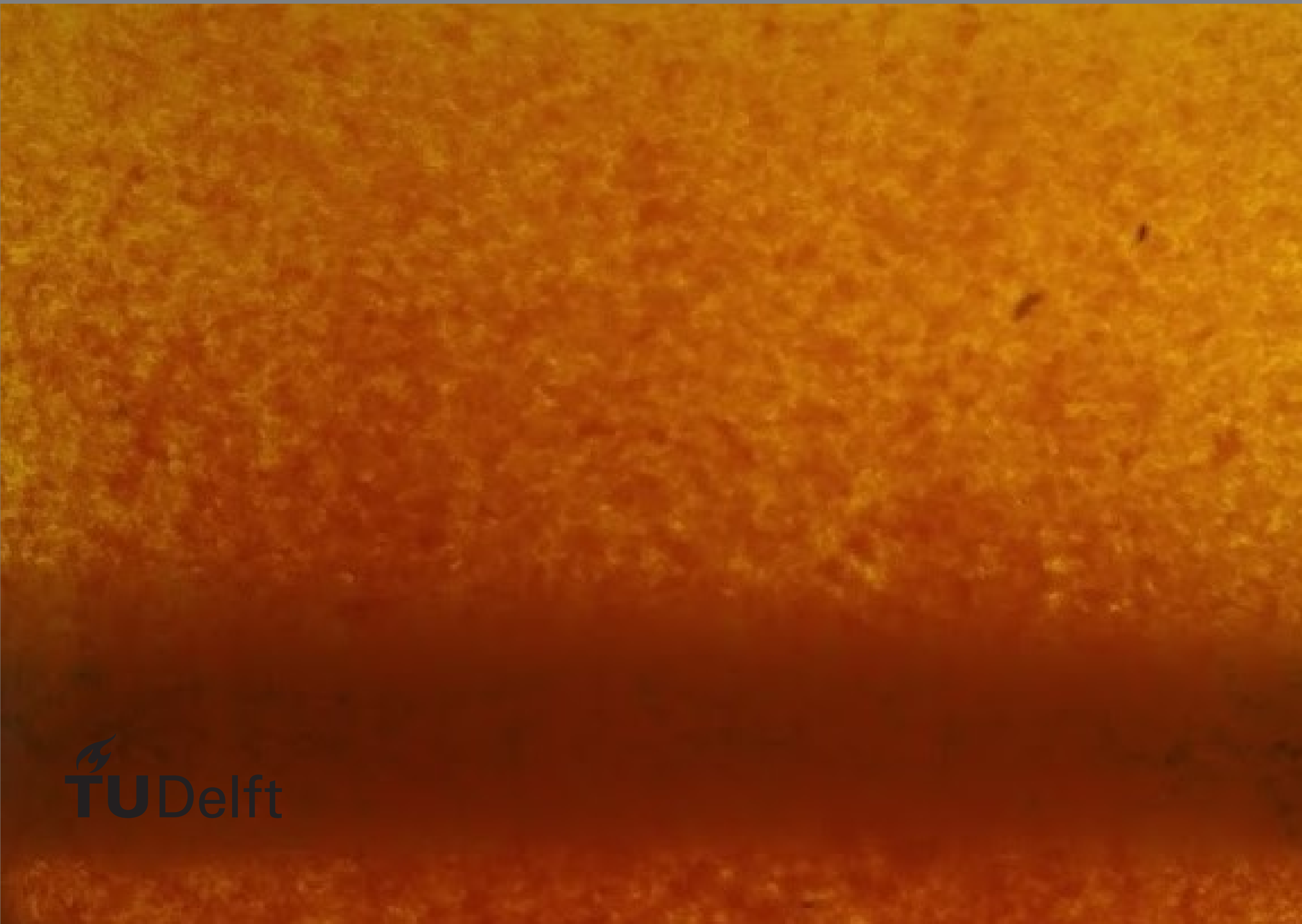
Disinfection of faecal indicator organisms by iron electrocoagulation for reuse of sewage water

B. van der Werff



Disinfection of faecal indicator organisms by iron electrocoagulation for reuse of sewage water

by

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Abstract

Due to the quick growth in human population and subsequently a high rate of urbanization, fresh water sources are under pressure. Furthermore, an estimated 1.8 billion people drink water from potentially sewage-contaminated sources with the risk of significant levels of pathogens. Also the presence of antibiotic resistant bacteria in sewage has made the improper treatment of wastewater effluent an emerging problem. Iron electrocoagulation (Fe-EC) is mentioned as a promising treatment technique for disinfection of wastewater effluent. Fe-EC has shown to be successful in reducing pathogen concentration, although to different extent for types of pathogens. Besides, the water quality was found to be of influence on the effectiveness. This study therefore aimed to determine the removal mechanism for both bacteria and virus as well as assess the effect of wastewater components on the Fe-EC treatment process. Two disinfection mechanisms were considered from literature; physical removal by iron precipitates and inactivation by reactive oxygen species. To distinguish the two mechanisms EC experiments were performed with and without the addition of TEMPOL, a well known scavenger of the superoxide radical, excluding inactivation. Further EC experiments were performed by adding phosphate, calcium, carbonate, and natural organic matter (NOM) individually or in combination, to observe the effect of these wastewater components. EC experiments were conducted in an aerated beaker with an electrolyte volume of 1L, continuously stirred. pH and current were kept stable at 7.5 and 200mA, respectively. Total electrolysis time was 15 minutes, resulting in a total charge dosage of 180 C/L. After electrolysis the samples were left to settle for 2 hours. Blank measurements for control were conducted in a 230 mg/L NaCl solution. Wastewater components were added in concentration of 4.0 mg/L for phosphate, 60 mg/L of calcium, and 10 mg/L of humic acids. *E. coli WR1* and ϕX174 were used as indicator organisms for bacteria and virus, respectively. From TEMPOL experiments is was concluded that contributing disinfection mechanisms for *E. coli* were inactivation and physical removal. During electrolysis in TEMPOL-free solutions a log removal of 3.8 log units was found, ascribed to inactivation. A further increase in removal to 6.0 log units was observed after settling. In the presence of TEMPOL only 0.3 log units removal were observed during electrolysis, but total removal after settling still amounted 5.5 log units. This indicates a larger contribution of physical removal without inactivation. For ϕX174 removal during electrolysis only accounted for 0.8 log unit both with and without TEMPOL addition, indicating the minor role of reactive species on virus. Disinfection after settling equaled 4.5 log units, when settling was complete. Settling was found to be dependent on mixing conditions, and ϕX174 removal was strongly correlated as well. Extended mixing promoted the formation of larger flocs and thereby enabled sweep flocculation for phage removal. The effect of phosphate on attenuation of *E. coli* could be observed either for inactivation, 2.0 log units, during electrolysis as for physical removal, 4.7 log units, after settling. Phosphate has high affinity for iron and competes with bacteria for surface area. Calcium as well inhibited abatement of *E. coli*. Inactivation only amounted for 1.4 log units and removal after settling equaled 2.1 log units. Calcium is expected to complex bacteria surface, increasing repulsive forces between *E. coli* and iron. The effect of carbonate was only seen during electrolysis, 1.3 log units. Total removal after settling was 5.2 log units. The formation of the carbonate radical is suggested as explanation for the decrease in effectiveness of reactive species. The effect of NOM on *E. coli* attenuation was less clear. Inactivation was inhibited to only 1.4 log units, but no effect was found on the total removal. Therefore, it can be concluded that NOM does not successfully compete with bacteria for iron surface. ϕX174 attenuation was not effected by addition of phosphate, calcium, or carbonate. On the other hand, the addition of NOM inhibited phage removal, only 0.6 log units in comparison to 4.0 log units for the blank. Adsorption of NOM onto iron surface is expected to negatively change the surface charge, inhibiting association between iron and virus.

In conclusion, this research concluded that *E. coli* potentially either can be removed by inactivation as well as physical removal, although physical removal has the upper hand when wastewater components are added. For ϕX174 inactivation plays a minor role and disinfection is mainly assured by physical removal. Bacteria attenuation is mainly influenced by phosphate and calcium, by competition for surface area and blocking association between iron and bacteria. Carbonate and NOM are not effectively competing with bacteria. For virus, NOM is the main inhibitor in attenuation. Also for phage, mixing conditions and subsequently floc formation, is an important factor for disinfection rate.

Contents

1

Introduction

1.1. Context

Over the last couple of decades, the human population has grown quickly. Subsequently, the world has seen a high rate of urbanization, growth of economic activity and a rapid change in land use. All these factors have put a pressure on natural fresh water sources. Nowadays, it is estimated that one third of the human population lives in water scarce regions [91]. Regions known for water scarcity coincide with regions of exponential population growth and therefore this number is expected to increase over the next decades. Fresh water is needed for drinking water, but also for agricultural purposes. To counter water shortage, wastewater is often mentioned as a potential source of fresh water [127]. To potentially be able to upgrade wastewater into drinking water or water for irrigation, reuse schemes have received a lot of attention in recent years.

Although water scarcity is mostly defined as a measure of water *quantity*, also the *quality* of water available is important. Access to safe drinking water is under pressure, again due to a lack of sufficient freshwater sources, but also due to pollution of the existing sources. Worldwide, only a small fraction of wastewater is treated extensively. Most wastewater is discharged either untreated or limitedly treated [127]. Nowadays, an estimated 1.8 billion people drink water from potentially sewage-contaminated sources [31]. Sewage usually contains high concentration of bacteria, protozoa, spores, and viruses, all being able to be pathogenic to humans. Microbial pollution of natural sources has an harmful impact by causing acute water borne diseases like diarrhea, cholera, and typhoid, leading to death, especially in developing countries [18, 41].

Another concern that has gained interest lately, is the spread of antibiotic resistant bacteria (ARB) and genes (ARG) through wastewater. Antibiotic resistance presents a threat to global public health by decreased effect of antibiotics against certain diseases. The usage of antibiotics has increased drastically worldwide. Wastewater possesses a combination of high concentration of microorganisms together with moderate levels of antibiotics, favoring the selectivity for resistance. The fact that conventional wastewater treatment plants only remove a small portion of the microorganisms has marked their outlets as one of the hot-spots in the spread of ARB and ARG [71].

The shortage on fresh water, the deterioration of natural waters by microbials and the spread of antibiotic resistance in bacteria have made the preservation of water resources being described as one of the biggest upcoming challenges for the next decades ahead [43]. To counter these problems, there is a need to develop new technologies that are able to treat wastewater up to higher standards at large scale and low cost. Conventional treatment methods used in drinking water production, removing microorganisms, are not efficient for the use on wastewaters. Conventional treatment normally consists of chlorination, filtration, or chemical coagulation processes. The issue with these treatment methods is the application on large scale with huge volumes and the safety and quality of the effluent cannot be guaranteed, when intended to be used for drinking- or irrigation water [38].

Therefore, electrocoagulation (EC) has recieved a lot of attention in recent years. EC is based on the in situ generation of metal ions that can form the base for floc formation, and afterwards be removed from the water by settling or filtration techniques. An EC system consists of a reactor with metal plates (electrodes). An electrical current is applied over these electrodes, creating an anode and

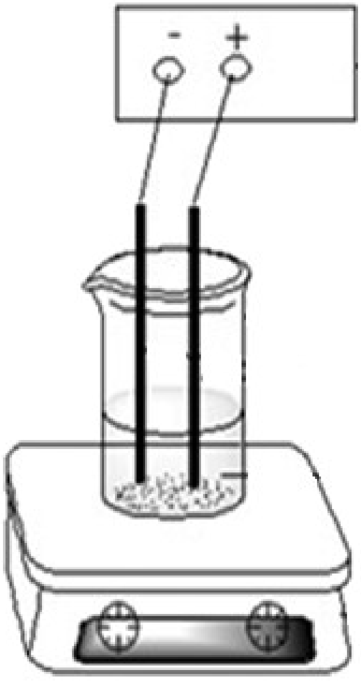


Figure 1.1: Schematic representation of an electrocoagulation reactor with one anode and cathode [97]

cathode. The anode will be sacrificial and introduce metal ions into the solution due to oxidation. EC electrodes are usually made out of iron or aluminum. Iron and aluminum are widely available metals with a relatively low price. Under normal conditions, the typical coagulation products are $Fe(OH)_3$ or $Al(OH)_3$, depending on the type of anode used [112]. Both metals have a high valence of +3, leading to efficient removal of pollutants [43]. Without treatment, ions and colloidal particles are kept in solution, because they are stable with a negative surface charge and therefore don't react with each other to settle or be removed by conventional filtration. By electrically introducing positively charged metal ions, released due to the electric corrosion, the outer layer of these particles is made unstable and they can start forming aggregates and form flocs together with coagulation products, due to which they can be removed more easily.

EC can remove a wide variety of substances, among other things organics, phosphates, free oil and grease (FOG), metals, bacteria, and viruses. This has made EC one of the promising treatment options to treat water for several pollutants at once. In conventional coagulation, a counter ion is added as well, decreasing the pH, so neutralization is needed afterwards. This increases the salinity of the water and reduces the quality for reuse [65]. EC does not require any counter ions being advantageous over conventional practices.

Several other advantages of electrocoagulation are that it needs minimal operator attention and operation is simple with a compact reactor; costs are lower than conventional treatment technologies; there is sludge minimization; and lower power consumption, making it an eco-friendly process which can run on renewable energy [38, 112].

1.2. Problem statement

Although iron electrocoagulation (Fe-EC) is mentioned as a promising technology for disinfection in future treatment of secondary municipal wastewater effluent , exact mechanisms are still unknown. Various studies have shown high removal rates of microbes, in application of relatively simple water matrices. On the other hand, when Fe-EC was applied for treatment of more complex types of water, removal efficiency dropped rapidly [22, 38, 45, 110]. Secondary wastewater effluent contains substantial concentrations of wastewater contaminants, such as phosphate, calcium, magnesium and organic matter, probably influencing efficiency of Fe-EC for disinfection. Besides, the exact removal mechanism is still under debate. It is concluded that pollutants can be removed from the water in several ways. It is often argued that they either can be physically removed by ending up in the metal precipitates formed in EC, or be killed by inactivation due to reactive species formed during iron oxidation. The tendency of particles to coagulate depends on the inter-particle forces, that is a combination of attractive and repulsive forces. Also the inactivation of microbes by oxidation is still under debate, and whether this is by direct oxidation at the anode or through oxidation by reactive species formed during oxidation of iron. On top of that, the exact effect of these components is until now still unknown for either of these removal mechanisms.

The suggested pathogen removal through EC technique implies two main stages participating simultaneously: (1) physical elimination produced by the adsorption of coagulant to cell membranes, which then favors the entrapment of microorganisms through electrochemically formed flocs and settling, and (2) demobilizing the cell membrane structure by ROSs produced by Fe^{2+} oxidation through dissolved oxygen existing in solution [38].

One of the major issues for EC is the lack of systematic knowledge on the interactions between water components and iron in the disinfection system. There are no models describing all interactions during electrolysis and settling, preventing operators to use and optimize the treatment method for application. Water types can be of very different nature, and testing all combinations would be very time consuming, it is important to have insight into the individual and combined effect of the most prominent wastewater components [43].

1.3. Research questions

The main focus of this research is to assess the influence of secondary municipal wastewater constituents on the disinfection capacity of iron electrocoagulation. In order to answer this question several sub questions have to be answered. Furthermore, mechanistic insight into the contribution of separate removal mechanism for specific microorganisms are assessed.

Main question What is the influence of wastewater components on the disinfection capacity of iron electrocoagulation?

subquestions

1. What are the main wastewater components?
2. Which removal mechanisms do contribute?
3. How do these mechanisms contribute and for what part?
4. How do wastewater components interfere with these mechanisms?
5. Are there differences for different types of micoorganisms?

1.4. Lay-out of the report

This is thesis is structed as follows. The first part provides information about the electrocoagulation technique and an overview of previous studies performed to assess electrocoagulation for disinfection. In the second chapter the research objectives are described together with the followed experimental methodology. The third part shows the results and in the fourth part these results are discussed. In the final chapter recommendations are made for further research and practice.

2

Literature

2.1. EC technology

Iron electrocoagulation is a relatively easy applicable water treatment method. In most basic form it only consist of an electrolyte and anode(s) and cathode(s). An electrical current is applied, causing oxidation and reduction at the electrodes surfaces. The anode is sacrificial and starts to corrode. In this way, metallic iron (Fe(0)) is oxidized and introduced in the water. In the past the oxidation form of iron has been debated. Nowadays, it is generally accepted that iron is dissolved as Fe^{2+} [67, 101]. Next to the in-situ generation of iron particles, at the same time water can be oxidized into O_2 and H^+. Both the anodic reactions, shown in the formula below, can happen simultaneously.

$$Fe^0 \rightarrow Fe^{2+} + 2e^-$$

$$2H_2O \rightarrow O_2(g) + 4H^+ + 4e^-$$

At the same time at the cathode, hydrogen ions are reduced to hydrogen gas as well as water molecules are reduced into hydrogen gas and OH^- ions.

$$2H^+ + 2e^- \rightarrow H_2(g)$$

$$2H_2O + 4e^- \rightarrow H_2(g) + 2OH^-$$

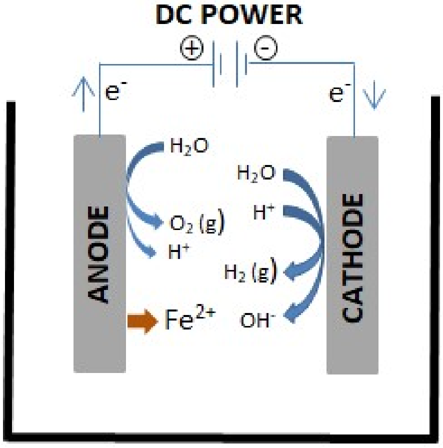


Figure 2.1: Basic electrocoagulation reactor layout with oxidation and reduction reactions

The ratio between the anodic reactions determines the efficiency of the current delivered. According to Faraday's law, the iron anode is sacrificed and dissolved into the liquid by a rate linked to the current applied. Faraday's law can be described by:

$$m = \frac{It\epsilon}{F}$$

in which m = the weight of metal in mg, I = the current applied in Ampere, t = the time in seconds, ϵ = the gram equivalent weight (27.92 mg/mol in the case of iron), and F = the Faraday constant of proportionality (96485 Coulombs). Dependent on conditions, the efficiency can vary. It has been seen that two main factors play a major role; current density and the pH of the electrolyte [54]. At higher current densities values were found to be below the value expected from Faraday. On the other hand, acidic pHs increase the iron generation. The difference is expected to be formed due to spontaneously occurring corrosion, which is higher in acidic circumstances and the portion of spontaneous corrosion is higher when a lower current and thus longer corrosion time is applied [54, 70]. At circumneutral pH and low currents, normally, the total dissolved iron is close to 100% of the theoretical value.

Subsequently, Fe^{2+} is oxidized by dissolved oxygen to Fe^{3+}. The oxidation rate is dependent on several factors; namely concentration of ferrous iron (Fe^{2+}), partial pressure of oxygen, and pH. According to the oxidation rate formula determined by Singer and Stumm (1970), pH has the strongest effect.

$$\frac{dmFe^{2+}}{dt} = k\ x\ P_{O_2}\ x\ [Fe^{2+}]\ x\ [OH^-]^2$$

Oxidation rate is important in electrocoagulation, since no residual Fe^{2+} is desired after treatment. Therefore, for efficient operation of Fe-EC, optimization measures can be taken. The measures can vary from increasing pH, increasing dissolved oxygen concentration, or applying longer residence times to ensure full iron oxidation. At alkaline conditions of pH 7.5 and higher in combination with oxygen rich conditions, this process takes place almost immediately [42] At lower pH, the rate will be much lower and below pH 4, oxidation will be negligible [83].

2.2. Removal pathways

Research in the past has assessed the effectiveness of Fe-EC for the removal of microbial pollutants in different waters [22, 45, 110]. These studies have shown promising results, but were not tested on waters like secondary municipal wastewater effluent, where concentrations of pollutants are generally higher compared to the ground-, and surface waters previously tested.

The application of current and the introduction of iron particles in the water unleashes a whole chain of reactions and interactions between substances in the water. All of these processes might contribute in different degrees to the attenuation of microbial pollutants. Four distinctive processes are mentioned in literature; physical removal through complex formation and settling, direct oxidation at the anode, indirect oxidation through reactive oxygen species (ROSs), and electron flooding [112]. All processes will be described more extensively in the next section.

2.2.1. Physical removal, particle entrapment

After introduction of iron, part of the iron will be in the form of Fe^{2+} and part will immediately oxidize into Fe^{3+}. Depending on oxygen and pH conditions, this rate will be determined. Both oxidation states of iron can form different species when introduced in the water. Activity graphs of both Fe^{2+} and Fe^{3+} are shown in Figure 2.2. Many different species can be formed, Fe^{2+}, $Fe(OH)^+$, and $Fe(OH)_2$ (s) for iron(II), and Fe^{3+}, $Fe(OH)^{2+}$, $Fe(OH)_2{}^+$, $Fe(OH)_3$ (s), and $Fe(OH)_4{}^-$ for iron(III) [33]. Over the whole range of pH, the iron hydroxide form $Fe(OH)_3$ (s) is dominant for iron(III). For iron(II), the solid state form $Fe(OH)_2$ becomes only dominant at pH above 6, and below mainly stays at the ionic form. The fact that $Fe(OH)_2$ is only dominant at high pH, combined with the positive correlation of iron oxidation rate and pH, would single out this species to be formed in oxygen rich conditions.Therefore, at neutral pH, it can be seen that predominately iron hydroxides ($Fe(OH)_3$) are formed, with poor solubility and therefore readily precipitate [43].

In principle, first iron hydroxides are formed, which later on can be transformed to dehydrated forms of rust. Depending on the conditions hematite, maghemite, magnetite, goethite and lepidocrocite can be formed [40, 82, 93].

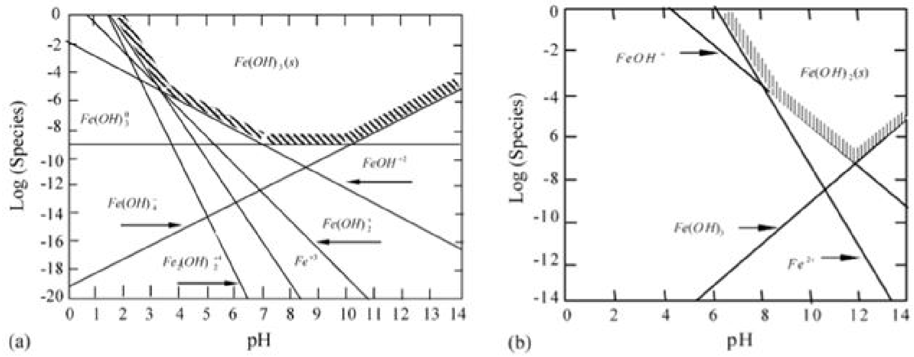


Figure 2.2: Activity diagrams of Fe(III) (a) and Fe(II) (b) according to pH [50]

$$2Fe(OH)_3 \rightarrow (\alpha,\ \gamma)\ Fe_2O_3 + 2H_2O\ (hematite,\ maghemite)$$

$$2Fe(OH)_3 \rightarrow Fe(OH)_2 + Fe_3O_4 + 4H_2O\ (magnetite)$$

$$Fe(OH)_3 \rightarrow (\alpha,\ \gamma)\ FeO(OH) + H_2O\ (goethite,\ lepidocrocite)$$

EC experiments with sodium electrolyte analysing the final product by X-ray absorption spectroscopy concluded poorly crystalline lepidocrocite to be formed [102, 119]. The presence of chemical impurities in natural systems generally blocks the formation of the more crystallized forms, and favors the formation of short-range-ordered Fe(III)-precipitates, the most prominent examples being hydrous ferric oxide (HFO) and ferrihydrite (Fh) [123]. The advantage of those poorly crystallized forms is that they are highly porous and have large surface area. Considering the larger surface area of HFO and Fh compared to more crystallized forms, they have a higher sorption capacity with beneficial characteristics for the treatment of polluted waters [25, 53, 73].

Generally, it is expected that mitigation of microorganisms is primarily through the adsorption to these iron species or the entrapment in flocs after coagulation and flocculation. Identical to conventional coagulation theory, particles are kept in solution, because of the repulsive forces between them. The opposing forces are the repulsive forces of the electrical double-layer and the attractive forces are the Van der Waals forces. Only when the attractive forces exceed the repulsive forces, colloids can coagulate. Colloidal particles are mostly negatively charged, and the similar electrical charge keeps them in suspension. A layer of oppositely charged particles is attracted to the colloid surface. The first layer around is called the Stern layer and is firmly attached to the particle. A second layer is formed on the outside of the Stern Layer. This second layer, or diffuse layer, is loosely associated with the particle. At the outer limit of the diffuse layer, the slipping plane, the zeta-potential can be measured. The fluid inside the slipping plane remains attached to the surface, while outside is mobile. An illustration of the electric double layer is shown in Figure 2.3. If the zeta potential in absolute number is greater than 30 mV, the particle is considered stable and insusceptible to coagulation [84].

Bacteria, viruses and protozoa carry, just like colloids, normally a negative charge and therefore similar behavior is expected [116]. In Fe-EC, the dosing of positively charged ions (Fe^{2+}) by corrosion of the iron anode, destabilizes the double layer around microorganisms and decreases the zeta potential or neutralizes the surface charge, enabling coagulation into bigger flocs. Via sedimentation or flotation those flocs, and so the colloids, are removed. Also during sedimentation/flotation particles can adsorb to the flocs, which is called sweep coagulation [51, 87]. Most bacteria are negatively charged, because of the predominance of anionic functional groups present within the cell wall, therefore the positively charged iron ions are a perfect base for particle deposition [116]. Another important factor associated with iron floc formation and removal of microorganisms is direct electrostatic or specific bonding of these functional groups and iron [23, 41]. For bacteria, four types of functional groups can be specified, namely amine-, carboxyl-, hydroxyl-, and phosphate groups [8, 88]. Adorption experiments of amino acids on goethite [90] and phenol on iron oxides [79] showed that amine- and hydroxyl groups do not have a strong affinity for iron particles, and therefore are not expected to interact greatly with iron hydroxides formed during EC. On the other hand, carboxyl- and phosphate groups have been shown to have a high affinity for iron precipitates. Especially the phosphate groups have been signaled to have

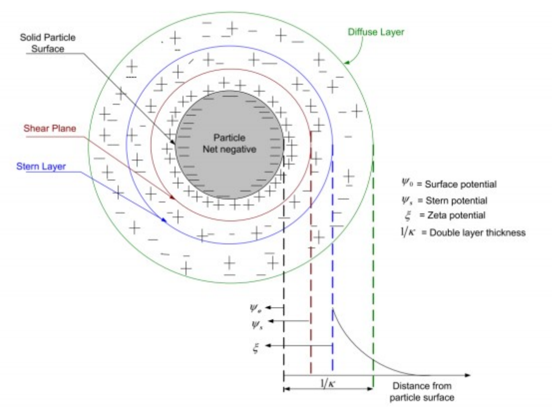


Figure 2.3: Demonstration of the electric double layer theory and zeta potential measuring point [85]

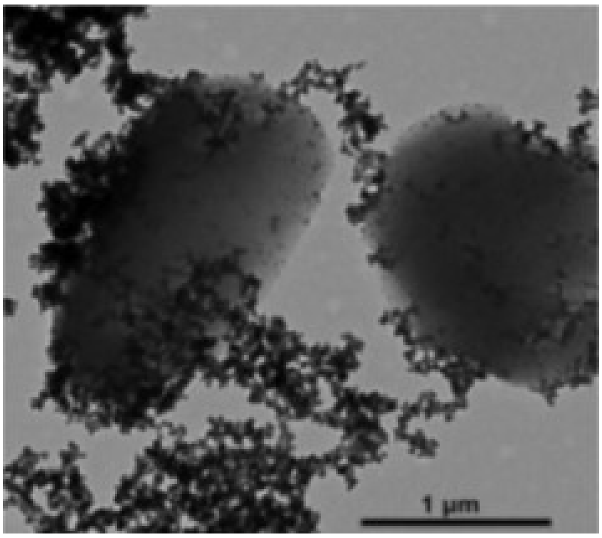


Figure 2.4: Transmission electron microscopy image illustrating the intimate association of EC precipitates and bacteria surfaces, with precipitates bridging two *E. coli* cells.[22]

great influence on the bonding of bacteria to iron hyroxides species [94, 95]. Research with ATR-FTIR spectrocopy on *Cryptosporidium parvum* has shown that carboxyl groups mediate in the adhesion to iron species [32]. Evidence is found that initially the bonding is caused by adhesion of the phosphate group to the iron species, and the strength of the bond is increased with reaction time by adhesion of carboxyl groups [28]. The relative small iron species that are formed during EC, bind to specific groups and finally by bridging connect bacteria in bigger flocs all together, as shown in Figure 2.4.

The exact contribution of the functional groups, charge neutralization and sweep flocculation are still unknown and might differ per type of microorganism. A schematic overview of the resulting system is shown in Figure 2.5.

The total physical removal can be described by the destabilization of primarily held stable substances in the water. Similar electrical charges keep them in solution. By addition of opposite charged ions (i) the electric double layer is compressed, (ii) ions are adsorbed to the surface and neutralize the charge, (iii) further adsorption and bridging between adsorbed particles, (iv) during precipitation a sludge blanket is formed, entrapping particles during settling (sweep flocculation) [35].

2.2.2. Inactivation - Oxidation by reactive oxygen species

Additionally to physical removal in flocs, indirect oxidation in EC has been getting more attention lately. The effect of reactive species in the abatement of microorganisms has been studied, and results show different outcomes on effectiveness. Therefore, the contribution of the process is generally quite un-

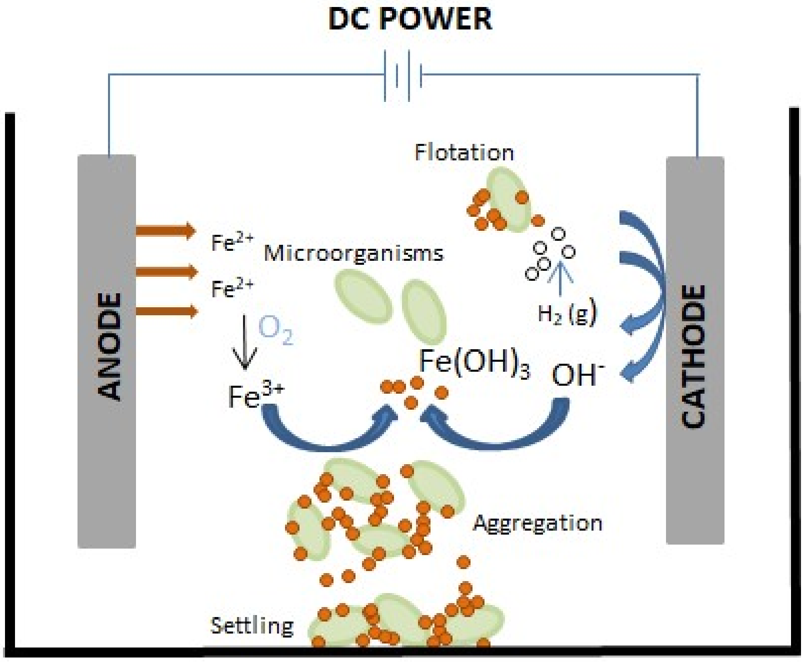


Figure 2.5: Illustration of the removal of microorganisms by formation of iron hydroxides and settling or flotation

known. The rate of inactivation by indirect oxidation is expected to be dependent on conditions, of which water composition is mentioned most often. Reactive species are produced step wise. The first step is the formation of the oxygen radical. Oxygen, responsible for the oxidation of Fe^{2+} into Fe^{3+}, gains one electron from the iron. This unleashes a whole chain of reactions and the production of different reactive species. In several steps, finally the hydroxyl radical is produced [109]. The reactions that occur in this process are expressed in the formulas below:

$$O_2 + Fe^{2+} \rightarrow {}^*O_2^- + Fe^{3+}$$

$${}^*O_2^- + Fe^{2+} + 2H^+ \rightarrow H_2O_2 + Fe^{3+}$$

$$H_2O_2 + Fe^{2+} \rightarrow {}^*OH + OH^- + Fe^{3+}$$

A different pathway is the production of a ferryl ion (Fe(iv)), which is an unstable intermediate, from the oxidation of Fe^{2+} and H_2O_2 [9, 72].

$$H_2O_2 + Fe^{2+} \rightarrow 2OH^- + Fe^{4+}$$

The ferryl iron then either can oxidize contaminants directly or via the production of reactive oxygen species [46]. The exact type of species produced is still debated. Figure 2.6 shows a graphical overview of the oxidation steps into radical production.

The impact of reactive species on microorganisms were assessed in the past. The oxidation by reactive oxygen species have shown to cause good ion permeability, interruption of the respiratory pathway and membrane rupture demobilizing the cell membrane [24, 122]. For bacteria cell death due to reactive oxygen species produced during EC is mainly coupled to either stable pores formation resulting destabilization of cell membranes or loss of essential cellular constituents. Virus reduction via reactive species was mainly ascribed to capsid damage, as was found for MS2 inactivation via ferrous iron. Genetic damage was not detected [59].

The hydroxyl radical has a low CT value and is an approximately 10^5 times more effective disinfectant than chlorine or ozone. So already in low concentrations the radical can inactivate all kinds of microorganisms to a great extent [15, 52].

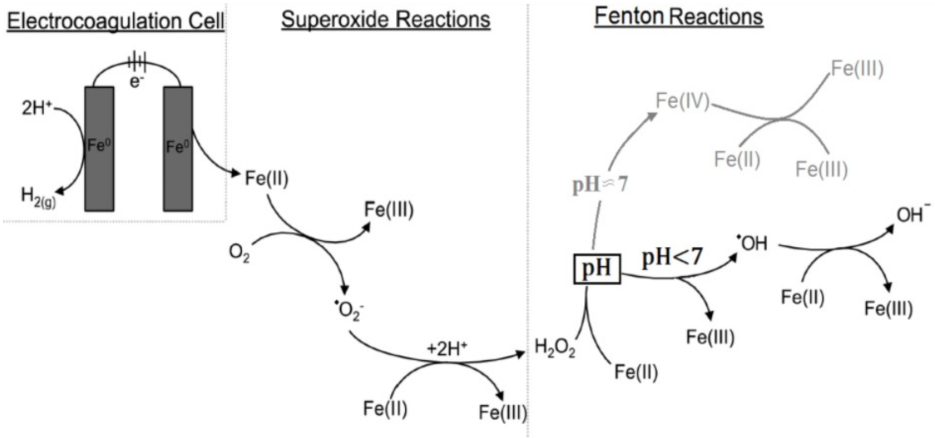


Figure 2.6: Illustration of the oxidation process of iron and the formation of reactive oxygen species [117]

2.2.3. Further suggested disinfecting mechanisms

Next to the two mechanisms described above, physical removal in flocs and inactivation by reactive species, several other mechanism have been described in literature. Although these mechanism have not been found by many other researchers.

In stead off indirect oxidation via production of reactive oxygen species after iron oxidation by oxygen, it is mentioned that direct oxidation at the anode can take place [80]. A study by Maher et al, found that direct anodic oxidation was the main driver for reduction in levels of estrogenic compounds. It was found that by switching both electrodes from anode to cathode, higher removal levels were achieved, indicating the effect of direct oxidation at the electrode surfaces [78]. Similar mechanism were described by Boudjema et al, who observed irreversible permeabilization of cell membranes after applying the electric field [10].

Furthermore, it was mentioned that the reduction of colloidal materials in the water after co-precipitation with iron flocs, create osmotic pressure for bacteria, cysts, and viruses, rupturing cell walls [112], but no evidence for this specific theory was deliverd.

Studies comparing iron coagulation with $FeSO_4$ and Fe-EC showed that no difference was seen on the reduction of *E. coli* in both systems, supporting the evidence of the lack in contribution of the electric field in the removal of microorganisms. Therefore, the two main mechanisms of attenuation are physical removal by co-precipitation with iron flocs and inactivation by reactive species formed during oxidation of $Fe^{2}+$ into $Fe^{3}+$.

2.3. Water composition

The composition of secondary municipal wastewater effluent is highly variable. The concentrations of components is mainly dependent on the influent quality and treatment method. Over the world, many different treatment methods are applied. In wealthy countries, wastewater is treated to high standards, but on average wastewater effluent quality is not strictly regulated. Especially, the reduction of phosphate and organic matter are highly influenced by treatment method. In this study, the main secondary wastewater effluent indicators are phosphates, silicates, calcium and magnesium, nitrates, carbonates and natural organic matter (NOM). It chosen to focus solely on dissolved components in secondary wastewater effluent. All of these have the potential to intervene in the redox reactions, complexation, acid/base and adsorption reactions that will occur.

When electrolysis starts, these particles in the water start interacting with the iron. Besides, pH may cause interactions between the particles in the water. Average concentrations have been determined on base of different secondary municipal wastewater effluent studies and shown in Table 2.1 [1–4, 69].

Table 2.1: Average composition of secondary municipal wastewater effluent

Component	Concentration (mg/L)
Phosphate	0.1 -5.0
Silicate	5.0 - 10.0
Calcium	50.0 - 150.0
Magnesium	5.0 - 70.0
Nitrate	5.0 - 25.0
Alkalinity (HCO_3)	40.0 - 80.0
Natural organic matter	1.0 - 25.0

2.4. Interactions

2.4.1. Phosphate and silicate

Phosphate

According to studies performed to measure the removal of phosphate by electrocoaculation, the interaction between phosphate and iron is strong [50, 65, 120]. The interactions and formation of species is dependent on certain conditions, of which pH and dissolved oxygen concentration are the main factors. But in every situation the removal of phosphate is generally higher than 95%. Phosphate can be removed in different insoluble forms. Phosphate-iron species can be formed directly in most cases two forms: $FePO_4$ (s) and $Fe_3(PO_4)_2$ (s). $FePO_4$ is the direct precipitation form of Fe^{3+} and a single phosphate, where $Fe_3(PO_4)_2$ consist of Fe^{2+} and forms the base for more hydrated crystalline forms as vivianite. The formation reactions of these are shown below:

$$3Fe^{2+} + 2PO_4^{3-} \rightarrow Fe_3(PO_4)_2\ (s)$$

$$Fe^{3+} + PO_4^{3-} \rightarrow FePO_4\ (s)$$

In the treatment of secondary municipal effluent, the appearance of $Fe_3(PO_4)_2$ is not expected. Normally, the pH will be above 7 and the some dissolved oxygen will be present. In that case, the iron will be oxidized quickly and Fe^{3+} species will be dominant. The formation of $FePO_4$ is pH dependent. As shown in Figure 2.7, the solubility of $Fe(OH)_3$ is lower in most ranges of pH. Especially, at higher pH ranges formation of $Fe(OH)_3$ is favored. When pH is low, below 6, insoluble $FePO_4$ can be formed and direct precipitation of phosphate can be seen. The optimum range for formation and removal of phosphate via $FePO_4$ is around pH 4.5 -5.5. So when pH is higher, mainly iron hydroxides will be formed, but on their surface phosphate adsorption can take place. At pH above 6, adsorption of phosphate onto the surface of iron precipitates is expected to be the main removal system [50, 65].

When phosphate is adsorbed onto the iron hydroxides surfaces, the crystallization process is hindered [5, 118]. Studies in the past have showed that crystallization of goethite was not completed in phosphate rich waters [6, 56]. In stead shorter range species are formed, of which in the presence of phosphate are mainly hydrous ferric oxides [99, 102].

Similar to the high affinity of phosphate functional groups on bacterial walls, phosphate ions are attracted to the iron surfaces in high rates [95]. Phosphate ratios in iron species can get as high as 0.7 P/Fe at low dosages of iron. At higher dosage the ratio is decreased, but this explains the high removal rates of phosphate. Phosphate is favored above other ions in the adsorption to iron. After most of the phosphate is removed other ions can be adsorbed [123]. Because of the high affinity to iron of phosphate ion, they are able to compete well with microorganisms for surface sites [126]. Comparison of synthetic groundwater containing phosphate and phosphate free water, showed a decrease of 1.6 log reduction difference for *E. coli* removal [23]. Furthermore, no interaction of phosphate with the production and quenching of ROSs is expected [48, 72]. Therefore, the main interacting mechanism of phosphate will be competition for surface sites. Although it is not expected to have influence on the production and quenching of ROSs, an effect on inactivation is still not completely ruled out. Due to occupation of the surface of iron and the change in interaction between iron and microorganisms, inactivation efficiency might change.

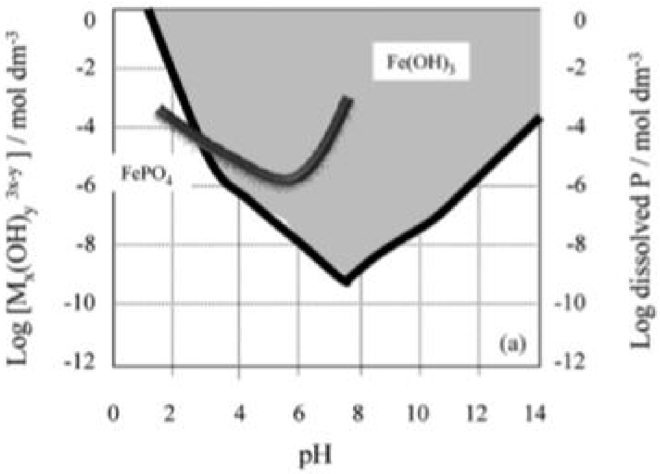


Figure 2.7: Solubility diagram for iron phosphate species and iron hydroxide as function of pH and concentration [65]

Silicate

The behavior of silicates is similar to phosphate, except no separate silica-iron species are formed. The effect of silicate on the ironhydroxide formation is just like phosphate a decrease of the crystallization ability [98]. The structure of the precipitate formed in silicate-rich, but phosphate free, solutions can be described as ferrihydrate-type of precipitates, covered with silicate [26]. The affinity of silicate to iron is lower compared to phosphate. In extensive studies performed in phosphate and silicate containing waters, phosphate showed P/Fe ratios of 0.55 and near-complete removal, whereas Silicate only was partially removed with the excess iron at a ratio of 0.24 Si/Fe. Even in solutions were silicate was dosed in higher concentrations compared to phosphate, phosphate showed higher ratios [123]. From this is can be concluded that the affinity of silicate is lower compared to the phosphate. Even though the affinity of silicate is lower, normally waters contain higher concentrations of silicate compared to phosphate, therefore making silicate of greater influence on the final species formed.

Also silicate is not expected to intervene with reactive species and limit the inactivation by indirect oxidation [48, 72]. But whereas phosphate has been shown to decrease the bacterial attenuation, for silicate this has not been reported. On the contrary, experiments showed that silicate did not have any influence on the removal of bacteria, even considering the changed iron species [23]. Taking into account that bacteria cell walls are densely packed with phosphate functional groups, and the affinity for phosphate to iron is higher, silicate is assumed not to be able to compete with bacteria for iron surface sites.

2.4.2. Cations: Calcium & Magnesium

Calcium and magnesium both are multivalent cations. Therefore, no interaction can take place between Ca/Mg and positively charged iron precipitates. Previous work has shown that the uptake of Ca/Mg in iron precipitates is negligible at neutral pH [57, 106]. Calcium and magnesium, on the other hand, are able to interact with bacteria surface sites. Both are able to bond directly to the functional groups on the cell wall, and by doing so complex the bacteria surface. Carboxyl and especially phosphate functional groups have been determined to be responsible in the strong bonding between iron and bacteria. Both functional groups are able to interact with calcium and magnesium. Binding between calcium and cell walls has been shown in previous studies [55, 96]. The binding of calcium and magnesium onto the cell walls, inhibits the potential reaction between bacteria and iron. Because calcium and magnesium are already present in the water before iron is dosed, bacteria cell walls are packed with Ca/Mg before being able to react with the iron. Evaluation of the zeta-potential of the bacterial surface indeed showed Ca/Mg to cause an increase, proving the strong interaction and showing a decrease in *E. coli* removal in waters containing Ca/Mg, with the inhibitory effect being larger for Mg [23]. No effect on reactive species is expected from bivalent ions, so the whole decrease in attenuation is ascribed to the complexation of bacterial surfaces.

In absence of oxyanions (phosphate and silicate) no interaction has been observed between the iron precipitates and Ca/Mg. In waters containing both phosphate and Ca/Mg, an increase in calcium and magnesium uptake was observed [102], in which the bridging between iron and calcium/magnesium is regulated by phosphate (Fe-P-Ca and Fe-P-Mg bonds). This effect has not been observed in the presence of silicate [119]. The addition of Ca/Mg also enhanced the coagulation process, resulting in an increase in particle size [73]. The effect of this bridging on bacterial removal was seen, countering part of the decrease in log removal by the addition of Ca/Mg [22].

2.4.3. Nitrates

Nitrate removal with the application of electrocoagulation has been studied. In this process two distinctive mechanisms are determined. The nitrate can be removed by accompanying the $Fe(OH)_3$ in precipitation or by direct reduction of nitrate at the cathode into N_2 gas [43, 64]. Generally, a high iron dosage is required to have sufficient nitrate removal. The affinity of nitrate for iron precipitates is therefore assumed to be low. And it is believed that nitrates don't play a big role in the EC process.

2.4.4. Carbonates

Carbonate/bicarbonate is one of the major water species and known to interact with many substances in the water. Past studies indicated improved removal of wastewater pollutants in the vicinity of CO_3^{2-}, showing increased removal of COD and nitrates [43]. The presence of CO_3^{2-}/HCO_3^{-} is mentioned to change the phase, size and surface charge of iron precipitates, enabling further adsorption and floc growth [22]. Besides, direct formation of siderite, $FeCO_3$, is observed [11] and found to be the dominant species. Research has shown that $FeCO_3$ has effect on the iron oxidation rate. Slower oxidation is observed when $FeCO_3$ is present [62]. On the other hand, when concentrations of HCO_3^{-} are below 1 mM, no significant effect is observed. Iron oxidation experiments with low concentration of CO_3 have shown that no effect is found and oxidation follows the formula presented previously [104, 109]. Although carbonate has been shown to improve the removal of pollutants like COD and nitrate, studies assessing the abatement of *E. coli* showed different results. The role of HCO_3^{-} in the decreased attenuation was not ascribed to the physical removal. No effect was observed, and also no change in zeta-potential was seen, indicating that HCO_3^{-} did not associate with the iron precipitates. Also zeta-potential of *E. coli* was unchanged, indicating no adsorption of HCO_3^{-} onto the surface [23]. The 1.2 log unit difference was ascribed to a decrease in inactivation, which is consistent with the observation of lower membrane permeabilization. Bicarbonate was found to be a scavenger for OH radical in aqueous solutions, inhibiting radical-type chain reactions [108] and quenching Fe(IV) and the OH radical. Besides, CO_3^{2-} radical is formed instead off OH radical, which is much shorter lived and therefore less effective [23].

2.4.5. Natural organic matter

Natural organic matter (NOM) is a collective name for a mixture of organic molecules. NOM mostly consist of humic acids (HA) followed by fulvic acids (FA). Where fulvic acids are soluble at all pH, humic acids precipitate at low pH. Humic substances originate from dead plant tissues, dead organisms, and excrement of living organisms [77]. NOM, especially humic substances, are responsible for the brownish-yellowish color of natural waters. Humic substances are heterogeneous and their composition and size vary immensely. Mixtures of humic compounds generally contain several functional groups, described in Figure 2.8 [115].

Of the main wastewater components, the effect of natural organic matter is least understood. In recent studies, a wide variety of mechanisms have been described to play a role in iron (electro)coagulation. The presence of organics was found not to affect the total iron production during electrolysis. Also pH during experiments was seen to be unaffected by organic matter [70]. On the other hand, an effect on the oxidation of iron is observed. The oxidation of iron was found to be slower in waters which contain substantial amounts of humic substances, including municipal wastewaters. Iron would be oxidized followed by the (partial) reduction again through organic matter. In this way, iron can form complexes with organic material and become effectively resistant for oxidation. Other suggest that the oxidation is not completely blocked, but only slowed down [19, 103, 114].

The attenuation of microorganisms in the presence of NOM is seen to be different. The influence on both inactivation and physical removal has been previously shown, but the results are conflicting for different studies. Sorption of organics can block the interaction between microorganisms and the iron precipitates. NOM can react with either microorganism surface as iron surface, as shown by sorption

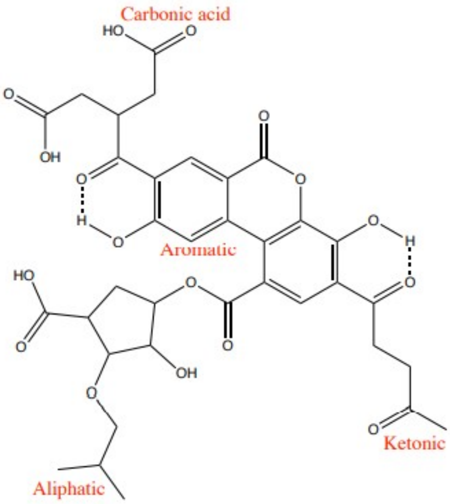


Figure 2.8: Typical functional moieties in humic substances [115]

experiments. The suggested mechanism for interactions are hydrophobic interactions for sorption of organics on bacteria, and electrostatic and ligand exchange for organics on iron species [53]. Hydroxyl and carboxylic functional groups are found to be the main facilitators of binding of NOM on iron species [29]. By sorption of organics, both iron and bacteria were complexed [76]. By adsorption of NOM on the surface, the electrostatic repulsion is increased and interactions between microorganisms and iron are diminished, causing the removal to decrease.

Also the effect of NOM on reactive species was mentioned, either the positive or negative. Relative low concentrations of organic carbon were mentioned as a facilitator of the formation of reactive species [13, 30, 100]. Although most research conclude that the presence of NOM had negative influence on the formation of reactive species. NOM is especially effective at scavanging hydroxyl radicals [47, 125]. Concentrations of 30 mg/L fulvic or humic substances decreased the formation of hydroxyl radical by 11% and 27% respectively [76]. This predicts that NOM can have dramatic influence on either the formation and effectiveness of reactive species. Less radicals will be formed and once formed species are scavenged, so only a small fraction of possible reactive species will be effective. The effect of this scavenging was also shown in previous works. [45, 59, 75].

2.5. Potential interacting mechanisms

All together, the influence of the water matrix can occur due to a wide variety of reasons. Wastewater components may intervene in several ways and combinations of wastewater components could work counter-effective again. A summary of the potential mechanisms is presented below.

- **Total iron dissolved** - The total dissolution of iron into the water is found to be dependent on the type of electrolyte and pH of the water. Spontaneous release and competition by other components in the water could cause the total amount of iron to be different from the theoretical value predicted by Faraday's law.

- **Iron oxidation rate** - Iron oxidation rate is strongly dependent on the pH of the water and oxygen concentration. Carbonate and NOM have been mentioned to influence the oxidation rate of iron during electrocoagulation. A lower oxidation rate is found to be more effective for the attenuation of microorganisms.

- **Competition for iron surface** - Microorganisms are attached to the surface of iron precipitates and are removed by settling. At the same time, wastewater components as phosphate, silicate, carbonate, and NOM compete for surface sites.

- **Complexation of microbial surface** - The bacterial surface functional groups have high affinity for iron. Calcium and NOM can adsorb to the surface groups and therefore inhibit interactions between the functional groups and iron species.

- **Inhibition of reactive oxygen species formation** - By complexation the formation of reactive species is inhibited. A decrease in formation of more than 20% was seen in the presence of NOM.
- **Scavenging of reactive oxygen species** - The effectiveness of reactive oxygen species on microorganisms is impacted by wastewater components. Reactive species can be scavenged before coming into contact with microorganisms.

The overview above shows the impact of wastewater components that have been mentioned in literature. Conflicting suggestions have been presented in literature. This research is designed to investigate these components, both individually and in combination, to unravel the mechanisms taking place in treatment of municipal wastewater effluent with electrocoagulation.

3

Methodology

3.1. Laboratory setup

Electrocoagulation experiments were performed in 1000 mL cylindrical glass beakers. Electrodes, anode and cathode, used in the reactor were made out of S235 steel plates (maximum percentages: 0.14% carbon, 0.10% silicium, 0.80% manganese, 0.025% phosphorus, 0.015% sulphur, 0.010% nitrogen, 0.20% copper, and 0.080% aluminum). Only a single anode and cathode were used with dimensions of the submerged steel plates being 4cm x 4 cm, totaling an active anode surface of 32 cm^2. A small strip of iron on top of the iron square was used to connect the iron plates by use of crocodile clip cables to the power source, as shown in Figure 3.1. The power source used was a 30V-3A TENMA 72-10500 bench DC power supply. Electrodes were being polished with coarse and fine sand paper before the start of each run. The electrodes were placed in parallel into the middle of the reactor with an inter-electrode distance of 1 cm, which is a often applied distance for EC experiments [78, 86].

The beaker was placed on a LABNICO L23 magentic stirrer and a PTFE coated bar was used to mix. To create a well-mixed solution and prevent local fluctuations in pH and dissolved oxygen content, the mixing was set to the highest possible stable rotation speed. Throughout the experiment oxygen was supplied through a pump with to ends. Oxygen outlets were placed on one side of the iron plates each to distribute oxygen most evenly. Three meters, pH, dissolved oxygen, and conductivity, were placed in the beaker and continuously monitored. The meters were placed in line along the wall of the beaker perpendicular to the electrodes. This location was chosen, because influences of the anode and cathode were least.

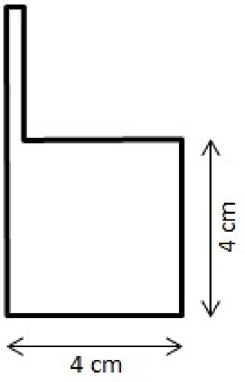


Figure 3.1: Dimensions and shape of iron electrodes used. Only the 4 x 4 square was submerged during EC

3.2. Chemicals

Different types of wastewaters were prepared to test the influence of components. As to measure the effect of a single component, a blank measurement was performed for comparison. The blank was prepared by adding NaCl to demineralized water. NaCl was solely added for conductivity. Without the addition of any ions and therefore low conductivity, power required to have a circuit is high. As a single-valent cation, sodium is not expected to interact in the iron oxidation, production and scavenging of reactive oxygen species, or in the floc formation of iron hydroxides. Chloride on the other hand is mentioned as accelerator of inactivation. Electrochemically-generated chlorine species are seen as a possible contributor to microbial attenuation [36], but experiments conducted with electrochemically dosing iron and dosing $FeSO_4$ have shown that chlorine species do not lead to additional removal of microbials [22]. Also within this study, no hypochloride was measured in any of the experiments. Furthermore, no effect is expected from chloride on either iron oxidation or floc formation.

As wastewater components oxy-anions, multivalent cations, carbonate and natural organic matter (NOM) are seen as the main factors in interactions with Fe-EC. Phosphate is selected as a representative for oxyanions. Phosphate is chosen over silicate, although silicate has greater influence on the final precipitate structure [119], because Si had no detectable effect on microbial attenuation [23]. The higher affinity of phosphate for iron and the theoretical possibility of governing bridges with other particles, favors the phosphate in the experimental procedure. Phosphate is added as K_2HPO_4. For multivalent cations, calcium is chosen as representative. It is argued that the effect of magnesium is stronger, but calcium is present in higher concentrations in secondary wastewater effluent, normally. Calcium is added as $CaCl_2$. Carbonate is added as $NaCO_3$.

The type of NOM (humic substances) added is retrieved from Evides groundwater. The NOM is described in this paper [12]. The NOM can be characterized as humic acids. Normally NOM mainly consist of humic and fulvic acids. Humic acids were chosen, because the effect of humic substances on the production of hydroxyl radical was found to be bigger compared to fulvic acids [76].

In all experiments that don't contain calcium, NaCl was added to increase conductivity. Standard concentrations of the components are added and are shown in Table 3.1.

Table 3.1: Standard amount of wastewater components added in experiments

Component	Concentration (mg/L)
Phosphate	4.0
Calcium	60.0
Alkalinity (HCO_3^-)	60.0
Natural organic matter	10.0

3.3. Microbial indicators

As indicator organisms two types are chosen. Both the effect on bacteria and virus are examined. For bacteria the most common type of indicator is *E. coli*. In this case *E.coli WR1* was selected, because it is commonly used for research and quality control of drinking water. For virus, bacteriophage ϕX174 was used. Bacteriophage ϕX174 represented the mitigation of the mammalian viruses best in a comparison study between ϕX174, P22, and MS2 [45]. Both indicators were prepared in high concentrations and at the start of the experiment the waters were spiked. Starting concentrations of *E. coli WR1* were maintained around 1 x 10^6 cfu/mL and for bacteriophage ϕX174 starting concentration was between 1 x 10^5 and 5 x 10 ^{5} pfu/mL.

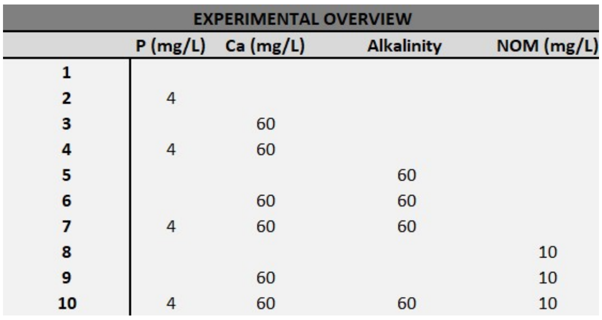
3.4. Experimental overview

First some preliminary experiments were performed to determine the further experimental conditions. In the first part the iron dissolution and oxidation for different types of electrolytes were considered. The total iron dissolution should be confirmed to be constant over all experiments to make a comparison. Also the iron oxidation was mentioned to influence the abatement of microorganisms, and should be confirmed to be similar for all cases. Besides, several different mixing procedures were tested to find one with full settling.

After preliminary tests, three types of experiments were performed to assess the removal mechanisms, the influence of the oxidation rate, and the impact of wastewater components added to the electrolyte, respectively. First, tests were performed to assess the contribution of the individual removal mechanisms. Experiments were performed with and without the addition of TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl). TEMPOL is a stable piperidine nitroxide of low molecular weight and known as a scavenger for the superoxide radical, $^{*}O_2^-$ [20, 66]. Inhibition of reactive oxygen species should single-out physical removal as the only removal mechanism. These experiments were performed with the blank and the synthetic water with only calcium added to see if results are similar for different types of waters.

Also the pH dependence of microbial attenuation was tested. By varying the pH, the iron oxidation rate was controlled and assessed for differences in log attenuation. Experiments with the blank at different constant pH levels were performed. Additional oxidation time was added to these experiments if after electrolysis not all iron was oxidized. During this extra time, power was switched of and no iron was added into solution, but oxygen was supplied to oxidize all residual Fe^{2+}.

Next, the influence of wastewater components specifically were tested. Both by individually adding the components, but as well by adding combinations of components to see the combined effect. The first part was about testing the settling capacity of the water. Phosphate, calcium, carbonate and NOM were added in separate experiments and tested under mixing conditions 1 and 4 explained above. Afterwards, the effect of wastewater components on the attenuation of microorganisms were tested. An overview of the experimental conditions is shown in Figure 3.2.



EXPERIMENTAL OVERVIEW				
	P (mg/L)	Ca (mg/L)	Alkalinity	NOM (mg/L)
1				
2	4			
3		60		
4	4	60		
5			60	
6		60	60	
7	4	60	60	
8				10
9		60		10
10	4	60	60	10

Figure 3.2: Overview of electrolytes tested for removal of *E. coli* and ϕX174

3.5. Operational Fe-EC parameters

Electrolysis operational parameters were similar for nearly all experiments. Fe-EC experiments were conducted at a constant current of 200mA (charge dosage rate of 12 C/L/min), corresponding to a current density of 6.25 mA cm^{-2}. The current was applied for 15 minutes, dosing a total charge dosage of 180 C/L. Based on previous work by Bicudo et al. [7], it was assumed that above total charge dosage of 180 C/L pathogen removal did not increase significantly. The conductivity of the electrolyte equaled about 500 μS/cm and using a voltage of about 22 Volts. In all experiments dissolved oxygen concentration was kept above saturation, to not have the partial oxygen pressure as a limiting factor for the iron oxidation rate. Besides, by applying oxygen at saturation, full oxidation is achieved more easily, and residual Fe^{2+} is undesirable for the quality of the supernatant afterwards. pH was controlled at 7.5 by adding drops of either HCl or NaOH, except for experiments in which the effect of pH was tested.

3.6. Preliminary experiments

Preliminary studies were performed to check whether the iron dissolution and oxidation conditions were similar for all types of waters, as well as mixing conditions were tested for optimal removal of iron.

3.6.1. Total iron production

It was assumed beforehand, the dissolution of iron is in the form of Fe^{2+}. As depicted in graph 3.3 the dissolution of iron during electrolysis follows the expected amount according to Faraday theory when iron is dissolved as Fe^{2+}. The total amount of iron produced ranged from 52 to 53.2 mg/L, which corresponds with a Faraday efficiency of 99.8% to 102.1% for all experiments performed at pH 7.5 with the blank water sample.

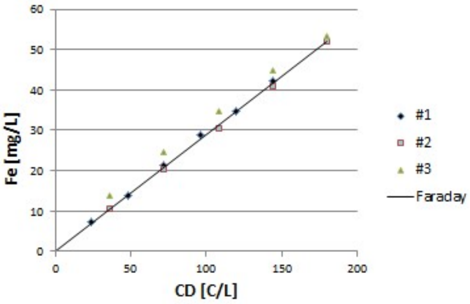


Figure 3.3: Iron production during electrolysis as function of charge dosage applied, in comparison with theoretical value based on Faraday

Besides the total iron, also the Fe^{2+} was measured. Fe^{2+} concentrations were fairly low for all experiments and all sampling moments. The concentration climbed somewhat towards the end of the experiment. At pH 7.5 and saturated conditions for dissolved oxygen, most iron was oxidized rapidly. The production of Fe^{3+} is also shown in Figure 3.4. This production is calculated based on the amount of total iron produced minus the amount of Fe^{2+}. Because the total iron production and the oxidation of iron are stable throughout the experiment, a constant production of Fe^{3+} can be observed.

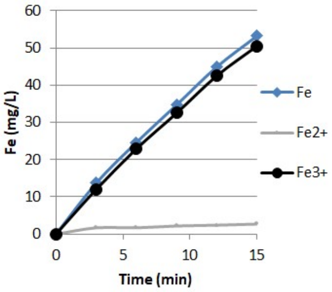


Figure 3.4: Measurement of total iron and Fe^{2+}, and the calculated value of Fe^{3+} production

After 15 minutes, when the power was switched off and the electrolysis phase was ended, the residual Fe^{2+} fraction was below 3.0 mg/L. The oxygen was generally applied a couple of minutes extra to oxidize all iron before settling. After two hours of settling, the residual concentration of Fe^{2+} was below detection limit of 0.2 mg/L, indicating full oxidation of iron.

Furthermore, the total iron dissolved during electrolysis was not found to be dependent on the type of electrolyte, as shown in Table 3.2. As mentioned in literature, the type of electrolyte could have influence on iron dissolution. In this study it was found that the Faraday efficiency for all types of water was between 95% and 105 % on average, indicating relatively small deviations from the Faraday theory.

For the individual addition of wastewater components no effect on the iron oxidation rate could be seen (A up to and including D in Figure A.1 in Appendix A). Iron oxidation was similar to the blank and so Fe^{2+} concentrations were low. Experiments were performed at pH 7.5, and according to Stumm and Lee (1970) oxidation was almost instant. Also at the end of the electrolysis phase, the residual Fe^{2+} concentration was negligible, and thus near fully oxidized into Fe^{3+}.

When calcium and carbonate were dosed in combination, a somewhat different pattern could be observed. At the start of the experiment, the Fe^{2+} concentration is higher compared to the blank (E and F in Figure A.1 in Appendix A). A delay in oxidation could be observed at the start. Meanwhile, during the dosing the concentration started to drop and at the end of the electrolysis phase Fe^{2+} concentrations were similar to other experiments. So, after the electrolysis phase, nearly all iron was oxidized. These findings underline that the effect of the type of electrolyte is not important for iron conditions and can be regarded as constant over all experiments.

Table 3.2: Amount of iron dissolved during electrolysis phase compared to the Faraday theoretical value

Type of water	Iron dissolution mg/L	Faraday efficiency
Blank	52.0 - 53.2	99.8 - 102.1%
P	49.9 - 50.1	95.7 - 95.8%
Ca	50.6 - 51.4	97.1 - 98.7 %
P / Ca	48.7 - 52.6	93.6 - 101.9%
CO3	52.0 - 54.2	99.8 - 104.0%
Ca / CO3	53.7 - 54.8	103.1 - 105.2%
P / Ca / CO3	51.9 - 53.6	99.6 - 102.9%
NOM	51.1 - 54.6	98.1- 104.8%
NOM / Ca	49.1 - 54.6	94.2 - 104.8%
NOM / P / Ca / CO3	50.9 - 51.8	97.7 - 99.4%

3.6.2. Mixing conditions

Different mixing conditions after electrolysis were tried to optimize settling and remove all iron from the water. With the blank sample four mixing conditions were tested;

1. **MIX1** mixing with magnetic stirrer during electrolysis, no enhancement and left to settle immediately after
2. **MIX2** mixing during electrolysis and 10 minutes of stirring with magnetic stirrer after power was switched of, then left to settle
3. **MIX3** mixing with magnetic stirrer during electrolysis followed by 5 minutes of stirring in jar test at 50 rpm
4. **MIX4** mixing with magnetic stirrer during electrolysis followed by 10 minutes of stirring in jar test at 100 rpm and 10 minutes of stirring at 50 rpm

After the 15 minutes of electroylysis and mixing time, the samples were left to stand for 2 hours of settling. As can been seen in Figure 4.1, only mixing with a magnetic stirrer and no further enhancement lead to minor settling. It was observed that iron flocs were small and mostly stayed in suspension. The residual iron concentration was about 45 mg/L, so just over 10% of iron had either settled or floated. When after electrolysis the magnetic stirrer was used for ten more minutes, the flocs had more time to grow and therefore settling conditions improved, giving a residual iron concentration of only 4.1 mg/L. The methods in which the beakers were placed in the jar test mixer, showed highest settling capacity for the iron. When only mixed for 5 minutes in the jar test mixer, it showed comparable effect to tests that had enhanced mixing with the magnetic stirrer. The residual iron in this case was 3.3 mg/L. Only the last method showed close to full removal of iron. Less than 1.0 mg/L of iron was left after settling, which was below detection limit of the kits. The water in this case looked clear, no turbidity could be observed. MIX4 was therefore chosen for all of the experiments.

3.7. Sampling and analyzing

Over the time of the experiment different samples are taken for analysis. Figure 3.5 gives an overview of all sampling events. pH, dissolved oxygen concentration and conductivity are continuously measured over the length of the iron dosing phase. The dissolved oxygen concentration was monitored all along, because saturation is desired for the length of the experiment. pH fluctuations, as described, were monitored and controlled to stay constant at the desired level. pH was also measured after 2 hours of settling. Both before the start of the experiment and after 2 hours of settling a 10 mL sample was obtained, to determine wastewater component concentration, phosphate, calcium, and NOM, and to measure microorganism concentrations. For microbials also a sample was taken immediately after electrolysis, to differentiate between removal during iron dosage and removal after settling. For wastewater components no additional analysis was done immediately after electrolysis phase, because no settling had taken place at that time and therefore no removal of components. Iron was measured at regular intervals of 3 minutes during iron dosage phase. Both Fe^{2+} and total iron were measured at every time interval and every sample was 1 mL. No measurement was needed before the power was switched on, because no iron was present at that moment. After 2 hours of settling, the residual iron concentration was measured as well.

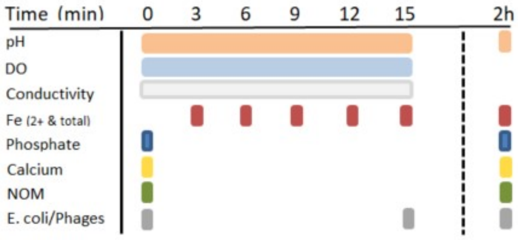


Figure 3.5: Overview of sampling times and measurements

Continuous ph, DO, and conductivity probes were used, that were placed in the water as described in laboratory set-up. Iron, phosphate, and calcium were measured with test kits. Total iron (Fe^{2+}, Fe^{3+}) and Fe^{2+} were measured using Spectroquant® Iron Cell Test (1-50mgFe/L), read in the Spectroquant® NOVA60 (Merck, Germany) photometer. Spectroquant® Calcium Cell Test (10-250mgCa/L) was measured using in the Spectroquant® NOVA60 (Merck, Germany) photometer as well. The concentrations of natural organic matter were determined by UV absorption method [105, 124]. 5mL samples were centrifuged for four minutes at 3000 rpm and 1 mL of supernatant acidified with 20 μL 0.1 M HCl were prepared. Then the UV absorption was measured. Wavelength of λ = 254 nm was applied, which is common for the determination of humic acids [124]. UV absorption values were recalculated into concentrations by use of a calibration curve, that was constructed beforehand as shown in Figure B.1 in Appendix B. To account for residual iron precipitates in the water, blank experiments were sampled in similar way and blank absorbance was compensated for in constructing the calibration curve.

3.8. Microbiological assays

E. coli samples were taken before the start of the experiment, after electrolysis, and after settling. Depending on the estimated concentration, a number of dilutions with PBS were created to get a final count of 30 to 300 colony-forming units (cfu). Spread plate technique was applied. 0.1 mL of sample was inoculated on chromocult agar plates and spread using sterile spreaders. If concentrations were below 30 colonies per 0.1 mL, filtration technique was used to increase the count. Samples were vacuum filtered using 0.45 micron filters. The filter then was placed on the chromocult agar. Samples were incubated for 18 - 22 hours at 37 °C.

ϕX174 assays were conducted according to ISO 10705-2 somatic coliphage assay. Concentrations were estimated and samples diluted to get in the range of 10 - 100 plaque-forming units (pfu) per mL. The sample is, together with a semi-solid nutrient medium and culture of host strain (*E. coli WG5*), plated on modified Scholten's agar plates. After 18 - 22 hours incubation at 37 °C, visible plaques are counted.

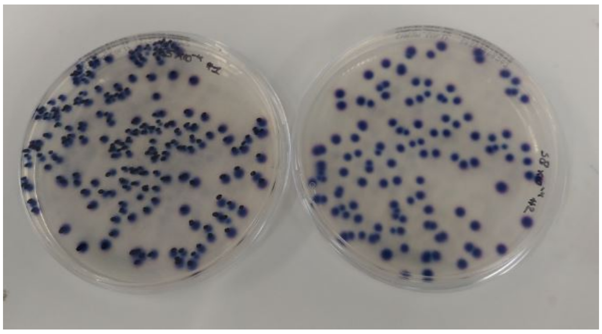


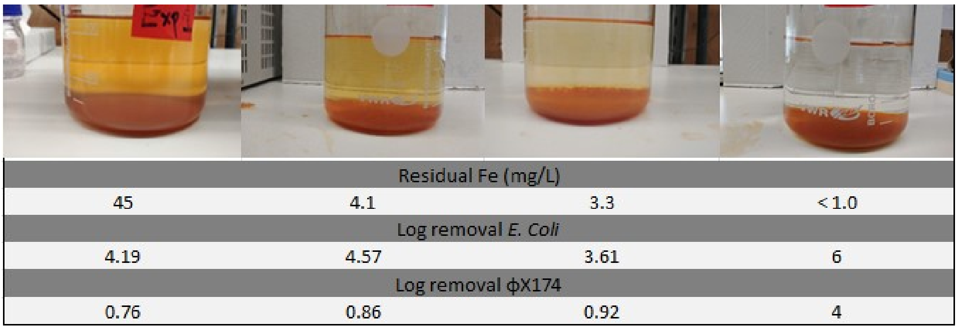
Figure 3.6: Example of plating of *E. coli WR1* and colony count

4

Results

4.1. Settling

Preliminary study on the mixing conditions showed besides improved iron settling, also an influence on the disinfection capacity of iron electrocoagulation. *E. coli* and ϕX174 removal results demonstrated difference in the removal rates depending on the settling efficiency. For *E. coli* the log removal equals 4 log units in case of minor settling as in MIX1. This removal stayed constant over the improved settling conditions in MIX2 and MIX3 in which iron is not fully settled. In MIX4, the log removal was increased and equals about 6 log units. For somatic coliphage ϕX174 the results were different. The log removal values for MIX1, 2, and 3 were below 1 log unit. So only low removal values were observed in the case where the iron settling was not near complete. In MIX4, with fully settled iron flocs, the removal of ϕX174 increased to 4 log units.



Residual Fe (mg/L)			
45	4.1	3.3	< 1.0
Log removal *E. Coli*			
4.19	4.57	3.61	6
Log removal ϕX174			
0.76	0.86	0.92	4

Figure 4.1: Settling after 2 hours for different mixing conditions. Depicting the residual iron concentration and total removal after settling

Also the influence of wastewater components on settling was tested. If samples were left to stand immediately after the dosing phase, without enhanced mixing, both samples containing phosphate and natural organic matter were not able to settle completely. Only a minor fraction settled in the case of phosphate and for NOM no settling was observed at all. For calcium, even without enhanced mixing the flocs were able to settle and low residual iron was measured after 2 hours (below 1.0 mg/L). For phosphate the effect of extensive mixing was clear and positive. All iron settled to the bottom and < 1.0 mg/L residual iron concentration was found. Calcium containing water was fully settled as well, but a difference could be seen in the speed of settling. In the case of additional mixing, the flocs settled much faster and only after 15 minutes the water was settled fully, as could be seen by the naked eye. In case of NOM, even though applying extended mixing, the water was hard to settle. A limited decrease in iron concentration of 60% was observed and as can be seen in Figure 4.2, only a small sludge layer formed on the bottom of the beaker. For all waters containing combinations of wastewater components, settling was near complete and residual iron concentrations were below 1 mg/L.

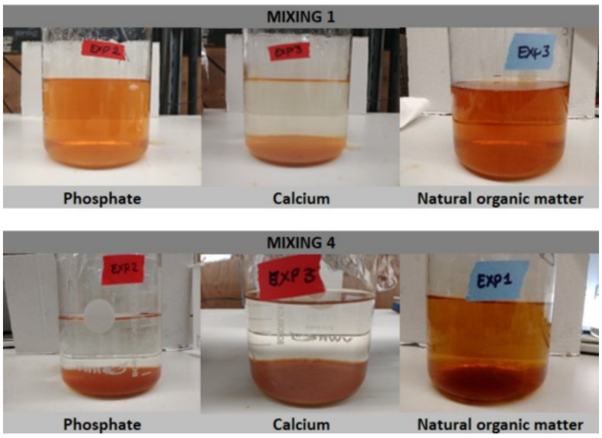


Figure 4.2: Settling at different mixing conditions for individually added wastewater components. Top row shows settling for MIX1, and the bottom row for MIX4

4.2. TEMPOL experiments

As has been shown in the figures in the Methodology, the production of iron follows the Faraday theory in all cases close to 100%. Iron was released as Fe^{2+}, and further oxidized in Fe^{3+}. Based on concentrations of Fe^{2+} below 3 mg/L observed during the experiments, it is concluded that iron is quickly oxidized during electrolysis. During the electrolysis phase the concentration of Fe^{2+} increased slightly from 1.5 mg/L after 3 minutes to about 2.5 mg/L at the end, but it can be seen that a constant oxidation took place, which means a steady production of Fe^{3+}. Linked to the formation of Fe^{3+} is the production of reactive species. The constant production of Fe^{3+} indicates a constant production of reactive species.

Presumed is the formation of hydroxyl radical and ferryl ion (Fe(IV)) via superoxide anion radical and hydrogen peroxide, but the formation of other reactive species is not excluded. Since oxidation at pH 7.5 is quick and only almost no residual Fe^{2+} was found after electrolysis time, the production of reactive species is not expected after current was switched off.

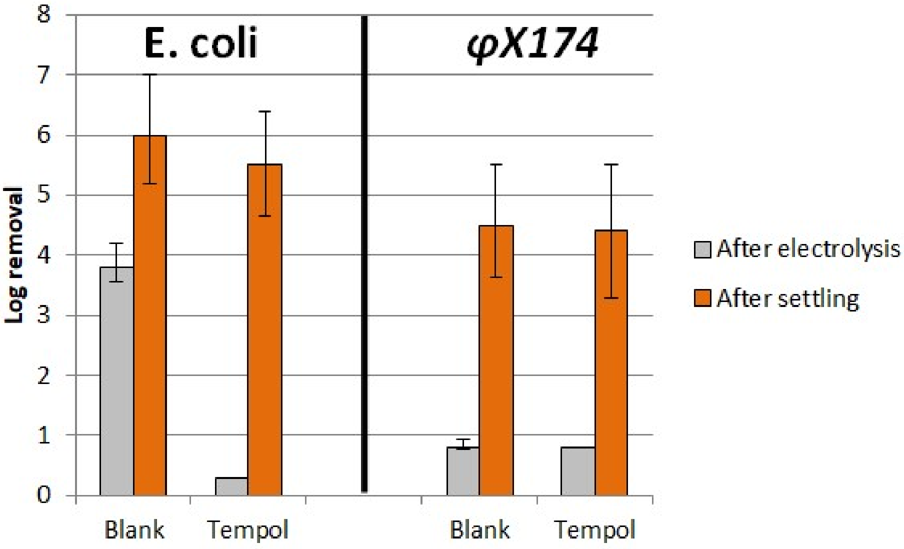


Figure 4.3: Resuls of *E. coli* and ϕX174 removal in experiments with and without the addition of Tempol. Experiments performed with charge dosage of 180 C/L

Results for *E.coli* without the application of TEMPOL showed a removal of 3.8 (±0.4) log units immediately after 15 minutes of electrolysis. After 2 hours of settling, this value increased with an additional 2.2 log units, to a total removal of 6.0 (±0.9) log units. This means that the major part of removal is already achieved in the first 15 minutes of the experiment. In the situation where TEMPOL was added, the log removal during electrolysis was diminished to only 0.3 log units. The total log removal on the other hand, stayed similar to the situation without TEMPOL added, and equals 5.5 (±0.8) log units. In this situation, most *E. coli* was removed during the 2 hours of settling.

A different pattern could be seen for somatic coliphage, ϕX174. Results of both experiments, with and without TEMPOL, were similar and resulted in a total removal of 4.0 (±1.0) log units. In TEMPOL-free experiments, the removal during electrolysis is determined to be only 0.8 log units, indicating that the removal during electrolysis only plays a minor role in the total removal of somatic coliphage. Whereas for *E. coli* the removal during the first phase was hampered by the addition of TEMPOL, in the case of ϕX174 no change could be observed, resulting in a log removal of 0.8 log units.

4.3. pH experiments

pH experiments were performed at three different pH levels. In literature the influence of pH on the dissolution of iron in EC was described, indicating a higher dissolution of iron at lower pH values. Low pH values would favor the spontaneous corrosion of iron, corrosion without applying current. In the experiments no difference was found on the total iron generation during EC, as shown in Figure 4.4.

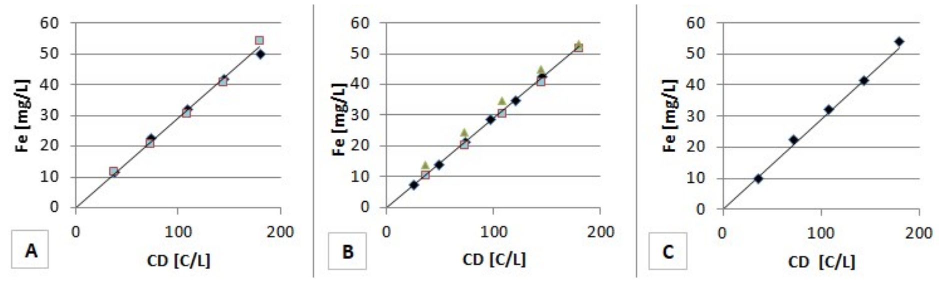


Figure 4.4: Total iron production for different levels of pH in comparison to Faraday theoretical value (a) pH 5.5, (b) pH 7.5, (c) pH 8.5

Although the total iron production is not dependent on pH, the oxidation rate of iron was affected by pH. According to research by Stumm and Lee (1970) the oxidation of iron is positively correlated to pH. As can be seen in Figure 4.5, the oxidation rate was indeed lower at lower pH. As was the case for pH 7.5, it can be seen that the oxidation rate at pH 8.5 was almost immediate. $Fe^{2}+$ concentrations for pH 8.5 were lower compared to pH 7.5, but in both cases the concentrations were below 3 mg/L. At pH 5.5 a difference can be observed. $Fe^{2}+$ concentrations are much higher and so the production of $Fe^{3}+$ was lower. At the end of the experiment only 31 (±3.5) mg/L of iron was oxidized in contrast to over 50 mg/L for pH 7.5 and 8.5. A residual $Fe^{2}+$ concentration of 20 mg/L was observed, indicating potential for further oxidation. As was observed, the oxidation of iron at pH 5.5 continued until the end of the electrolysis period. After switching of the power, the oxidation of iron was stopped as well.

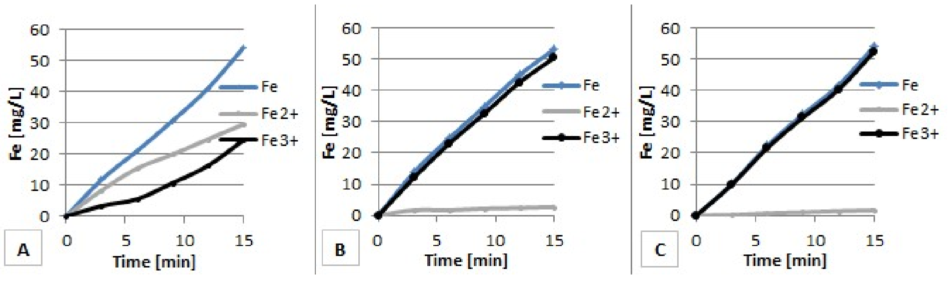


Figure 4.5: Production of $Fe^{3}+$ at different pH conditions (a) pH 5.5, (b) pH 7.5, (c) pH 8.5

Total removal of both *E. coli* and ϕX174 was not affected, even though at pH 5.5 not all iron is oxidized into $Fe^{3}+$ and residual $Fe^{2}+$ concentration of 22 mg/L was measured after the experiment. Furthermore, especially for *E. coli* the removal during electrolysis is much higher for lower pH values. Whereas at pH 7.5 a log removal of 4 was observed, at pH 5.5 this was 5 log units and for pH 8.5 only 1.4 log units. For ϕX174 the trend was less clear. Although the removal during electrolysis was 1.5 log unit for pH 5.5 and only 0.5 log unit for pH 8.5, most of the removal still was observed after the settling phase.

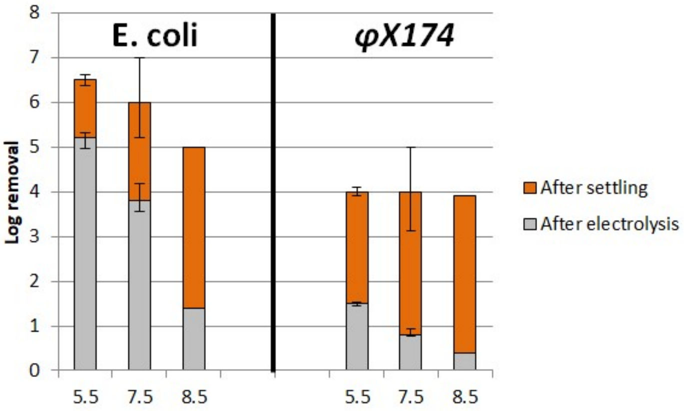


Figure 4.6: Resuls of *E. coli* and ϕX174 removal in experiments with different constant pH

4.4. Calcium

Measurement of the initial and final concentration of calcium indicated that no interaction was observed between iron flocs and the calcium as expected, since iron and calcium both are multivalent cations. On average 2% of calcium was removed from the solution. Waters containing only calcium were generally easy to settle, even without enhanced mixing.

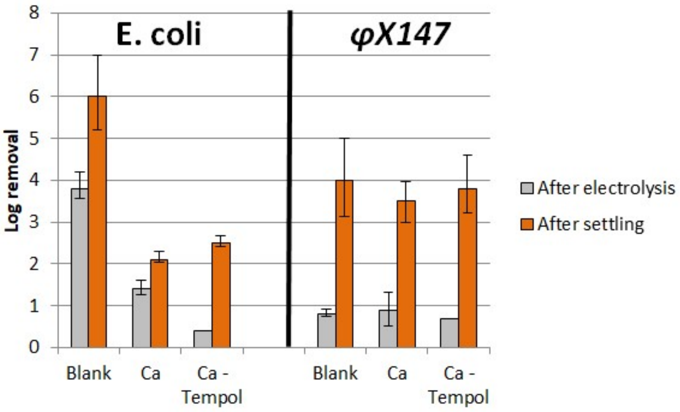


Figure 4.7: Resuls of *E. coli* and ϕX174 removal in experiments with and without the addition of Tempol

E. coli - The addition of calcium showed a significant decrease in the disinfection capacity. Results are shown in Figure 4.7. The removal during electrolysis decreased and became about 1.4 (±0.2) log units. Especially, the total removal was hampered and only became 2.1 (±0.2). For the situation containing TEMPOL, the removal during electrolysis for *E. coli* was decreased after addition of TEMPOL. The attenuation dropped from 1.4 log units to below 0.5 log unit. The total mitigation of *E. coli* was not strongly affected comparing both situations, although having much lower values compared to the blank.

ϕX174 - For calcium, the disinfection during electrolysis was slightly lower compared to the blank, although not found to be significant. An average of 3.2 log units removal was observed, which is on the low boundry of the removal in the blank. For ϕX174, similar to the blank, no differences could be observed due to TEMPOL. The disinfection after electrolysis is still similar and removal after settling is even slightly improved, but falls within the margin of error compared to the situation without TEMPOL.

4.4.1. Phosphate

Measurements of the initial and final concentration of phosphate, respectively before the experiment and after settling, showed high removal rates for phosphate. On average, the removal of phosphate was 98%, which was near complete. The influence of phosphate could also be observed on the floc formation. Without proper and enhanced mixing conditions, the iron flocs were not able to settle. Only when mixing was performed for 10 minutes after the electrolysis in the jar test configuration, the water was able to settle. The simultaneous addition of phosphate and calcium was tested as well. The measurement of initial and final phosphate and calcium concentration showed a somewhat different results compared to addition of both components separately. Phosphate removal was 95% on average, which is still near complete, and the amount of calcium removed increased in comparison with calcium only, which gave a removal of 6% of the initial concentration. The settling capacity of the flocs formed in addition of phosphate and calcium together was higher and flocs appeared bigger and could best be described as cloudy.

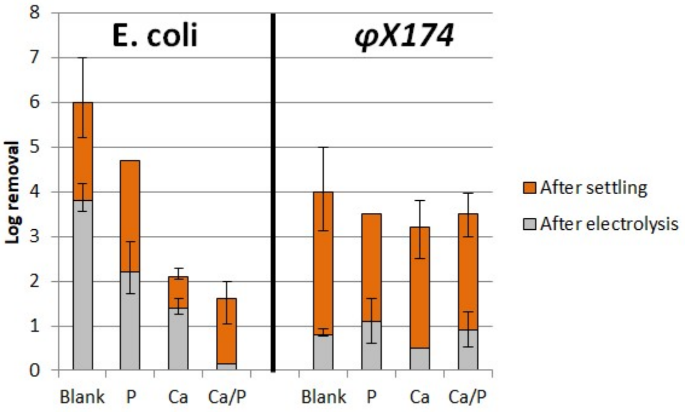


Figure 4.8: Results of *E. coli* and ϕX174 removal

E. coli - Addition of phosphate had influence on the removal of *E. coli*, as depicted in Figure 4.8. During electrolysis, removal values decreased from 3.8 to 2 (±0.6) log units compared with the blank electrolyte. The total removal after adding phosphate also decreased. It went down to 4.7 log units, compared with 6 log units for the blank. The effect of both calcium and phosphate together was even stronger on *E. coli* removal compared to the addition of both wastewater components separately. The removal during electrolysis went down to close to zero log units. Total removal in this case was 1.6 (±0.5) log units.

ϕ**X174** - The effect as described for *E. coli* was not seen for phages. Both removal during electrolysis and total removal results were similar to the blank experiments. For total removal the results also felt within the error margin of the blank, and equaled 3.5 log units. No effect was observed when phosphate and calcium were added separately and the same result was achieved when both components are combined. Behavior, both during the electrolysis phase and the settling phase, was similar to the results of the blank.

4.5. Carbonate

The settling capacity was not affected by the addition of CO_3^-. The residual iron concentration was below detection limit of 1.0 mg/L Fe after settling, with and without enhanced mixing.

E. coli - When carbonate was added to the initial water the removal during electrolysis was affected greatly, but the total removal was not hampered. Removal during electrolysis decreased to 1.3 (±0.8) log units, whereas the total removal was 5.2 (±0.4) log units. In comparison to the blank, the total removal was not significantly different. When carbonate was added in combination with calcium, the results became less effective in comparison with carbonate only. The removal during electrolysis was decreased even more and total removal was decreased from 5.2 to 3.8 (±0.1) log units. Although the removal has been shown to be less effective compared to the carbonate only situation, an improvement was observed compared to the water with calcium only. The removal during electrolysis was still generally lower, but total removal improved substantially from calcium only.

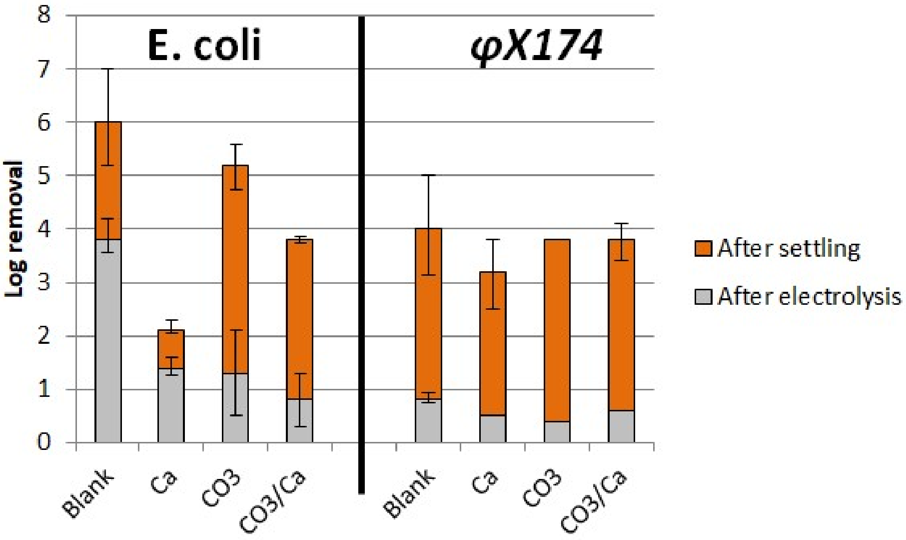


Figure 4.9: Results of *E. coli* and ϕX174 removal

ϕX174 - Where carbonate had a strong inhibiting influence on the removal of *E. coli* during the dosing phase, no strong effect was observed for phages. Removal during the electrolysis phase for phages was already low. Total attenuation of phages in the presence of carbonate was not affected, similar to *E. coli*. No effect was observed when calcium and carbonate were added separately and the same result is achieved when both components are combined. Behavior during the electrolysis phase and the settling phase are close to the results of the blank.

4.6. Natural organic matter

The method applied to settle all waters, was not able to fully settle the suspension when NOM was added individually. A residual iron concentration of 13 mg/L was observed. From visual observations, it could be seen that the flocs formed were dense and relatively small. No cloudy flocs were seen and the water had a strong orange color after settling. In contrast to the water only containing NOM, the water containing calcium and phosphate as well was able to settle. The residual iron concentration was below 1.0 mg/L Fe. Calcium was removed in higher concentration compared to the calcium alone situation, namely 10 % (up from 3%) and 70% of NOM was removed after settling. Phosphate removal, just as in previous cases, was high with an average removal of 97%. Based on observations of the flocs during and after settling, the flocs seemed to be smaller and darker compared to other waters without the addition of NOM.

E. coli - A strong effect of natural organic matter (NOM) on the removal of *E. coli* was observed. Both the removal during electrolysis as well as the total removal was significantly decreased by the addition of NOM. The removal during electrolysis was only 1.4 (±0.1) log units, in contrast to a 4 log unit removal in the blank. The total removal by addition of NOM was 2.2 log units. In the case calcium was added to the water with NOM, the removal slightly improved. Although the removal during electrolysis became negligible, the total removal was 2.9 (±0.2) log units. Test performed by adding all of phosphate, calcium, carbonate and natural organic matter, represented the real wastewater effluent best. As can be seen in Figure 4.10 the removal of *E. coli* was improved from the situation with only calcium or only natural organic matter. In this case, the removal during electrolysis was equal to 0.8 (±0.3) log units and total removal was 3.5 (±0.4) log units.

ϕX174 - While phosphate, calcium, and carbonate were found to have negligible effect on the mitigation of phages in EC, the effect of NOM was strong. The deleterious effect could be seen already during the electrolysis phase, where almost no removal was observed (0.2 log units). Besides, as shown before, the settling capacity of water containing NOM was low and no additional removal was observed after 2 hours of settling. Whereas the water only containing NOM was hard to settle, the water with calcium and NOM was able to settle. In this case the influence of NOM could be better observed when comparing it with the situation of only calcium. The introduction of NOM decreased

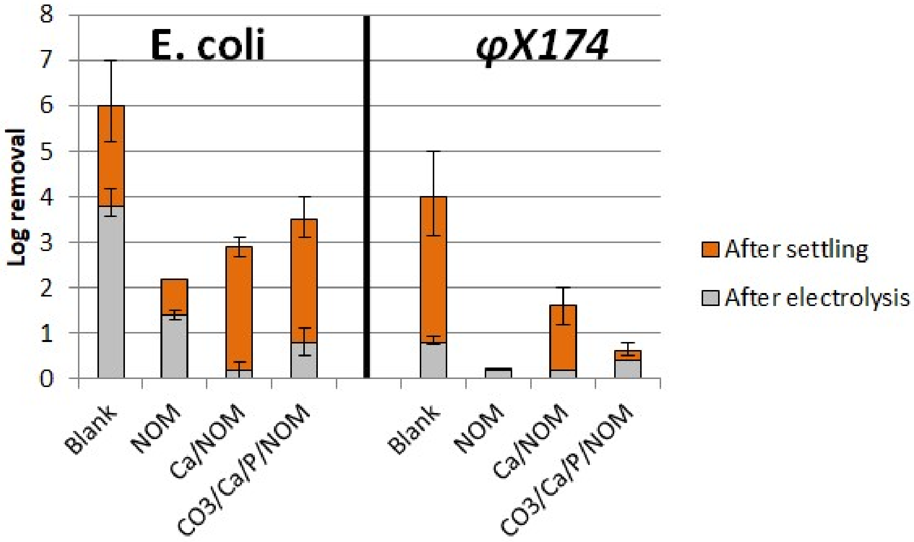


Figure 4.10: Resuls of *E. coli* and ϕX174 removal

average removal of phages from 3.2 log units with only calcium to 1.6 (±0.4) log units. The addition of all wastewater components resulted in a poor result for phage removal. During electrolysis only a removal of 0.4 log units was observed. Total attenuation of phage resulted in 0.7 (±0.2) log units.

5

Discussion

5.1. Disinfection mechanisms

5.1.1. Mechanisms *E. coli*

Experiments performed under different mixing conditions showed different settle ability of the iron flocs. The settle ability of the iron precipitates are related to the treatment of bacteria and phages, although in varying degree. For *E. coli* even in the case with hardly any settling, log removal units of above 4 were observed. With improvement of the mixing conditions, causing bigger flocs to be created, the removal of *E. coli* was increased to above 6 log units. It therefore is concluded that for *E. coli* apart from physical removal, a distinctive removal mechanism is contributing. Furthermore, the improved mixing conditions showed, that physical removal is an important factor in *E. coli* attenuation as well.

Results obtained from the experiments with TEMPOL, scavenger of superoxide radical, showed that the removal during the electrolysis phase was inhibited. The fact that *E. coli* removal was inhibited in the phase where, as previously showed, ROSs were formed proves that *E. coli* is affected by ROSs, as observed before by different studies [16, 49, 68]. Since no settling had taken place during electrolysis, the about 4 log units removal is fully ascribed to inactivation by ROSs.

The fact that similar total removal was observed in the presence of TEMPOL, indicates that both inactivation by ROSs and physical removal are not independent systems. Both mechanisms can not be added up together, to get the total removal. Since the total removal is similar without inactivation, it is assumed that physical removal will always remove similar amount of *E. coli*, independent whether these are active or inactive. In that way, inactivation can be seen as a first line of treatment, with physical removal as back-up mechanism.

Similar experiments, but with the addition of calcium, showed lower attenuation for both situations with and without adding TEMPOL. Both inactivation and total removal were hampered. Again, the inactivation was completely diminished by addition of TEMPOL, showing the effect ROS. Already without adding TEMPOL, the inactivation part decreased from about 4 log units to only 1.5 log units, compared to the situation without calcium. Calcium itself is not expected to scavenge reactive species [48, 98]. So this drop in efficiency can only be described by the lesser interaction between iron and *E. coli*. Calcium is known to complex bacteria surface and hinder interaction because of electrostatic repulsion with iron. Therefore, it is suggested that *E. coli* can only be inactivated when in close association with iron. This assumption is based on the high chemical reactivity of hydroxyl radicals, which therefore have a very short life time in aqueous solutions [49] and oxidants produced in the bulk are not effective at inactivating *E. coli* [17, 61, 63]. Furthermore is it suggested that reactive species are effective when iron is oxidized within the bacterial cell. Transmission electron microscopy (TEM) images showed dark spots along the inside of the cell wall and inside cytoplasm, providing evidence that iron had entered the cell before being oxidized [68]. Total removal is not affected by addition of TEMPOL, compared to the situation without, showing that the contribution of inactivation can be compensated by physical removal.

So for *E. coli* it is proposed that both inactivation and physical removal are possible removal mechanisms. Part of the *E. coli* is strongly bonded to iron and is sensitive for inactivation by reactive species. These inactivated *E. coli* are removed by settling as well. Further disinfection can be observed with enhanced mixing conditions, indicating a weaker form of bonding, and additional removal by sweep flocculation. Sweep flocculation is the process in which iron flocs are growing under mixing conditions and aggregate into bigger flocs. Mixing conditions are an important parameter in the growth of these flocs. Rapid mixing promotes the formation of sweep flocs, while under slow mixing conditions these sweep floc continue to agglomerate. Because sweep flocs are weak and vulnerable for breaking, a rapid mixing step for quick formation and a slow mixing step for growth should be combined for optimal result [37]. When forming sweep flocs, microorganisms are entrapped in the structures and swept out from the solution, promoting attenuation [111].

5.1.2. Mechanisms phages

For phage ϕX174, the results gave a different result. In case of hardly any settling, only small abatement of phage was observed. Even further improvement of the settling conditions didn't immediately show much higher removal values. Relatively small concentrations of residual iron, less than 10%, showed that removal was still below 1 log unit, namely 0.86 and 0.92 for 4.1 mg/L and 3.3 mg/L, respectively. Only when residual iron concentration was below 1 mg/L, removal increased to 4 log units. The low removal results in case without proper settling were endorsed by findings in the experiments with TEMPOL. In both situations, removal during electrolysis was 0.8 log units. Attenuation by ROSs of phages was much lower compared to *E. coli*, which might be explained by the smaller size of phages, thicker capsid structure or less interaction between surfaces of iron and phages. Also the thicker capsid structure on bacteriophage compared to bacteria could make phages more resistant for the effectivity of ROSs [45]. Furthermore, there was no effect seen of the addition of TEMPOL, indicating no effect on phages of reactive species. Previous studies, on the other hand, have shown inactivation by reactive species for different bacteriophages [45, 46, 59, 60]. Similar to findings in this study, previous researchers have concluded that addition of radical scavengers had no impact on the inactivation [59]. As mentioned before, close contact between iron and microorganisms is expected to be required for effective inactivation [89]. Reactive oxygen species produced in the bulk are not affecting microorganisms. Therefore, it is suggested that TEMPOL does not intervene in the association between phages and iron and does not inhibit inactivation. For *E. coli*, TEMPOL is able to scavenge ROSs, suggesting influence on the interaction between iron and bacteria surface.

Overall, results show that there is a strong dependence of phage removal on settling characteristics. Also poor attenuation of virus in combination with moderate settling reveals the weak direct interaction between virus and iron. Although iron particles are positively charged and phage ϕX174 negatively no strong bonding occurs. This might be explained by the charge of the capsid structure of ϕX174. Positively charged nobs on the capsid might repulse ϕX174, although strong attractive forces are expected by the net charge [81]. With enhanced mixing conditions, and so bigger flocs, phage removal is improved, probably because of the sludge blanket that is formed in which virus are trapped (sweep flocculation). Furthermore, inactivation could play a role in phage removal, although at pH 7.5 only a minor contribution of inactivation is observed, most likely explained by the weak interaction between phages and iron.

5.2. pH

According to iron oxidation theory, pH is a major factor in the oxidation rate. Both at pH 7.5 and 8.5, iron oxidation was near immediate and complete during electrolysis phase. At pH 5.5, the oxidation rate should be very limited. Although in the EC experiments at pH 5.5 even though oxidation rate was much lower compared to pH 7.5, iron was still oxidized at moderate rate, in contrast to expectations. Oxidation was only observed during the electrolysis phase. After switching off the power supply, even in the presence of dissolved oxygen at saturation, no further oxidation took place. The reason only oxidation was observed during electrolysis was ascribed to the fact that different pH zones can be formed. Especially around the cathode, where OH^- is formed, pH was higher. Mixing breaks these barriers around these zones, but still differences in pH might be present in the mixture [44].

At lower pH, it was demonstrated that inactivation is more effective. Especially for *E. coli* the efficiency is significantly higher, as has been found in literature as well [22, 46]. For ϕX174 the inactivation is slightly increased, but still physical removal is the major contributor. As previously mentioned, reactive species produced in the bulk are not effective for inactivating microorganisms. Close contact between the iron and microorganisms during oxidation is necessary for effective treatment. The decrease in oxidation rate offers more time for iron to associate with microorganism surface and therefore treatment becomes more efficient. Furthermore, it is mentioned that (1) the production of reactive species is dependent on pH, favoring the lower range [76], and (2) different speciation of reactive radicals might influence the effectiveness. At pH below neutral, mainly OH radical is formed, and at higher pH the formation of Fe(IV) species is suggested [58], although the activity of Fe(IV) is still debated [92]. Generally, the quicker oxidation rate, and so the shorter contact time, is assumed to be the main reason for lower inactivation at higher pH. This could also best explain the difference between removal at pH 7.5 and 8.5. Impact on total removal was not seen.

At pH 5.5 not all dissolved iron is oxidized. A significant concentration of Fe^{2+} was found at the end of the experiment. After settling the water seemed clear, and all color was removed, but still high levels of iron were found. Residual Fe^{2+} does not aggregate and precipitate. High residual iron concentrations deteriorates water quality for further reuse. Therefore, it is important to have all iron oxidized and be settled. Besides, experiments showed that total removal is not strongly affected by pH and would even favor low pH. Less Fe^{3+} is produced and so less iron can settle. From this, it can be concluded that a lower dose of iron would be sufficient as well for similar removal. As has been concluded before, good mixing conditions that promotes floc growth is most important.

5.3. Interactions *E. coli*

As described above, *E. coli* and ϕX174 are dependent on different removal mechanisms. Also the effect of wastewater components therefore is different. In this part, first the effect of wastewater components on *E. coli* is explained. In the next section, the effect on phage ϕX174 is described. Figure 5.1 gives an overview of both removal by inactivation and total removal for all tested wastewater types.

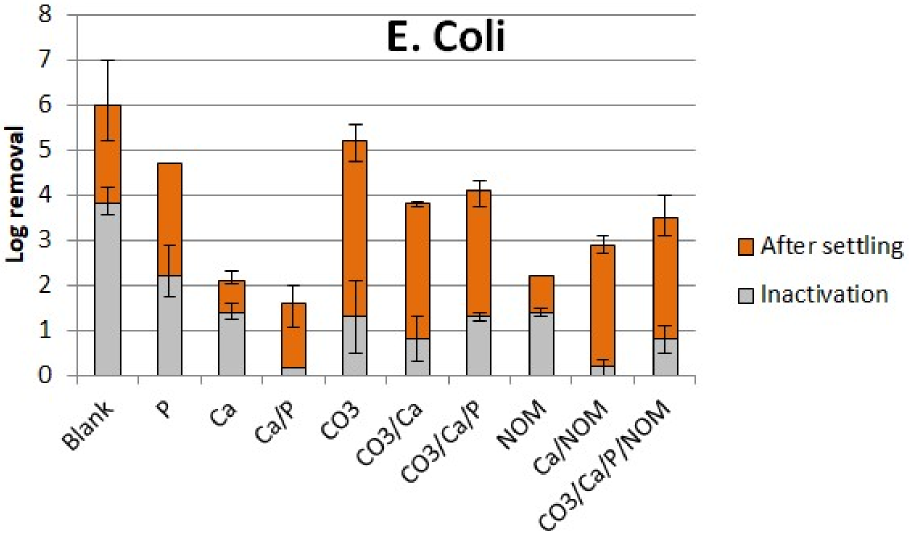


Figure 5.1: Resuls of *E. coli* and ϕX174 removal in experiments with different constant pH

5.3.1. Phosphate

The high affinity of phosphate for iron is known, and also in this study high removal of phosphate from the electrolyte could be seen, indicating strong bonding between iron and phosphate. Based on PHREEQC modeling, potential of precipitation of strengite ($FePO_4$) was determined to be low under experimental conditions, see Appendix C. The uptake of phosphate impacted the particle formation, and iron flocs became harder to settle. Smaller and more compact particles were seen in comparison with the blank electrolyte. Mainly short-ranged hydrous ferrous oxides are expected to be formed [102]. The effect of phosphate uptake was also seen in the removal of *E. coli*. Both the inactivation during electrolysis and total removal were decreased. The drop on physical removal in the presence of phosphate has been found before and is ascribed to the competitive adsorption between free phosphate ions and phosphate functional groups on the surface of bacteria cell walls [23]. In comparison to previous studies the drop in attenuation is rather small, only about 1 log decrease, and this could be explained by the relatively low concentration of phosphate present. Besides, as has been found at experiments at pH 5.5, in which not all iron was oxidized into Fe^{3+}, a lower dose of iron was just as effective in removal of *E. coli* in the blank water. So not all of the iron was needed for the full removal and competition between free phosphate ions and phosphate functional groups did not immediately impact removal. The effect on inactivation by reactive oxygen species was not according to expectation. No effect on inactivation was expected based on previous research [48, 98]. Although phosphate is also mentioned as either promoter [107] as well as inhibitor [108, 121] of radical formation. As mentioned previously, the decrease in inactivation is not expected from the phosphate ion either as promoter or inhibitor, but by the decreased in interaction between the iron and bacterial surface. Part of the iron is covered with phosphate, increasing repulsive forces. Close contact is partly avoided and less of the reactive species are effective. Due to good mixing conditions, bigger flocs can be formed and physical removal partly takes over inactivation.

5.3.2. Calcium

As expected no calcium uptake was seen in the iron precipitates, found by the low removal of calcium (only 2%). Repulsive forces between both positively charged calcium and iron are a clear explanation for this. On the other hand, calcium has affinity for both phosphate and carboxyl functional groups on the bacterial cell wall. Calcium then is known as complex agent for bacterial cell walls. By association of calcium onto the surface, the bacterial surface is becoming more positively charged, repelling iron from close interaction. In this was, calcium was found to inhibit *E. coli* removal significantly. Also inactivation is blocked. Similar to phosphate, calcium is not expected to affect reactive species by scavenging [48]. Therefore, similar mechanism as described for phosphate is expected. The close contact between iron and bacteria is blocked by sorption of calcium onto the bacterial surface making reactive species less effective and die off in the bulk.

Whereas with the addition of phosphate, formed flocs where seen to be smaller and harder to settle, in combination with calcium this effect was not observed. Also the low uptake of calcium, when added solely, was increased in combination with phosphate. PHREEQC modeling in this case excluded the precipitation of hydroxyapatite. Both factors together indicate bonding between calcium and phosphate. The calcium removal increased from 2% to 6% on average. Previous research has also shown increased uptake of phosphate and calcium when added simultaneously, and was suggested to be caused by electrostatic interactions and direct bonding of calcium to phosphate [123]. Even though bigger flocs are formed and calcium is able to bond between phosphate groups, no increase in *E. coli* attenuation was observed compared to the situation with only phosphate or calcium. Especially, no improvement compared to the electrolyte with only calcium is unexpected. Explanation could be found in the ratio between phosphate and calcium. Calcium concentration is much higher compared to phosphate for secondary wastewater effluents. Before the addition of iron, it is expected that all phosphate and carboxylic functional groups on bacteria surface are packed with calcium. But also the free phosphate ions could be bonded by calcium, inhibiting bridging between phosphate and calcium, because both phosphate groups are packed with calcium.

Furthermore, inactivation was hardly observed. The combination of phosphate and calcium has probably led to a further decrease in interaction between iron and bacteria surface. The effectiveness of reactive oxygen species was already found to be low when there was no direct association between the two surfaces. Overall, it is expected that calcium does interact with free phosphate ions and both phosphate and carboxyl functional groups before introduction of iron. Only via bridging a connection is made between iron and the bacterial surface.

5.3.3. Carbonate

Analyses of the addition of carbonate to the electrolyte indicates that there is no major effect. The removal of *E. coli* was slightly lower compared to the blank. Inactivation was inhibited in the presence of CO_3^-/HCO_3 compared to the blank. As expected, carbonate was presumably out competed by phosphate functional groups, which have a higher affinity for iron precipitates [23]. This does explain the minor effect of carbonate on the physical removal of *E. coli* and does not indicate any change in precipitation structure. On the other hand, the inhibition of inactivation by carbonates does indicate interaction in the association between iron and bacteria surface. Reactive species produced in the bulk were not found to be effective against microorganisms [17, 61], indicating that scavenging of reactive species in the presence of carbonates had to take place close to the bacterial surface. Therefore, co-adsorption of CO_3^- is suggested, enabling formation of the carbonate radical which is much shorter lived an less effective compared to the hydroxyl radical [23, 108].

The simultaneous addition of calcium and carbonate influenced the disinfection. In comparison to calcium only, the removal increased with addition of carbonate. Calcium and carbonate are known to form $CaCO_3$, which can associate with iron and block reactive surface sites [43, 123]. Also precipitation of $CaCO_3$ and so removing hardness is mentioned [21]. Based on the decrease in calcium concentration, neither mechanism is found in these experiments. Only 3% of calcium was removed. The high affinity of calcium for carbonate and the formation of $CaCO_3$ [113] left less calcium available to interact between phosphate groups on bacteria surface and iron, explaining the improved disinfection compared to calcium alone.

Further addition of phosphate increased the disinfection of *E. coli*. Also the uptake of calcium was increased to 6%, comparable to the situation of phosphate and calcium without carbonate. In this situation, part of the calcium is expected to be associated with carbonate. The part that is left, is available for bridging between free phosphate ions and bacteria surface. The removal, in this situation, improved slightly by addition of phosphate. Whereas without carbonate, the disinfection was lowered when phosphate and calcium were combined. It is concluded that calcium is mainly associated with carbonate, and $CaCO_3$ is not able to compete with phosphate for iron surface. Also the uptake of calcium is not ascribed to carbonate, but solely to phosphate. The characteristics of the precipitates is not affected by addition of carbonate, as has been found earlier [123]

5.3.4. NOM

The addition of organics caused a significant drop in disinfection for *E.coli*. The effect was also seen in the floc formation and capability of settling, as has been found before [73]. When natural organic matter was added individually, the water was unable to settle. It is suggested that NOM is adsorbed to the surface of both iron species and bacteria surface, as has been found in previous sorption experiments [53]. Unexpectedly NOM was able to compete with bacterial phosphate groups for iron surface, which was excluded before [22]. NOM mainly contains carboxyl and hydroxyl functional groups, that have been found to have a lower affinity for iron surface compared to phosphate groups on bacteria cells [14, 27]. It is suggested that the presence of NOM before introducing iron favors adsorption of NOM on bacterial surface, explaining the difference with sorption experiments in which iron oxide-bacteria composites had lower maximum adsorption compared iron oxides and bacteria individually [53]. The possible adsorption on both iron precipitates and bacterial surface increases the repulsive forces, inhibiting floc growth and binding of bacterial cells to the iron flocs, similar to studies that used iron nano-particles covered with NOM and observed less direct association between bacteria and iron [74]. Inactivation was also lowered to only 1 log unit. It is unclear whether this drop is caused by scavenging of reactive species or due to complexation and the decreased direct contact between iron and bacteria surface [110]. The relative high share of inactivation compared to total removal indicates no big scavenging effect of NOM itself.

Addition of calcium showed an improvement in the disinfection efficiency for *E. coli*. Most notably, the introduction of calcium in combination with NOM, led to good settleability of the iron precipitates. Also an increase in calcium removal was seen in combination with NOM. Calcium removal increased to 10%, compared to at highest 6% in the absence of NOM. In contrary to other researchers [22], that have not observed increased uptake of calcium in the presence of NOM, bridging of calcium and NOM is expected [39, 73]. The co-existance of Ca and NOM in the water, neutralized the charge of iron complexes and offered binding positions to bacterial cells. Bridging with calcium and lower direct interaction between iron and bacterial cells, was immediately seen in the effectiveness of inactivation. The inactivation fraction became almost zero.

5.3.5. Full synthetic wastewater (Ca, P, carbonate, NOM)

Further addition of phosphate and carbonate to the electrolyte imitated synthetic domestic wastewater effluent. Presence of phosphate and carbonate did not have influence on the settling. Full iron removal was observed. Phosphate removal was still high at 98%. For NOM and calcium a small decrease was observed, 62% and 9%, respectively. The decrease in NOM uptake is ascribed to the competition for iron surface with phosphate and similar results were found in the presence of CO_3 [39, 53]. In general, the removal of *E. coli* was slightly improved by adding phosphate and carbonate and disinfection was

3.5 log units (±0.5).The improvement is mainly ascribed to the presence of carbonate, that partly associates with calcium, decreasing it's disinfection inhibition. Good disinfection is achieved when both cations and (oxy)anions are in balance. The contribution of inactivation is below 1 log unit. In the presence of several wastewater components, direct contact between oxidizing iron and bacteria surface is not achieved and reactive species are not effective in oxidizing microorganisms. Besides, as was shown in experiments containing TEMPOL, the fraction of inactivation is compensated for by physical removal. Therefore, it is concluded that *E. coli* removal in domestic wastewater effluent is mostly dependent on physical removal by iron precipitate formation.

5.4. Interactions phages

The influence of wastewater components on the removal of ϕX174 is described in this part. As concluded before, the main disinfection mechanism is physical removal in iron precipitates. The contribution of inactivation peaked at 1 log unit removal. Figure 5.2 shows an overview of the disinfection capacity of the tested electrolyte types.

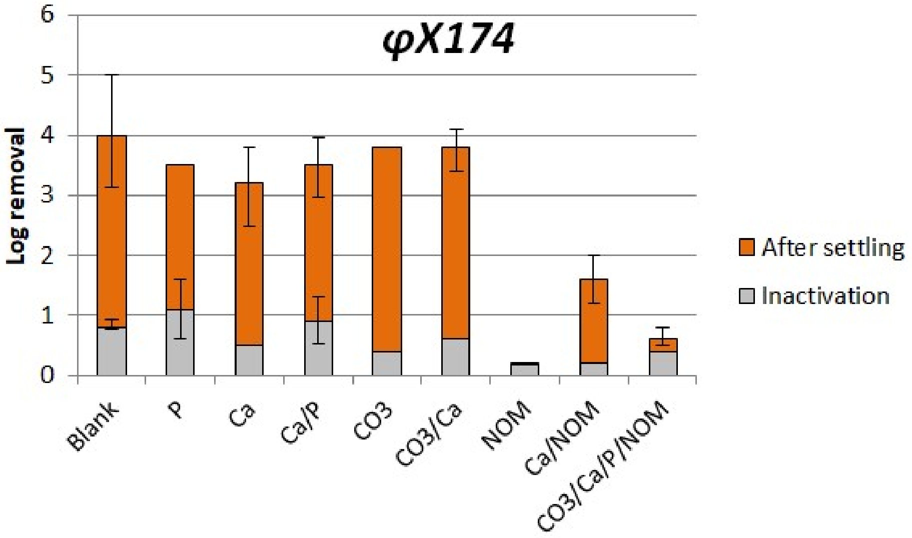


Figure 5.2: Resuls of *E. coli* and ϕX174 removal in experiments with different constant pH

It can be clearly seen that the effect of wastewater components on the removal of ϕX174 is completely different in comparison to *E. coli*. No significant effect on the disinfection was observed in the addition of phosphate, calcium, and carbonate. Disinfection was stable when adding these components individually as well as in combinations. For all these electrolytes, settling was complete. ϕX174 was seen to be vulnerable for even a slight downturn in settling conditions. Furthermore, addition of these components did probably not significantly change the charge of iron precipitates [110]. Phosphate concentrations were relatively low in comparison with iron dosage. As shown by experiments at pH 5.5, similar removal could be reached by only 60% of iron oxidized and removed by settling. Calcium individually does not interact with iron, and in combination with phosphate neutralizes charge of iron precipitates, compensating the uptake of phosphate. CO_3 individually did not associate with iron and uptake of calcium was not seen in the presence of calcium simultaneously. Also the inactivation was not strongly impacted. Inactivation in the blank was 0.8 log units (±0.1), and results in this case were varying from 0.4 to 1.1 log units.

In contrast to other components, the addition of organics had big impact on the disinfection of ϕX174. Whereas attenuation of 3.5 log units were reported for synthetic waters without organics, electrolytes containing NOM all showed lower reductions. Loose bonding and sweep flocculation were signaled as the main contributor in phage removal. This explains the low attenuation in case of NOM alone. This electrolyte was not able to settle, and therefore it can be expected that removal of phages would be low. Only a small reduction during the electrolysis phase indicates minor effect of reactive species on phages. In contrast to NOM only experiments, the other types of water were well able to settle. But also in these cases the disinfection was impacted strongly. This effect was observed before and mainly ascribed to scavenging of or reduction of effectiveness of reactive species [59, 110]. Since inactivation was already a minor contributor in this study for disinfection of ϕX174, this effect can't be only explained by impact on reactive species, although inactivation is lowered in comparison to the average of waters not containing NOM.

The adsorption of NOM on iron precipitates is expected to change the charge to become more negative. No strong bonding with iron is present on phage surface, and the fact that NOM is generally negatively charged, impacts the attraction between iron and phages [46]. Adsorption of NOM is favored over phages. NOM contains carboxyl and hydroxyl functional groups, of which especially carboxyl groups have affinity for iron [23, 59]. The presence of NOM may shield phages for interaction with iron, and in that way inhibit inactivation as well as sweep flocculation of phages [47, 103].

6

Conclusion

Iron electrocoagulation has shown potential for disinfection of pathogens. The aim of this study was to gain more insight into the removal mechanism of faecal indicator organisms and study the influence of wastewater components present in the secondary wastewater effluent. Better understanding will enlarge the potential of iron electrocoagulation for disinfection and help to minimize the limitations for reuse of wastewater effluent. *E. coli WR 1* and ϕX174 are used as indicators for bacteria and virus, respectively. Research into the main disinfection pathways resulted in different outcome for both indicators. *E. coli* was found to be highly susceptible for inactivation by reactive oxygen species as well as removed by physical floc formation and subsequent settling. The majority, 4 log units, of the removal for *E. coli* in electrolytes with a simple water matrix already took place within the electrolysis phase, which was ascribed to inactivation. Further attenuation was achieved by physical removal to a total removal of 6 log units. Experiments using scavengers for reactive species demonstrated similar total disinfection, indicating the subordinate role of inactivation. Inactivation can be seen as first line of defense, able to be compensated by physical floc removal. For somatic phage ϕX174, different behavior was observed. During the electrolysis period, disinfection only accounted for about 1 log unit of the total removal. Furthermore, the efficiency of ϕX174 removal was highly dependent on the degree of settling. Without near complete iron settling, the removal of somatic phages was below 1 log unit. Enhancing mixing and floc growth lead to improvement to 4 log units. Therefore, it is concluded that ϕX174 attenuation is mainly based on physical removal. A minor role is ascribed to inactivation.

Influence of wastewater components are observed on the disinfection of *E. coli*. All had impact on the efficacy of reactive species for inactivation, differing in magnitude. Scavenging often is mentioned as the main mechanism for decrease in inactivation, but both phosphate and calcium are not expected to quench reactive species. Therefore, the suggested process for decreased efficacy is the reduced direct contact between iron and bacteria surface. Phosphate is known to compete with bacterial functional groups for iron surface area, and calcium complexes the surface of bacteria. For carbonate and NOM both quenching and a decrease in contact might explain the findings. The competition between phosphate functional groups and free phosphate ions is also observed in physical removal, lowering attenuation in the presence of phosphate. Calcium complexation of the bacterial surface has even stronger effect on the attenuation, while carbonate is not associated with changes in physical removal. NOM individually impacts disinfection strongly, but just like phosphate and carbonate offers bridging sites between calcium and iron. The bridging between calcium and iron reduces the effect of calcium. Full synthetic wastewater containing phosphate, calcium, carbonate, and natural organic matter was able to removal *E. coli* up to 3.5 log units.

For somatic phages the impact of wastewater components was completely different. No strong bonding is formed between phages and iron, and so disinfection for phage is dependent on good settling conditions and floc formation. No significant impact was seen by addition of phosphate, calcium and carbonate. NOM on the other hand strongly prevented removal of phages. The adsorption of NOM on iron precipitates is expected to change the charge and to become negative. The fact that natural organic matter is generally negatively charged, impacts the attraction between iron and phages. The presence of NOM may shield phages for interaction with iron, and in that way inhibit inactivation as well as sweep flocculation of phages.

7

Recommendations

1. Based on the result of this research it can be concluded that iron electrocoagulation is able to remove bacteria from wastewater to significant levels. The removal of virus on the other hand, is much harder, limiting the possibilities for reuse. Especially NOM inhibited the disinfection of virus. Further research is needed on how exactly NOM does inhibit virus removal and how to avoid the severe effect.
2. The role of inactivation in the blank was dominant for *E. coli*, but the main mechanism for both indicators in treatment of synthetic wastewater effluent was physical removal. To enhance removal, further focus should be on improving floc formation. Better floc formation has been shown to greatly improve physical removal.
3. pH experiments showed that a slower oxidation rate was beneficial for inactivation. This was only tested for the blank, but might work for other types of water as well. By decreasing either dosing speed, pH, or oxygen level the oxidation rate can be lowered.
4. The results of the experiment at pH 5.5 showed not all iron was oxidized into Fe^{3+}, but removal after settling was similar to experiments at higher pH, with full oxidation of the dosed iron. It can be concluded that a lower iron dosage might result in similar disinfection and optimization is possible.
5. Mixing conditions were of great influence on the floc formation and settling capability of the different types of waters. Enhanced mixing conditions were tried, in order to be able to settle most waters in this study. Extensive research on mixing conditions might offer optimized solutions and higher removal per mg of iron dosed.
6. Another remarkable phenomenon are the two stages of disinfection for phages, as has been seen before in other studies [59, 60]. Half of the inactivation takes places within the first minute of electrolysis. A possible explanation is that phages are aggregated into formed flocs after some iron is oxidized, a become shielded for further disinfection by 'fresh' iron [34]. Another explanation for the two stage inactivation is direct oxidation at the anode in the first minute of the experiment, as has been seen in estrogenic compounds research [78]. No conclusive evidence has been found for this assumption. Therefore, further experiments can be performed with consecutive electrolysis phases alternated with settling.
7. Combined addition of wastewater components cancelled out the inhibiting effect of some. Balancing the concentrations of constituents could create beneficial conditions. Therefore, additional experiments should be performed with changing concentrations of wastewater components. Also modeling these interactions would give the chance to predict disinfection efficiency for a specific type of water.
8. A pilot plant experiment should be conducted to assess applicability of EC technology for disinfection on large scale. Also experiments with continuous reactors, coupled to settling ponds or filters to remove iron, could improve applicability of EC technology.

8

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A

Iron oxidation

NOM and carbonate were mentioned to decrease the oxidation rate and phosphate was named as a catalyst, but for neither of the parameters a change in oxidation rate was observed in this study [19, 62, 109]. Only the simultaneous addition of calcium and carbonate introduced a delay in the oxidation rate at the start of the experiments, although at the end of the electrolysis phase only low concentration of Fe^{2+} was found. Carbonic species were found to increase the oxidation rate, $Fe(CO_3)_2^{2-}$ was found to be the most determining trace species for iron oxidation [62]. In case of calcium addition simultaneously, $CaCO_3$ can be formed and associate with iron precipitates. Thereafter, formation of $CaCO_3$ can decrease the formation of trace species. Furthermore, $CaCo_3$ is able to block reactive sites [123].

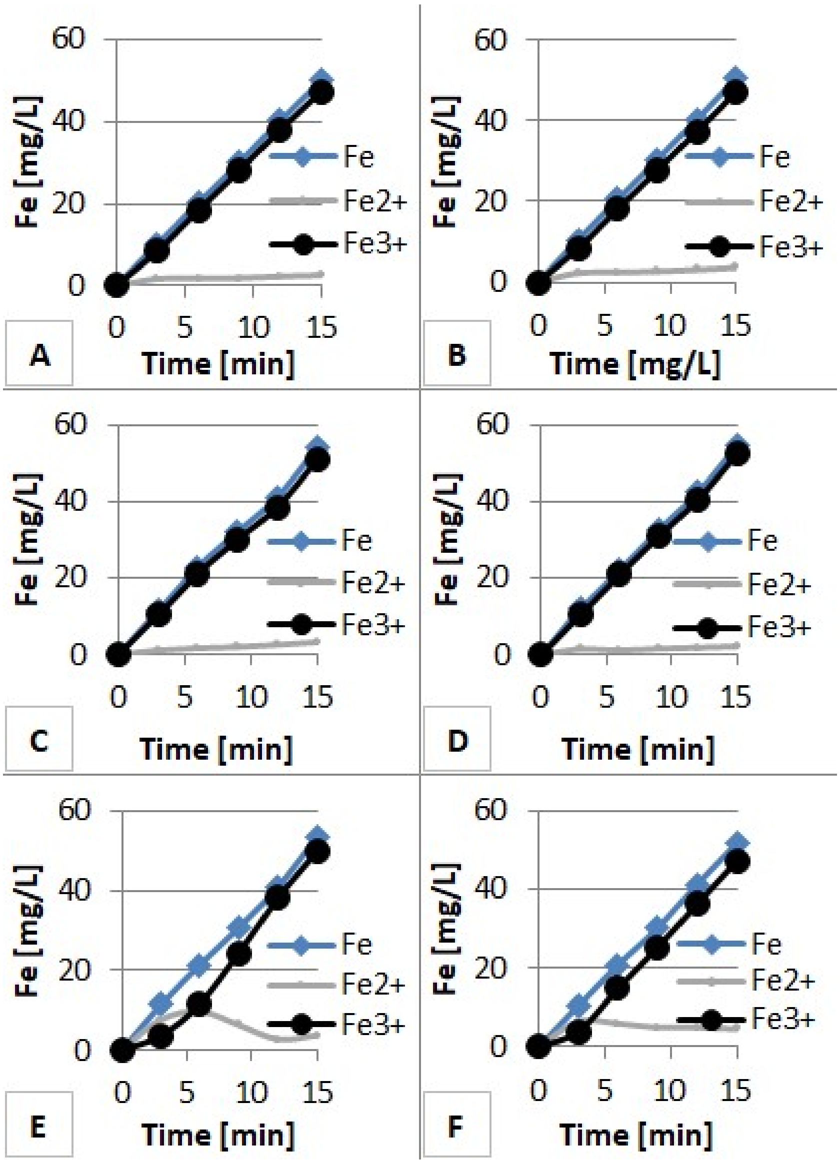


Figure A.1: Iron oxidation rate in different types of electrolytes. A) Phosphate only, B) Calcium only, C) Carbonte only, D) NOM only, E) Phosphate, calcium, and carbonate, F) Phosphate, Calcium, Carbonate, and NOM

B

NOM curve

The concentration of natural organic matter is calculated based on UV adsorption tests. UV adsorption is recalculated into a concentration based on the calibration curve. The curve is shown below.

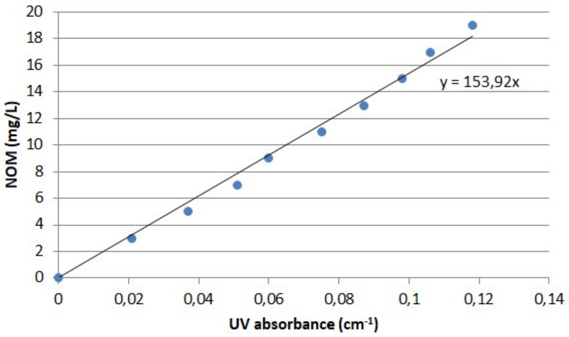


Figure B.1: Calibration curve for the determination of the NOM concentration

C

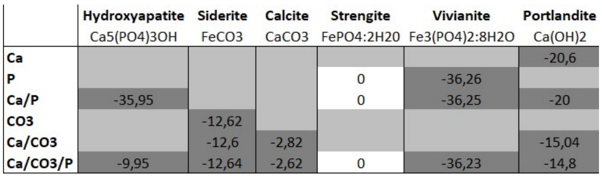
PHREEQC - saturation indices

Precipitation behavior is analyzed using aqueous calculations model PHREEQC. The model was run to analyze saturation indices. The main species that were analyzed were; calcite ($CaCO_3$), siderite ($FeCO_3$), hydroxyapatite ($Ca_5 5(PO_4)_3(OH)$), strengite ($FePO_4$:$2H_2O$), vivianite ($Fe_3(PO_4)_2$:$8H_2O$), and portlandite ($Ca(OH)_2$). Equilibrium reactions were imported from PHREEQC database *wateq4f.dat* and are shown below.

Equilibrium reactions:

Hydroxyapatite: Ca5(PO4)3OH + 4H+ = 5Ca+2 + 3HPO4-2 + H2O
log_k -3.421
delta_h -36.155 kcal
Siderite(d)(3): FeCO3 = Fe+2 + CO3-2
log_k -10.45
Calcite: CaCO3 = Ca+2 + CO3-2
log_k -8.48
delta_h -2.297 kcal
Strengite: FePO4:2H2O = Fe+3 + PO4-3 + 2H2O
log_k -26.4
delta_h -2.030 kcal
Vivianite: Fe3(PO4)2:8H2O = 3Fe+2 + 2PO4-3 + 8H2O
log_k -36.0
Portlandite: Ca(OH)2 + 2H+ = Ca+2 + 2H2O
log_k 22.8
delta_h -31.0 kcal

With the use of EQUILIBRIUM_PHASES command and based on the concentrations after completing electrolysis phase, the saturation indices were calculated using PHREEQC. According to expectations, except for strengite none of the end solutions was saturated and so promoted the precipitation of one of the aforementioned species. Precipitation of strengite, about 1x10^{-5}, was much smaller than that of iron species, about 1x10^{-3}, and mainly formation and precipitation $Fe(OH)_3$ was seen. Therefore, precipitation of strengite only accounts for a small fraction of the iron concentration.



	Hydroxyapatite	Siderite	Calcite	Strengite	Vivianite	Portlandite
	Ca5(PO4)3OH	FeCO3	CaCO3	FePO4:2H20	Fe3(PO4)2:8H2O	Ca(OH)2
Ca						-20,6
P				0	-36,26	
Ca/P	-35,95			0	-36,25	-20
CO3		-12,62				
Ca/CO3		-12,6	-2,82			-15,04
Ca/CO3/P	-9,95	-12,64	-2,62	0	-36,23	-14,8

Figure C.1: Saturation indices per type of water for main species

D

Statistical test - ANOVA

ANOVA (analysis of variance) statistical test was used in order to determine if data series of *E. coli* and somatic coliphage attenuation on the addition of individual wastewater components were statistically significant. Tests were performed in duplicates and microbial screening for each experiment was performed in triplicates. Individually addition of each wastewater component was compared to the results of the blank. The selected alpha level was 0.05. Critical F-value was 18.51.

Table D.1: F-value from ANOVA test for *E. coli* after electrolysis phase in comparison with blank results

F value	Ca	P	CO3	NOM
	61,49	24,60	7,79	53,56

Table D.2: F-value from ANOVA test for *E. coli* after settling in comparison with blank results

F value	Ca	P	CO3	NOM
	22,33	2,52	0,86	22,52

From calculations it could be concluded that the addition of calcium and NOM caused significant changes from the blank both for inactivation as well for total removal for *E. coli*. Phosphate and carbonate only caused significant deviation for inactivation.

Table D.3: F-value from ANOVA test for somatic coliphage after settling in comparison with blank results

F value	Ca	P	CO3	NOM
	0,44	0,45	0,08	19,60

For somatic coliphage the critical significance value was only exceeded for NOM, indicating no significant impact of either calcium, phosphate, and carbonate.