of granular activated carbon (GAC). The objective of this thesis is to prove the principle of this FBE disinfection technology and to reveal the main components of the underlying mechanisms.

Chapter 2 describes the proof-of-principle of the FBE system as a new device for disinfection. In the FBE system, GAC particles were suspended, and an Alternating Current radio frequency electric field (AC field) was applied over the suspended bed. 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 hours. Studies with non-pathogenic luminescent bacterium *Escherichia coli* YMc10 demonstrated that disinfection with FBE requires both
the presence of GAC particles and the application of an AC field. 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.

In **Chapter 3**, the proof-of-principle of FBE disinfection was extended for several pure microbial cultures: *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 AC field amplitude and shape were kept constant and the effect of AC field frequency on disinfection performance in FBE was investigated. From the bacteria tested, Gram negative strains were more susceptible for FBE disinfection than Gram positive strains and the efficiency of disinfection is AC field frequency and microbial strain dependent. During 6 hours of disinfection, the decrease above 2 Log colony forming units (CFU) were 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.

The role of the FBE key component GAC on metabolic activity and survival of a pure culture *Escherichia coli* YMc10 is described in **Chapter 4**. The change in *E.coli* concentration and metabolic activity was studied with 5 media differing in organic, mineral and salt content and exposed to 5 different GAC concentrations between 23 and 350 g GAC /L (without applied AC field). The changes in *E. coli* concentration and metabolic activity were related to changes in the media such as COD (organic concentration), osmotic balance (salt concentration) and buffer capacity. The results show that *E. coli* is sensitive to changes in organic concentrations. The adsorption of organics on GAC is proportional to the GAC concentration present in the suspensions. Severe pH variations were observed in phosphate buffered saline when exposed to GAC suspensions. *E. coli* suspended in 350 g/L of GAC in media with low organics and low salt concentrations resulted in 3.5 Log CFU decay after 6 hours exposure. The presence of GAC alone does not result in disinfection in rich media, but establishes unfavorable conditions which can enhance adverse effects on microorganism survival.
The mechanistic role of GAC in FBE disinfection was investigated in Chapter 5. Ten different types of GAC were tested in FBE for *E.coli* YMc10 disinfection. The disinfection performance of only GAC, and of GAC combined with an AC field were quantified. Seven GACs showed poor to intermediate and three GACs: Norit RB3 (2.7 Log CFU *E.coli* decrease), Sorbonorit 3 (2.8 Log CFU *E.coli* decrease) and Rx 3 Extra (3.4 Log CFU *E.coli* decrease) showed substantial disinfection. The results suggest a relation between the pH$_{pzc}$ of the GAC and the disinfection performance in FBE. Furthermore, disinfection performance increased with larger GAC particles and decreasing conductivity. Therefore, these physico-chemical and physical properties of GAC are important factors controlling the disinfection performance in the FBE systems.

The results from the previous chapters showed a synergistic effect of the combination of GAC and AC field in the FBE system. To determine whether a similar synergistic effect could be achieved through combination of FBE with another physical disinfection technique, we investigated ultrasound (US) combined with an AC field and/or suspended GAC in Chapter 6. The disinfection and energy consumption were compared for 7 different combinations of process components (US, GAC and AC field). Through combination of US and AC field, the disinfection achieved was 2.5 Log CFU *E.coli* reduction with 0.82 kWh/L energy input. A synergistic disinfection effect was observed when combining AC field and US without GAC. However, the lowest energy input of 0.05 kWh/L and highest disinfection of 3.7 Log CFU *E.coli* reduction was achieved with the combination of US and GAC.

Finally, Chapter 7 describes the contributions of the key components of the FBE system to the disinfection mechanism. Based on the findings described in the previous chapters, the disinfection mechanism seems to have mainly a physical origin. Possibly, GAC particles both function as adsorbent for organics and microbial species, and as conductive component that locally enhances the electric field strength. This locally enhanced electric field might increase the effect of various stresses that have been previously described in literature such as cell rotation causing mechanical wear and cell membrane permeability. To develop an effective disinfection technology, the energy
consumption and disinfection time needs to be decreased and the general or specific applicability for FBE disinfection of various microbial species needs to be demonstrated. Nevertheless, the use of low cost reactor materials and consumables (GAC) make FBE a potentially attractive solution for disinfection of water for reuse.
Nederlandse Samenvatting

Zoetwaterschaarste is een groot probleem voor de volksgezondheid. Ongeveer 10 % van de wereldbevolking heeft geen toegang tot veilig drinkwater en 36 % van de bevolking heeft geen basis sanitaire voorzieningen. Het grootste deel van deze mensen leeft in waterschaarse gebieden en hergebruik van water is relevant om te voorzien in de benodigde hoeveelheid zoet water. Om meer water te hergebruiken zou behandeld afvalwater gebruikt kunnen worden voor bijvoorbeeld irrigatie. Disinfectie van hergebruikt afvalwater is dan belangrijk om de veiligheid van mens en dier te waarborgen.

Reeds bestaande water disinfectietechnologieën hebben diverse nadelen, zoals het gebruik van gevaarlijke chemicaliën, een hoog energieverbruik, de vorming van desinfectiebijproducten, een hoge mate van complexiteit met intensief onderhoud en een soms beperkte desinfecterende werking. Er is dus vraag naar alternatieve technologieën die weinig energie, chemicaliën en materialen gebruiken, geen desinfectiebijproducten vormen en simpel en robuust functioneren.

Een mogelijke alternatieve manier van desinfectie is gebaseerd op het gebruik van elektrische velden. Sterke elektrische velden (bijvoorbeeld als gepulseerde elektrische velden) voor desinfectie van water worden al enige decennia toegepast. Echter, deze technologie maakt gebruik van een sterk elektrisch veld, wat problematisch is voor troebel water die veel organische stof bevatten. Dit kan worden vermeden door gebruik te maken van een zwak, wisselend elektrisch veld gecomcombineerd met een geïntegreerde bed elektrode (FBE) van granulaire actieve kool (GAC). Het doel van dit proefschrift is om het principe aan te tonen van deze FBE desinfectietechnologie en om de belangrijkste componenten van het onderliggende mechanisme te beschrijven.

In hoofdstuk 2 wordt het principe van het FBE systeem als een nieuwe desinfectietechnologie aangetoond. Het FBE systeem bestond uit gesuspendeerde GAC
deeltjes waarover een wisselstroom (AC) elektrisch veld werd aangelegd. De mate van desinfectie is onderzocht bij verschillende frequenties tussen 80 en 200 kHz bij een elektrische veldsterkte van 6 ± 0.5 V/cm gedurende 6 uur. Proeven met niet-pathogene, luminiscerende Escherichia coli Ymc10 toonden aan dat zowel GAC deeltjes als een AC elektrisch veld nodig zijn om te kunnen desinfecteren met het FBE systeem. De grootste afname in de concentratie van levende E.coli bacterieën (van 10⁸ tot 10⁶ CFU/mL ) was bereikt bij de optimum frequentie van 140 kHz.

In hoofdstuk 3 is het bewijs van het FBE principe uitgebreid voor verscheidene microbiële culturen: Bacillus subtilis, Bacillus subtilis subsp. subtilis, Enterococcus faecalis als vertegenwoordigers van gram positieve bacterieën en Erwinia carotovora, Pseudomonas luteola, Pseudomonas fluorescens en Escherichia coli YMc10 als vertegenwoordigers van gram negatieve bacterieën. Het effect van de frequentie van het AC elektrische veld op de mate van desinfectie in FBE is bestudeerd, waarbij de amplitude en vorm van het AC elektrische veld constant werden gehouden. Gram negatieve bacteriën waren meer vatbaar voor FBE desinfectie dan gram positieve bacteriën en de efficiëntie was afhankelijk van de AC frequentie en microbiële cultuur.

De invloed van de FBE component GAC op de metabolische activiteit en overleving van een pure Escherichia coli Ymc10 culuur wordt beschreven in hoofdstuk 4. De verandering in E.coli concentratie en metabolische activiteit is bestudeerd in 5 verschillende media die varieerden in concentratie van organische stof, mineralen en zouten en in 5 verschillende GAC concentraties tussen 23 en 350 g GAC/L (zonder aangelegd AC elektrisch veld). De veranderingen in E.coli concentratie en metabolische activiteit zijn vergeleken met veranderingen in COD (organische stof concentratie), osmotische balans (zout concentratie) en buffer capaciteit van de media. De resultaten toonden aan de E.coli gevoelig is voor veranderingen in organische stofconcentratie. De adsorptie van organische stof aan GAC is proportioneel met de GAC-concentratie van de suspensie. Sterke pH variaties waren zichtbaar voor de fosfaat buffer als deze was blootgesteld aan GAC. E.coli gesuspendeer in 350 g/L GAC in media met lage organische stof en zout concentraties resulteerde in 3.5 Log CFU afname na 6 uur. Enkel de
aanwezigheid van GAC leidt niet tot desinfectie in rijke media, maar resulteert in ongunstige condities die het overleven van micro-organismen negatief beïnvloedt.

De mechanistische rol van GAC in FBE desinfectie is bestudeerd in hoofdstuk 5. Tien verschillende typen GAC zijn getest in het FBE systeem voor *E.coli* Ymc10 desinfectie. De mate van desinfectie van enkel GAC en van GAC gecombineerd met een AC elektrisch veld zijn gekwantificeerd. Zeven GACs resulteerden in beperkte tot matige desinfectie en drie GACs: Norit RB3 (2.7 Log *E.coli* afname), Sorbonorit 3 (2.8 Log *E.coli* afname) en Rx 3 Extra (3.4 Log Ecoli afname) resulteerden in substantiële desinfectie. De resultaten suggereren een relatie tussen de pH\textsubscript{pzc} van het GAC en de mate van desinfectie in FBE. Bovendien nam de desinfectie toe voor grotere GAC deeltjes en met afnemende geleidbaarheid van de GAC deeltjes. Deze fysisch-chemische en fysische eigenschappen van GAC zijn belangrijke factoren voor de mate van desinfectie.

De voorgaande hoofdstukken toonden een synergetisch effect aan voor de combinatie van GAC met een AC elektrisch veld in het FBE systeem. Om te bepalen of er een vergelijkbaar synergetisch effect kon worden bereikt door het FBE systeem te combineren met een andere fysische desinfectie techniek, hebben we in hoofdstuk 6 ultrageluid (US) gecombineerd met een AC elektrisch veld en/of een gesuspendeerd GAC bed. De mate van desinfectie en het energieverbruik zijn vergeleken voor 7 verschillende combinaties van proces componenten (US, GAC en AC elektrisch veld). De combinatie van US en AC elektrisch veld resulteerde in 2.5 Log *E.coli* CFU afname bij een verbruik van 0.82 kWh/L. Er was een synergetisch effect voor de combinatie van het AC elektrisch veld met US (zonder GAC). Het laagste energieverbruik (0.05 kWh/L) en de hoogste mate van desinfectie (3.7 Log *E.coli* afname) echter, werd bereikt middels de combinatie van US en GAC.

In hoofdstuk 7 tenslotte, worden de bijdragen van de verschillende componenten van het FBE systeem aan de desinfectie beschreven. Gebaseerd op de voorgaande hoofdstukken lijkt het desinfectie mechanisme van fysische aard te zijn. GAC deeltjes functioneren mogelijk zowel als adsorbens voor organische stof en microben en als elektrisch geleidende component die lokaal het AC elektrische veld versterkt. Dit
versterkte elektrische veld zorgt voor een toename in stress op bacteriële cellen zoals eerder in de literatuur beschreven, bijvoorbeeld een toename van mechanische stress als gevolg van celrotatie en een toename in membraanpermeabiliteit. Om een effectieve desinfectie technologie te ontwikkelen moeten de energieconsumptie en de desinfectietijd omlaag en de algemene of specifieke toepasbaarheid van FBE worden aangetoond. Desalniettemin, vanwege het gebruik van goedkope reactormaterialen is het FBE systeem een potentieel aantrekkelijke technologie voor desinfectie van water voor hergebruik.
Geriamo vandens trūkumas pasaulioje kelia grėsmę žmonių sveikatai ir didelį susirūpinimą visuomenės sveikatos specialistams. Kokybiškas ir saugus geriamas vanduo yra neprieinamas 10 % pasaulio gyventojų, o dar didesnė dalis žmonijos (36 %) negali patenkint pagrindinių sanitarinių sąlygų. Dauguma šių gyventojų gyvena vietovėse su skurdžiais vandens ištekliais, ir pakartotinas vandens naudojimas padėtų spręsti geriamo vandens trūkumo problemą. Siekiant sumažinti naudojamo geriamo vandens kiekį, nuotekos galėtų būti naudojamos tokiems poreikiams kaip, pavyzdžiui, laukų laistymas, teritorijų priežiūra, skalbimas. Tačiau naudojant nuotekas šiems tikslams, iškyla pavojus žmonių ir galvijų sveikatai dėl mikrobiologinės taršos, todėl prieš pakartotinai naudojant išvalytas nuotekas jos turėtų būti dezinfekuojamos.

Nors šiuolaikinės vandens dezinfekavimo technologijos yra efektyvios, tačiau jos turi ir trūkumų. Keletas iš jų: toksiškų cheminių medžiagų išsiskyrimas, didelės energijos sąnaudos, ribotas procesų intensyvumas dezinfekavimo metu ir susidarantys šalutiniai produktai, sudėtingi įrenginiai, reikalaujantys pastovios priežiūros. Todėl būtina ieškoti alternatyvių technologijų, kurios būtų efektyvios, patvarios, patikimos, paprasto veikimo; taipogi ieškoti aplinką tautosą sprendimų, kad sumažėtų elektros energijos, cheminių medžiagų ir išteklių suvartojimas, būtų išvengta žalingų reakcijos produktų susidarymo.

Viena iš galimų alternatyvų galėtų būti dezinfekcija elektromagnetiniais laukais. Stiprių elektromagnetinių laukų (pvz. impulsinis elektromagnetinis laukas) naudojimas dezinfekcijai yra gerai žinomas ir plačiai naudojamas jau keletą dešimtmečių, tačiau stiprūs elektromagnetiniai laukai yra sunkiai pritaikomi jeigu valomas vanduo yra drumstas ir su didele ištirpusiu organinių medžiagų koncentracija. Šių trūkumų galima būtų išvengti, jei dezinfekcijai butų naudojami silpni elektromagnetiniai kintamos srovės laukai, generuojami pseudo-verdančioje aktyvuotos anglies įkrovoje. Šios technologijos pritaikymas dezinfekcijai yra aptariamas šiose tezėse.
Antrame skyriuje aptariamas periodinio veikimo, pseudo-verdančio sluoksnio reaktoriaus, veikiančio kintančios srovės elektriniame lauke (FBE) poveikis dezinfekcijai, tyrimų įranga, sąlygos ir pirminiai rezultatai. Pseudo-verdančio sluoksnio įkrovai naudotos aktyvuotos anglies granulės (GAC), o kintančios srovės generavimui buvo panaudoti įkrovoje panardinti elektrodai (AC field). Dezinfekcijos efektyvumas buvo tiriamas keičiant elektromagnetinės bangos dažnius (80 - 200 kHz diapazone) ir palaikant elektrinio lauko stiprumą 6±0.5 V/cm ir šešių valandų poveikio trukmę. Dezinfekcijos, atliktos su nepatogeniniais liuminescenciniais mikroorganizmais *Esherichia coli* YMc10 (*E.coli*), lygis parodė, kad dezinfekcijai įvykti buvo reikalingas ir kintančios srovės laukas, ir granuliuota aktyvuota anglis. Atlikti tyrimai leido nustatyti efektyviausiai vekiantį dažnį. Didžiausias dezinfekcijos efektyvumas buvo pasiektas esant 140 kHz elektromagnetinės bangos dažniui, kai *E.coli* koncentracija sumažėjo nuo $10^8$ iki $10^6$, t.y. 2.7 Log kolonijų formavimo vienetais (CFU).

Trečiame skyriuje aprašytas FBE dezinfekcijos veikimo principas buvo išbandytas ir su kitoms mikroorganizmų rūšims - Gram teigiamų bakterijų rūšies atstovais *Bacillus subtilis*, *Bacillus subtilis* subsp. *subtilis*, *Enterococcus faecalis* ir Gram neigiamų bakterijų rūšies atstovais *Erwinia carotovora*, *Pseudomonas luteola*, *Pseudomonas fluorescens*, *Esherichia coli* YMc10. Šiame tyrome buvo vertinta tik kintamosios srovės elektromagnetinės bangos dažnių įtaka, o kintamosios srovės elektrinio lauko stiprumas ir bangos pobūdis buvo pastovūs. Eksperimentas parodė, kad Gram neigiamos bakterijos buvo labiau pasiduodančios dezinfekcijai nei Gram teigiamos bakterijos, be to buvo konstatuota, kad dezinfekcijos procesas buvo priklausomas nuo elektromagnetinių bangų dažnio. Per šešias dezinfekcijos valandas *P. luteola* ir *E. coli* koncentracija sumažėjo daugiau nei 2 Log CFU, esant 10 kHz dažniui ir 10 kHz bei 140 kHz dažnių digubiems moduliuotiems laukams.

Ketvirtame skyriuje yra aprašytas granuliuotos aktyvuotos anglies įkrovo, įtakos tyrimas *E. coli* bakterijų medžiagų apykaitos intensyvumui ir jų išgyvenimui. *E. coli* koncentracijos ir medžiagų apykaitos pokyčiai buvo tirti penkiuose skirtinguose tirpaluose su skirtomis organinių medžiagų ir druskų koncentracijomis bei skirtomais aktyvuotos anglies kiekiais (23-350 g/L). *E. coli* koncentracijos ir medžiagų apykaitos intensyvumo
Pokyčiai buvo susieti su COD (organinių medžiagų koncentracijos), osmosinės pusiausvyros (druskų koncentracija) ir buferiškumo (pH pusiausvyra) pokyčiais. Gauti rezultatai parodė, kad *E. coli* bakterijos yra jautrios organinių medžiagų koncentracijos pokyčiams. Savo ruožtu, organinių medžiagų koncentracijos pokyčiai tirtame tirpale yra priklausomi nuo aktyvuotos anglies granulių koncentracijos. Dideli pH svyravimai buvo pastebėti fosfato buferiniame tirpale, kai juo buvo užpilta aktyvuotos anglies granulė įkrova. Kai *E. coli* suspensija buvo užpilta fosfato buferiniu tirpalu su granuliota anglimi (350 g/L koncentracija), *E. coli* kiekis per šešias tyrimo valandas sumažėjo 3.5 Log CFU. Todėl, galima teigti, kad granuliota anglis nesukelia bakterijų dezinfekcijos organiniuose tirpaluose, tačiau prisideda prie neigimų sąlygų bakterijoms sukūrimo, kurios prisideda prie dezinfekcijos.

**Penktame skyriuje** yra aprašytas mechaninis granuliotos aktyvuotos anglies poveikis dezinfekcijai FBE sistemos įkrovoje. FBE sistemoje buvo tirta dešimt skirtingų rūšių aktyvuotos anglies ir jvertintas jų poveikis *E. coli* YMC10 dezinfekcijai. Tyrimai buvo atliekami, vertinant visų skirtingų aktyvuotų anglies poveikį; bei bendrą kintamosios srovės ir aktyvuotų anglų poveikį. Naudojant septyni aktyvuotos anglies tipai turėjo žemą/vidutinę įtaką dezinfekcijos efektyvumui, o trys aktyvuotos anglies tipai: Norit RB3 (2.7 Log *E. coli* CFU sumažėjimas), Sorbonorit 3 (2.8 Log *E. coli* CFU sumažėjimas), Rx 3 Extra (3.4 Log *E. coli* CFU sumažėjimas) - turėjo teigiamą įtaką FBE dezinfekcijos efektyvumui. Rezultatai parodė, kad veikiant FBE sistemai yra sąsaja tarp dezinfekcijos intensyvumo ir anglies savybių, tokių kaip pH_pzc (paviršiaus krūvio), aktyvuotos anglies granulių dydžio ir elektrinio laidumo. Dezinfekcijos intensyvumas buvo didesnis, kai buvo naudojamos stambesnės aktyvuotos anglies granulės ir kai jų elektrinis laidumas buvo mažesnis.

**Šeštame skyriuje** buvo aptartas dezinfekcijos veiksnių sinergizmas. Ankstesniais skyriuose buvo nustatytas sinergizmas, kai kintamoji elektrinė srovė ir aktyvuota anglis buvo naudojami pseudo-verdančio sluoksnio reaktoriuje dezinfekcijos tikslais. Norėdami įvertinti ar yra galimi panašūs sinergizmo požymiai, naudojant FBE kartu su kita fizinio poveikio dezinfekcijos priemone, mes tyrėme, kokį vienalaikį poveikį dezinfekcijos
efektyvumui gali turėti ultragarso (US) ir kintamosios srovės (AC field) bei/arba aktyvuotos anglių (GAC) panaudojimas pseudo-verdančiame sluoksnyje.

Dezinfekcijos intensyvumas ir energijos suvartojimas buvo lyginami septynių procesų metu, parinkus komponentus (US, AC field, GAC) skirtingais deriniais.

Tačiau intensyviausia dezinfekcija pseudo-verdančiame sluoksnyje vyko tuomet, kai vienu metu buvo naudojami ultragarasas ir aktyvuotos anglių granulės. E.coli koncentracija sumažėjo 3.7 Log CFU ir buvo sunaudota tik 0.05 kWh/L elektros energijos.

Galiausiai septintame skyriuje yra apibendrinti visų skyrių duomenys ir aptarti visi esminiai dezinfekcijos mechanizmo FBE sistemoje elementai. Analizuojant gautus rezultatus šiose tezėse, galima teigti, kad, FBE dezinfeckijos principas yra fizinės prigimties. Tikėtina, kad aktyvuotos anglių granulės veikia ir kaip organinių medžiagų bei bakterijų adsorbentas, ir kaip laidusis komponentas, lokalai padidinantis elektrinio lauko stiprumą. Taip pat tikėtina, kad šis lokalai sustiprintas elektrinis laukas - sustiprina kitus poveikius kurie literatūroje traktuojami kaip sukeliantis „stresą“ mikroorganizmams. Pavyzdžiui, didinantis ląstelių membranos pralaidumą ar ląstelių sukimasi (rotaciją), o tai savo ruožtu sukelia mechaninį nuovargį.

Siekiant išvystyti efektyviai veikiančią dezinfeckijos sistemą, turi būti sumažintos energijos ir laiko sąnaudos reikalingos pasiekti dezinfekcijai, o taip pat reikia nuodugniau ištirti FBE tinkamumą mišrių mikroorganizmų dizinfekcijai.

Tačiau mažos žaliavų sąnaudos, paprasta reaktoriaus konstrukcija ir galimybė panaudoti šią sistemą dezinfekuoti nuotekoms skirtoms antriniam vandens panaudojimui, padaro FBE sistemą potencialiai patrauklia technologija.
I want to express my gratitude to a lot of people that directly or indirectly contributed to this book and development of my personal and professional skills.

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About the Author

Justina Račytė was born on 6th of August 1981 in Kaunas, Lithuania. In 1999 she finished S.Neris secondary school and the same year was admitted to Kaunas University of Technology (KTU), at the faculty of Chemical technology. She received her Bachelor degree as Environmental protection engineer in 2003, which was followed by an international MSc degree in “Environmental Management and Cleaner Production” at the Institute of Environmental Engineering “Apini” (KTU). During her study years she was actively involved in student society “Vivat Chemija”. During the MSc studies she came to the sub-department ETE at Wageningen University for an Erasmus exchange program and did an MSc project for 8 months under the supervision of Harry Bruning and Wim Rulkens. Both MSc theses at ETE and Apini were related to the textile industry. The former dealt with treatment of textile effluents contaminated with reactive dyes and the latter dealt with developing a cleaner production approach in small and medium textile enterprises. She did her internship in the textile company “Trys sezonai” in Kaunas. After her MSc graduation, she was working in the European social fund agency in Vilnius, Lithuania as a project manager for 2 years. In Vilnius she got admitted to a sandwich PhD program with Vilnius Gediminas Technical University and the sub-department ETE at Wageningen University. After a year of sandwich PhD she switched to a full PhD position at Wetsus, and was working from 2008-2013 on her PhD project. The results of this project are described in this thesis.
List of publications

In peer-reviewed journals:
Racyte, Justina; Yntema, Doekle R.; Bernard, Severine; Bruning, Harry; Paulitsch-Fuchs, Astrid H.; Rijnaarts, Huub H.M. Gram positive and gram negative bacteria disinfection with low strength alternating field and granular activated carbon. Accepted for publication in Water research.


International conference proceedings:

The Netherlands Research School for the Socio-Economic and Natural Sciences of the Environment (SENSE), declares that

**Justina Račytė**

born on 6 August 1981 in Kaunas, Lithuania

has successfully fulfilled all requirements of the Educational Programme of SENSE.

Wageningen, 20 September 2013
The SENSE Research School declares that Ms. Justina Račytė has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 68 ECTS, including the following activities:

**SENSE PhD Courses**
- Environmental Research in Context
- Biological processes in environmental technology

**Other PhD Courses**
- Anthropogenic pollution and public health
- General technology of wastewater treatment
- Environmental mathematical modelling
- Instrumental methods in electrochemistry
- Pedagogy
- Teaching and supervising Thesis students
- Techniques for Writing and Presenting a Scientific Paper
- Effective presenting
- Matlab Introductory course
- Advanced course: guide to scientific artwork
- Training Nederlands NT2-1 and NT2-2
- Competence Assessment

**Didactic Skills Training**
- UV/H2O2 - **Advanced oxidation processes possibilities for textile reactive dyes decolorization.** 7th International Conference on Environmental Engineering, 22-23 May 2008, Vilnius, Lithuania
- Disinfection by combination of ultrasound and fluidized bed electrodes. Wetsus Internal Congress, 24 November 2011, Leeuwarden
- Alternating electric fields combined with activated carbon for disinfection of gram negative and gram positive bacteria. Ecotechnologies for Wastewater Treatment. Technical, Environmental and Economic Challenges, 25-27 June 2012, Santiago de Compostela, Spain
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