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A conceptual framework modeling of functional microbial communities in wastewater treatment electro-bioreactors

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ABSTRACT

Understanding the microbial ecology of a system allows linking members of the community and their metabolic functions to the performance of the wastewater bioreactor. This study provided a comprehensive conceptual framework for microbial communities in wastewater treatment electro-bioreactors (EBRs). The model was based on data acquired from monitoring the effect of altering different bioreactor operational parameters, such as current density and hydraulic retention time, on the microbial communities of an EBR and its nutrient removal efficiency. The model was also based on the 16S rRNA gene high-throughput sequencing data analysis and bioreactor efficiency data. The collective data clearly demonstrated that applying various electric currents affected the microbial community composition and stability and the reactor efficiency in terms of chemical oxygen demand, N and P removals. Moreover, a schematic that recommends operating conditions that are tailored to the type of wastewater that needs to be treated based on the functional microbial communities enriched at specific operating conditions was suggested. In this study, a conceptual model as a simplified representation of the behavior of microbial communities in EBRs was developed. The proposed conceptual model can be used to predict how biological treatment of wastewater in EBRs can be improved by varying several operating conditions.

Key words | conceptual framework, electro-bioreactors, microbial communities, modeling, wastewater

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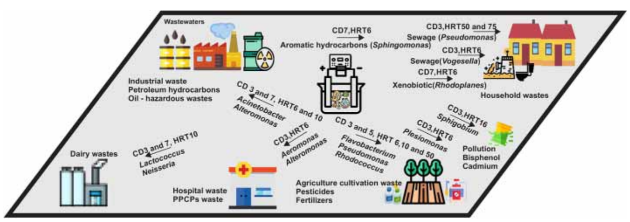
HIGHLIGHTS

- A conceptual framework for microbial communities in wastewater EBRs is proposed.
- Manipulation of operating conditions to specific types of wastewater is suggested.
- The conceptual model could assist in retrofitting wastewater EBRs.

GRAPHICAL ABSTRACT



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INTRODUCTION

Various wastewater treatment processes have been developed to reduce water pollution and improve drinking water quality. One of these wastewater treatment technologies is the wastewater electro-bioreactor (EBR), which has been the focus of many research studies elucidating the microbial community present in the bioreactors and electro-bioreactors (ElNaker *et al.* 2018a, 2019). Several developments and efforts have been made to functionally characterize the microorganisms associated with the removal of organic and inorganic nutrients from wastewater (Juretschko *et al.* 1998; Daims *et al.* 2015). The integration of electrochemical processes into membrane bioreactors (MBRs) combines biodegradation, electrochemical and membrane filtration processes into one system, achieving high effluent quality compared to conventional MBRs and activated sludge processes (Giwa & Hasan 2015a, 2015b, 2017; Giwa *et al.* 2016a, 2016b; Ahmed & Hasan 2017). Bioelectrochemical systems have been reported to treat recalcitrant wastewaters (Xu *et al.* 2016; Chen *et al.* 2019). These studies have confirmed that coupling bio-electrochemical systems with up-flow anaerobic sludge blanket (UASB) has resulted in high removal efficiency.

Models are a standard tool for engineers to describe important processes and functionally defined populations in wastewater treatment plants (WWTPs) (Derco *et al.* 1990; Henze 2008; Valentín-Vargas *et al.* 2012). Many models have been developed, such as the activated sludge models: ASM1, which focused on the degradation of organic carbon material and nitrogen removal (nitrification and denitrification); ASM2, which included biological phosphorus removal; ASM2d, which included the denitrifying phosphorus accumulating organisms (PAOs); and ASM3, which recognizes the importance of storage polymers in the heterotrophic activated sludge conversions (Henze *et al.* 2015; Arif *et al.* 2018).

Other dynamic and simulation models have also been developed for the modification of the activated sludge process and to solve the complexity of several wastewater treatment processes, including moving bed bioreactors (Charfi *et al.* 2018a, 2018b; Leyva-Díaz *et al.* 2020), MBRs (Giwa *et al.* 2019; Mannina *et al.* 2020), membrane fouling control (Charfi *et al.* 2018a, 2018b; Song *et al.* 2020) and integrated MBR model for greenhouse gas emission (Mannina *et al.* 2018, 2020). These models are a sophisticated way to evaluate process alternatives, optimize design, and analyze and evaluate costs.

Conceptual modeling is a representation of a system that uses ideas and concepts to formulate such a schematic. Conceptual models are developed after process generalization or conceptualization. A recent research study proposed a conceptual ecosystem model of an enhanced biological phosphorus removal (EBPR) system based on the microbial communities in full-scale EBPR plants in Denmark (Nielsen *et al.* 2010). Their results revealed that a limited number of identical species constitute the majority of communities in all plants. Their model encompasses knowledge about core species in different microbial functional groups and their specific physiological properties.

Conceptual frameworks and designs were developed to facilitate the complexity of wastewater treatment processes. Previous models included either both the analysis and optimization of the process design or modeling of the full scale of microbial communities' structure in WWTPs (Yuan & Blackall 2002; Daims *et al.* 2006; Comas *et al.* 2008; Henze 2008). Hu *et al.* (2019) developed a neural network for an up-flow bio-electrochemical system for treating b-lactams pharmaceutical wastewater under different hydraulic retention times (HRTs) (Hu *et al.* 2019). Indeed, theoretical and mathematical modeling of EBRs with a focus on the system operational conditions, performance and optimization has been investigated in previous studies; yet there has been limited investigation of microbial community structure and function. However, these models do not integrate the electro-bioreactor operating conditions, performance, and microbial community structure and function.

Focusing on this approach, our laboratory investigated the impact of applying electric fields at different current densities (CD) of 3, 5 and 7 A/m^2 for EBRs operated in aerobic conditions. These operating conditions were selected based on our previous study (Zeyoudi *et al.* 2015), and industrial scale MBR WWTPs (Tchobanoglous *et al.* 2003). The operation of the electrochemical process under aerobic conditions in EBRs enhances the removal efficiency and diversifies the functional microbial community where oxygen, as an electron acceptor, has a major role in the process of oxidation of organic matter and bacterial metabolism.

The identification and analysis of microbial communities in control bioreactors (CBRs) and EBRs operated for 24 hours at CDs of 3, 5 and 7 A/m^2 and HRT of

6 hours has previously been published (ElNaker *et al.* 2018a, 2018b, 2019). Average abundance of major functional bacterial groups at the phylum level in both CBRs and EBRs at different CDs and HRTs are summarized in Figure 1. A quick summary of various bacterial phyla demonstrated that Proteobacteria and Bacteroidetes accounted for the largest fraction (33.2–35.3% and 30.4–32.3% average abundance, respectively, across all samples), followed by Chloroflexi (10.0–10.6%), Planctomycetes (9.9–10.5%), Verrucomicrobia (7.1–7.5%) and Nitrospirae (4.2–4.5%) (ElNaker *et al.* 2018a, 2018b).

The main objective of this study was to present a conceptual model for the microbial communities in wastewater EBRs operated at different CDs and HRTs with efficient removal of soluble chemical oxygen demand (sCOD), phosphorus and nitrogen. The model was based on our recent comprehensive quantitative data on the microbial community structure of EBRs as well as a review of data from other relevant literature. To the best of our knowledge, this study is the first microbial community conceptual model that extends simple community characterization approaches and provides a framework that can aid engineers in the design and operation of wastewater EBRs. The conceptual framework proposed in this study will be useful to assist in the management of the WWTPs treating wastewaters around the world.

METHODS

EBR design, microbial community analysis and wastewater characterization

The conceptual model presented here was based on quantitative data on microbial populations present in CBRs that did not use electric current as part of the treatment process and EBRs following the methodology shown in Figure 2 (ElNaker *et al.* 2018a, 2018b, 2019). All the experiments utilized activated sludge samples collected from the MBR plant in Masdar City (Abu Dhabi, United Arab Emirates), which was used as inoculum in batch EBRs operated for 24 hours. Fresh collected activated sludge was fed with synthetic wastewater (0.2% glucose, 1.5 mM ammonium sulfate, 270 μM potassium phosphate, 160 μM magnesium sulfate, 20 μM manganese sulfate, 1.47 μM iron (III) chloride, 20 μM calcium chloride, 330 μM potassium chloride, 300 μM sodium bicarbonate) in aerobic batch CBRs and EBRs. Dissolved oxygen (DO; mg/L), pH, temperature (T; °C) and electrical conductivity (EC; μS/cm) of the synthetic wastewater were analyzed using a HACH HQ40d single-input multi-meter (Hach Company, Loveland, CO, USA). An aluminum anode and a stainless steel cathode were used as the electrodes and were connected to DC power supplies. A 50-mL sample was collected from each reactor cell and centrifuged for 15 min at 3885xg. The supernatant was analyzed by measuring sCOD, phosphorus (as

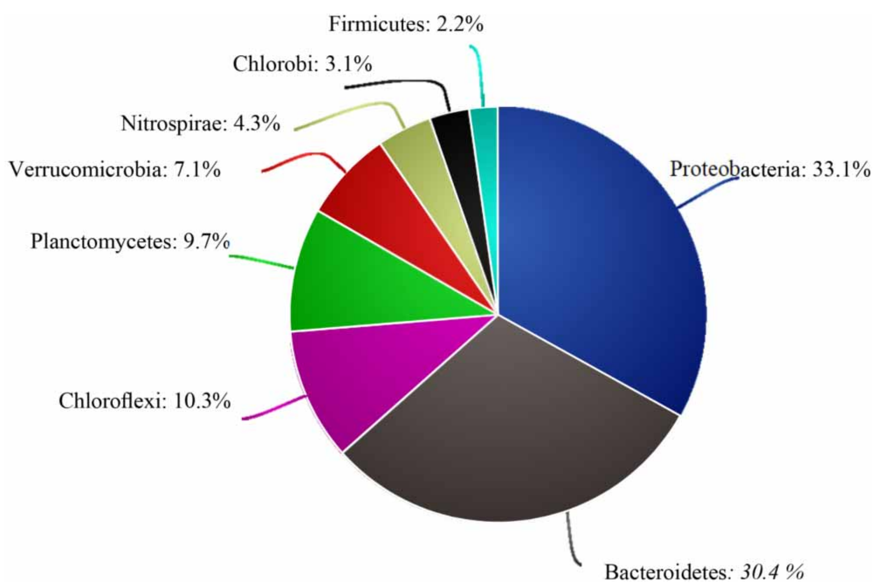

Figure 1 | Average abundance of major functional bacterial groups at phylum level in both CBRs and EBRs at different CDs and HRTs.

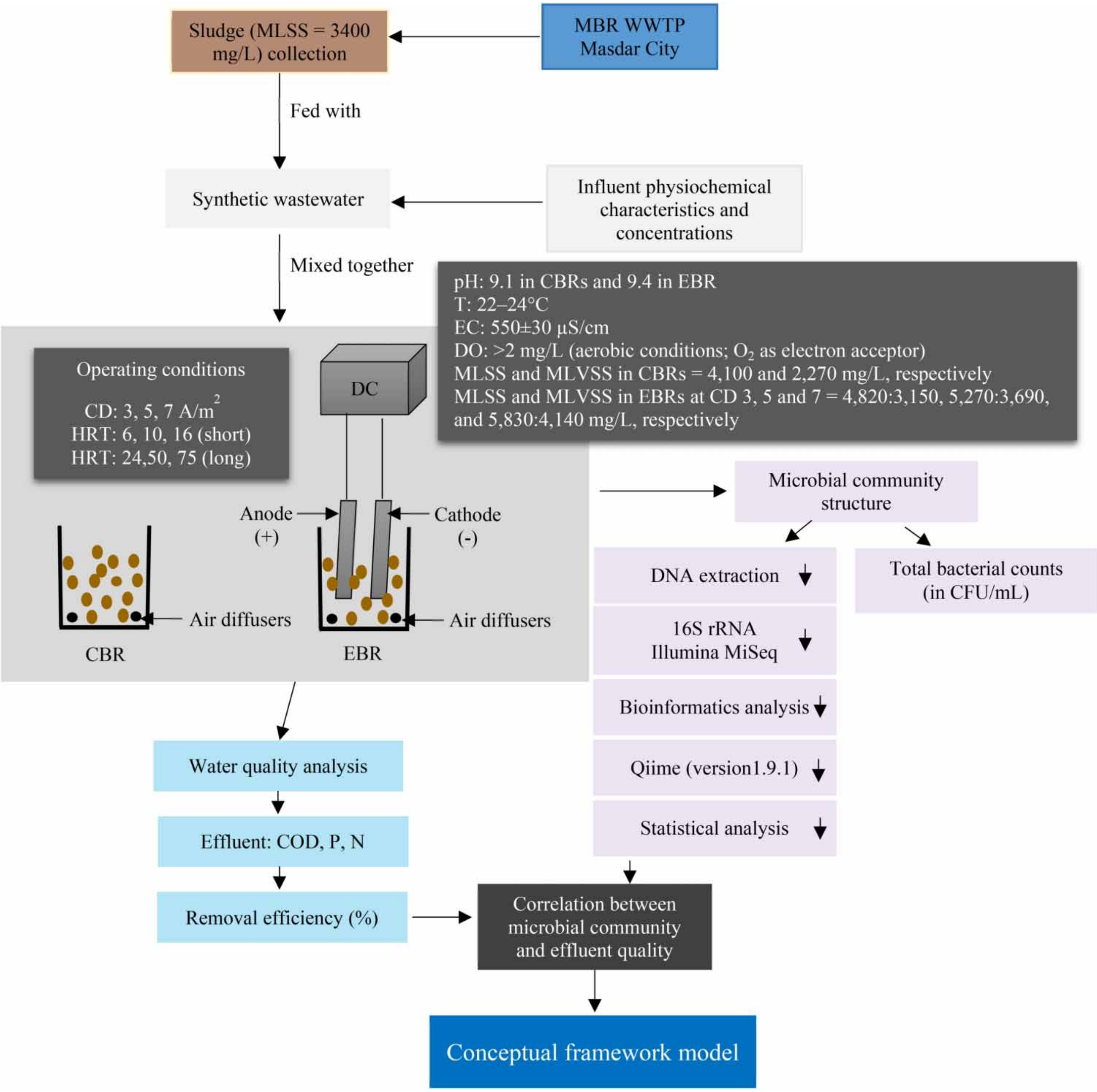

Figure 2 | Methodology flowchart of EBR and CBR.

PO_4^{3-}-P), ammonia (as NH_4^+-N) and nitrate (as NO_3^--N) using HACH vials LCK 314-1014, LCK 348-349, LCK 303-304 and LCK 339-340, respectively, according to manufacturer's instructions. The EBRs were operated at CDs of 3, 5 and 7 A/m^2 and a HRT of 6 hours. The effect of varying HRT on CBR and EBR performance was evaluated at HRTs of 6, 10, 16, 24, 50 and 75 hours.

Conceptual framework establishment, components and assumptions

Conceptual models consist of four main components based on Robison's framework (Robinson 2002): objectives (experiment objective), inputs (experimental factors), outputs (responses/outcomes) and model content (scope and level of detail), identifying any assumptions and simplifications. These fundamentals were implemented to develop the conceptual model presented in this study. An overview diagram of how our model was developed is presented in Figure 3. Operating conditions, such as CD and HRT, were the inputs, with outputs being the microbial communities that developed under different parameter configurations and the pollutant removal efficiencies.

RESULTS AND DISCUSSION

Conceptual framework modeling of EBRs and CBRs

Biological wastewater treatment models are founded on the number of functional microbial groups with associated kinetics, and the concentrations of electron donors (such as COD) and electron acceptors (such as oxygen, nitrate, nitrite) (Henze 2008; Nielsen *et al.* 2010). This study is the first to present a model developed for EBRs, as previous conceptual models and designs focused on either the reactor performance or the microbial communities, with limited integration of the two. The conceptual model proposed is anticipated to provide an overview of the functional microbial communities in wastewater EBRs by including:

(a) Microbial communities in CBRs and EBRs operated at different CDs and HRTs with efficient COD, P and N removal.
(b) Functional microbial communities enriched in CBRs and EBRs.

Conceptual framework modeling of microbial communities present in EBRs and their correlation with bioreactor function

The conceptual model included all functional microbial communities enriched and depleted in the EBRs at CDs of 3 A/m^2-EBRshort (3-EBRshort), 5 A/m^2-EBRshort (5-EBRshort) and 7 A/m^2-EBRshort (7-EBRshort) and a short HRT at a CD of 3 A/m^2 (3-EBRshort) and a long HRT at a CD of 3 A/m^2 (3-EBRlong) in relation to nutrient removal is presented in Figure 4(a). The functional

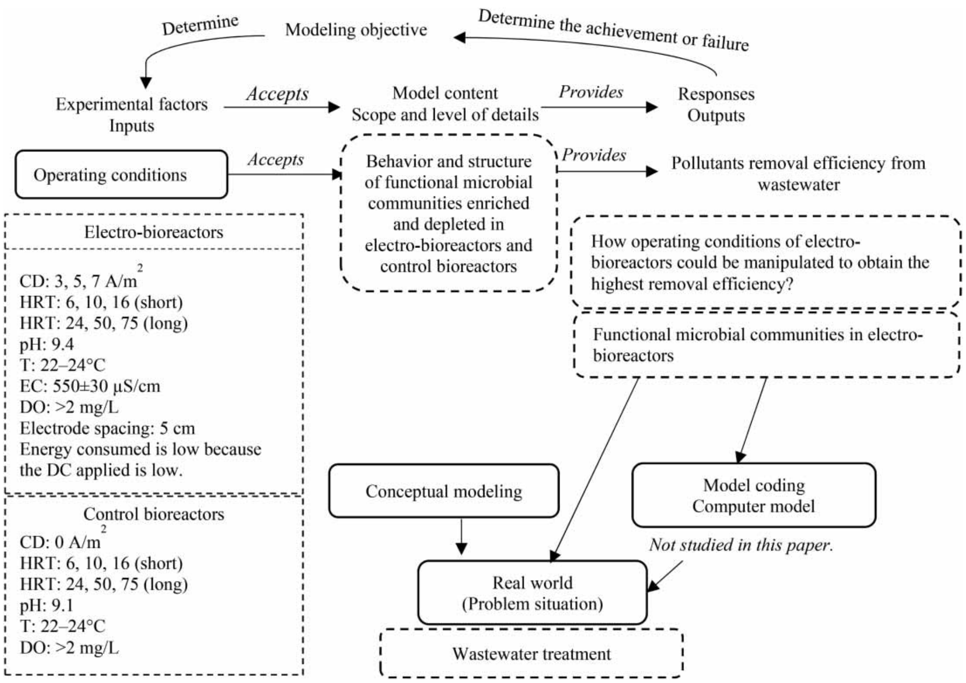

Figure 3 | Overview of conceptual model establishment, development, factors and assumptions.

Assumptions:
The conceptual model developed in this study was clearly based on our previous experimental research studies and thus it relies on the operating conditions of the electro-bioreactors and control bioreactor. The impact of pH, EC, DO, and sludge retention time (SRT) is significant, as widely reported in the literature, so the operation of electro-bioreactors and control bioreactor under pH, EC, DO, and SRT was adjusted during all experimental investigations. It was hypothesized that CD and HRT were the most significant parameters affecting the performance of electro-bioreactors, hence were the main focus of this study. For simplification, voltage and energy consumed were not included in the model since electro-bioreactors operated at 3, 5 and 7 A/m^2, which required very low DC and therefore resulted in low energy consumption.

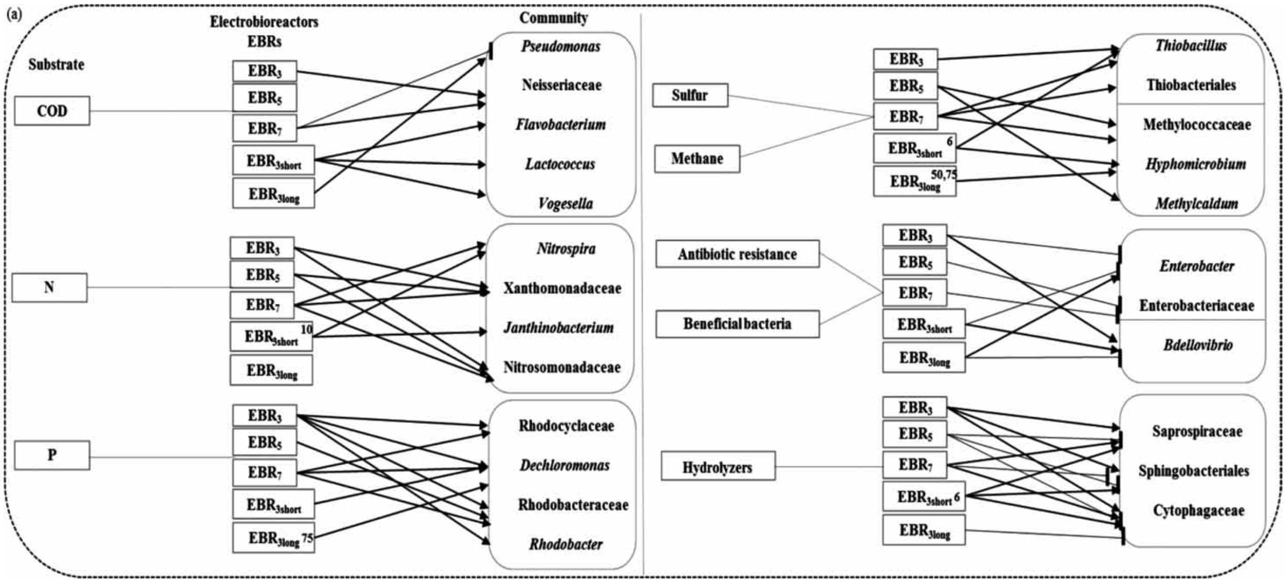

Figure 4 | (a) Conceptual model of how different microbial species associated with the degradation of pollutants in wastewater are affected by varying CD and HRT in EBRs: linkage between substrate removal, EBRs operated at CDs of 3, 5 and 7 A/m^2 (EBR$_{3, 5, 7}$) and HRTs (short: 6, 10 and 16 hours and long: 24, 50 and 75 hours) and involved bacterial groups or species are shown. To obtain the highest removal efficiency of a given substrate, EBRs should be operated as illustrated in the model and consequently bacterial groups listed are either enriched (i.e. high abundance) or depleted (i.e. low abundance). For example, an EBR operated at a short HRT at 3 A/m^2 enriches the population of *Flavobacterium*, *Lactococcus* and *Vogesella*, which are all associated with efficient COD removal. Enriched (high abundance) → Depleted (low abundance) ——┫.

genera are listed in Table 1. Various concentrations of contaminants in the mixed liquor suspended solids (MLSS) and synthetic wastewater components were presented as input variables (Giwa *et al.* 2015). The mechanisms involved simultaneously are microbe-mediated biodegradation of organic pollutants and electrochemical coagulation via the application of an electric field (Giwa & Hasan 2015a, 2015b). In WWTPs, quantitative changes between autotrophic and heterotrophic microbial communities are affected by wastewater characteristics, and the type and operation of the technological system (Hu *et al.* 2012; Ju & Zhang 2014; Muszyński *et al.* 2015).

The observations of bacterial groups enriched or depleted in EBRs associated with sCOD, N and P removal are shown in Figure 4(a). A summary of the model is provided below:

- In wastewater EBRs, sCOD removal was high (95–98%) and improved as the CD increased. Functional bacterial species Neisseriaceae (Treimo *et al.* 2006; Porwal *et al.* 2015) associated with COD removal were enriched in both 3-EBR and 7-EBR. *Pseudomonas* species were depleted in 7-EBR and enriched in 3-EBRlong. *Flavobacterium*, *Lactococcus* and *Vogesella* (Nielsen *et al.* 2012) were enriched in 3-EBRshort.
- Efficient N removal (99%) was directly correlated with the abundance of *Nitrospira*, Xanthomonadaceae, *Janthinobacterium* and Nitrosomonadaceae (Weissbrodt *et al.* 2014; Wells *et al.* 2014). Populations of Nitrospira, Xanthomonadaceae and Nitrosomonadaceae were enriched in 7-EBR and 5-EBR, which might explain the high removal efficiency in both of these EBRs compared to 3-EBR. *Nitrospira* and *Janthinobacterium* sp. were enriched in 3-EBRshort specifically operated at an HRT of 10 hours. In general, bacterial species associated with N removal were enriched in EBRs with higher CDs and shorter HRTs.
- P removal (97%) was directly correlated with the abundance of Rhodocyclaceae, *Dechloromonas*, Rhodobacteraceae and *Rhodobacter* (Bond *et al.* 1995) bacterial communities, which were enriched in 3-EBR and 7-EBR compared to 5-EBR. *Dechloromonas* species (Ahn *et al.* 2007; Zhang *et al.* 2017) were enriched in 3-EBR, 7-EBR and 3-EBRlong operated at HRT of 75 hours. This suggests that *Dechloromonas* species favored a wide range of operating conditions of EBRs, which correlates to the high removal efficiency of P in EBRs.
- Sulfur reducing bacteria, *Thiobacillus* and Thiobacteriales (Madigan *et al.* 1997), were enriched in all EBRs, but were present at low levels (low OTU counts) (ElNaker *et al.* 2019). *Thiobacillus* species were enriched in 3-EBR and 7-EBR, while Thiobacteriales favored 7-EBR only. Both species favored 3-EBRshort operated at an

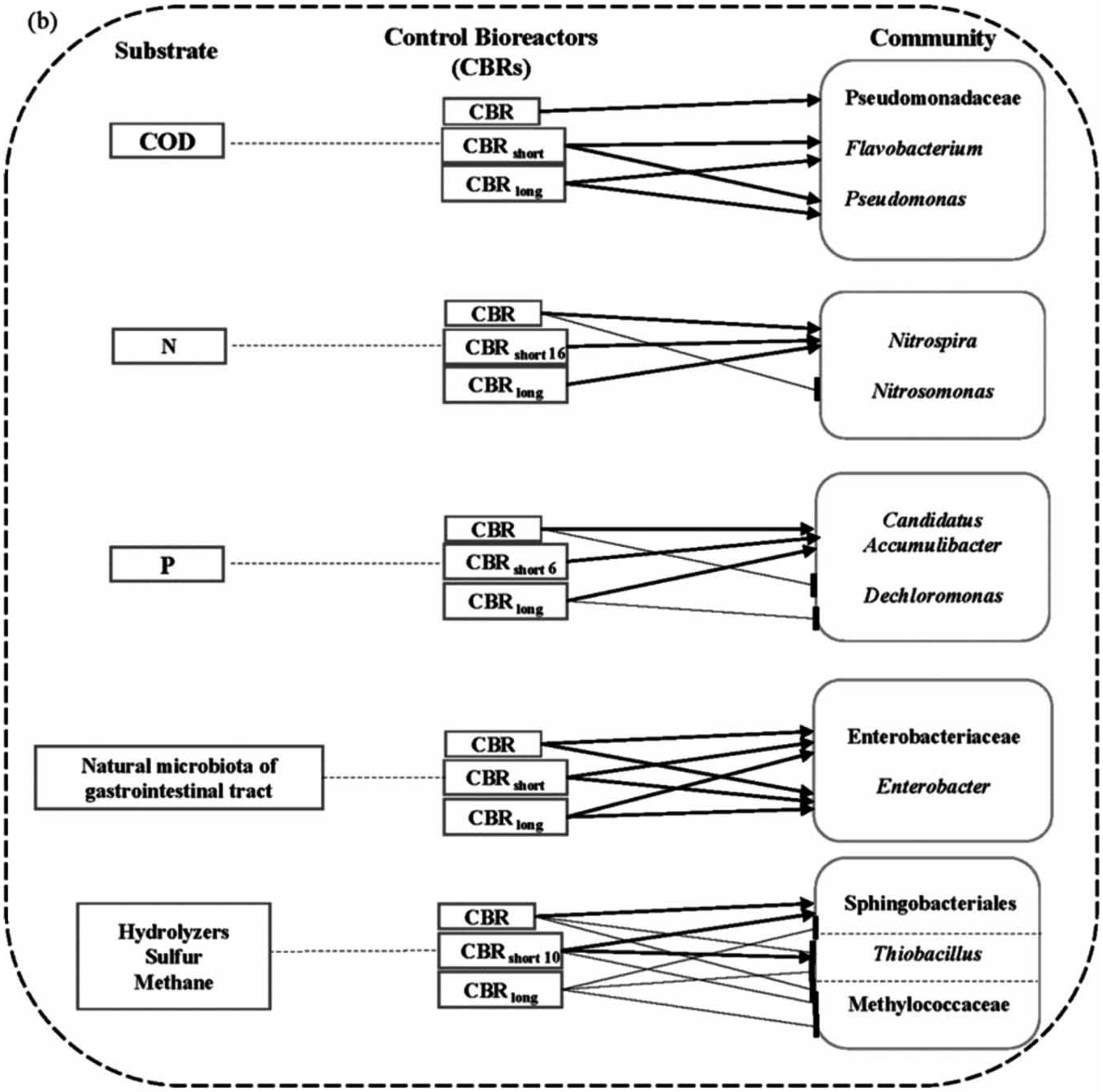

Figure 4 | (b) Conceptual model of how different microbial species associated with the degradation of pollutants in wastewater are affected by varying HRT in CBRs. The model can be read the same way as the model for EBRs illustrated in (a).

HRT of 6 hours. The low abundance of these bacteria could be due to the aerobic conditions maintained in these reactors as they have been reported to be physiologically active during anaerobic periods or in sludge storage tanks (Nielsen *et al.* 2010).

- Methanotrophs (methane oxidizing bacteria) Methylococcaceae, *Hyphomicrobium and Methylocaldum* (Bowman 2006) were detected in our EBRs with low average relative abundance (0.26%) across EBRs in comparison to other bacterial functional groups. Methylococcaceae and *Methylocaldum* were enriched in 5-EBR and 7-EBR while *Hyphomicrobium* favored 3-EBRshort operated at an HRT of 6 hours and 3-EBRlong operated at an HRT of 50 and 75 hours.
- Antibiotic resistance associated bacterial species *Enterobacter* (Lee *et al.* 2014) were depleted in all EBRs except for 3-EBRlong. Beneficial bacterial species *Bdellovibrio* (El-Shanshoury *et al.* 2016) were enriched in 3-EBR at all short HRTs. It is possible that enrichment of *Bdellovibrio*, which are predators of Gram-negative

Table 1 | Functional bacterial genera in EBRs versus CBRs

Genus	EBR	CBR
Dechloromonas	↑	↓
Nitrospira	↑	↓
Lactococcus	↑	↓
Pseudomonas	↑	↓
Hyphomicrobium	↑	↓
Janthinobacterium	↑	↓
Streptococcus	↑	↓
Bdellovibrio	↑	↓
Thiobacillus	↑	↓
Enterobacter	↓	↑
'*Candidatus Accumulibacter*'	↓	↑
Plesiomonas	↑	↓
Tolumonas	↓	↑

microbes, might explain the decrease in *Enterobacter* species, but that cannot be determined conclusively from the data collected. The data do suggest that using EBR technology over conventional MBR technology could potentially lead to a decrease in the spread of wastewater mediated antibiotic resistance. Future experiments should be developed to confirm this possibility.

- Hydrolyzers such as Saprospiraceae, Sphingobacteriales and Cytophagaceae (Bond *et al.* 1995; Kong *et al.* 2007; Xia *et al.* 2008; Zhang *et al.* 2018) species were enriched in 3-EBR. Sphingobacteriales favored 3-EBR and was depleted in 5-EBR and 7-EBR. Hydrolyzers were depleted in 5-EBR and 3-EBRlong. Cytophagaceae species favored both 3-EBR and 7-EBR.

From the model discussed in Figure 4(a), it could be noted that EBRs operated at CDs of 3, 5 and 7 A/m^2 have impacted the bacterial groups in various ways, which is a major finding for future EBR design. The conceptual modeling approach allowed a clear simplification of the interaction between the multiple processes that occur in EBRs, showing the impact of applying an electric field to microbial community structure, function and organic pollutant removal efficiency. This model can be updated as more data are collected, which in turn could be implemented to predict and improve microbial community structure and function, thus effectively removing organic pollutants and nutrients from wastewater.

Conceptual framework modeling of microbial communities present in CBRs and their correlation with bioreactor function

A conceptual model of functional microbial communities detected in CBRs operated at CBRshort (HRTs of 6, 10 and 16 hours) and CBRlong (HRTs of 24, 50 and 75 hours) is shown in Figure 4(b). A summary of the model is provided below:

- In CBRs, sCOD removal did not exceed 92% across all bioreactors. Two bacterial species associated with COD removal, *Pseudomonas* and *Flavobacterium*, (Xu *et al.* 2018a, 2018b), were enriched in both CBRshort and CBRlong.
- Efficient N removal in CBRs was correlated to *Nitrospira* species, which were enriched in CBRshort at HRTs of 6 and 16 hours and CBRlong at all HRTs tested. *Nitrosomonas* species were depleted in all CBR operating conditions.
- P removal in CBRs was associated with enrichment of '*Candidatus Accumulibacter*' (Stokholm-Bjerregaard *et al.* 2017) in CBRshort at an HRT of 6 hours and CBRlong while *Dechloromonas* species (Terashima *et al.* 2016) were depleted in all CBRs tested.
- *Enterobacter* species, which are the natural microbiota of gastrointestinal tracts and tend to be associated with the spread of antibiotic resistance, were enriched in all CBRs operated.
- Sulfur reducing bacteria *Thiobacillus* species (Ma *et al.* 2015) increased in abundance in CBRshort at an HRT of 10 hours while they decreased in the other CBRs tested.
- Methanotrophs (methane oxidizing bacteria) Methylococcaceae (Hatamoto *et al.* 2014) were inactive in all CBRs tested.
- Of the hydrolyzers, only Sphingobacteriales species were stimulated and enriched in CBRshort at HRTs of 6 and 10 hours and were depleted at CBRlong.

In general, it was observed that the absence of an electric field in CBRs as compared to EBRs resulted in the development of communities with lower relative abundances of bacterial species that have been shown in many previous studies to correlate well with removal of N and P containing nutrients. This observation also supported the hypothesis that the mechanism of action in EBRs includes the development of a superior water treatment microbial community in addition to electrochemical coagulation reactions that remove nutrients from wastewater, as thoroughly discussed in the next section.

Hypothesis of the assumed mechanisms of nutrient removal in EBRs

Operating conditions such as CD, HRT, aeration intensity and nutrients play a vital role in the microbial composition of the functional groups in EBRs. These parameters and variables most likely work together to affect the microbial community structure through different mechanisms. First, the electric field directly affects bacterial community structure through impacting the growth rates or survival rates of individual bacterial species in different ways, changing the available enzymatic milieu that then acts to degrade various impurities in the wastewater. Second, the presence of the current might alter substrate uptake kinetics as well as cellular uptake rates (ElNaker *et al.* 2019), which could also be a contributing factor for the enrichment and depletion of different bacterial species. Third, the bacterial community itself undergoes changes that result from interspecies interactions facilitated by the shift in environment (caused by the electric field or enzymatic action). An example of this is the depletion of *Enterobacter* species, which, as mentioned earlier, could result from an enrichment of *Bdellovibrio* species. These interactions do not have to be predator/prey interactions and can instead be interactions where one species produces molecules or byproducts that are either toxic or required for the growth of another species in the community.

Finally, the electrocoagulation process, where organic compounds form coagulates that precipitate out of the wastewater, indirectly affect the community by changing the concentrations of available nutrients in the wastewater. In combination with the electrocoagulation process, there is a biodegradation process in which microorganisms stimulated by the current oxidize or sequester these organic compounds. Aerobic conditions favor the formation of reactive oxygen species that are detrimental to living cells, but it appears that aerobic biofilms possess an arsenal of enzymes to protect them against oxidative stress (Erable *et al.* 2012; Sun *et al.* 2019). Accordingly, a slight layer of microbial biofilm could precipitate on the aluminum anode, which could have enhanced phosphorus removal. However, the EBRs operated in aerobic conditions reported an increase in permeate flux, enhancement of microbial growth and activity, and reduction of filtration resistance and sludge extracellular polymeric substances (EPS) content (Ensano *et al.* 2016). The forces exerted by the electric field, microbial community and nature of the wastewater work together to convert the wastewater into higher quality effluent water (ElNaker *et al.* 2018a, 2019) An illustration of the possible mechanisms for the removal of N, P, and COD when integrating the electrochemical process with biological treatment is shown in Figure 5. Key points from the illustration are summarized below:

- N removal in CBRs and EBRs could be achieved via two main mechanisms (Grady *et al.* 2011). The first is nitrification, conducted in a two-step process in which ammonia (NH_4^+) is oxidized into nitrite (NO_2^-) and nitrite is oxidized into nitrate (NO_3^-). This is achieved in CBRs by the nitrifiers; bacterial family Nitrospiraceae; *Nitrospira* genera (ammonia oxidizing bacteria) and *Nitrosomonas* (nitrite oxidizing bacteria) (Juretschko *et al.* 1998; Daims *et al.* 2015). The second is denitrification, which involves the biological oxidation of many organic substrates in wastewater treatment using nitrate or nitrite as an electron acceptor instead of oxygen. This could be carried out by microbes such as *Dechloromonas* and other species present in both CBRs and EBRs, which in turn result in effective nitrogen removal. In EBRs, *Nitrospira* and *Dechloromonas* species have been stimulated and increased in abundance in the presence of an electric field. It has been reported that the genome of the chemolithoautotrophic nitrifying bacteria encodes the pathways both for ammonia and nitrite oxidation, which are consequently activated during growth by ammonia oxidation to nitrate (Juretschko *et al.* 1998; Daims *et al.* 2015).
- P removal in CBRs is biologically accomplished by PAOs (Stokholm-Bjerregaard *et al.* 2017), which proliferate and store large quantities of inorganic phosphate as polyphosphate, thereby allowing phosphorus removal from wastewater via bioaccumulation. Multiple scenarios have been postulated to explain PAO metabolism, and they differ primarily in the source of the reducing power needed to form poly-β-hydroxyalkanoate (PHA) (Ahn *et al.* 2007). When the wastewater and biomass are under aerobic conditions, they have oxygen as an electron acceptor, so the PAOs carry out normal aerobic metabolism for growth by using the stored PHA as their carbon and energy source, generating ATP through electron transport phosphorylation.
- Among PAOs, members of *Dechloromonas* have also been reported as denitrifying PAO that can uptake acetate and act as denitrifiers. During denitrification, these species can take up phosphorus with nitrate acting as an electron acceptor. This genus likely contributes to denitrification and P removal in the reactors

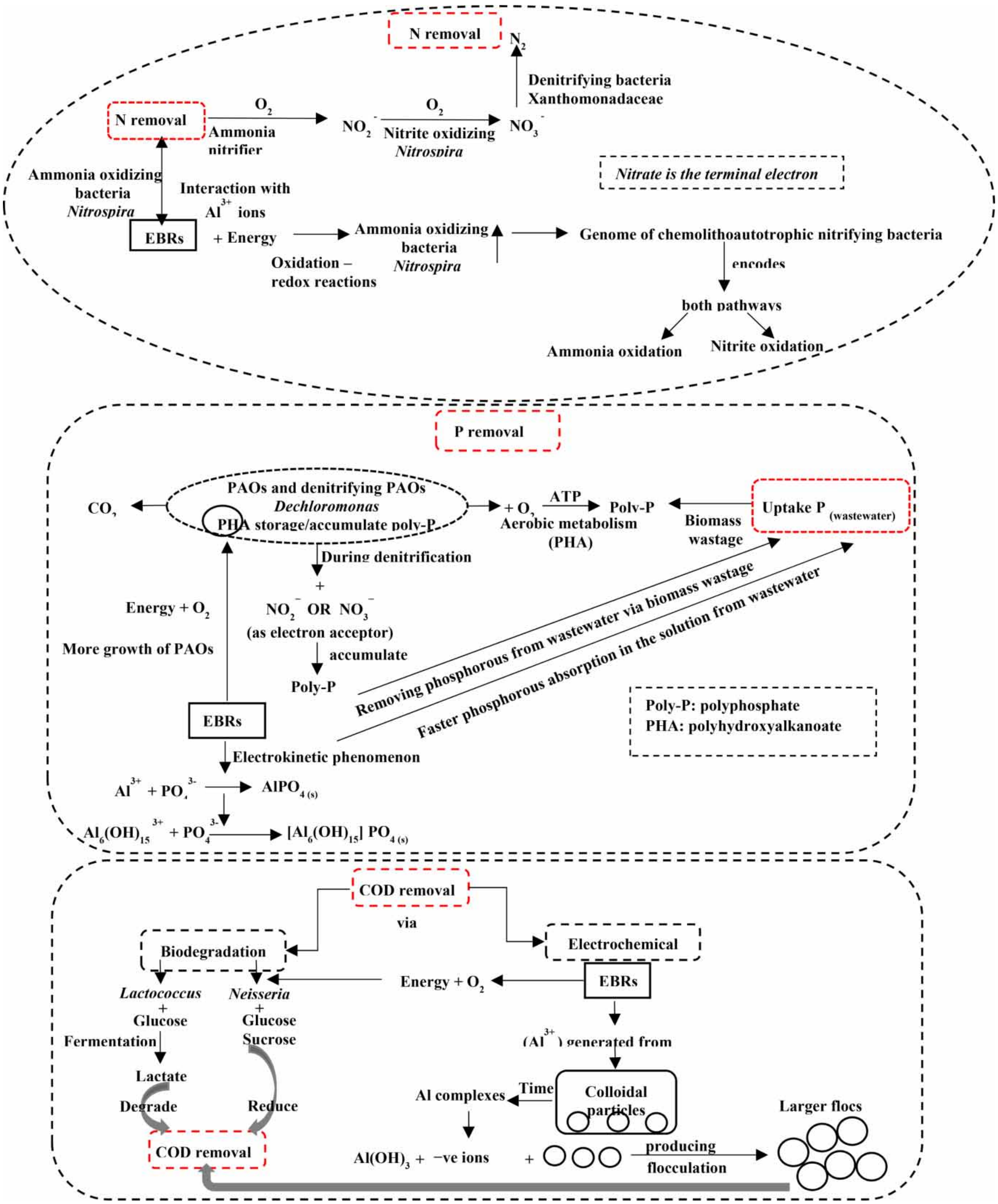

Figure 5 | Hypothetical illustration of the mechanisms of nutrient efficient removals when integrating the electrochemical process with biological treatment.

tested. In EBRs, P removal most likely occurs due to electrocoagulation in addition to electric current stimulation effects on bacterial communities associated with P removal.

- COD removal is complex and occurs via various processes: biodegradation and electrochemical oxidation and absorption. Biodegradation could be carried out by *Lactococcus* and *Neisseria* species that have been stimulated and increased in abundance in EBRs; they can acquire energy from the electrochemical oxidation that occurs due to the presence of an electric field in aerobic conditions. Those species have been reported to reduce and degrade COD in wastewaters typically discharged by the dairy industry (Porwal *et al.* 2015). Alongside the biodegradation process, when the electric field is applied, aluminum ions (Al^{3+}) are released in situ from the anode into the reactor, causing coagulation and flocculation of the negatively charged colloidal particles (Drews 2010; Bouamra *et al.* 2012). Consequently, long chains of Al hydroxide complexes are formed as larger flocs precipitating contaminants such as organic matter and P.

Manipulating operating conditions in EBRs that favor the enrichment of functional bacteria tailored to treat specific types of wastewater

Altering the operating conditions of a bioreactor will contribute to the evolution of different functional microbial communities, which in turn will aid in the removal of different nutrients from wastewater. This means that if engineers can predict how a parameter affects the microbial community, protocols with regards to operating conditions can be set up to match the characteristics of the wastewater being treated. Based on the conceptual models we have developed in this study, we have created a first draft of such a protocol, where recommendations are made for how to treat various types of wastewater, as illustrated in Figure 6. The suggested protocols are summarized below:

- Industrial wastewater, containing petroleum hydrocarbons, oil and hazardous materials are generally degraded by *Pseudomonas*, *Acinetobacter* and *Alteromonas* species (Yu *et al.* 2017; Xu *et al.* 2018a, 2018b; Chung *et al.* 2020). These species were enriched at CD3/HRT6, CD7/HRT6 as well as CD3/HRT10 (HRT in hours). Aromatic hydrocarbons could also be removed by *Shingomonas* species (Zylstra & Kim 1997), which were slightly enriched at CD7/HRT6.
- Household municipal wastewater containing sewage is degraded by *Pseudomonas* and *Vogesella* species (Yan *et al.* 2015). *Pseudomonas* species were enriched at CD3/HRT50 and CD3/HRT75, and *Vogesella* species were enriched at CD3/HRT6. In addition, Rhodoplanes species (Ma *et al.* 2015) were enriched at CD7/HRT6 and are reported to degrade xenobiotic substances (Zhu *et al.* 2019), which belong to a relatively new class of contaminants in municipal wastewater called emerging pollutants.
- Pollution that is caused by hazardous compounds such as bisphenol A and heavy metals, such as cadmium, could be treated by *Sphigobium* and *Plesiomonas* species (Sasaki *et al.* 2005; Zielińska *et al.* 2014), which increased in abundance at CD3/HRT16 and CD3/HRT6, respectively.

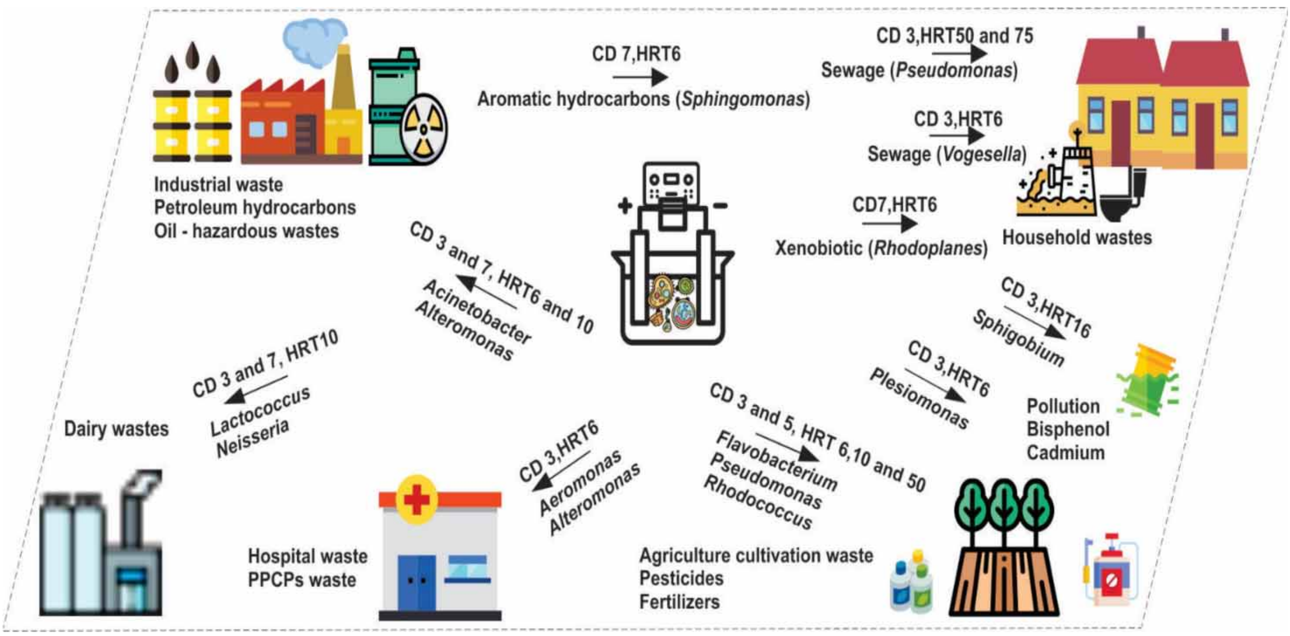

Figure 6 | Schematic diagram showing manipulation of the operating conditions that favor functional bacteria relevant to specific types of wastewater.

- Agricultural wastewater, which typically contains fertilizers and pesticides, could be treated by bacterial species such as *Flavobacterium*, *Pseudomonas* and *Rhodococcus* (Huang *et al.* 2018), which were enriched in EBRs operated at CD3 and CD5 at HRTs of 6, 10 and 50 hours.
- Wastewater generated by hospitals, the pharmaceutical and the personal care products (PPCP) industries contain heavy metals and toxic chemical compounds such as Cu, Fe, Cd, Pb, Hg, Ni, Pt, cyanide, phenol and others. This type of wastewater could be biodegraded by *Aeromonas* and *Alteromonas* species (Cruz *et al.* 2007; Selvin *et al.* 2009; Matyar *et al.* 2010; Lee *et al.* 2014; Mohamed 2016), which favored EBRs operated at CD3/HRT6.
- Wastewater generated by the dairy industry could be treated by *Lactococcus* and *Neisseria* species, which are reported to degrade sCOD and dairy products (Treimo *et al.* 2006; Dong *et al.* 2011; Porwal *et al.* 2015). Both species increased in abundance at CD3/HRT10 and CD7/HRT6.

Taken together, operating an electro-bioreactor with specific combinations of CD and HRT (and under fixed pH, solids retention time, EC and DO as indicated earlier) can boost the electrocoagulation and bioactive process leading to evolution of numerous functional bacterial communities and result in a more efficient WWTP.

CONCLUSION

In this study, a new conceptual framework model for EBRs based on the detailed structure and function of microbial communities of EBRs operated at different CDs (3, 5, and 7 A/m^2) and HRTs (short: 6, 10 and 16 hours; and long: 24, 50 and 75 hours) was proposed. Specific bacterial groups were associated with efficient sCOD, N and P removal. The model is a first attempt at analyzing data from many previous studies in order to provide guidance for future studies of the structure and function of microbial communities exposed to electric fields. The model also allows for future experiments to test if altering the parameters of an electro-bioreactor can be tailored to the type of wastewater being treated. The model provides different mechanisms for the integration of electrocoagulation and biodegradation processes in efficient nutrient removal from wastewater. This conceptual model provides a framework for developing mathematical models in order to validate and predict how the treatment efficiency of EBRs can be improved by varying operating conditions. Finally, the model lays the foundation for the development of a more comprehensive quantitative model when more data is collected and published by different groups operating in this field.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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