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Low voltage iron electrocoagulation as a tertiary treatment of municipal wastewater: removal of enteric pathogen indicators and antibiotic-resistant bacteria

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ABSTRACT

In this paper we analyse the feasibility of low voltage iron electrocoagulation as a means of municipal secondary effluent treatment with a focus on removal of microbial indicators, Antibiotic Resistant Bacteria (ARB) and nutrients. A laboratory scale batch unit equipped with iron electrodes was used on synthetic and real secondary effluent from a municipal wastewater treatment plant. Synthetic secondary effluent was separately assayed with spiked *Escherichia coli* WR1 and with bacteriophage ΦX174, while real effluent samples were screened before and after treatment for *E. coli*, Extended Spectrum Betalactamase-producing *E. coli*, Enterococci, Vancomycin Resistant Enterococci, *Clostridium perfringens* spores and somatic coliphages. Charge dosage (CD) and charge dosage rate (CDR) were used as the main process control parameters. Experiments with synthetic secondary effluent showed $>4\log_{10}$ and $>5\log_{10}$ removal for phage ΦX174 and for *E. coli* WR1, respectively. In real effluents, bacterial indicator removal exceeded $3.5\log_{10}$, ARB were removed below detection limit ($\geq 2.5\log_{10}$), virus removal reached $2.3\log_{10}$ and *C. perfringens* spore removal exceeded $2.5\log_{10}$. Experiments in both real and synthetic wastewater showed that bacterial removal increased with increasing CD and decreasing CDR. Virus removal increased with increasing CD but was irresponsive to CDR. *C. perfringens* spore removal increased with increasing CD yet reached a removal plateau, being also irresponsive to CDR. Phosphate removal exceeded 99%, while total nitrogen and chemical oxygen demand removal were below 15% and 58%, respectively. Operational cost estimates were made for power and iron plate consumption, and were found to be in the range of 0.01 to 0.24€/m^3 for the different assayed configurations. In conclusion, low voltage Fe-EC is a promising technology for pathogen reduction of secondary municipal effluents, with $\log_{10}$ removals comparable to those achieved by conventional disinfection methods such as chlorination, UV or ozonation.

1. Introduction

At present, one-third of the world's population lives in water-stressed countries and by 2025 the figure is expected to rise to two-thirds (Elimelech, 2006). Discharge of untreated wastewater into the water sources can degrade water quality, increasing the risk to human health and ecosystem degradation. In this context, water reuse is often recognised as a solution with great potential in reducing the gap between availability and demand. Agriculture is currently the largest consumer of reclaimed water, providing an all year round water source with an estimate 200 million farmers worldwide using either raw or treated wastewater for irrigation of over 2000 km^2 (Qadir et al.,2007, Raschid-sally & Jayakody, 2008), constituting roughly 8% of all irrigated land in the planet, most of which happens in Asia (Howell, 2001).

The United Nations agenda for Sustainable Development Goals targets improvement in water quality by reducing pollution, eliminating the discharge of polluted waters, halving the proportion

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of untreated wastewater and increasing safe water reuse globally (Anfruns-Estrada et al., 2017). Despite reclamation being an attractive concept, municipal wastewater harbours a wide range of enteric pathogens such as virus, bacteria, protozoa, parasitic worms and eggs, and its (re)use calls for careful management of its associated health risks. Such risks depend on the type of water to be recycled, the type and concentration of pathogens, and in particular, the ability of such pathogens to survive treatment, as well as the type of exposure of susceptible community members to such waters. The required level of pathogen reduction in reclaimed water depends on the nature of reuse application and potential for human exposure to water.

In this context, the feasibility of Iron(0) Electrocoagulation (Fe-EC) for microbial attenuation in municipal secondary effluents was explored. Previous studies have demonstrated the effect of Fe-EC in the removal of a wide range of microorganisms from bacteria to viruses in different water matrices, mainly for drinking water applications (Ghernaout et al., 2019, Heffron et al., 2019a, Heffron et al., 2019c, Delaire. 2016,). The application of Fe-EC for the reclamation of secondary municipal effluents provides interesting advantages, since their higher conductivities reduce electrolysis cost, plus residual iron is a lesser concern than for drinking water. In spite of this, only few Fe-EC studies focussed on real secondary effluents (Ding et al., 2017, Anfruns-Estrada et al., 2017, Llanos et al., 2014) and to the best of our knowledge, none investigated the removal of Antibiotic Resistant Bacteria (ARB).

The main objective of the present research is to evaluate the performance of low voltage Fe-EC during the treatment of municipal secondary effluents (for reclamation purposes) in the removal of microbial pathogen indicators (*Escherichia coli*, enterococci, somatic coliphages and *Clostridium perfringens* spores) and Antibiotic Resistant Bacteria (Extended Spectrum Beta Lactamase (ESBL) and Carbapenem Resistant (CRE) -*E. coli* and Vancomycin Resistant Enterococci-VRE). Other parameters of importance such as nutrients, COD, turbidity, colour, pH and residual iron were also analysed (SI Table S4). Charge Dosage (CD) and Charge Dosage Rate (CDR) were selected as the main process-control parameters for the iron dosage, directly linked to the electrolysis time and current intensity, respectively (Ghernaout et al., 2019,Van Genuchten et al., 2017, Delaire et al., 2015, Amrose et al., 2013, Gu et al., 2009). CD is defined as the total electric charge per unit volume applied to a given water sample, while CDR is defined as the speed of application of electric charge (Charge Dosage per unit time). In this way CD [C/L] represents the total dose of current, while CDR [C/L/min] represents the current dosing speed. The influence of CD and CDR in the microbial removal of municipal secondary effluents using Fe-EC is central to this research.

2. Materials and Methods

2.1. Field sampling and selection of microbial indicators

Prior to the beginning of the EC experiments and in order to collect data on the expected background levels on pathogenic indicator organisms and ARB in secondary effluents, a six month sampling campaign (June-Dec 2018) was conducted in a municipal wastewater treatment plant (WWTP) from the Netherlands. This facility is of the activated sludge type, with primary and secondary treatment and no disinfection. Grab samples from raw sewage and secondary effluent were collected approximately every two weeks and screened for diverse indicators, namely *E. coli*, enterococci, Extended-Spectrum Betalactamase-producing *E. coli* (ESBLE), Carbapenemase-producing-*E. coli*, (CRE) Vancomycin Resistant Enterococci (VRE), *C. perfringens* spores and somatic coliphages. The premise underlying such selection was to cover bacterial, viral and protozoan indicators, as well as ARB. The screening was culture-based, using selective agar medium for each one of the aforementioned indicators. This microbial toolkit is described in further detail in Supporting Information (SI Table S1).

2.2. Laboratory setup

Parallel experiments in cylindrical glass beakers (0.5 L for synthetic effluents and 1 L for real effluents) were conducted in the laboratory, as depicted in Fig. 1. The dual power source was a 30V-3A TENMA 72-10500 bench DC power supply, connected with crocodile clip cables to two S235 steel plates (maximum percentages: 0.14% carbon, 0.10% silicium, 0.80% manganese, 0.025% phosphorous, 0.015% sulphur, 0.010% nitrogen, 0.20% copper and 0.080% aluminium). Dimensions of the steel plates were 6cm x 4cm, of which 4cm x 4cm were submerged (2cm emerging to connect the clip cables), being polished with coarse and fine sand paper before each use. The plates were mounted in the end of a plastic tube with carved parallel slots ensuring the plates remained parallel and spaced approximately 1cm as described elsewhere (Heffron et al., 2019b, Anfruns-Estrada et al., 2017, Ndjomgoue-Yossa et al., 2015, Merzouk et al., 2009). The beakers were mounted on identical LABNICO L23 magnetic stirrers and fitted with PTFE coated bars for stirring purposes. During each individual experiment, only two process-control parameters were varied, namely CD and CDR, by controlling the electrolysis time and the supplied amperage, respectively. These parameter combinations were selected beforehand for each experiment. Stirring was provided by the magnetic stirrers and the speed was set to 100rpm for all experiments.

For each assay, after the current was applied, the iron plates were removed from the beaker, and the latter was covered with aluminium foil to prevent the entry of dust. Particles were left to settle overnight (as reported by Delaire et al., 2016, Delaire et al., 2015), after which the supernatant was carefully harvested with the use of a sterile 50ml serological pipette. Supernatant was collected from the surface until only a 1-2cm layer of water over the sediments was left. The harvesting procedure was conducted carefully, in order not to generate ripples that could disturb the settled particles. The collected supernatant was transitorily deposited in a clean container, and used immediately for microbiological and physical/chemical characterization.

2.3. Synthetic and real secondary effluents

The formula for the synthetic secondary effluent was based on the specifications from the Organisation for Economic Co-operation and Development guidelines (OECD, 2001) and then adjusted based on a preceding water sampling campaign at the WWTP. Based on these samples, readily biodegradable COD was replaced by less degradable compounds as expected in secondary effluents: yeast extract was replaced by starch, and peptone was replaced by microcrystaline cellulose, which also provided particulates. The nitrogen and phosphorous sources (urea and dipotassium phosphate, respectively) were adjusted following the same principle. Sodium chloride was increased in order to provide a conductivity of approximately 1000μS/cm, similar to that of the grab samples collected from the municipal WWTP. Adopting this composition as the baseline, two variants were produced: one medium with higher nutrients than the baseline (HNM), the other with lower (half) the level of nutrients (LNM) (Table 1).

Two non-pathogenic organisms were used to spike the synthetic effluents, namely *E. coli* WR1 (NCTC 13167) and somatic coliphage ΦX174 (ATCC 13706-B1), bacterial and viral indicators respectively. It is worth noting that experiments were conducted either with *E. coli* or with coliphage ΦX174, but not both simultaneously to prevent *E. coli* from being infected by the coliphage. *E.*

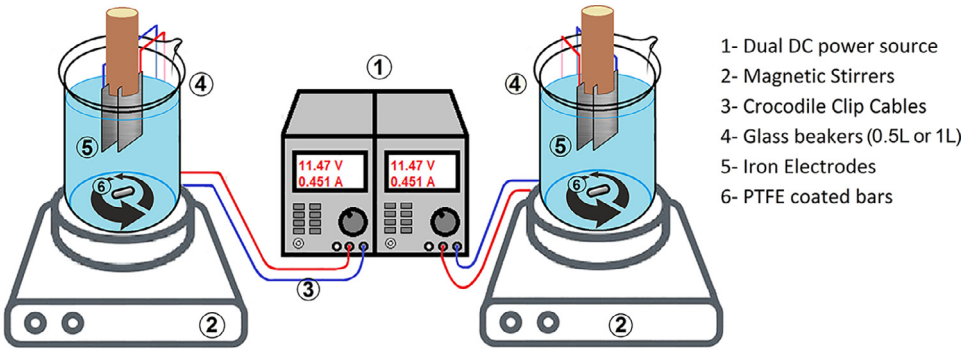

Figure 1. Bench scale EC laboratory setup.

Table 1
Composition of synthetic effluent (OECD 2001) and synthetic effluent medium with high nutrients (HNM) and low nutrients (LNM).

	Concentration (mg/L)		
Compound	OECD	HNM	LNM
Yeast Exctract	22	-	-
Peptone	32	-	-
Starch	-	8	8
Microcrystaline Cellulose	-	5	5
Urea	6	8.6	4.3
Dipotassium Phosphate	28	5.4	2.7
Sodium Chloride	7	60	60
Calcium chloride dihydrate	4	4	4
Magnesium Sulphate Heptahydrate	2	2	2

coli WR1 was propagated in TYGB broth for 3h at 37°C to concentrations of approximately 2×10^8cfu/ml, while phage ΦX174 was propagated following the ISO 10705-2_2000 method, to concentrations of approximately 1×10^{12}pfu/ml. *E. coli* WR1 and ΦX174 were dosed into the test liquid at initial concentrations of approximately 1×10^5(cfu/pfu)/L in order to match the concentrations in the real secondary effluent detected during the sampling campaign.

Experiments involving real secondary effluents were conducted with unaltered grab samples (20-30L) collected downstream from the secondary settlers of the aforementioned WWTP during the months of May-June 2019. All assays on these samples were performed immediately upon arrival in the laboratory during the same day of collection. The complete physical/chemical characteristics of these samples can be observed in SI Table S2 .

2.4. Operational Fe-EC parameters

Fe-EC experiments conducted on the spiked synthetic secondary effluents and real secondary effluents followed the configurations of CD and CDR described in Table 2. For synthetic medium, conditions apply for both kinds of effluent (HNM and LNM) and for both spiked indicators (*E. coli* WR1 and somatic coliphage ΦX174). All Fe-EC experiments with synthetic effluent were performed in 0.5L beakers equipped with iron electrodes as described in 2.2. Synthetic medium was freshly prepared every day before the assays. Experiments using real secondary effluent were conducted in 1L beakers, using *E. coli*, ESBL-*E. coli*, enterococci, VRE, Somatic coliphages and *C. perfringens* spores as indicators. The experiments described in this section were conducted on four different days, and hence under slightly different real secondary effluent qualities. The characteristics of the real secondary effluent and the scheduling for the different days and microbial groups can be found under SI Tables S2 and S3. The theoretically dosed iron concentration Fe_{theo} was calculated according to Faraday's law (SI).

2.5. Analytical methods

Microbiological screening and quantification was performed either by spread plate method or by membrane filtration according to APHA-Standard Methods for the Examination of Water and Wastewater, 23rd Edition, depending on the expected microbial load of the sample. The specific culture media used for each indicator is detailed in SI Table S1. Screening of somatic coliphages was performed by pour plate technique following ISO 10705-2.

Analysis of ions such as NO_2^-, NO_3^{2-}, NH_4^+, PO_4^{3-} and Cl^- in filtered water samples was carried out with Metrohm 881 basic IC plus and 883 compact IC pro Ion chromatography. For the analysis of total nitrogen, Spectroquant® nitrogen cell test were used, with digestion at 120°C for 1h, followed by reading in a Spectroquant® NOVA60 photometer (Merck, Germany). Total iron (Fe^{+2}, Fe^{+3}) was measured using Spectroquant® Iron Cell Test (1-50mgFe/L), read in the aforementioned Spectroquant® NOVA60 (Merck, Germany) photometer. COD analysis was performed using HACH-Lange test kits (LCK314, 15-150mgO_2/L) with 2h digestion at 148°C and reading in a HACH DR3900 spectrophotometer. Total suspended solids analysis of samples was carried out according to APHA- Standard Methods for the Examination of Water and Wastewater 23rd Edition. Turbidity was measured using Turb 430IR multimeter. Colour was analysed in both unfiltered and filtered samples using UV-VIS spectrophotometer at a 410nm wavelength.

2.6. Methodology for Fe-EC cost estimation

Simplified operational cost estimates for each individual Fe-EC experiment were performed considering as inputs the consumed electric power and metallic iron. As for 2019 average energy cost in The Netherlands for a medium size consumer = 0.0679€/kWh (Eurostat, 2019), and the steel S235 cost = 0.21€/kg (MEPS International Ltd, 2019).

For a given Fe-EC experiment, in which U is the applied voltage (v), I is the current intensity (mA), t is the treatment time (h) and V is the volume of the cell (m^3), then the consumed power can be estimated as:

$$P\left[\frac{kW.h}{m^3}\right] = \frac{U.I.t}{V} \quad (1)$$

Then, the operational cost for each particular experiment is determined by the amount of consumed power and the amount of dosed iron (described in SI-Faraday's equation), multiplied by their respective unit costs:

$$\text{Cost}\left(€/m^3\right) = P\left[\frac{kW.h}{m^3}\right] \times 0.0679€/\text{kWh} + m_{Fe}(\text{kg}) \times 0.21€/\text{kg} \quad (2)$$

Table 2
Operational parameters for synthetic and real secondary effluent assays.

Water type	Electrode area (cm^2)	Vol (L)	CD (C/L)	CDR (C/L/min)	Dosed Fe_{theo} (mg/L)
Synthetic secondary effluent	32	0.5	10	5 - 7.2 - 36 -72	2.9
			20	5 - 7.2 - 36 -72	5.8
			50	5 - 7.2 - 36 -72	14.5
			75	5 - 7.2 - 36 -72	21.8
			150	5 - 7.2 - 36 -72	43.9
			200	5 - 7.2 - 36 -72	58.1
Real secondary effluent	32	1	50	7.2	14.5
			100	7.2	29.0
			200	7.2	58.1
			400	7.2	116.1
	40	1	50	36	14.5
			100	36	29.0
			200	36	58.1
			400	36	116.1

2.7. Data Analysis

Data series of somatic coliphage and *E. coli* attenuation in synthetic effluents was analysed with the statistical test ANOVA (analysis of variance) in order to determine if CDR introduced significant logarithmic removal variations for the different CDs assayed. In this case, the obtained data was comprised by duplicate microbial sampling in duplicate assays (n=4). For real effluent samples, obtained data was comprised by duplicate microbial samples in single assays (n=2). For microbial indicators presenting removal stagnation, Tukey's (honest significance) range test was used to verify the so called "removal plateau". Faradaic efficiency determination in real effluent experiments was determined by the use of the least square function approximation.

3. Results

3.1. Microbial indicators in raw sewage and secondary effluent

The average concentrations of target microbial indicators in the WWTP influent were found in the 1×10^5-1×10^8cfu/L (or pfu/L) range, while for the effluent, average values were between 1×10^2 and 1×10^5cfu/L (or pfu/L). The observed 2-3log_{10} removal is typical for activate sludge-based treatment systems (Hata et al., 2013, De Luca,et al., 2013, Fu et al., 2010, Tanji et al., 2002, Rose et al., 1996). Concentrations of bacterial indicators (*E. coli* and enterococci) exceeded that of ESBLE and VRE by 2-3log_{10} in both influent and effluent, indicating that ARB were present in lower numbers (Fig. 2). Also the removal of ESBLE and VRE by secondary treatment was comparable to that of *E.* coli and enterococci. The levels of the selected indicators in the WWTP influent and effluent streams were in-line with published literature (Schmitt et al., 2017, Karon et al., 2016, Harwood et al., 2005, Lodder & De Roda Husman, 2005, Noble et al., 2004, Hot et al., 2003, Cetinkaya, et al., 2000, Lasobras et al., 1999, Gantzer, et al., 1998); therefore, the WWTP was selected as source of real wastewater for further laboratory experiments. Of particular interest were the levels of *E. coli* and somatic coliphages in the effluent (approximately 1×10^5cfu/L and 1×10^4pfu/L respectively), as these were used to determine the spike concentration values for *E. coli* WR1 and somatic coliphage ΦX174 in the synthetic effluent during Fe-EC experiments (section 2.3).

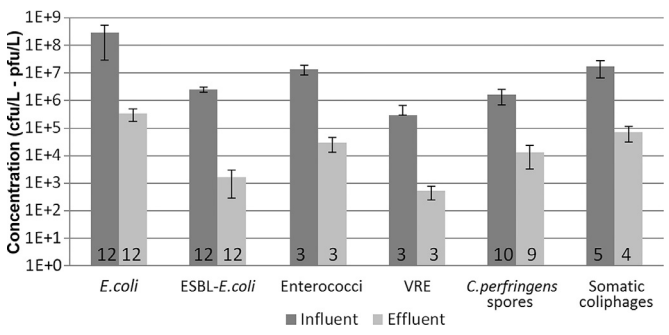

Figure 2. Microbial indicators *E. coli*, ESBL-*E. coli*, enterococci, VRE, *C. perfringens* spores and somatic coliphages in raw influent and secondary effluent of a conventional Dutch WWTP. Number of samples analysed for each indicator is noted on the foot of each bar. Error bars represent standard deviation.

3.2. E. coli WR1 and ΦX174 removal from synthetic effluent

Experiments assessing the removal of *E. coli* WR1 are shown in Fig. 3. For both synthetic water types, removal of *E. coli* WR1 increased gradually with increasing CD. In the experiments using LNM (Fig. 3a), the effect of CDR in the removal appears negligible, with no clear pattern for microbial attenuation. ANOVA tests conducted for all CDs in LNM using CDR as the independent variable, produced p-values consistently below 0.05 for CDs≥50C/L, meaning that although for a given CD (≥50C/L) removal variations seem unimportant in operational terms (≤0.5log), the influence of CDR in removal is statistically present. In the experiments using HNM (Fig. 3b), similar ANOVA tests were conducted, once again producing for CDs ≥50C/L, p-values below 0.05, meaning that the influence of CDR in removal is statistically significant, with greater removal values associated to lower CDRs. Obtained *E. coli* removal using HNM was lower than with LNM, reaching 4.9log_{10}, hence suggesting that the presence of higher nutrient concentration negatively affected *E. coli* removal.

Somatic coliphage ΦX174 attenuation during LNM experiments (Fig. 4a) and HNM experiments (Fig. 4b) displayed in qualitative terms a very similar behaviour; removal being <1log_{10} in the 10–75C/L range without any significant variation, and levelling off at 150-200C/L, reaching a plateau of 3.0-4.0log_{10} removal. ANOVA tests conducted for all CDs in both water matrices using CDR as the independent variable produced for all cases p-values above 0.05, meaning that for somatic coliphages the influence of CDR in removal is statistically insignificant throughout the whole range of dosed charge, irrespective of the considered water matrix. For the aforementioned plateaus in removal observable at 150C/L and 200C/L, Tukey tests were performed taking all 16 bars comprising both plateaus, as independent variables. No combination of 2 bars yielded a Tukey-p value <0.05, meaning that all 16 bars constitute the same plateau. Hence, the concentration of nutrients

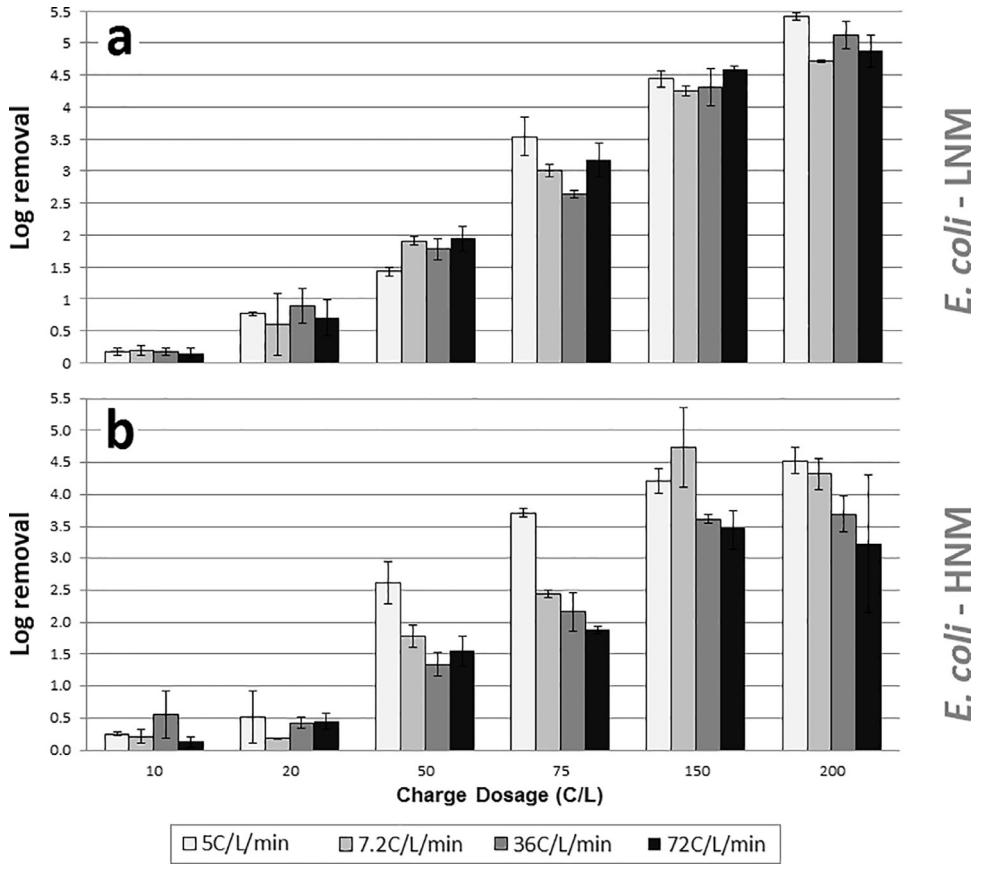

Figure 3. *E. coli* WR1 removal during Fe-EC experiments in LNM (a) and HNM (b) with increasing CD (10, 20, 50, 75, 150 and 200C/L) and CDRs (5, 7.2, 36, 72C/L/min). Each bar represents the average of four values (duplicate microbial screening in duplicate assays), error bars represent standard deviation.

was not found to affect the response of coliphages to the iron dosing speed (CDR), nor to the value of the maximum removal (plateau).

3.3. Microbial and physical chemical attenuation in real effluents

Following the experiments using spiked synthetic secondary effluent, real secondary effluent from the WWTP was assayed. Two CDRs (7.2 and 36C/L/min) in combination with four different CDs (50, 100, 200 and 400C/L) were assayed. For all experiments here described, a single assay was conducted in which duplicate samples were screened for microbial indicators. The results for *E. coli* and ESBL-*E. coli* are depicted in Fig. 5. The removal was found to increase with increasing CD, for both assayed CDRs. *E. coli* removal spanned from $0.5\log_{10}$ at CD 50C/L, to a maximum of $3.7\log_{10}$ at 400C/L. This is considerably lower than the results obtained for *E. coli* in synthetic effluent, since removals of $5.4\log_{10}$ were achieved using half of the iron dose (200C/L). CDR showed a significant effect on *E. coli* attenuation, with removal rates of $3.7\log_{10}$ and $2.4\log_{10}$ at 7.2 and 36C/L/min, respectively (ANOVA p-values between same CDs and either CDR$<$0.05 for 50, 100 and 200C/L). A similar behaviour was observed during Fe-EC experiments with ESBL-*E. coli*, with increasing removal following increases in CD, and favoured by the lower CDR of 7.2C/L/min over 36C/L/min. Minimum removal of $0.2\log_{10}$ was observed for 50C/L, while a maximum surpassing $2.6\log_{10}$ was estimated for 400C/L. It is noteworthy that experiments with CD 200 and 400C/L involving ESBL-*E. coli* achieved removal rates high enough to hinder detection through culture based methods, meaning that the filtered samples were below the limit of detection (LOD). For these experiments, the minimum removal efficiency for ESBL-*E. coli* was calculated on a mathematical basis, considering the method appreciation of 1cfu and the filtered volume in each case. From a comparison perspective, obtained ESBL-*E. coli* removal under 7.2C/L/min was lower than that of sensitive E. coli (ANOVA p-values$<$0.05) while for 36C/L/min removal was equal for 50C/L (ANOVA p-value$\approx$0.2) but lower for 100C/L (ANOVA p-value$<$0.05). This suggests that sensitive *E. coli* is a conservative indicator for ESBL-*E. coli*, since sensitive *E. coli* is removed equally or less than ESBL-*E. coli*. The fact that ESBL-*E. coli* was removed below LOD for CD 200 and 400C/L while sensitive *E. coli* was not, should not be misread as ESBL-*Ecoli* being better removed, since concentrations of ESBL-*E. coli* were 3 orders of magnitude lower than sensitive *E. coli* in the real secondary effluent samples as depicted in Fig. 2.

For enterococci and VRE (Fig. 6) removal also increased with CD for both CDRs. Enterococci removal spanned from $0.4\log_{10}$ for CD of 50C/L, to a maximum of $3.6\log_{10}$ at 400C/L, with attenuation of enterococci being very similar to that of *E. coli* (Fig. 5). CDR also showed a major effect on enterococci attenuation, with removal rates at 7.2C/L/min being up to $0.9\log_{10}$ higher than those at 36.0C/L/min (ANOVA p-values between same CDs and either CDR$<$0.05 for all CDs). Regarding VRE, attenuation levels were in the same range as those of ESBL-*E. coli*, with a minimum of $0.3\log_{10}$ achieved at 50C/L (36.0C/L/min) and a maximum exceeding $2.5\log_{10}$ at 200-400C/L (7.2C/L/min), respectively. VRE removal for 200 and 400C/L at 7.2C/L/min, and 400C/L at 36C/L/min were also estimated minimum values since the resulting concentration for these experiments was below LOD. Enterococci and VRE behave in a very similar way, with VRE being removed in almost identical ratios to that of sensitive enterococci, at least for the samples with

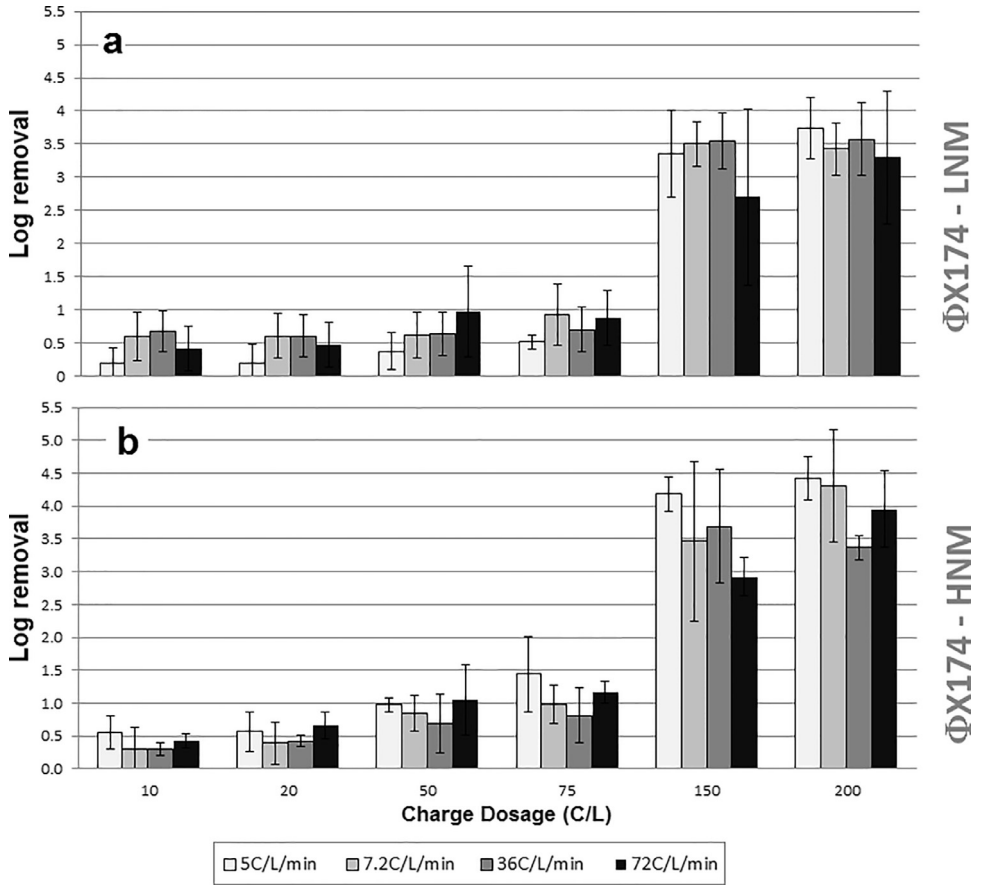

Figure 4. Somatic coliphage ΦX174 removal during Fe-EC experiments in LNM (a) and HNM (b) with increasing CD (10, 20, 50, 75, 150 and 200C/L) and CDR (5, 7.2, 36, 72C/L/min). Each bar represents the average of four values (duplicate microbial screening in duplicate assays), error bars represent standard deviation.

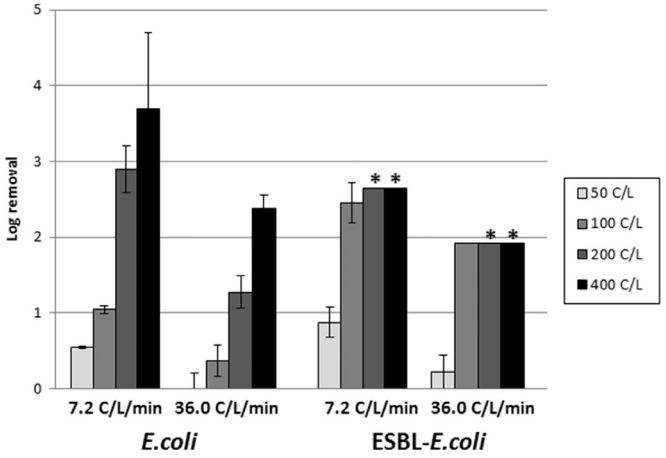

Figure 5. *E. coli* and ESBL-*E. coli* removal during real secondary effluent Fe-EC experiments. Note: Bars marked with an asterisk (*) indicate a minimum estimated removal, due to effluent concentration below LOD. Each bar represents the average of two values (duplicate microbial screening, single assay). Error bars indicate standard deviation.

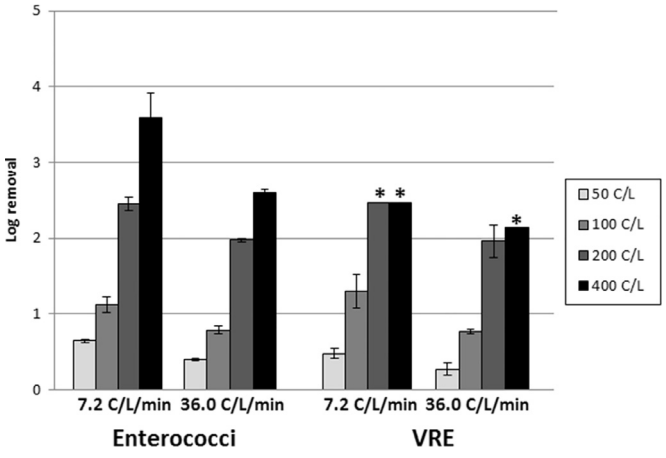

Figure 6. Enterococci and VRE removal during real secondary effluent Fe-EC experiments. Note: Bars marked with an asterisk (*) indicate a minimum estimated removal due to effluent concentration below LOD. Each bar represents the average of two values (duplicate microbial screening, single assay). Error bars indicate standard deviation.

concentrations above LOD (ANOVA p-values >0.05 for both CDRs). This suggests that removal of enterococci can be used as a proxy for removal of VRE, due to the observed similarities in their attenuation patterns.

C. perfringens spores showed the most complex behaviour under Fe-EC (Fig. 7). From a CD perspective, removal increased with CD yet appeared to stagnate under 200 and 400C/L reaching over $2\log_{10}$. ANOVA test between the 7.2 and 36C/L/min series revealed no considerable statistical difference between them (indicating that CDR plays no major role in *C. perfringens* spore removal), while Tukey test indicated two statistically different groups, namely the 50C/L samples, and the 100, 200 and 400C/L samples (confirming the existence of the removal stagnation). This behaviour was not observed for any other indicator organism in this study.

Table 3
Simplified operation cost calculation for experiments conducted using real secondary effluent and associated microbial removal.

Settings				Costs			Microbial log removal					
CD (C/L)	CDR (C/L/min)	Intensity (A)	Voltage. (v)	Electric (€/m^3)	Metal Iron (€/m^3)	Operation (€/m^3)	*E.coli*	ESBL-*E.coli*	Enterococci	VRE	*C. perfringens* Spores	Somatic Coliphages
50	7.2	0.12	7.5	0.007	0.003	**0.010**	**0.6**	**0.8**	**0.6**	**0.5**	**1.0**	**0.2**
100			7.5	0.014	0.006	**0.020**	**1.0**	**2.5**	**1.1**	**1.3**	**2.2**	**0.5**
200			7.6	0.029	0.012	**0.041**	**2.9**	**>2.6**	**2.5**	**>2.5**	**2.0**	**1.0**
400			7.7	0.058	0.024	**0.082**	**3.7**	**>2.6**	**3.6**	**>2.5**	**2.2**	**>2.3**
50	36.0	0.60	27	0.025	0.003	**0.028**	**0.0**	**0.2**	**0.4**	**0.3**	**0.4**	**0.4**
100			27	0.051	0.006	**0.057**	**0.4**	**1.9**	**0.8**	**0.8**	**1.8**	**0.6**
200			27	0.102	0.012	**0.114**	**1.3**	**>1.9**	**2.0**	**2.0**	**2.8**	**0.9**
400			28	0.211	0.024	**0.236**	**2.4**	**>1.9**	**2.6**	**>2.1**	**2.8**	**2.1**

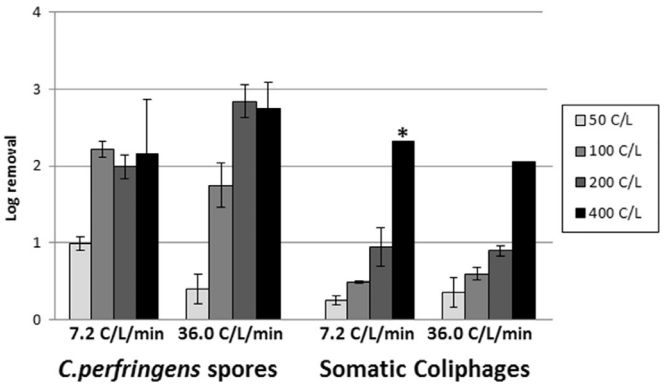

Figure 7. *C. perfringens* spores and somatic coliphage removal during real secondary effluent Fe-EC experiments. Note: Bars marked with an asterisk (*) indicate a minimum estimated removal due to effluent concentration below LOD. Each bar represents the average of two values (duplicate microbial screening, single assay). Error bars indicate standard deviation.

For somatic coliphages, removal below 1log$_{10}$ was observed for the three lower CDs assayed (50, 100 and 200C/L), yet increasing to over 2log$_{10}$ for 400C/L under both CDRs. CDR seems to play a less significant role for this indicator compared to bacteria, with no major difference between 7.2 and 36C/L/min in terms of achieved removal (ANOVA p-values>0.05).

3.4. Nutrient removal in synthetic and real effluents

Together with the removal of microbial indicators, PO_4^{3-}, COD, and TN removal was also measured for each of the samples under each configuration of CD and CDR in either synthetic or real effluent experiments. For synthetic HNM and LNM experiments, PO_4^{3-} removal increased rapidly with increasing CD to values below LOD (>98% removal), while TN removal displayed a decreasing trend with increasing CD for all CDRs (SI Figure S1 and S2). In real secondary effluents (Fig. 8), removal of PO_4^{3-} was high, dropping from 1.50mg/L to values below detection with associated removal rates above 99%. COD removal reached 30-40% for the higher CDs, in particular for CDR of 36C/L/min, while TN achieved a maximum removal of 15.4% for CD 200C/L and CDR 36C/L/min. The influence of CDR on phosphate removal was not conclusive, since very high removal values were quickly reached, irrespective of CDR. Interestingly, COD and TN exhibit higher removal for higher CDR, opposite of what is observed for most microbial indicators.

3.5. Cost estimation

The operational cost of the Fe-EC process for the real secondary effluent experiments, and their achieved microbial removal for each indicator are shown in Table 3. The calculations indicate lower costs at lower CDRs, due to a reduced power consumption for all CDs. Operational costs from 0.01 to 0.08€/m^3 were obtained for the experiments using real secondary effluent under a CDR of 7.2C/L/min, while for CDR of 36C/L/min, estimations range from 0.03 to 0.24€/m^3. It is worth noting that lower CDRs produce better outcomes in terms of microbial removal, and also result in lower operational costs due to lower required operational voltage. From these estimates, although the iron electrode cost is the same for both scenarios, the power cost is the most important factor in the total operating cost, with an impact of about 70% for CDR of 7.2C/L/min and 90% for CDR of 36C/L/min. For the experimental conditions explored in this research, the combination that produced the best outcome from a microbial perspective was CD 400C/L and CDR 7.2C/L/min, involving an associated cost of 0.082€/m^3.

Faradaic efficiencies during real secondary effluent assays were calculated using least square method, with reporting values of 113% for CDR of 7.2C/L/min and 114% for 36.0C/L/min (SI Figure S3).

4. Discussion

4.1. Effect of water matrix on removal efficiencies

The present study confirms the influence of water matrix, i.e., synthetic versus real secondary effluent, for bacteria and virus indicator removal by EC, as well as nutrient removal . In real secondary effluents, *E. coli* removal was 1-2log$_{10}$ lower than that observed for *E. coli* in synthetic effluents, even when the dosage of iron was doubled. Similar observations were made regarding phage ΦX174, with removal also dropping by 1-2log$_{10}$ in real secondary effluents. Although the response obtained with synthetic and real secondary effluent was similar in qualitative terms, removal obtained for *E. coli* and phage ΦX174 still differs by orders of magnitude.

It was hypothesised that the complexity of the water matrix from real secondary effluents, and its higher concentration of organic matter, iron-scavenging anions and complexation agents (such as phosphates, citrates, carbonates and sulphates) are responsible for substantially reduced coagulant generation or microbial removals, coinciding with the observations of Heffron et al., (2019a), Van Genuchten et al., (2017), and Ghernaout et al., (2019). These compounds are recognized as responsible for hindering Fe^{+2} oxidation into insoluble Fe^{+3}, hence reducing coagulant precipitation and subsequent sweep flocculation. *C. perfringens* spore removal was only assessed in real secondary effluents, showing similar characteristics both in quantitative and qualitative terms with previous Fe-EC research conducted in real sewage and secondary effluents by Anfruns-Estrada et al., (2017). In the mentioned study they determined the maximum removal of *C. perfringens* spores to 2.79log$_{10}$, with removal stagnating as the dosage of current progressed. This behaviour was also observed in the present research, particularly at lower CDRs underlining that increasing CD

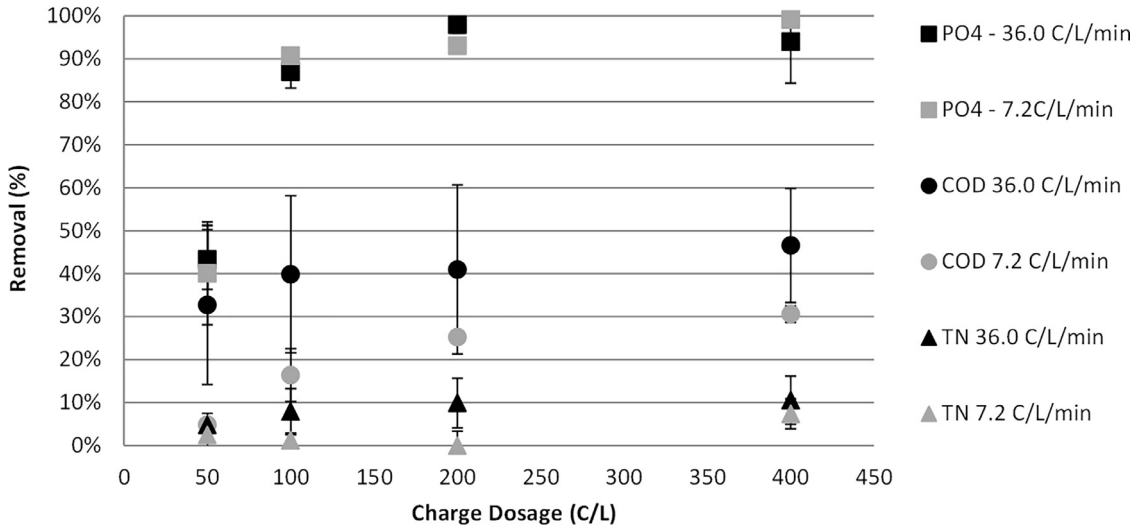

Figure 8. Removal of phosphate, COD and TN for real secondary effluent experiments. Each data point represents the average of four values (duplicate chemical screening in duplicate assays). Error bars indicate standard deviation.

will not enhance spores removal beyond a certain plateau, whatever the CDR. In terms of PO_4^{3-} COD and TN removal, the obtained results are well within range of previous research, where high PO_4^{3-} removal ranging from 50% to 98%, 26% to 85% for organics and less than 30% for TN was observed (Zaleschi et al., 2013, Ikematsu, et al., 2006, Inan & Alaydin, 2014, Lacasa et al., 2011, Malinovic et al., 2016).

4.2. Microbial attenuation mechanisms

Removal of all bacterial indicators was in general terms very similar, irrespective of their resistance to antibiotics or their gram classification, with removal being strongly dependent on the amount and speed of iron dosage. Similar conclusions are also valid for somatic coliphages (although appearing less sensitive to the speed of dosage), but do not fully apply for *C. perfringens* spores. This evidences a differing response to the Fe-EC process for each type of microorganism. The aim of this research was not to look into the mechanisms of removal for each one, yet the body of literature recognizes three pathways for microbial attenuation, namely: a) entrapment or adsorption in the metallic hydroxide flocs, and removal by sedimentation; b) inactivation due to formation of Reactive Oxygen Species (ROS) or disinfectants; and c) inactivation due to electrical current.

According to most researchers, entrapment seems to be the dominant removal mechanism for bacteria (Ghernaout et al., 2019, Delaire et al., 2016, Delaire et al., 2015), mainly due to the affinity of their surface functional groups such as teichoic acids and phospholipids, with the EC precipitates. These functional groups are found in similar amounts in gram positive and gram negative bacterial cell walls (Delaire et al., 2016, Borrok, et al., 2005). Virus removal has been attributed to both iron hydroxide floc entrapment (Heffron et al., 2019a, Tanneru & Chellam, 2012), and inactivation by either ROS or chlorine-based oxidants formed by reduction in the anode (Heffron et al., 2019). Formation of Cl_2 was ruled out as a mechanism of disinfection in our experiments, since Cl^- concentration was measured by ICP-MS before and after the application of current, and no variations were detected in any of the samples, also in line with the findings of Delaire et al., (2015) and Diao et al., (2004). Inactivation due to the effect of electric current has been the least reported biocidal pathway, with the research from Jeong et al. (2006) giving it a larger relevance at high current densities, from 33 to 100mA/cm^2. It is noteworthy that throughout the experiments conducted in this publication, current density never exceeded 20mA/cm^2, for which this pathway of removal is not regarded as dominant.

In terms of CDR in promoting either of the aforementioned mitigation mechanism, Heffron et al., (2019b) correlated ferrous iron oxidation rate and bacteriophage removal, concluding that fast oxidation of Fe^{+2} leads to a shorter exposure time and hence poorer contact between the phages and the reactive iron species, yielding a less important disinfection. In other words, implying that ROS generated during ferrous iron oxidation are a major contributor in the disinfection during Fe-EC, with the effect of these being stronger for slowly occurring oxidations. However, the selected overnight settling for all experiments likely influences oxygen diffusion into the effluent promoting the slow oxidation of ferrous iron, hence potentially impacting in the removal (besides from it having a reduced practicality on a municipal scale). The present research observed for both synthetic and real effluents an increasing removal efficiency for bacterial and viral indicators under decreasing CDRs (less prominent for viruses), even when the amount of dosed iron (and coagulation products) was identical. Although this study did not look into the mechanistic aspects of the microbial removal via Fe-EC, the observed dependency of microbial removal on CDR suggests that the production of ROS could in fact be a contributing factor during Fe-EC. The aerobic oxidation of Fe^{+2} released during the anode oxidation produces a cascade of reactive species which includes superoxide ion ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH^-$) (Van Genuchten & Peña, 2017, Hug & Leupin, 2003, Rush, et al., 1990), all of which are known to have disinfectant properties (Tanneru & Chellam, 2012, Jeong et al., 2006, Elena Pulido 2005). This implies that microbial removal by Fe-EC could be in fact a combination of physical separation and chemical inactivation, and not just an adsorption-sedimentation phenomenon. It could also explain why spores (dormant bacterial structures, highly resistant to chemical attack) are significantly less affected than bacterial indicators by varying CD or CDR.

4.3. Fe-EC as a disinfection technology

When compared against typical wastewater disinfection technologies in terms of pathogen removal, Fe-EC performs in a similar way to that of conventional alternatives such as chlorina-

tion, ozonation or UV light. Typical removal values for chlorination range from 2-6$\log_{10}$ for bacteria, 0-4$\log_{10}$ for viruses and 0-3$\log_{10}$ for protozoa. Disinfection values for ozonation include 2-6$\log_{10}$ for bacteria, 3-6$\log_{10}$ for viruses and >2$\log_{10}$ for protozoa. For UV, expected performance is 2-4$\log_{10}$ for bacteria, 1-3$\log_{10}$ for viruses and 2-3$\log_{10}$ for protozoa (Collivignarelli et al., 2018, USEPA, 2012, Bitton, 2005, USEPA, 2003, Rose et al., 1996, WHO, 1989). This means that Fe-EC can in fact be regarded as an unconventional chemical-addition free disinfection technology, based on comparable performance to other classically accepted disinfection methods. Abou-Elela et al. (2012) provides reference O&M cost values for municipal secondary effluent disinfection using chlorine (32mg/L, 15 minutes contact time), ozone (15mg/L, 15 minutes contact time) and UV irradiation (164mWs/cm^2, 15 minutes contact time). Cost estimates are 0.024€/m^3 for chlorine, 0.030€/m^3 for ozone and 0.044€/m^3 for UV irradiation. For the lowest studied CDR of this research (7.2C/L/min), the obtained operation costs (0.01 to 0.082€/m^3) fall within comparable range to those obtained by Abou-Elela et al. (2012), although a proper comparison should be made on a basis of equal microbial inactivation.

5. Conclusions

- Low voltage Fe-EC is a promising technology for microbial removal in secondary municipal effluents, with $\log_{10}$ removal comparable to those achieved by conventional disinfection methods such as chlorination, UV or ozonation.
- For real secondary effluents, achieved bacterial removal exceeded 3.5$\log_{10}$, ARB removal reached or exceeded 2.5$\log_{10}$, spores were removed between 2-3$\log_{10}$ and virus elimination reached or exceeded 2.3$\log_{10}$. In synthetic secondary effluent, bacterial and virus $\log_{10}$ removal was consistently higher with 1-2 orders of magnitude.
- Microbial removal was found to increase with CD, while decreasing CDRs showed a higher mitigation of bacteria, yet no significant effect on viruses or spores. The latter showed a different removal trend, with elimination reaching a plateau for medium-high CDs, this being slightly higher for higher CDRs.
- Sensitive *E. coli* and Enterococci appear as conservative indicators for ESBL-*E. coli* and VRE respectively, although it must be noted that ARB determination was limited by relatively low concentrations in the secondary effluent.
- Iron plates and electric consumption were the main components contributing to the costs, with the latter having the largest impact (70-90%) for the assayed conditions. For the most favourable microbial removal set of conditions (CD 400C/L, CDR 7.2C/L/min) the estimated unit cost of the process is 0.08€/m^3, within comparable range to other conventional disinfection technologies as chlorine, UV or ozone.
- Besides from microbe removal, Fe-EC offers additional benefits over traditional disinfection methods, such as nutrient and COD removal.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2020.116500.

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