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# **Constructed wetlands operated as bioelectrochemical systems for improvement and control of wastewater treatment**

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Thesis submitted in fulfilment of the requirements for the  
European Joint Doctorate (PhD) in

**Environmental Engineering**

Universitat Politècnica de Catalunya·Barcelona Tech

&

**Bioscience Engineering: Environmental Sciences and  
Technology**

Ghent University

European Joint Doctoral Training Programme on Sustainable  
Product, Energy and Resource Recovery from Wastewater  
(SuPER-W)

Dutch translation of the title:

Helofytenfilters opgezet als bio-elektrochemische systemen voor verbetering en controle van afvalwaterzuivering

Illustration on the cover: **xxxx**

Please refer to this work as:

Hartl M. (2020) Constructed wetlands operated as bioelectrochemical systems for improvement and control of wastewater treatment. PhD thesis, UPC Barcelona, Spain and Ghent University, Belgium.

ISBN: **XXXX**

This work is part of an European joint doctorate programme called SuPER-W (Sustainable Product, Energy and Resource Recovery from Wastewater) funded by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 676070. This communication reflects only the authors' view and the Research Executive Agency of the EU is not responsible for any use that may be made of the information it contains.

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## List of notations and abbreviations

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Anammox	Anaerobic ammonia oxidation
AC	Activated carbon
API	Active pharmaceutical ingredient
ATP	Adenosine triphosphate
BES	Bioelectrochemical systems
BOD	Biochemical oxygen demand
CBZ	Carbamazepine
CE	Coulombic efficiency
CEC	Contaminant of emerging concern
CF	Carbon felt
COD	Chemical oxygen demand
CV	Cyclic voltammetry
CW	Constructed wetland
CW-BES	Constructed wetland operated as bioelectrochemical system
CW-MFC	Constructed wetland operated as microbial fuel cell
CW-MEC	Constructed wetland operated as microbial electrolysis cell
DIET	Direct interspecies electron transfer
DO	Dissolved oxygen
DEET	Direct extracellular electron transfer
DNB	Denitrifying bacteria
DNRA	Dissimilatory nitrate reduction to ammonium
EAB	Electrochemically active bacteria
EET	Extracellular electron transfer
EDC	Endocrine-disrupting chemicals
EIS	Electrochemical impedance spectroscopy
$E_{an}$	Anode potential
$E_{cat}$	Cathode potential
$E_{cell}$	Measurable cell potential
$E_{emf}$	Maximum attainable potential
Emf	Electromotive force
FDA	Fluorescein diacetate
FWS	Free water surface

GF	Graphite felt
GAC	Granular activated carbon
H <sub>2</sub>	Hydrogen
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HF	Horizontal flow
HLR	Hydraulic loading rate
HRT	Hydraulic retention time
I	Current
IBU	Ibuprofen
MEET	Mediated extracellular electron transfer
MET	Microbial electrochemical technology
MEC	Microbial electrolysis cell
MFC	Microbial fuel cell
N <sub>2</sub>	Dinitrogen gas
N <sub>2</sub> O	Nitrous oxide
NADH	Nicotinamide adenine dinucleotide
NH <sub>4</sub> <sup>+</sup> -N	Ammonium-nitrogen
NO <sub>2</sub> <sup>-</sup> -N	Nitrite-nitrogen
NO <sub>3</sub> <sup>-</sup> -N	Nitrate-nitrogen
NPX	Naproxen
OCV	Open circuit voltage
OLR	Organic loading rate
OMP	Organic micropollutant
LCA	Life cycle assessment
MPP	Maximum power point
PAH	Polycyclic aromatic hydrocarbon
PC	Polarization curve
PCB	Polychlorinated biphenyl
PCR	Polymerase chain reaction
PE	Person equivalent
PEM	Proton exchange membrane
PMFC	Planted microbial fuel cell
PO <sub>4</sub> <sup>3-</sup> -P	Orthophosphate
PPCPs	Pharmaceuticals and personal care products
R <sub>ext</sub>	External resistance



$R_{int}$	Internal resistance
SMFC	Sediment microbial fuel cell
SND	Simultaneous nitrification and denitrification
SS	Suspended solids
SSF	Subsurface flow
SSM	Stainless steel mesh
TEA	Terminal electron acceptor
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorus
TSS	Total suspended solids
VF	Vertical flow
VFA	Volatile fatty acids
WW	Wastewater
WWTP	Wastewater treatment plant



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## Abstract

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The combination of constructed wetlands (CWs) and bioelectrochemical systems (BES) offers several opportunities. First of all, BES require a redox gradient between anode and cathode in order to drive bioelectrochemical processes, and CWs exhibit a pronounced natural redox gradient within the filter bed, especially when the CW is designed with a horizontal subsurface flow (HF) hydraulic regime. Electrochemically active bacteria (EAB) in BES utilize the energy gain from this redox gradient in order to act as catalysts and transfer electrons - derived from organic and inorganic matter oxidation - to the anode of the BES from where they flow to a higher redox potential at the cathode, creating a current in the opposite direction. Hence BES and CW-BES enable contaminant removal via a bioelectrochemical pathway while treating wastewater. If the BES are operated as microbial fuel cells (MFCs), a resistor or load is connected between anode and cathode, which additionally recovers a small amount of electric energy from the energy contained in the oxidized substrate. If the BES are operated as microbial electrolysis cells (MECs) an additional power source is applied instead of a load or resistance, hence no electricity is produced but otherwise thermodynamically unfavorable reactions can be achieved. Another advantage of MECs is that only an additional voltage of 0.2-0.8 V is required for water electrolysis to occur (usually 1.8-3.5 V are required), due to the current produced through the activity of EAB at the anode.

Therefore, CWs operated as BES such as MFC (CW-MFC) and MEC (CW-MEC), profit from each other's inherent features. The resulting synergies have the potential to improve contaminant removal and potentially allow for a broader range of contaminants to be treated. As a consequence, the relatively large required surface area per person equivalent (PE) of CWs could be reduced, which until now is one of the weak points of the technology. Furthermore, MFCs and CW-MFCs could potentially be used as a biosensor, since the produced current in an MFC is to some extent dependent and therefore correlated to the organic matter concentration in the wastewater.

Hence, the main objective of this work was the improvement and control of wastewater treatment using CW-MFCs and CW-MECs. For this purpose, eight meso-scale CW-BES systems with 0.2 m<sup>2</sup>

surface area were constructed, each with three BES in a row along the flow path, and liquid and gravel sampling ports in each transect in order to be able to observe processes within the filter beds. Unlike the majority of earlier research in the field, all systems received real urban wastewater and were operated in a realistic HF hydraulic regime.

The first experiment investigated the application of CW-MFC systems as a biosensor for chemical oxygen demand (COD) at the influent of the systems. Triplicate meso-scale CW-MFCs were periodically fed with real urban wastewater and showed good bio-indication responses between week 3 and 7 of operation (between an accumulated organic loading of ca. 100-200 g COD/m<sup>2</sup>). The majority of increases (75-80%) in COD concentrations at the influent could be detected after a response time of 2-4 h, however, the signal did not respond well to decreasing COD concentrations. Therefore, the CW-MFCs were suggested to be used as an “alarm-tool” for sudden increases in COD or contamination events. Nevertheless, the application of the assessment tool would require more research concerning the biosensor design and operation, especially in regards to the prolongation of functionality with acceptable bioindication ranges, response times and sensitivity.

The remaining experiments pivoted around improving contaminant removal using CW-BES. Initially duplicates of closed-circuit CW-MFC (CW-MFC+) were compared to an open-circuit CW-MFC (CW-MFC-) control and a conventional CW-control over a period of 23 weeks. The CW-MFC+ fed with a continuous flow produced an extremely statistically significant higher current density than the CW-MFC+ with intermittent flow, leading to the use of a continuous flow regime for the remaining experiments. Contaminant removal results showed no significant differences between tested organic loading rates (4.9±1.6, 6.7±1.4 and 13.6±3.2 g COD/m<sup>2</sup>·day), hydraulic regimes (intermittent vs. continuous flow) or different electrical connections. However, on average, CW-MFC+ with continuous flow outperformed other experimental conditions. In more detail, CW-MFC+ exhibited around 5% and 22% higher COD and ammonium removal, respectively, compared to conventional CW-control systems. Correspondingly, overall bacteria activity, as measured by the fluorescein diacetate technique, in CW-MFC+ was higher by 4% to 34% when compared to CW-control systems.



For the next experiment, a duplicate of CW-MEC systems was added to the CW-MFC+, CW-MFC- and CW-control duplicates, and wastewater treatment performance was assessed again. Results showed that average ammonium and COD removal was higher in CW-MEC (by 18% and 9%, respectively) and CW-MFC+ (by 16% and 6%, respectively) when compared to CW-control, while CW-MFC- performed similarly to the CW-control. This time also a microbial community analysis was performed and showed statistically significant differences in microbial structural composition of CW-MEC anodes and cathodes when compared to all other treatments. The most abundant species was *Sphingobium yanoikuyae* which has not been reported in CW-MEC, or in general in BES such as MFC or MEC before. However, the closely related genera *Sphingomonas* and *Sphingopyxis* were reported in other CW-MEC system. Probably due to the sampling method at the anode, only cathode samples of CW-MFC+ showed a microbial community significantly different from CW-MFC- and CW-control with relative high abundance of the species *Lysinibacillus boronitolerans*, which is closely related to *Lysinibacillus sphaericus*, a species which was also found in other MFC systems and was even identified to be electrochemically active.

During the final investigation CW-MEC, CW-MFC+ and CW-control duplicates were fed with wastewater spiked with organic micropollutants, more precisely the four pharmaceuticals carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBU) and naproxen (NPX). Higher removal rates were obtained for three out of the four compounds (CBZ, DCF and NPX) with an increase of 10-17% in CW-MEC and 5% in CW-MFC systems, compared to the CW-control. However, no statistically significant differences were found. IBU removal was similar amongst treatments.

Taken all together, the initial experiment on the use of CW-MFCs as a COD assessment tool revealed interesting results but more research in terms of strategies in order to maintain functional stability, as well as a reasonable response time, detection range and sensitivity are suggested in order to advance the technology. In terms of contaminant removal, CW-BES systems showed promising results especially for ammonium and certain organic micropollutants which are recalcitrant and not easily treated with other technologies. In any case, it is suggested to continue research and identify the precise processes, conditions and microbial communities which are responsible for these

improvements, in order to be able to design tailor-made CW-BES systems for the respective treatment goals in the future.

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## Resumen

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Los sistemas naturales de tratamiento de aguas residuales (tales como los humedales construidos – CWs) no sólo requieren de largos tiempos de retención hidráulicos debido al dominio de las reacciones anaeróbicas de eliminación de los contaminantes, si no que debido a su simplicidad de operación (ausencia casi completa de sistemas electro-mecánicos), también carecen de elementos de control del proceso. La combinación de CWs y sistemas bioelectroquímicos (BES) describe un escenario de combinación de tecnologías en el contexto del tratamiento de aguas residuales urbanas que permitiría no sólo la mejora del proceso de depuración, si no también incrementar los elementos de control del mismo. La combinación de CWs y BES (CW-BES) es posible ya que los CWs ofrecen de forma natural los tres elementos clave para que un BES pueda funcionar: *i)* la presencia natural de los elementos de catálisis de las reacciones bioelectroquímicas (bacterias electroactivas – EAB); *ii)* la presencia de materia orgánica como fuente de electrones (agua residual) y, *iii)* gradientes de oxidación- reducción (redox) marcados que representan la fuerza electromotriz de las reacciones cinéticas de las EAB. Las EAB utilizan la existencia del gradiente redox entre los electrodos del CW-BES como motor para las reacciones de oxidación de la materia orgánica. Las EAB cederán los electrones producto de la degradación de la materia orgánica en la zona de bajo potencial redox del CW-BES (ánodo) y éstos fluirán a través del circuito del sistema hasta la zona de potencial más alto del CW-BES (cátodo), donde se combinarán biótica o abióticamente con un aceptor de electrones (generalmente el oxígeno). Los sistemas BES implementados en CW pueden ser operados como celda microbiana de combustible (MFC) o como celda microbiana de electrolisis (MEC). En la operación de CW-BES como MFC, se requiere un circuito eléctrico que opere bajo condiciones de carga (generalmente mediante la implementación de una resistencia externa) mientras que la operación de un CW-BES como MEC se requiere la adición de una fuente de energía externa (fuente de alimentación). Los CW-BES operados como MFC (CW-MFC) permiten la generación de energía, mientras que los CW-BES operados como MEC requieren de la adición de energía externa para conseguir reacciones que son termodinámicamente desfavorables. Una ventaja de la operación de CW-BES como sistemas MEC (CW-MEC) es que éstos sólo requieren de una adición pequeña de energía para

conseguir una diferencia de potencial adicional (0,2-0,8 V) que permita conseguir procesos electrolíticos que en otras condiciones requerirían de la energía suficiente para llegar a diferencias de potenciales mayores para la electrolisis (1,8-3,5 V).

En Los CW-BES, las EAB degradan la materia orgánica en condiciones anaeróbicas, pero obtienen más energía que las bacterias anaeróbicas tradicionales ya que su aceptor final de electrones es el oxígeno. Así mismo, el flujo de electrones que se obtiene en un CW-BES, es proporcional a la cantidad de combustible presente en el sistema (materia orgánica del agua residual). Por tanto, un CW-BES permite acelerar el proceso de depuración y actuar como elemento de control de estado de tratamiento (biosensor autónomo).

El objetivo principal de esta tesis es cuantificar la mejora y el grado de control del proceso de tratamiento de aguas residuales que podemos conseguir utilizando CW-MFC o CW-MEC. Para este propósito, ocho sistemas de humedales construidos de flujo horizontal a meso-escala fueron construidos y operados en distintos periodos experimentales como CW-MFC o CW-MEC. Cada sistema experimental se compone de tres BES en línea con el flujo de agua y cuyos electrodos están compuestos por grava (anodo) y fieltro de grafito (cátodo). Los sistemas experimentales fueron diseñados y construidos para obtener muestras de medio granular y muestras de agua intersticial a lo largo del proceso de tratamiento del agua. A diferencia de la mayoría de los estudios realizados por otros grupos de investigación sobre este tema, los sistemas experimentales aquí considerados fueron operados bajo condiciones realistas de flujo hidráulico de humedales construidos y utilizando agua residual urbana real.

Para abordar el objetivo principal planteado anteriormente se han realizado un total de cuatro experimentos encaminados a determinar: i) La capacidad bioindicación de las condiciones de carga orgánica de un sistema CW-MFC (Capítulo 4); ii) La mejora en la eficiencia de tratamiento de contaminantes convencionales en un sistema CW-MFC comparado con un sistema CW convencional (Capítulo 5); iii) La mejora en la eficiencia de tratamiento de contaminantes convencionales en un sistema CW-BES operado como MFC y MEC comparado con un sistema CW convencional; iv) La mejora en la eficiencia de tratamiento de contaminantes

convencionales en un sistema CW-BES operado como MFC y MEC comparado con un sistema CW convencional (Capítulo 6); y La mejora en la eficiencia de tratamiento de micro-contaminantes en un sistema CW-BES operado como MFC y MEC comparado con un sistema CW convencional (Capítulo 7). Cabe destacar, que para esclarecer las bases de los resultados obtenidos también se han realizado análisis de la actividad bacteriana y análisis de la comunidades microbianas de los sistemas experimentales (Capítulo 5 y 6 respectivamente).

A continuación se describen los experimentos realizados y los resultados principales obtenidos que dan lugar a los capítulos 4,5,6 y 7 de esta tesis doctoral.

El primer experimento abordó la aplicación de CW-MFC como biosensor de la DQO de entrada a los sistemas experimentales (Capítulo 4). Para tal efecto, tres CW-MFCs a meso-escala fueron periódicamente alimentados con agua residual urbana real. Los resultados de bioindicación fueron buenos entre las semanas 3 y 7 de operación (equivalente a cargas orgánicas acumuladas desde 100 a 200 g COD/m<sup>2</sup>. La mayoría de los episodios de carga orgánica (70-80% de los casos) fueron acompañados de un cambio significativo en la señal eléctrica, demostrando un gran potencial para la bioindicación. Sin embargo, no fue el caso para la disminución de las concentraciones, donde la señal eléctrica presentó un desfase respecto a la concentración de DQO de entrada que dificulta su utilización como herramienta de bioindicación. Debido a estos resultados (capacidad de bioindicación a cambios bruscos en la entrada de materia orgánica) se concluyó que los CW-MFC pueden ser utilizados como un “sensor alarma” de condiciones excepcionales de carga (episodios de vertido). Sin embargo, cabe destacar que se requiere más investigación en el diseño y operación de CW-MFC como herramienta de biosensorización para mejorar su funcionalidad, tiempo de respuesta y sensibilidad.

El siguiente experimento aborda la utilización de CW-BES para la eliminación de contaminantes (Capítulo 5). Inicialmente se utilizaron duplicados de CW-MFC en modo circuito cerrado (CW-MFC+) y se compararon con sistemas control donde los elementos físicos de los sistemas BES estaban presentes, pero el circuito no estaba cerrado (CW-MFC-) y con sistemas CW convencionales sin los elementos físicos del BES. En el diseño experimental también se incluyó el efecto

del tipo de flujo hidráulico (continuo vs discontinuo) y la carga orgánica. Este estudio se realizó durante un periodo de 23 semanas. Los sistemas CW-MFC+ alimentados en régimen hidráulico continuo produjeron una señal eléctrica significativamente más elevada que los CW-MFC+ operados bajo régimen hidráulico discontinuo. La eliminación de contaminantes no mostró ninguna diferencia significativa entre los diferentes sistemas operados a distintas cargas orgánicas ( $4.9 \pm 1.6$ ,  $6.7 \pm 1.4$  and  $13.6 \pm 3.2$  g COD/m<sup>2</sup>·dia), diferentes regímenes hidráulicos o tipo de conexión eléctrica. Sin embargo, aún y la ausencia de diferencias estadísticas, los sistemas conectados (CW-MFC+) operados en modo hidráulico continuo mostraron mejores rendimientos que el resto de condiciones testadas (5% y 22% mayores en relación a la eliminación de materia orgánica y amonio que el sistema control – CW). Durante este experimento, y con el objetivo de determinar si las mejoras en las eficiencias de tratamiento observadas se debían a una causa biológica, también se analizó la actividad bacteriana mediante la técnica de la FDA. Los resultados obtenidos del análisis de la actividad mostraron que efectivamente, los sistemas conectados (CW-MFC+) presentaban valores de actividad que superaban a los sistemas control (CW) entre un 4 y un 34%.

Durante el tercer experimento realizado (Capítulo 6), se utilizaron sistemas en alimentación en continuo operados como CW-MEC, CW-MFC+, CW-MFC- y CW, todas las condiciones por duplicado. En total, 8 sistemas experimentales fueron analizados en relación a la eliminación de contaminantes convencionales. Los resultados mostraron un promedio mayor en la eliminación de DQO y amonio para la condición CW-MEC (9 y 18% mayor que el sistema control CW, respectivamente). Los sistemas CW-MFC+ funcionaron un 16% y 6% mejor que los CW respecto a la eliminación de amonio y DQO, y los sistemas CW-MFC- funcionaron muy parecidos a los sistemas control CW. En este caso, y con el objetivo de determinar posibles diferencias a nivel de población bacteriana, se realizó un estudio poblacional sobre las comunidades microbianas en los ánodos y cátodos de todos los sistemas. Los resultados fueron que las poblaciones microbianas de ánodo y cátodo de los sistemas CW-MEC así como las muestras de ánodo de CW-MFC eran significativamente diferentes al resto de condiciones experimentales consideradas. En este sentido, se vió que que la especie más abundante para el caso de las CW-MEC fue *Sphingobium yanoikuyae*, especie que hasta el momento no se ha descrito en sistemas BES de

ningún tipo pero que está cerca de los géneros *Sphingomonas* y *Sphingopyxis* que sí han sido descritas anteriormente en CW-MEC. Debido probablemente a dificultades en el muestreo de la zona anódica (dificultades en realizar un muestreo homogéneo de la zona conectada del sistema), sólo las muestras de cátodo de los sistemas CW-MFC+ mostraron diferencias significativas en cuanto a composición microbiana se refiere respecto del resto de condiciones experimentales (CW-MFC- y CW). En este caso, las especies dominantes fueron *Lysinibacillus boronitolerans*, que está cerca de la especie *Lysinibacillus sphaericus*, que se ha descrito anteriormente en sistemas MFC convencionales e incluso se ha descrito como especie electroactiva.

Durante el experimento final de la tesis (Capítulo 7), se abordó la eliminación de microcontaminantes en condiciones CW-MEC, CW-MFC+ y CW. Todas las condiciones estudiadas en duplicado. Para el estudio de la eliminación de microcontaminantes se enriqueció el agua residual real utilizada con 4 productos farmacéuticos de uso común y que están ampliamente descritos en aguas residuales y son considerados de difícil biodegradación: i) Carbamacepina (CBZ), ii) Diclofenaco (DCF), iii) Ibuprofeno (IBU) y iv) Naproxeno (NPX). Los sistemas CW-BES (CW-MFC+ y CW-MEC) mejoraron la eliminación de 3 de los 4 compuestos considerados (CBZ, DCF y NPX). Más concretamente, la mejora de la eliminación de estos microcontaminantes fue entre un 10-17% mayor y alrededor de un 5% mayor para los sistemas CW-MEC y CW-MFC+, respectivamente, en relación a los sistemas control (CW).

Taken all together, the initial experiment on the use of CW-MFCs as a COD assessment tool revealed interesting results but more research in terms of strategies in order to maintain functional stability, as well as a reasonable response time, detection range and sensitivity are suggested in order to advance the technology. In terms of contaminant removal, CW-BES systems showed promising results especially for ammonium and certain organic micropollutants which are recalcitrant and not easily treated with other technologies. In any case, it is suggested to continue research and identify the precise processes, conditions and microbial communities which are responsible for these improvements, in order to be able to design tailor-made CW-BES systems for the respective treatment goals in the future.





## Samenvatting

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De combinatie van *constructed wetlands* (CW's) of helofytenfilters en bio-elektrochemische systemen (BES) biedt verschillende mogelijkheden. Allereerst hebben BES een redoxgradiënt nodig tussen anode en kathode om bio-elektrochemische processen mogelijk te maken, en CW's vertonen een uitgesproken natuurlijke redoxgradiënt in het filterbed, vooral wanneer de CW is ontworpen volgens het horizontaal, ondergronds doorstroomd principe (HF). Elektrochemisch actieve bacteriën (EAB's) in BES gebruiken de energiewinst van deze redoxgradiënt om als katalysatoren te fungeren en elektronen - afkomstig van oxidatie van organische en anorganische stoffen - over te brengen naar de anode van de BES vanwaar ze naar een hogere redoxpotentiaal stromen bij de kathode, waardoor een stroom in de tegenovergestelde richting gecreëerd wordt. Daarom maken BES en CW-BES verwijdering van verontreinigingen mogelijk via een bio-elektrochemische route terwijl afvalwater wordt gezuiverd. Als de BES worden gebruikt als microbiële brandstofcel (MFC), wordt een weerstand of belasting verbonden tussen anode en kathode, die een kleine hoeveelheid elektrische energie terugwint uit de energie in het geoxideerde substraat. Als de BES gebruikt worden als microbiële elektrolysecel (MEC), wordt een extra stroombron toegepast in plaats van een belasting of weerstand, waardoor er geen elektriciteit wordt geproduceerd, maar waardoor anders thermodynamisch ongunstige reacties mogelijk worden. Een ander voordeel van MEC's is dat alleen een extra spanning van 0,2-0,8 V nodig is om water elektrolyse te laten plaatsvinden (meestal is 1,8-3,5 V vereist), vanwege de stroom die wordt geproduceerd door de activiteit van EAB's aan de anode.

Daarom profiteren CW's met een BES component, zoals MFC (CW-MFC) en MEC (CW-MEC), van elkaars inherente kenmerken. De resulterende synergieën hebben het potentieel om de verwijdering van verontreinigingen te verbeteren en mogelijk een breder scala aan verontreinigingen te behandelen. Als gevolg hiervan zou de relatief grote vereiste oppervlakte per inwoner equivalent (IE) van CW's kunnen worden verminderd, wat tot nu toe een van de zwakke punten van de technologie is. Verder kunnen MFC's en CW-MFC's mogelijk worden gebruikt als een biosensor, omdat de geproduceerde stroom in een MFC tot op zekere hoogte afhankelijk is van en daarom gecorreleerd met de concentratie organische stof in het afvalwater.

Daarom was het hoofddoel van dit werk de verbetering en beheersing van afvalwaterzuivering met behulp van CW-MFC's en CW-MEC's. Voor dit doel werden acht mesoschaal CW-BES-systemen gebouwd, elk met drie BES in een rij langs het stroompad, en vloeistof- en grindmonsterpoorten in elk transect om processen binnen de filterbedden te kunnen observeren. In tegenstelling tot de meeste eerdere onderzoeken ontvingen alle systemen echt stedelijk afvalwater en werden ze bediend in een realistisch HF hydraulisch regime.

Het eerste experiment onderzocht de toepassing van CW-MFC-systemen als biosensor voor de chemische zuurstofvraag (CZV) in het influent van de systemen. CW-MFC's in drievoud op mesoschaal werden periodiek gevoed met echt stedelijk afvalwater en vertoonden goede bio-indicatiereducties tussen week 3 en 7 van bedrijf (tussen een geaccumuleerde organische belasting van ca. 100-200 g CZV / m<sup>2</sup>). De meeste toenames (75-80%) in CZV-concentraties bij het influent konden worden gedetecteerd na een responstijd van 2-4 uur, maar het signaal reageerde niet goed op afnemende CZV-concentraties. Daarom werd voorgesteld de CW-MFC's te gebruiken als een "alarmtool" voor plotselinge toename van CZV of besmetting. Desalniettemin zou de toepassing van het beoordelingsinstrument meer onderzoek vereisen naar het ontwerp en de werking van de biosensor, vooral met betrekking tot de verlenging van de functionaliteit met acceptabele bio-indicatiebereiken, responstijden en gevoeligheid.

De resterende experimenten draaiden rond het verbeteren van de verwijdering van verontreinigingen met CW-BES. Aanvankelijk werden duplicaten van CW-MFC met gesloten circuit (CW-MFC+) vergeleken met een controle CW-MFC (CW-MFC-) met open circuit en een conventionele CW-controle gedurende een periode van 23 weken. De CW-MFC+ gevoed met een continue influentstroom produceerde een extreem statistisch significant hogere stroomdichtheid dan de CW-MFC+ met intermitterende influentstroom, wat leidde tot het gebruik van een continu stroomregime voor de resterende experimenten. Resultaten van verwijdering van verontreinigende stoffen toonden geen significante verschillen tussen de geteste organische belastingen ( $4,9 \pm 1,6$ ,  $6,7 \pm 1,4$  en  $13,6 \pm 3,2$  g CZV / m<sup>2</sup> · dag), hydraulische regimes (intermitterende versus continue stroom) of verschillende elektrische verbindingen. Gemiddeld presteerde CW-MFC+ met continue stroom echter beter dan de andere opstellingen. Meer specifiek vertoonde CW-MFC+ respectievelijk ongeveer 5% en 22% hogere CZV- en

ammoniumverwijdering in vergelijking met conventionele CW-systemen. Dienovereenkomstig was de totale bacterieactiviteit, zoals gemeten met de fluoresceïnediacetaattechniek, in CW-MFC+ 4% tot 34% hoger in vergelijking met CW-controlesystemen.

Voor het volgende experiment werden twee CW-MEC-systemen toegevoegd aan de CW-MFC+, CW-MFC- en CW-controle duplicaten en werden de prestaties van de afvalwaterzuivering opnieuw beoordeeld. De resultaten toonden aan dat de gemiddelde verwijdering van ammonium en CZV hoger was in CW-MEC (met respectievelijk 18% en 9%) en CW-MFC+ (met respectievelijk 16% en 6%) in vergelijking met CW-controle, terwijl CW-MFC- gelijkaardige resultaten toonde als de CW-controle. Deze keer werd ook een microbiële gemeenschapsanalyse uitgevoerd; deze toonde statistisch significante verschillen in microbiële samenstelling van CW-MEC-anoden en kathoden in vergelijking met alle andere behandelingen. De meest voorkomende soort was *Sphingobium yanoikuyae* die niet eerder is gemeld in CW-MEC, of in het algemeen in BES zoals MFC of MEC. De nauw verwante geslachten *Sphingomonas* en *Sphingopyxis* werden echter gerapporteerd in andere CW-MEC-systemen. Waarschijnlijk als gevolg van de bemonsteringsmethode bij de anode, vertoonden alleen kathodemonsters van CW-MFC+ een microbiële gemeenschap die aanzienlijk verschilde van CW-MFC- en CW-controle. Hierbij bleek een relatief grote overvloed van de soort *Lysinibacillus boronitolerans*, die nauw verwant is aan *Lysinibacillus sphaericus*, een soort die ook in andere MFC-systemen werd gevonden en waarvan zelfs werd vastgesteld dat deze elektrochemisch actief was.

Finaal werden CW-MEC, CW-MFC+ en CW-controle duplicaten gevoed met afvalwater met daarin organische micropolluenten, meer bepaald de vier geneesmiddelen carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBU) en naproxen (NPX). Hogere verwijderingspercentages werden verkregen voor drie van de vier verbindingen (CBZ, DCF en NPX) met een toename van 10-17% in CW-MEC en 5% in CW-MFC-systemen, vergeleken met de CW-controle. Er werden echter geen statistisch significante verschillen gevonden. IBU-verwijdering was vergelijkbaar bij alle behandelingen.

Alles bij elkaar genomen heeft het eerste experiment met het gebruik van CW-MFC's als een CZV-beoordelingstool interessante resultaten opgeleverd, maar meer onderzoek naar strategieën om functionele stabiliteit te behouden, evenals een redelijke responstijd,

detectiebereik en gevoeligheid worden voorgesteld om de technologie vooruit te helpen. Wat betreft het verwijderen van verontreinigende stoffen, toonden CW-BES-systemen veelbelovende resultaten, met name voor ammonium en bepaalde organische microverontreinigingen die recalcitrant zijn en niet gemakkelijk met andere technologieën kunnen worden verwijderd. In ieder geval wordt voorgesteld om het onderzoek voort te zetten en de precieze processen, omstandigheden en microbiële gemeenschappen te identificeren die verantwoordelijk zijn voor deze verbeteringen, om in de toekomst op maat gemaakte CW-BES-systemen te kunnen ontwerpen voor de respectieve behandelingsdoelen.

# CHAPTER 1

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## Introduction, objectives and thesis outline

The overarching context of this thesis is a paradigm shift from energy intensive wastewater treatment plants to sustainable resource recovery plants. Recoverable resources from wastewater include energy (e.g. electrical, thermal, biofuels, biochemical), nutrients (e.g. phosphate, direct irrigation), asset recovery (e.g. building materials, metals, operating costs) and of course water itself, which can be reused in industry, irrigation, aquifer recharge, recreational purposes and after sanitizing even for potable water use (Wallis-Lage et al., 2011). The global pressures behind this shift to a more circular economy include water scarcity (9% of the world population lack improved drinking water), lack of sanitation (23% of the world's population do not have access to basic sanitation), depletion of resources (e.g. Phosphorus), pollution and destruction of the environment, and the associated health

risks which arise as a result (WHO and Unicef, 2015). The increase in world population and human activities all around the globe intensify these pressures (Hoekstra and Wiedmann, 2014). A UN report from 2017 called wastewater the “untapped resource” and stated that probably over 80% of the wastewater produced worldwide was still discharged to the environment without adequate treatment. In the recent decades a variety of technologies have been developed which could support and drive the above-proposed paradigm shift and tap wastewater resources, however, only few of them have been implemented due to technical (e.g. scalability) and non-technical bottlenecks such as economics, value chain development, environment and health, as well as societal and policy issues (Kehrein et al., 2020).

Constructed wetlands (CWs) are a well-established nature-based solution for wastewater treatment and have the potential to tap this resource and thereby aid the paradigm shift from wastewater treatment to resource recovery (Capodaglio, 2017). CWs treat wastewater from a wide range of sources, such as domestic, industrial and agricultural wastewater or landfill leachate, and are operated in different climate zones around the world (Langergraber and Haberl, 2001; Molle et al., 2005). They emulate processes occurring in natural wetland systems and utilize them for the conversion and removal of contaminants via physical, chemical and biological processes (García et al., 2010). In doing so, microbial removal processes are key for the removal of a variety of contaminants, including conventional contaminants in urban wastewater such as COD and ammonium (Faulwetter et al., 2009). The involved microbial communities are usually extremely diverse due to the variety of microenvironments within a CW bed, which depend on factors such as redox, pH, nutrient availability or pollutant concentration (Weber and Legge, 2011). Redox conditions are especially diverse on a spatial scale in the case of horizontal flow (HF) CWs, with aerobic zones at the top of the bed down to anoxic and anaerobic zones at the bottom.

Benefits of CWs include a relatively low cost, low operation and maintenance needs, low energy consumption, no requirement for chemicals as well as the possibility to use construction materials which are available locally in most parts of the world (García, 2001; Kivaisi, 2001; Puigagut et al., 2007b). Due to the utilization of natural processes they generally have a limited energy demand (Vymazal, 2011). A disadvantage of CWs is their relatively high area requirement per

person equivalent of 1-10 m<sup>2</sup>/PE (Kadlec and Wallace, 2009). Over the last decades several CW intensification strategies have been developed and implemented in order to lower the required area and to further improve contaminant removal, especially targeting total nitrogen and more recently also several emerging contaminants such as organic micropollutants. Most prominently artificial aeration has been applied widely, as well as recirculation of treated effluent. However these strategies increase the energy demand significantly and complicate the design and operation of the originally low-tech systems (Austin and Nivala, 2009).

A more recent and relatively new research field is a group of technologies called bioelectrochemical systems (BES), which include Microbial Fuel Cells (MFCs) and Microbial Electrolysis Cells (MECs). BES use electrochemically active bacteria (EAB) as catalysts in order to produce current from the oxidation of organic and inorganic compounds (Logan et al., 2006). If the BES are operated as microbial fuel cells (MFCs), a resistor or load is connected between anode and cathode, producing electricity from the energy contained in the wastewater. A microbial electrolysis cell (MEC) is basically a modified MFC, with the main difference that no electricity is produced but instead an external power is supplied in order to achieve otherwise thermodynamically unfavorable reactions at the cathode (Rozendal et al., 2006). An important aspect is that in an MEC, due to the already supplied electrons from the oxidation of (in)organic matter catalyzed by EAB at the anode, only an additional voltage of 0.2-0.8 V (e.g. solar panels would be sufficient) between the electrodes is required in order to overcome the thermodynamic barrier for water electrolysis to occur (usually 1.8-3.5 V are required) (Lu and Ren, 2016).

Both BES technologies, MFC and MEC, are able to use wastewater as a substrate and remove the contained contaminants at the same time. The combination of CWs and BES (CW-BES) results in a favorable synergy of the two technologies. First of all, the redox-gradient which is necessary between BES electrodes occurs naturally in CWs, especially if they are operated with a horizontal subsurface flow (HF) hydraulic regime (Corbella et al., 2014). Furthermore, recent research showed that CW-BES improved contaminant removal (Corbella and Puigagut, 2018), probably due to several mechanisms, including synergies between EAB and different microbial species (such as fermentative species) (Kiely et al., 2011b), and in the case of CW-

MEC due to direct and indirect effects related to electrolysis in the systems (Gao et al., 2017). Hence, the increased contaminant removal would result in a reduced required CW treatment area, and additional treatment pathways could enable the removal of emerging contaminants such as organic micropollutants (Corbella and Puigagut, 2018; Katuri et al., 2011). As a consequence, the combination of CW and BES would offer a greater potential for resource recovery from wastewater.

Another important factor in wastewater treatment and resource recovery is the control of the conditions and processes within the systems. The current which is produced by MFC and CW-MFC is a consequence of oxidation of organic and inorganic compounds catalyzed by EAB at the anode. Therefore it has been possible to correlate the MFC and CW-MFC signal with the influent COD concentration (Corbella et al., 2019; Di Lorenzo, 2015). The resulting advantages include the possibility to monitor systems on-line, in-situ and in real-time without the need of time-consuming analysis in a laboratory and without the negative side-effect of producing chemical waste from the used reactants.

The majority of earlier CW-BES studies used artificial wastewater and lab-scale reactors (often up-flow and batch fed), which is advantageous for the study of fundamental processes, but reflects real conditions to a limited extent only. The research in this work was conducted using real urban wastewater which was continuously fed to meso-scale HF systems and thus forms part of a further stage in the research on CW-BES systems for wastewater treatment and control.

The main **objective** of this work was the **improvement and control of wastewater treatment** using constructed wetlands operated as Microbial Fuel Cells (CW-MFCs) and Microbial Electrolysis Cells (CW-MECs). To date there are various research groups around the world working on this topic using similar or very different system architectures and operational strategies, which will be described in detail in the state of the art (**Chapter 3**), including basic information on the design and processes with regards to CWs and bioelectrochemical systems (BES) such as MFC and MEC.

The specific objective of **chapter 4** was to investigate constructed wetland microbial fuel cells (CW-MFCs) as a **biosensor** for bioindication, more precisely as a COD assessment tool. The



hypothesis was that the CW-MFC signal can be correlated to the influent COD content.

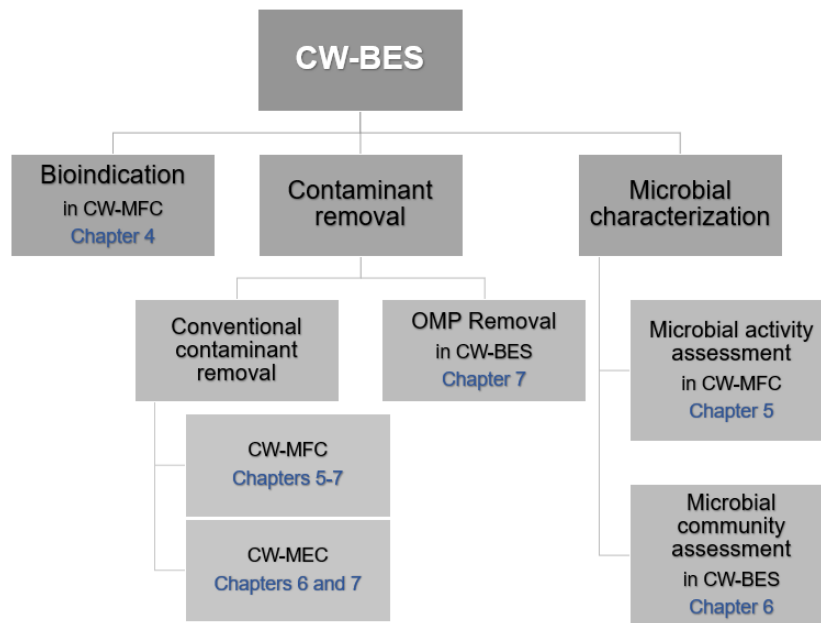
**Chapter 5** compared the **wastewater treatment performance of CW-MFCs** with the different electrical connections CW-MFC+ (closed-circuit) and CW-MFC- (open-circuit), to a conventional CW-control treatment. In the course of these experiments the specific objectives were to test **different operational conditions** such as hydraulic regime (continuous vs. intermittent) and different organic loading rates (OLRs). Furthermore, overall **bacteria activity**, as measured by the fluorescein diacetate technique, was assessed for different treatments. The hypotheses were that CW-MFC+ will outperform control treatments in terms of COD and ammonium removal, that continuous flow and low OLR will benefit contaminant removal, and that bacterial activity can be correlated to the treatment performance.

In a further stage, in **chapter 6**, another duplicate of systems using **CW-MECs** was added to the open- and closed-circuit CW-MFC and the CW-control, and again the wastewater treatment performance was compared in terms of conventional contaminants such as COD, ammonium, orthophosphate and sulfate. During this experiment, a **microbial community analysis** was performed in order to investigate differences in microbial composition within anodes and cathodes across treatments. The hypotheses were that CW-MEC and CW-MFC+ will outperform all other treatments due to the involved bioelectrochemical processes. Further hypotheses were that the microbial community will differ in CW-MEC and CW-MFC+ as compared to control systems.

After investigating the effect of CW-BES on conventional wastewater contaminants, **chapter 7** investigated the effect of CW-MEC, CW-MFC on selected **organic micropollutants** compared to the CW-control. The hypothesis was that CW-MEC and CW-MFC+ will improve organic micropollutants removal as compared to the CW-control system

Finally, in **chapter 8** the combined work is **discussed** in a wider context and **chapter 9** presents the **conclusions** as well as a future outlook and recommendations.

Figure 1.1 provides an overview on the principle thesis topics described above, together with an indication in which chapter they are addressed.



**Figure 1.1.** Schematic overview of the main thesis topics and the corresponding chapters

# CHAPTER 2

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## State of the art

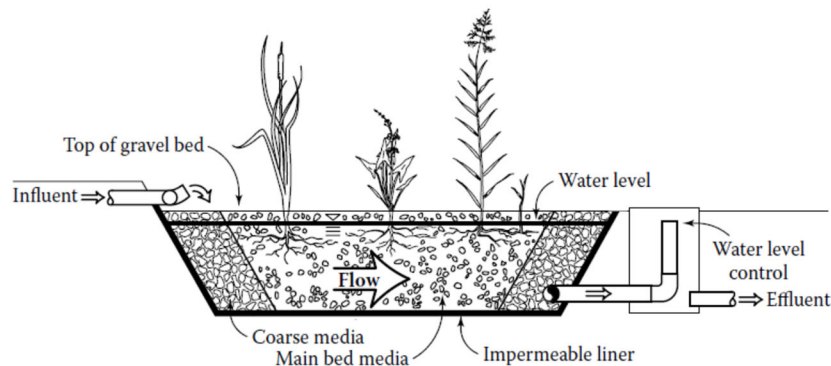
## 2.1 Constructed wetlands

### 2.1.1 General description

Constructed wetlands (CWs) for wastewater treatment are a well-established nature based solution (Vymazal, 2011). The utilized processes for the conversion and removal of contaminants are of physical, chemical and biological nature (García et al., 2010). The treatment beds consist of shallow lined basins filled with filter media (generally gravel or sand) and are commonly planted with aquatic macrophytes. CWs treat wastewater from a wide range of sources, such as domestic, industrial and agricultural wastewater or landfill leachate, and are situated in different climate zones around the world (Langergraber and Haberl, 2001; Molle et al., 2005). The systems are characterized by their low external energy demand, comparatively low cost, easy operation and maintenance as well as the possibility to use local materials and manpower. Moreover, the treated water can potentially be reused for different applications depending on the effluent quality and prevailing regulations (Arden and Ma, 2018; Masi and Martinuzzi, 2007; Nivala et al., 2019a). Hence, they have a strong potential for application as an alternative to conventional intensified systems in small communities, as well as small settlements in rural areas and emerging countries (García, 2001; Kivaisi, 2001; Puigagut et al., 2007a). Another advantage is that this treatment technology adds an aesthetic value to a landscape or urban environments and provides a variety of ecosystem services (Liquete et al., 2016). A disadvantage of CWs is their relative high area demand of ca. 1 to 10 m<sup>2</sup> per person equivalent (PE), depending on the type of wastewater and wetland configuration (Kadlec and Wallace, 2009). CWs can be distinguished and categorized by a multitude of different parameters. The most important are based on hydrology, macrophytic growth and flow path (Kadlec and Wallace, 2009). Three main types of CWs can be distinguished: free water surface (FWS), vertical subsurface flow (VF) and horizontal subsurface flow (HF) wetlands. HF CWs for urban wastewater treatment will be described in more detail in the following section, since this is the type used in the presented study.

### 2.1.2 Horizontal flow constructed wetlands

HF systems are the CW configuration most widely applied worldwide, and are commonly used for secondary treatment of wastewater from single-family homes, small cluster systems or small communities (Kadlec and Wallace, 2009). In the case of urban wastewater, an effective primary treatment is needed. The wastewater is fed to the HF CW through an inlet and flows horizontally through a filter bed containing a medium such as gravel (see Figure 2.1.1). The water table is kept ca. 10-15 cm below the bed surface. In this way, the wastewater is not exposed to the atmosphere which lowers the risk of pathogen transfer to humans or wildlife (Kadlec and Wallace, 2009).



**Figure 2.1.1.** Basic scheme of a HF CW (From Kadlec and Wallace (2009))

The HF wetland bed is normally around 0.3 to 0.6 m deep. Due to the water-saturated condition in the bed, anaerobic degradation processes are dominant because oxygen release by roots is too weak to facilitate aerobic processes in the lower parts of the bed. This creates a marked redox gradient between the aerobic zone at the interface with the atmosphere and the anaerobic lower filter bed zones. Further aspects which influence the treatment in HF CWs include design parameters such as organic loading rate (OLR), width to length ratio, size of the granular media and water depth (García et al., 2003). The resulting area requirement per person equivalent ( $\text{m}^2/\text{PE}$ ) for HF CWs is around 3-10  $\text{m}^2/\text{PE}$  (Hoffmann et al., 2011).

### **2.1.3 Contaminant removal processes**

CW systems exhibit a diverse set of removal processes and pathways which can facilitate a reduction or close to complete removal of conventional contaminants as well as trace metals, pathogens, viruses and other pollutants. These processes include microbially mediated processes, sedimentation, filtration, accretion, volatilization, sorption and plant uptake, which for the most part happen simultaneously (Dotro et al., 2017). The following section will describe removal processes of contaminants which were discussed in this work in detail and continue to focus on HF CWs for the treatment of urban wastewater.

#### **2.1.3.1 Organic matter**

The main removal processes for organic matter are sedimentation and filtration of particulate organic matter and biological degradation of dissolved organic matter. Retained particulates are further hydrolyzed and add onto the dissolved organic matter load. The following biological degradation of the dissolved fraction can be performed by a multitude of biological pathways, whereas anaerobic processes dominate HF CWs. In general, the dissolved organic matter serves as the electron donor in a chemical reaction which is induced by microorganisms. The electron is passed on to a specific compound which serves as the electron acceptor and usually defines the removal pathway. During this process energy is released which serves for the microorganisms cell growth (Dotro et al., 2017). Therefore, the redox state (aerobic, anoxic, anaerobic) is very important in determining the removal pathways (García et al., 2010). In the mostly anaerobic HF CWs, most energy can be theoretically released via (in order of decreasing energy release); denitrification, sulphate reduction and methanogenesis. Generally, most energy could be released via the aerobic respiration pathway which is the dominant process in other systems with more aerobic conditions, such as VF CWs. Removal rates of organic matter in HF CWs are usually >80% (Dotro et al., 2017).

### 2.1.3.2 Nitrogen

The majority of nitrogen in urban wastewater appears in the form of organic N and ammonium ( $\text{NH}_4^+\text{-N}$ ) and can be removed by a variety of processes, including microbial degradation, sorption and plant uptake (Vymazal, 2007). However, the pathways with a significant and dominant contribution to nitrogen removal in CWs involve a chain of microbially mediated conversion processes called nitrification and denitrification, which lead to the formation of nitrite ( $\text{NO}_2^-\text{-N}$ ), nitrate ( $\text{NO}_3^-\text{-N}$ ), nitrous oxide ( $\text{N}_2\text{O}$ ) and dissolved elemental nitrogen or dinitrogen gas ( $\text{N}_2$ ). First, during nitrification,  $\text{NH}_4^+\text{-N}$  is oxidized to nitrite ( $\text{NO}_2^-\text{-N}$ ) and further to nitrate ( $\text{NO}_3^-\text{-N}$ ), requiring aerobic conditions and a source of inorganic carbon as well as alkalinity and several micronutrients. After nitrification, denitrification would be necessary for full nitrogen removal. Denitrification is facilitated by facultative heterotrophic bacteria which reduce nitrate over several steps to nitrogen gas. The process requires not only relatively high amounts of carbon from dissolved organic matter and anoxic/anaerobic conditions but is also dependent on pH, redox potential, filter bed media and also requires a relatively high retention time within the bed. In regards to HF CWs, especially nitrate can be limiting for this process, since it is usually not present in sufficient amounts in the influent when using urban wastewater, and generated only to a very limited extent through autotrophic nitrification in HF CWs due to the lack of oxygen (García et al., 2004; Haberl et al., 2003). Also more unusual pathways like anaerobic ammonia oxidation (anammox) have been observed (Pelissari et al., 2016; Saeed and Sun, 2012). Most jurisdictions require the conversion of ammonium to nitrate. However, increasingly more jurisdictions expect total nitrogen (TN) removal from wastewater (Dotro et al., 2017). Therefore, multi-stage systems (e.g. VF and HF CWs in series) are widely used offering aerobic as well as anaerobic conditions in order to facilitate nitrification, denitrification and consequent high TN removal. HF CWs typically remove around 20 to 30% ammonium and 30 to 50% TN (Dotro et al., 2017). Nevertheless, some HF CWs have been reported to remove nitrogen to a relatively high extent with values of up to 68% (Puigagut et al., 2007b) and even above 80% in systems with shallower beds used by Caselles-Osorio and García (2007). The removal rates are also affected by factors like applied loading rate, temperature, pH or vegetation type (Akratos and Tsihrintzis, 2007; Kuschek et al., 2003).

### 2.1.3.3 Phosphorus

Main removal processes for phosphorus in CWs encompass chemical precipitation, sedimentation, sorption, as well as plant and microbial uptake. The majority of phosphorus occurs as organic phosphorus and orthophosphate ( $\text{PO}_4^{3-}\text{-P}$ ) in urban wastewater, whereas the organic phosphorus is mostly converted to orthophosphate as well. Secondary treatment of urban wastewater was only modest in HF CWs, with only 10% to 20% in the long term (Dotro et al., 2017). Phosphorus removal is generally limited in CWs due to the low sorption capacity of filter material like gravel or sand (Vymazal, 2005). Even reactive media especially made for the purpose of phosphorus removal have a finite capacity. If phosphorus removal is needed, an option is to build an additional, mostly unplanted, bed from which the reactive media can be replaced after being saturated (Dotro et al., 2017). In some areas it is common practice to dose chemical salts (iron or aluminum based) in the sedimentation tank upstream from the CW bed and filter out the precipitates (Brix and Arias, 2005; Lauschmann et al., 2013). This way more than 90% of phosphorus can be removed. However, the dosing makes the treatment more expensive and less environmentally friendly and thus has to still be optimized.

### 2.1.3.4 Sulfate

Urban wastewater contains sulfur originating from the potable water supply and different waste products. Also atmospheric deposition can play a role. Removal processes of sulfur in CWs include a multitude of possible interconversions depending on the conditions in the different zones and micro-regions within the bed, such as sulfide oxidation, sulfate reduction, adsorption, precipitation or volatile emissions. Under aerobic conditions sulfur is found in oxidized forms such as sulfite, sulfate and thiosulfate. Since HF CWs are rather anoxic/anaerobic with low redox, the sulfur will mainly be present in reduced forms such as sulfide, bisulfide and elemental sulfur. The conversion into volatile forms of sulfur, namely hydrogen sulfide and methylated sulfur, may promote the removal through loss to the atmosphere. However, the microbial processes leading to the formation of these volatile forms require a very low redox, i.e. very anaerobic conditions (Kadlec and Wallace, 2009). Hence, HF CWs sulfate removal rates were reported to



be in the range of 24% up to 88% (Huang et al., 2005), higher as compared to a median of only 14% in other CW systems (Kadlec and Wallace, 2009). Plant storage seems to only play a minor role. Although sulfur removal is normally not a treatment goal, sulfur processes are not independent from other nutrient cycles and can be very important in other contaminant removal processes, such as metal precipitation with metal sulfides. Due the dependency on sufficient biodegradable organic matter sulfate reduction can be in competition with similarly dependent processes, like nitrogen removal (Wiessner et al., 2005).

### 2.1.3.5 Organic micropollutants

Micropollutants encompass organic and inorganic substances with the potential of causing negative effects for the environment already at very low concentrations, i.e. in the order of micro, nano or pico-grams. Organic micropollutants (OMPs) include a large array of substances, such as pharmaceuticals, personal care products (PPCPs), hormones, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), endocrine-disrupting chemicals (EDCs) or pesticides. Inorganic micropollutants on the other side include “heavy metals” (e.g. Cd, Pb, Cu), “trace metals” (e.g. Fe, Mn) and metalloids (e.g. As, V).

This section will focus on pharmaceuticals within OMPs, as their removal is also investigated and discussed in chapter 7. Pharmaceuticals enter our environment mainly via human intake and consequent excretions, but can also originate from livestock excretions, domestic animals or manufacturing operations. In general, the human excretions arrive via sewer systems to wastewater treatment plants (WWTP) or septic systems and reach the wastewater effluent due to incomplete degradation along the way. Unfortunately, there is still a big gap of knowledge regarding the potential ecotoxicological effects of the active pharmaceutical ingredients (APIs) and their metabolites to the environment and human health (Barbosa et al., 2016; Garcia-Rodríguez et al., 2014). Traces of APIs were as well already found in drinking water, however, the low concentrations indicate that the risk to human health is low (Leung et al., 2013; Schwab et al., 2005). Some of these pollutants are also being considered as contaminants of emerging concern (CECs) or emergent contaminants, due to their

continuous release, presence in water bodies and potential ecotoxicological effects. Although there are no legal discharge limits for OMPs, some regulations have been published for several of them, in the case of European policy for example in the Decision 2015/495/EU of 20 March 2015 amending earlier regulations, which included OMPs such as the pharmaceutical diclofenac (DCF) as well as several antibiotics, hormones and pesticides (Barbosa et al., 2016).

CWs have shown to be able to remove a variety of pharmaceuticals from wastewater with promising results (Verlicchi and Zambello, 2014). Removal and treatment processes include microbial degradation, photodegradation, hydrolysis and plant uptake, while plants seem to affect the removal of some compounds mainly through root exudates in the rhizosphere (Y. Zhang et al., 2016). The removal efficiency of pharmaceuticals in CWs varies with design, operation and type of CW employed. In general, VF CWs and intensified (aerated) CWs have shown to be more efficient in removing readily biodegradable pharmaceuticals, such as caffeine, ibuprofen (IBU), or naproxen (NPX) through aerobic biodegradation (Nivala et al., 2019b). In the case of HF CWs, the removal of pharmaceuticals ranges from poor (e.g. compounds which prefer aerobic biodegradation) to comparatively efficient (e.g. the recalcitrant pharmaceutical carbamazepine (CBZ)) or very efficient, depending also on characteristics such as bed depth, media size, loading frequency or potential clogging (Ávila et al., 2014b; Matamoros and Bayona, 2006; Nivala et al., 2019b). Various CW intensification strategies have been developed over the last decades and were also tested for the treatment of pharmaceuticals, with promising results especially for biodegradable pharmaceuticals, but further research is still needed (Ávila et al., 2014b; Nivala et al., 2019b; Zhang et al., 2014).

### 2.1.4 Role of aquatic macrophytic plants

CWs are commonly planted with aquatic macrophytes which are generally recognized to have a positive effect on treatment performance, not only in HF CWs but CWs in general (Kadlec and Wallace, 2009; Tanner, 2001). Subsurface flow CWs are exclusively planted with emergent aquatic macrophytes, whereas FWS CWs may also be planted with submerged (e.g. *Potamogeton crispus* and *Littorella uniflora*) or floating aquatic macrophytes (e.g. *Potamogeton gramineus* and *Hydrocotyle vulgaris*). Examples for commonly used emergent aquatic macrophytes are common reed (*Phragmites australis*), bulrushes (*Scirpus* spp.) and cattails (*Typha* spp.), whereas each is adapted to different water depths and shows different depth penetration of roots and rhizomes (Brix et al., 1996a; Brix and Schierup, 1989). These plants are specialized to grow in saturated conditions and use large internal air spaces for oxygen transport to their extensive submerged roots and rhizome systems in order to survive in the reduced environment. As a consequence they stimulate decomposition of organic matter and nitrification by creating oxidized conditions in microenvironments within the otherwise anoxic/anaerobic lower areas of HF filter beds (Brix, 1997; Caselles-Osorio and García, 2007). The amount of oxygen released depends on the redox potential in the bed (Faulwetter et al., 2009) and the used plant species (Brix, 1997; Stottmeister et al., 2003). Furthermore, root exudates in the rhizosphere contain a diverse assortment of enzymes and carbon-containing metabolites, which seem to play an important role in the removal of some contaminants including organic micropollutants (Bais et al., 2006; Y. Zhang et al., 2016). Plants can also influence the height of the water table due to increased evapotranspiration (Mann and Wetzel, 1999). As a consequence the filter bed above the lowered water table is aerated and the redox potential increased (Pedescoll et al., 2013).

Initially it was assumed that plants are the major cause of contaminant removal in CWs, due to their direct uptake and sequestration of pollutants. However, that is only true for some pollutants such as certain heavy metals and special organic compounds, as well as in low-loaded systems (especially FWS). For the remaining contaminants plant uptake may play a role during the establishment and initial growing phase but in the long-term these contaminants are mainly transformed and removed by microbial and

physical processes as described in the previous Chapter 2.1.3 (García et al., 2010; Haberl et al., 2003; Kadlec and Wallace, 2009).

It also has to be noted that contaminants incorporated in macrophytes will only leave the system if they are harvested, otherwise they return during plant decomposition (Haberl et al., 2003). The presence of plants has several additional indirect benefits for CWs, such as bed stabilization, erosion control through vegetation cover, filtration effects (most important in FWS systems), roots providing an immense surface area for biofilm growth, root growth maintaining hydraulic properties of bed substrate (mostly in VF systems), preventing algae growth through shading and insulation against frost during winter (Brix, 1994; Haberl et al., 2003).

### 2.1.5 *Microbial community characterization*

As mentioned above, microbially mediated processes are the main driver for many contaminant removal mechanisms in CWs (Faulwetter et al., 2009). Microbial communities also play an important role for the system's hydrological development, due to the biofilm growing within the CW's pore system, where they are also in close interaction with plant roots and the rhizosphere (Weber and Legge, 2013). Microbial communities in CWs are usually extremely diverse on a spatial and temporal scale. On the spatial scale communities can differ due to the many microenvironments within a CW bed which depend on factors such as pH, redox, DO, nutrient availability or pollutant concentration (Weber and Legge, 2011). For example, inside the bed of a HF CW, the microenvironment within the distance of 1 mm from a root can be largely aerobic while the bed is generally dominated by anaerobic processes (Truu et al., 2009). On a temporal scale CW microbial communities usually change the most during the initial start-up period of a system and the involved establishment of plants as well as due to climatic or seasonal changes (Bernard and Lauve, 1995; Goulet and Roy, 2000; Samsó and García, 2013; Sims et al., 2012; Weber and Legge, 2011).

Several techniques are being used in order to characterize microbial communities. Generally they can be grouped in techniques for (Weber and Gagnon, 2014):

- **enumeration**; counting of colony forming units, dry weight measurements of total organic matter, direct microscopical counting and/or identification. More recently also microbial staining techniques, flow cytometry, and real-time polymerase chain reaction (RT-PCR).
- **activity assessment**; soil respiration rates ( $O_2$  utilization or  $CO_2$  production), direct/indirect quantification of adenosine triphosphate (ATP – provides energy for cellular metabolism) or nicotinamide adenine dinucleotide (NADH – involved in redox reactions in the cellular metabolism), as well as the quantification of extracellular enzyme activities (e.g. Fluorescein diacetate method (FDA), as used in chapter 5).

- **assessment of function**; mRNA (indication of specific active function), qPCR and fluorescence in-situ hybridization (indication for specific function), community level physiological profiling (CLPP) (metabolic activity in relation to 31 to 95 carbon sources), and microarrays (assess presence of 20,000-60,000 genes) which have the potential to assess full enzymatic pathways but can be costly.
- **assessment of structure**; fatty acid methyl ester (FAME), and phospholipid-derived fatty acid (PLFA) analysis (both for microbial community indication based on make-up of prokaryote cells), terminal restriction fragment length polymorphism (TRFLP), amplified rDNA (Ribosomal DNA) restriction analysis, ribosomal intergenic spacer analysis (RISA), length heterogeneity PCR (LH-PCR), and random amplification of polymorphic DNA (RAPD), and of course several methods are based on the characterization of PCR amplified DNA segments (most common 16S ribosomal RNA, as used in chapter 6). More recently, different high-throughput sequencing platforms using different techniques have been developed allowing for the simultaneous relative quantification and sequencing of all targeted genes within a sample. These provide a whole picture of a microbial community's structure using just one method but are very costly, which prohibits their widespread use.

Altogether these techniques allow to analyze spatial and temporal dynamics in microbial communities within CWs and correlate them with analysis of treatment performance relevant parameters in order to uncover the role of microbial communities quantitatively (Weber and Gagnon, 2014). For practical purposes, the gained information could be used in order improve CW design approaches such as regression equations or simplified first-order decay models (Langergraber, 2007; Rousseau et al., 2004; Samsó and García, 2013).

An important aspect in regards to microbial community analysis is the sampling procedure. Since the biofilm within the pore space of

the CW beds is responsible for the majority of transformation and treatment processes, it is crucial to take a representative sample from these areas including the biofilm, and not from the interstitial pore space water or simply from an effluent water sample (Weber and Gagnon, 2014). However, such representative samples are difficult to obtain in a non-destructive manner, therefore a practical method is to include sampling tubes in the design of CW systems if possible (as used in this work, see chapters 5 and 6).

### **2.1.6 Intensified constructed wetlands**

Even though a major advantage of CWs is their low external energy demand and use of straight forward building materials, a shift from completely passive treatment systems to more and more sophisticated engineered systems, so-called intensified CWs, has taken place in the last decades.

Probably the most prominent and widely used intensification strategy is the artificial aeration of subsurface flow CWs. The advantage of aerated CWs is the increased oxygen availability in the filter bed which generally is not sufficiently high enough to meet the oxygen demand of major wastewater removal processes (Kadlec and Wallace, 2009; Nivala et al., 2013). Aeration of subsurface flow CWs allows for higher and more robust (e.g. to seasonal variations) removal rates of contaminants such as COD and ammonium and also reduces the area requirement per person equivalent of a CW. These advantages come at the cost of an increased energy demand, as well as more complex design and operation (Austin and Nivala, 2009). A variant of continuous artificial aeration is the intermittent aeration which can be more effective in nitrogen removal since it not only allows for nitrification during aeration periods but also denitrification during non-aerated periods. Another way to increase the oxygen transfer is the use of a tidal flow operation, with multiple periodical flood and drain cycles per day. Drain cycles draw air into the bed and oxygenate the biofilm in the soil pores which then gets in contact with the wastewater again during the flooding (Green et al., 1997; Sun et al., 2006). These tidal flow systems have shown to be able to provide advanced biological treatment with less energy requirement than activated sludge system while requiring only half the energy of an aerated CW (Austin and Nivala, 2009). The energy reduction results from being more efficient in bringing the oxygen to the cation exchange sites by moving the water rather than forcing the oxygen into the bulk water via artificial aeration. This advantage of tidal flow systems is also shared by pulse-fed (also called intermittently loaded) VF CWs, which allow the influent wastewater to drain and rest for some time after each wastewater pulse feeding (Austin and Nivala, 2009; Molle et al., 2005). Intermittently fed VF CWs built according to the Austrian design standard require 4 m<sup>2</sup>/PE (Langergraber et al., 2007).



Another strategy to increase contaminant removal (especially nitrogen) which has been tested and applied widely is recirculation of the treated effluent to the pre-treatment or sedimentation tank (Arias et al., 2005), which can also be combined with intermittent or continuous aeration (Foladori et al., 2013) and other intensification strategies. A disadvantage of recirculation is the resulting increased hydraulic load to the system and energy demand for pumping. Area demand can be as low as 1.1 m<sup>2</sup>/PE, while still performing well in terms of contaminant removal (Ilyas and Masih, 2017).

An additional way of achieving nitrification and denitrification using CWs is to combine different systems to so-called hybrid, combined or multistage CWs in order to improve nitrogen removal as well as improve the robustness of CWs when facing fluctuating and peak loads (Brix et al., 2003; Foladori et al., 2012; Langergraber et al., 2008).

Apart from the above described intensification in regards to operation strategies, innovative designs and configurations could also be considered as intensifications of CWs. These designs and configurations include circular-flow corridor wetlands, towery hybrid CWs or baffled subsurface CWs (Wu et al., 2014).

In recent years, bioelectrochemical systems (BES) such as microbial fuel cells (MFCs) combined with CW systems have received increasing attention and produced an incremental number of publications each year. The first to develop and publish a hybrid of CWs and MFCs (CW-MFC) were Yadav et al. in the year 2012. Since then other BES such as microbial electrolysis cells (MECs) have been investigated in combination with CWs. This type of intensification is the topic of this work and the basics of BES like MFC and MEC, as well as their combination with CWs, will be introduced in more detail in the following section.

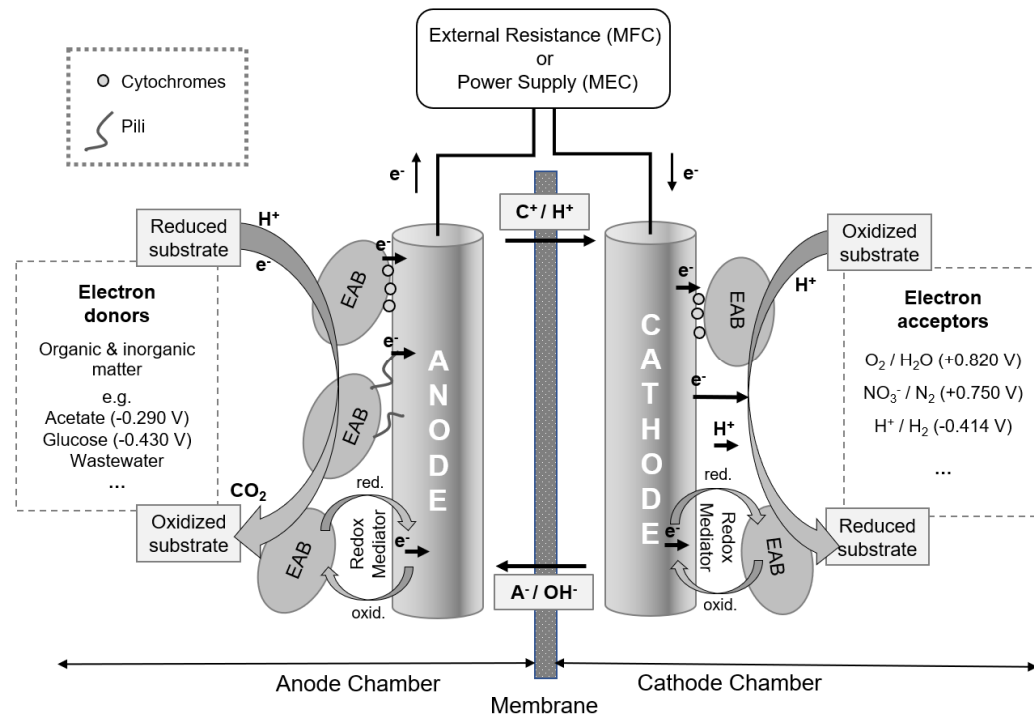
## 2.2 Bioelectrochemical systems

### 2.2.1 Overview

Bioelectrochemical systems (BES) encompass a set of technologies on the interface of microbiology and electrochemistry which utilize the interactions of living microbial cells and electrodes for different purposes (Schröder et al., 2015). Technologies in this framework are elsewhere also referred to as microbial electrochemical technologies (METs). Probably the most prominent BES are the microbial fuel cells (MFCs) which is able to convert the chemical energy contained in organic and inorganic matter into electric power (Logan et al., 2006; Schröder et al., 2015). The principles of microbial fuel cells have already been explored in the 19<sup>th</sup> and early 20<sup>th</sup> century, and it was Potter in 1911 who described the “electrical effects accompanying the decomposition with organic compounds” for the first time in a device which only nowadays is called a MFC. The difference to a commercially available battery or a chemical fuel cell is that the reactions at least at one of the electrodes are catalyzed by electrochemically active bacteria (EAB) (Logan et al., 2006; Rosenbaum et al., 2011a). A microbial electrolysis cell (MEC) is basically a modified MFC, with the main difference that no electricity is produced but instead an external power is supplied in order to achieve otherwise thermodynamically unfavorable reactions at the cathode (Rozendal et al., 2006). A variant of MEC are microbial desalination cells (MDCs) in which a third compartment in between the anodic and cathodic compartment is installed and filled with salt water which is desalinated by the created electrical field (Cao et al., 2009).

BES use either a membrane (bipolar or anion/proton exchange membrane) between anode and cathode, or the cathode is placed in an aerobic and the anode in an anaerobic/anoxic environment in order to create a redox gradient and a potential difference between the electrodes which is utilized by the EAB (Jang et al., 2004). Such systems are also called membrane-less BES and the same principle can also be applied in CWs due to the inherent redox gradient, resulting in a CW-MFC. Besides MFCs, CWs can also be operated as MECs (CW-MECs). In the following section, MFC, MEC and their principle mechanisms and influencing factors will be described in more detail as a basis for the later described CW-MFC and CW-MEC systems. In this chapter the term BES will be used unless a specific type of system is

discussed. Figure 2.2.1 show a scheme of a BES which can be operated in MFC and MEC mode.



**Figure 2.2.1.** Scheme of a bioelectrochemical system (BES) and its main processes when operated as either a microbial fuel cell (MFC) or microbial electrolysis cell (MEC). EAB: electrochemically active bacteria,  $e^-$ : electron,  $C^+$ : cation,  $A^-$ : anion. The scheme is adapted from Arends et al. (2012).

### 2.2.2 Electrochemically active bacteria

Electrochemically active bacteria (EAB), are also known as exoelectrogens, electrogens, electricogens, exoelectrogenic or anode respiring bacteria, and act as electrocatalysts for the reactions which are happening on the electrodes (Logan, 2009). These EAB are able to transfer electrons in and out of their cell in a process called extracellular electron transfer (EET). EET can be distinguished based on two different pathways 1) direct extracellular electron transfer (DEET) and 2) indirect extracellular electron transfer (MEET). A variation of DEET is direct interspecies electron transfer (DIET), in which electrons use other cells as terminal electron acceptor (TEA), forming syntrophic relationships in a biofilm acting as an electroactive aggregate (Malvankar et al., 2012; Shrestha and Rotaru, 2014). Those EET pathways can be observed as well in nature, with dissimilatory metal reducing bacteria amongst the most studied ones, utilizing insoluble minerals as electron acceptors via DEET and MEET processes (Lovley et al., 2004; Rosenbaum and Franks, 2014). For DEET the EAB have to be in direct physical contact with the electrode surface, either via outer membrane redox proteins (c-type cytochromes) that form an electron transport chain (Holmes et al., 2006; Shi et al., 2007), or via so-called nanowires and pili which are complexes of proteins including filaments (Gorby et al., 2006; Reguera et al., 2006, 2005) (see Figure 2.2.1). EAB performing MEET (e.g. *Pseudomonas aeruginosa* or *Shewanella oneidensis*) are capable of transferring electrons indirectly to an electrode by using redox mediators (Marsili et al., 2008; Rabaey et al., 2005). Redox mediators can be produced by EAB (e.g. pyocyanin by *P. aeruginosa*), but also natural compounds like humic acids are able to act as mediator for EET (Masuda et al., 2010; Venkataraman et al., 2011). MEET allows the involved EAB to compete for the limited access to electron acceptors and facilitates the build-up of relatively thick electroactive biofilm layers, since DEET alone would only allow for EAB in the first monolayer at the anode to be electrochemically active (Lovley, 2006; Mao and Verwoerd, 2013; Patil et al., 2012).

Koch and Harnisch (2016a) reported 94 confirmed bacteria species to be capable of EET and expected this number to increase significantly in the future. Out of the total 94, 69 species displayed capacity for anodic EET, 45 for cathodic EET, so twenty are able to perform EET at both electrodes. Furthermore, 33 perform DEET and 42

MEET, whereas for the latter only 19 produced the mediator themselves. The reported species belonged to fifteen different phylogenetic classes in the phyla *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Deferribacteres*, *Firmicutes*, *Proteobacteria* and *Euryarchaeota*, whereas the majority of 62 species are *Proteobacteria*. Further meta-analysis by Koch and Harnisch (2016a) indicated that there is not a single niche for EAB (77 out of 94 were found in multiple habitats), while many species were discovered in soil, sediment or sludge. The most studied model organisms in pure culture in regards to EAB are *Shewanella* (Gorby et al., 2006; Marsili et al., 2008; Ringeisen et al., 2006) and *Geobacter* (Call and Logan, 2011; Holmes et al., 2006; Richter et al., 2008; Rollefson et al., 2011). While pure cultures of EAB are ideal for the study of fundamental processes in BES, many BES investigations have been conducted using mixed cultures encompassing a variety of known and probably also unknown species which perform MEET and DEET (as well as possible unknown further EET processes), as well as a multitude of species that do not perform EET at all. Interactions between these communities may be synergistic, for example when fermentation end products are utilized rapidly by EAB, and in turn the removal is making the fermentative step faster and more energetically favorable (Kiely et al., 2011b). On the other hand, if organic loads are getting too high, methanogenesis might impede the BES performance by diverting electrons away from the electrode (Rosenbaum and Franks, 2014). The exoelectrogenic biofilm formed at the anode by EAB and non EAB reflects the type of substrate provided. Especially in complex mixed culture substrates, such as urban wastewater, hierarchical community structures indicate complex interactions of microbes with different metabolisms including EAB and non-EAB and seem to allow for a more rapid conversion of these complex wastewater streams (Kiely et al., 2011b). Probably due to these syntrophic and synergistic effects, mixed cultures were found to be more robust and resilient, and power densities were mostly higher than in pure cultures (Freguia et al., 2008; Min et al., 2005; Nevin et al., 2008; Rabaey et al., 2004; Rabaey and Verstraete, 2005).

### 2.2.3 Microbial fuel cells

BES such as MFC utilize EAB as catalysts in order to oxidize organic and inorganic matter, whereas the electrons produced by the EAB are transferred to an anode from where they flow via a conductive material and a resistor (or operated under a load) to a cathode where an electron acceptor with a higher potential, such as oxygen, is reduced. Thereby a current flow in the opposite direction of the electron flow is generated (Logan et al., 2006; Rabaey et al., 2007). However, there are also numerous ways certain EAB can interact with the cathode i.e. not through chemical catalysis (like with oxygen) but through biocatalysis in the case of a microbial bio-cathode (Rosenbaum et al., 2011b; Rozendal et al., 2008; Xing et al., 2010). At the same time protons diffuse from the anode to the cathode (see Figure 2.2.2).

MFCs only generate electricity if the overall reaction is thermodynamically favorable. Hence, an important feature of the MFC is to either use a proton exchange membrane between anode and cathode, or to place the cathode in an aerobic environment and the anode compartment in an anaerobic environment, creating a redox gradient and a potential difference between the electrodes (Jang et al., 2004). The thereby created electromotive force (emf) results in the maximum attainable cell voltage  $E_{emf}$  (V), being the difference between the cathode potential ( $E_{cat}$ ) and the anode potential ( $E_{an}$ ) (see Eq. 2.1).

$$E_{emf} = E_{cat} - E_{an} \quad \text{Eq. 2.1}$$

For example, if acetate is oxidized at the anode and oxygen used as TEA at the cathode the result would be an  $E_{emf}$  of  $1.101 \text{ V} = 0.805 (E_{cat}) - (-0.296) (E_{an})$ .

This theoretical maximum voltage of  $1.101 \text{ V}$  is lower in reality. The open-circuit voltage (OCV) resulting from operation without current (i.e. anode and cathode are not externally connected via a resistor or load) is between  $0.6\text{-}0.8 \text{ V}$  whereas the real voltage under a load (producing a current) is  $>0.62 \text{ V}$ . This measurable cell voltage ( $E_{cell}$ ) is considerably lower than the theoretically maximum voltage  $E_{emf}$ . The reduction results from several losses including overpotentials at the anode ( $\eta_a$ ) and cathode ( $\eta_c$ ) as well as ohmic losses ( $IR_\Omega$ ) (Logan et al., 2006) (see Eq. 2.2).

$$E_{cell} = E_{emf} - (\sum \eta_a + |\sum \eta_c| + IR_\Omega) \quad \text{Eq. 2.2}$$

In an MFC the voltage is measured over an external resistor ( $R_{ext}$ ) and the current ( $I$ ) can be calculated from Ohm's law (see Eq. 2.3)

$$I = \frac{E_{cell}}{R_{ext}} \quad \text{Eq. 2.3}$$

Where  $R_{ext}$  is the external resistance. In a further step electric power ( $P$ ) can be calculated (see Eq 2.4)

$$P = \frac{E_{cell}^2}{R_{ext}} \quad \text{Eq. 2.4}$$

The maximum attainable power is calculated using a Polarization Curve (PC) (see Chapter 2.2.6). Another way to describe the overall performance of an MFC is the coulombic efficiency (CE), which is the ratio of coulombs (unit for electric charge) actually transferred to the anode from the substrate (based on COD), to the maximum theoretically possible coulombs if all substrate i.e. COD would have been converted. Eq. 2.5 shows the CE formula in case of a continuously fed system.

$$\epsilon_{Cb} = \frac{MI}{Fbq\Delta COD} \quad \text{Eq. 2.5}$$

where  $M = 32$ , the molecular weight of oxygen,  $F$  is Faraday's constant,  $b = 4$ , the number of electrons exchanged per mole of oxygen,  $q$  is the volumetric inflow rate and  $\Delta COD$  the difference between inlet and outlet COD.

The majority of EAB utilize simple carbohydrates such as monosaccharides (e.g. glucose) or organic acids (e.g. acetic acid) for their metabolism (Koch and Harnisch, 2016a). These may be already present in the substrate or originate from microbial degradation of more complex organic substrates, allowing for simultaneous wastewater treatment and energy recovery via electricity production. Since wastewater treatment in wastewater treatment plants comes with a significant cost (mainly due to high energy need for air blowers etc.) the possibility to turn this waste into a resource by producing electricity has been investigated in a number of studies (Du et al., 2007; Lefebvre et al., 2011; Liu and Logan, 2004; Min and Logan, 2004; Clare E. Reimers et al., 2001). However, to date the highest achieved power density has been  $11,220 \text{ W/m}^3$ , using a miniaturized MFC reactor with a volume of only  $50 \mu\text{L}$  and a three-dimensional graphene anode (Ren et al., 2016). Even this extreme example of MFC power density is still two magnitudes lower than that of other power sources/converters. Lab-



scale MFCs fed with wastewater have achieved power densities in the order of magnitude around  $12 \text{ W/m}^3$  (Logan and Rabaey, 2012), while an up-scaled example of a stackable MFC with a volume of 250 L achieved  $0.47 \text{ W/m}^3$ , a significant decrease mainly due to the increased internal resistance but also due to factors such as voltage reversal in individual cells, nutrient limitation at anode or inactive cathode surface area (Feng et al., 2014). Hence, electricity production of up-scaled MFC is still a couple of magnitudes below commercially available photovoltaic systems with  $175 \text{ W/m}^2$  (Panasonic HIT® Photovoltaic Module, 2012).

In any case, even if electricity production with MFCs does not seem to be efficient enough at the moment and in the foreseeable future, wastewater treatment is still a great benefit. Examples of urban wastewater treatment using single-chamber air-cathode MFC systems (closest MFC design to CW-MFC) showed promising results. A system by Puig et al. (2010) showed long-term COD removal rates of  $77 \pm 6\%$  (organic removal rate of  $1.9 \text{ kg COD/m}^3 \cdot \text{day}$  and resulting power density of  $1.8 \text{ W/m}^3$ ) under an optimal tested pH of 9.5, while at a higher pH anodic bacteria were negatively affected. Ahn and Logan (2010) reached 26% COD removal when applying a high organic load of  $54 \text{ kg COD/m}^3 \cdot \text{day}$  (power density of  $12.8 \text{ W/m}^3$ ). Di Lorenzo et al. (2010) reported values of 89% COD removal for an organic loading rate (OLR) of  $16 \text{ kg COD/m}^3 \cdot \text{day}$  and a power density of  $1.8 \text{ W/m}^3$ .

However, besides relatively good COD removal rates an issue arises with nitrogen removal and denitrification, which is highly dependent on sufficient organic matter in relation to nitrogen ratio (C/N). Autotrophic nitrate and nitrite removal allows for denitrification at very low C/N ratios, whereas Clauwaert et al. (2007) demonstrated nitrate removal at the cathode performed by microorganisms which were supplied by electrons from the EAB oxidation of acetate at the anode. In addition, Puig et al. (2011) accomplished autotrophic nitrite reduction to dinitrogen gas at the cathode with removal rate of 76 and  $135 \text{ g N/m}^3 \cdot \text{d}$  in two tests (removal efficiency 30% to 37%, respectively), which lowers the energy and carbon demand of the nitrogen removal process greatly. Both these experiments were done using closed airtight anode and cathode chambers since oxygen presence at the cathode would oxidize nitrite via biological or electrochemical processes (Puig et al., 2011). A more recent study using a stacked air-cathode MFC (five units in series) for urban wastewater treatment with

an HRT of just 2.5 h achieved 85% and 94% COD and TN removal, respectively (effluent concentrations of  $20.7 \pm 2.5$  mg COD/L and  $1.7 \pm 0.1$  mg TN/L, respectively) over long-term (8 months), with a power density of  $6.3 \text{ W/m}^3$  (Park et al., 2017). However, upscaling still remains an issue to be solved.

MFCs have been also investigated for the use as biosensors for onsite and online water monitoring. Biosensors are analytical devices which convert a biological response into a quantifiable and processable signal (Di Lorenzo, 2015). Investigated parameters using MFC biosensors include organic matter content (e.g. BOD, COD) (Chang et al., 2005, 2004; Chee, 2013; Di Lorenzo et al., 2009; Kim et al., 2003; Peixoto et al., 2011a; Zhang and Angelidaki, 2011), microbial activity (Zhang and Angelidaki, 2011), dissolved oxygen (DO) (Zhang and Angelidaki, 2012a), volatile fatty acids (VFA) content (Kaur et al., 2013), or bioactive toxic substances such as organophosphorus compounds, Pb, Hg, or PCBs (Kim et al., 2007). The use of MFCs for bioindication will be discussed in more detail in Chapter 2.3.2.5 and Chapter 4).

### 2.2.4 Microbial electrolysis cells

A microbial electrolysis cell (MEC) is basically a modified MFC, with the main difference that an external power source is supplied to achieve thermodynamically unfavorable reactions at the cathode (Rozendal et al., 2006). Hence, MEC use the same principle as MFC, with EAB oxidizing a substrate and transferring electrons to the anode, which wander to the cathode via a resistor, but with the difference that additional power is supplied in order to enable reactions such as hydrogen production at the cathode. Hence, the MEC's purpose is not to produce electric power but creating organic compounds at the cathode, or, as shown in the following, simultaneous advanced treatment of wastewater. An important aspect is that MECs require, due to the supply of electrons and protons from the oxidation of organics by EAB to the anode, only an additional voltage of 0.2-0.8 V between the electrodes in order to overcome the thermodynamic barrier for water electrolysis to occur (usually 1.8-3.5 V are required) (Lu and Ren, 2016), producing oxygen at the anode and hydrogen at the cathode. Hence it could be sufficient to use a photovoltaic solar panel in order to provide this additionally required voltage (Kadier et al., 2016).

MECs have been investigated for a variety of applications. Wagner et al. (2009) treated swine wastewater using a MEC (applied voltage of 0.5 V) showing COD removals of 69% to 75% with a hydrogen production rate of 0.9-1.0 m<sup>3</sup> H<sub>2</sub>/m<sup>3</sup>·d (overall recovery from COD of 28±6%). The produced gas composition was up to 77% hydrogen and up to 13% methane. Further MEC applications include hydrogen peroxide production with 1.9±0.2 Kg H<sub>2</sub>O<sub>2</sub>/m<sup>3</sup>·d (Rozendal et al., 2009), caustic soda production with 3.4 wt% NaOH using an applied voltage of 1.77 V (Rabaey et al., 2010) or ethanol production of 1.82 mM EtOH and co-production of 0.012 m<sup>3</sup> H<sub>2</sub>/m<sup>3</sup>·d (applied voltage of -0.55 V at the cathode) (Steinbusch et al., 2010). Additional developments such as microbial desalination cells (MDC) achieved 0.16 m<sup>3</sup> H<sub>2</sub>/m<sup>3</sup>·d production with an applied voltage of 0.55 V (Mehanna et al., 2010).

However, also urban wastewater can be used as a substrate for MEC systems, with some of the investigated systems reaching energy neutrality (electricity input is equal or lower to the equivalent of hydrogen energy produced). Cusick et al. (2010) compared MFC and MEC (applied voltage of 0.9 V) systems treating winery and urban

wastewater and found that MFC were more efficient in energy recovery and COD removal (MFC achieved  $65\pm 7\%$  and  $83\pm 10\%$  COD removal compared to MEC with  $47\pm 3\%$  and  $58\pm 3\%$  COD removal treating winery and domestic wastewater, respectively). However, hydrogen produced from MEC ( $0.17$  and  $0.28 \text{ m}^3 \text{ H}_2/\text{m}^3\cdot\text{d}$  for winery and domestic wastewater, respectively) was potentially cost effective, at least at the system's scale. Heidrich et al. (2014) treated raw urban wastewater using a 100-L pilot-scale MEC (applied voltage of 1.1 V), where 12-month observations showed that hydrogen gas production ( $0.8 \text{ L H}_2/\text{d}$  with 89-99% purity) declined over time but was not affected by temperature (between  $1 \text{ }^\circ\text{C}$  and  $22 \text{ }^\circ\text{C}$ ), resulting in an average production of only half of what would be needed for energy neutrality. Suboptimal cell design, pumping problems, and large overpotentials were identified as major limitations, with the effect that average COD removal was only 44% and did not reach required local UK standards ( $125 \text{ mg/L COD}$ , or 75% removal). Brown et al. (2014) investigated a prototype MEC (technical scale size 16 L reactor) which treated continuously fed primary settled urban wastewater (and acetate spiked wastewater treatment plant effluent) achieving average removal rates of 67% for COD ( $210 \text{ mg/L COD}$  effluent concentration) and 40% for ammonium ( $31 \text{ mg/L ammonium}$  effluent concentration). Gil-Carrera et al. (2013) achieved 60% to 85% COD removal and limited hydrogen production due to substrate limitation and poor cathode performance. More recently, Baeza et al. (2017) investigated a 130-L pilot-scale MEC with an applied potential of 1.1 V, achieving  $4.2\pm 0.7 \text{ L H}_2/\text{d}$  while removing ca. 6% to 24% of COD depending on the used hydraulic retention time (HRT) (OLRs of 0.25 to  $0.5 \text{ kg COD}/\text{m}^3\cdot\text{d}$ ). Major identified problems were application of electrical potential and material deterioration. Cotterill et al. (2017) investigated a 175-L pilot with a scalable "cassette-design" and an applied voltage of 0.9 V, achieving a hydrogen production of  $0.8 \text{ L H}_2/\text{d}$  (93% purity) and COD removal of 64% (average  $124.7 \text{ mg COD/L}$  achieving EU effluent standards) treating low temperature urban wastewater (between  $9 \text{ }^\circ\text{C}$  and  $16 \text{ }^\circ\text{C}$ ) with an HRT of only 5 h. In general, Escapa et al. (2014) estimated that MECs could help to reduce ca. 20% of the energy consumption of WWTPs.

However, at the current state, MECs seem to be insufficient as a stand-alone technology for urban wastewater treatment but would require further treatment or an integration in other processes in the main stream or a side stream of a WWTP (Katuri et al., 2019). MECs have

been incorporated in (or hybridized with) other existing systems such as anaerobic digestion (Liu et al., 2016; Yu et al., 2018), membrane technologies (Katuri et al., 2016, 2014), as well as CWs (Ju et al., 2014a) (see Chapter 2.3).

### 2.2.5 Performance factors

Biological factors influencing performance include biological limitations due to the EAB needs in terms of physical environmental conditions (e.g. pH, temperature) and microbial metabolism limitations concerning their growth, nutrient uptake and electron transfer (Arends et al., 2012). Biofilm characteristics like thickness, density, structure and composition affect the transport of substrate and electrons in between the bulk liquid, the biofilm and the electrodes, whereas thicker biofilm on anodes has shown to produce higher power (Nevin et al., 2008). Remarkably cathodic biofilms have shown the opposite effect (Behera et al., 2010).

Generally, losses from the theoretically reachable cell voltage  $E_{emf}$  to the measurable cell voltage  $E_{cell}$  can be grouped in (Logan et al., 2006; Rozendal et al., 2008);

- **Ohmic losses** (or ohmic overpotential) include the resistance to the electron flow through electrodes and conductive interconnections, as well as the resistance to the ion flow through the proton exchange membrane (PEM), if used, and the anodic and cathodic electrolytes. Influencing factors on ohmic losses include type of electrode material, substrate (electrolyte) composition and their conductivity (too high conductivity can have negative effects on bacteria). Decreasing electrode spacing and improving proton movement from and to electrodes decrease ohmic losses as well. However if electrodes are too close, the anode gets contaminated with oxygen from the cathode zone which can lead to lower performance. Urban wastewater has relatively low conductivity and therefore causes high ohmic losses. Lower current densities also decrease the proportion of ohmic losses.
- **Activation losses** (or activation polarization) are a consequence of the activation energy needed for the reduction and oxidation reactions at the electrodes. They increase with increasing current and can be lowered by increased electrode surface area, improved electrode chemical properties (e.g. increase positive charges on anode), improved electrode catalysis, increased

temperature and improved electrochemical activity of biofilms as well as facilitation of electron transfer between EAB and electrodes.

- **Bacterial metabolic losses** occur due to the fact that bacteria generate metabolic energy by transporting electrons from a low potential substrate to a final electron acceptor at a higher potential, such as oxygen or nitrate, or in the case of an MFC, to the anode. The higher this metabolic gain, the lower is the attainable MFC voltage, therefore the anode potential should be kept as low as possible, but not so low that fermentation takes place and electron transport is inhibited.
- **Concentration losses** (or concentration polarization) occur due to limited mass transfer of a species from or to the electrode causing a lower reaction rate, e.g. insufficient supply of reduced species or limited discharge of oxidized species at the anode or the reverse at the cathode. These losses occur mainly under high current densities. Mass transport losses from the bulk liquid in poorly mixed systems affect as well the substrate availability for the biofilm and increase losses. Another problem is limited proton flux between electrodes which causes an increase in pH, resulting in limited EAB activity at the anode, and a decrease in pH at the cathode, thereby lowering the voltage generation. Hence it is important to provide a sufficient buffer capacity.

Electrode materials in BES ideally have a high surface area for attachment of microorganisms and enable high interaction rates. They can be used in various forms such as granules, felt, cloth, brushes, solid blocks or sheets and are generally made from carbon or metal based materials such as graphite or stainless steel (Arends et al., 2012; Koch and Harnisch, 2016b). The effectiveness of electrodes also depends greatly on the operating conditions especially if non-synthetic substrates such as real urban wastewater are used, since unwanted fouling may occur, especially on air-cathodes. A remedy for fouling of carbon felt could be treatments with ethylenediamine or nitric acid which led to power density increases of 25% and 58%, respectively. These

increases were attributed to changing the surface attributes of the felt (Zhu et al., 2011). The performance of electrode materials can also be enhanced by modification, for example with  $Mn^{4+}$  and  $Fe^{3+}$  doped graphite anodes and cathodes, respectively (Park and Zeikus, 2003), also Pt was commonly being used as a catalyst on cathodes (Cheng et al., 2006; Min et al., 2005). Nanoparticles and nanotubes or conductive polymers have been investigated as electrode materials in BES (Zhou et al., 2011). In MEC systems nickel powders and gold or (biogenic) palladium nanoparticles were used in order to enhance performance (De Gusseme et al., 2012; Fan et al., 2011; Hennebel et al., 2011; Y. X. Huang et al., 2011; Selembo et al., 2010).

Of course the architecture and design of BES have an impact as well on their performance. Influential design parameters include electrode spacing, flow patterns, reactor volumes and electrode surface areas (Arends et al., 2012). Generally there are single chamber, dual chamber and stacked MECs. Dual chamber BES consist of a separate anodic and cathodic chamber which are separated by a PEM (e.g. Nafion or Ultrex) and are often built in an “H-shape” out of materials like glass, polycarbonate or plexiglass (Du et al., 2007). Single chamber BES do not use a PEM. If oxygen is used as TEA in the cathode chamber of MFCs, it can either be provided by artificial aeration (i.e. bubbling) of the water in the cathodic chamber or by using an air-cathode (cathode is half submerged and therefore in contact with atmospheric oxygen). Another option are stacked BES in which several BES units are connected in series or parallel in order to increase voltage or current, respectively, and are utilized in larger scale applications (Aelterman et al., 2006; Kadier et al., 2016).



### **2.2.6 Characterization techniques**

Some of the performance indicators and above described losses of BES can be characterized with the help of analysis techniques. Cell voltage can be measured quite easily with common voltage meters and multimeters. For the measurement of a single electrode's potential an additional reference electrode is needed and for more complex analysis such as cyclic voltammetry (CV) a potentiostat is required. CV can aid in providing information on electrochemical activity of microbial strains or consortia, determining the standard redox potentials of redox active components, studying mass transfer influences and distinguishing between adsorbed or diffusive natures of mediators (Harnisch and Freguia, 2012; Logan et al., 2006). Electrochemical impedance spectroscopy (EIS) would require a potentiostat equipped with a frequency response analyzer which can be used to measure ohmic and internal resistances in order to identify contributions of different components amongst other uses (Zhang et al., 2011). EIS showed in several studies that solution and membrane resistance were dominant and accounting for 95% of the resistance in two-chamber designs (Ramasamy et al., 2008) as well as more than 50% in an upflow and a tubular shaped membrane-less air-cathode MFC (He et al., 2006; You et al., 2007).

Techniques using a potentiostat with a two-electrode setup (working electrode is connected to cathode and both the counter and reference electrode are connected to the anode) include the current interrupt technique (to determine ohmic resistance) and the polarization curve (PC) analysis.

PC analysis will be described in more detail since it was used in the following Chapters 5-7. Besides using a potentiostat with a two-electrode setup (or a galvanostat) PC analysis can also be performed more primitively by hand, using a voltmeter or multimeter and several different types of resistors or a resistor-box to set various external loads (however, with far fewer possible measurements and therefore a much lower resolution). A PC depicts the cell voltage output as a function of current density loading. The current density loading is dependent on the applied load (or resistance between anode and cathode) which is automatically gradually increased (in very small steps) in the potentiostat or if done manually by changing the resistors which act as the load. When changing manually, one has to wait for some time until

the voltage gets more or less stable i.e. pseudo-steady-state conditions have been established. On a typical PC chart the increasing current density (due to the increasing load) is plotted on the x-axis and the resulting measured voltage is plotted on the y axis. Typically, three zones can be distinguished in a PC with 1) a very steep decrease of the voltage after starting from open-circuit voltage at zero current (OCV, cell voltage that can be measured after some time in the absence of current) where activation losses are dominant, then 2) a zone in which the voltage drop is less steep and more or less linear in which ohmic losses are dominant and 3) the final zone with another rapid fall of the voltage in which concentration losses (mass transfer losses) are dominant (Logan et al., 2006) (see Supplementary Information (SI) Figures S5.1, S6.1 and S7.1). The internal resistance ( $R_{int}$ ) can be calculated from the PC (in case it is linear) since it is equal to the slope of the PC (see Eq. 2.5)

$$R_{int} = \frac{-\Delta E}{\Delta I} \quad \text{Eq. 2.5}$$

where  $\Delta E$  is the cell 's potential drop and  $\Delta I$  the current drop. Principally, the potential maximum power is achieved when internal and external resistances are close to each other (Lefebvre et al., 2011).

Furthermore a power curve (power density) can be derived from the PC and plotted on a second y axis as a function of the current density. At OCV, without any current the power is zero as well, but with increasing current also the power increases up to a maximum power point (MPP), after which the power drops again due to increasing ohmic losses and overpotentials until the produced power approaches zero again (short-circuit conditions) (Logan et al., 2006) (see SI, Figures S5.1, S6.1 and S7.1).

## 2.3 Constructed wetlands operated as bioelectrochemical systems

### 2.3.1 Overview

As mentioned above, membrane-less BES require a redox gradient between the electrodes in order to create a potential difference between them and drive the bioelectrochemical reactions catalyzed by EAB. This redox gradient also occurs in natural environments such as aquatic sediments (e.g. marine sediments on the seafloor) where MFC systems were implemented in order to power sensors in these mostly very remote locations (C E Reimers et al., 2001; Rezaei et al., 2007; Tender et al., 2002), with the major advantage of making onsite battery changes unnecessary and the technology very cost effective. These sediment MFC (SMFC) systems rely on a passive supply of reduced substrate at the anode which limits their performance. This limitation can be overcome by placing MFCs in planted sediments (PMFCs) (such as rice paddy fields) in which the roots supply nutrients via rhizodeposits and exudates to the anode (De Schamphelaire et al., 2008; Kaku et al., 2008; Strik et al., 2011; Venkata Mohan et al., 2011).

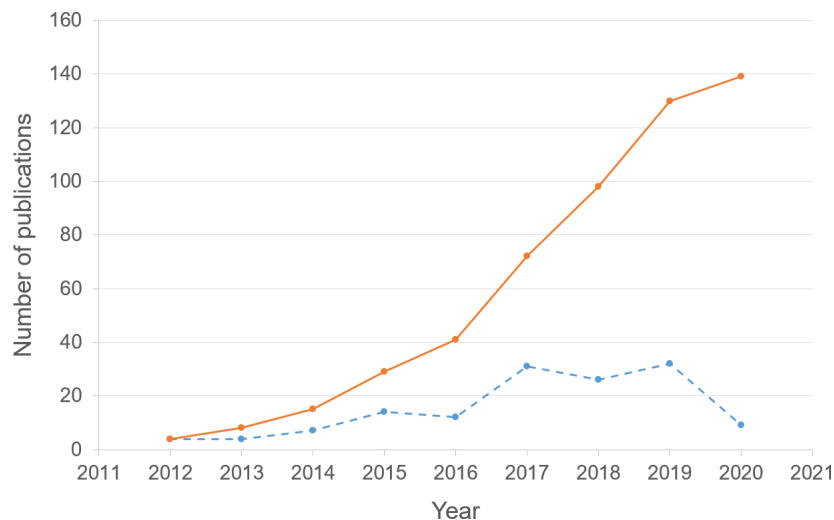
However, this redox gradient also occurs in CWs, especially when operated in HF (around 0.5 V vs. SHE) (García et al., 2003). Hence, it is possible to integrate BES into CWs by placing the anode into the lower anaerobic/anoxic section of the bed and the cathode in the upper aerobic section of the CW bed (at the interface with the atmosphere), with the advantage over SMFC or PMFC of a steady wastewater influx supplying the anode with organic and inorganic matter. The first publication of an MFC incorporated in CWs was published in 2012 by Yadav et al. (2012), performing simultaneous nutrient removal and electric power generation. CWs operated as BES, such as MFC or MEC, will hereafter be referred to as CW-BES, CW-MFC and CW-MEC, respectively.

If CW-BES achieve a higher contaminant removal in comparison to CW system, the specific area requirement per PE could be reduced, mitigating one of the few weak points of CWs. Ideally also contaminants which are not so easily removed or need intensification or additional steps, like total nitrogen or phosphorus removal would be enhanced by CW-BES, adding value to the systems.

### 2.3.2 CWs operated as MFCs

#### 2.3.2.1 General information

Since the first publication in 2012 by Yadav et al., publications on CW-MFCs were increasing steadily for the first few years and plateaued at an output of ca. 30 new publications each year between 2017 and 2019. Note that 2020 is lower because only publications until 9 February 2020 were considered, however, the number was already relatively high with 9 publications in a little more than just a month (see Figure 2.3.1).



**Figure 2.3.1.** Number of publications on the topic “constructed wetland” and “microbial fuel cells”. Number per year shown as dashed line and accumulated number per year shown as solid line. Data collection via Scopus on 9. February 2020.

To date, the total accumulated number of CW-MFC publications reached 139, out of which the vast majority originated from research groups in China (86 publications), followed by Ireland, India, the United States and Spain (13-16 publications each). Hence, the number of publications on the topic tripled within the last 4 years.

### 2.3.2.2 CW-MFC design and operation strategies

Table 2.3.1. shows a selection of different CW-MFCs designs, sizes and operational strategies. The majority of CW-MFCs investigated for wastewater treatment used artificial wastewater in lab-scale reactors (often tubular upflow and/or batch fed) (Fang et al., 2013a; Liu et al., 2014; S. Liu et al., 2013; Oon et al., 2018; Rathour et al., 2019; Xie et al., 2018; L. Xu et al., 2018b; Yakar et al., 2018), which is advantageous for the study of fundamental processes, but less realistic than the use of real urban wastewater and an upscaled reactor. An upflow hydraulic regime is rarely implemented on full-scale in conventional CWs. However, it is predominantly applied in lab-scale CW-MFC research and has the advantage of wastewater entering at the bottom where it first flows through the anaerobic anodic section where the substrate is oxidized by EAB and then continuous upwards to the aerobic cathodic section where heterotrophic bacteria oxidize the remaining organic matter using oxygen as electron acceptor. Thereby, the dissolved oxygen transported to the anode by the applied wastewater is minimized while the cathode only gets in contact with the wastewater when the majority of the organic load is already reduced, resulting in better redox conditions in the bed and MFC (Doherty et al., 2015b). So far there are no meso- or pilot-scale CW-MFC systems using an upflow hydraulic regime. In this study, meso-scale is defined as systems with >40 L volume or >15 L effective liquid phase volume, everything below is lab-scale, whereas pilot-scale is usually starting from around 10% of full-scale inflow. Meso-scale systems were implemented already quite early in 2013 by Villaseñor et al., using a HF regime and are increasingly investigated more recently (Hartl et al., 2019; Saz et al., 2018; Tang et al., 2019).

In terms of cathode design, in most publications a partially submerged air-cathode was used, which is probably the most economical solution, since atmospheric oxygen is utilized as a final electron acceptor. Nonetheless, some investigations also experimented with different forms of artificial forced/aeration, which improved nutrient removal but also increased operational costs and energy demand (Oon et al., 2018, 2017, 2016, 2015; Zhao et al., 2013). Investigated cathode and anode materials were mostly carbon based, including carbon felt (CF), granular activated carbon (GAC) or graphite, but also metal based materials such as stainless steel plates or mesh (SSM) were used (see Table 2.3.2). More unusual materials like foamed nickel were used as

well for electrode construction (J. Wang et al., 2017b, 2016a). The advantages of carbon and graphite based materials are high electrical conductivity, a non-oxidative nature, and their high specific surface area and porosity for attachment and growth of biofilm (Li and Sheng, 2012). Electrode spacing, i.e. the distance between anode and cathode is a key issue in MFC performance (Liu et al., 2008) and has also been investigated in CW-MFCs, where the general goal is to decrease ohmic resistances as far as possible by reducing electrode spacing but at the same time maintain a high enough redox gradient for satisfactory MFC performance (Doherty et al., 2015a). Although CW-MFC might provide a sufficiently high redox profile for MFC implementation without the use of a separator such as glass wool (Corbella et al., 2014; Fang et al., 2013a; W. L. Liu et al., 2013) the potentially relatively high electrode spacing, especially in the view of upscaling, may create also high ohmic losses, with measured internal resistances of 500-4300 ohms (Doherty et al., 2015c; Fang et al., 2017; Oon et al., 2015). As a comparison conventional MFC fed with wastewater produced an internal resistance of only 33 ohms (Ahn and Logan, 2012). CW-MFC internal resistances are higher due to the complexity of the systems. A possible solution for keeping the electrode spacing small and still achieve a sufficient redox gradient are glass wool separators placed horizontally between anode and cathode in order to prohibit oxygen to penetrate from the upper aerobic in the lower anaerobic compartments (Yadav et al., 2012; Zhao et al., 2013) resulting in internal resistances of 100-200 ohms for example (Hartl et al., 2019). However, Doherty et al. (2015c) mentioned that the use of such separators might promote long-term clogging problems due to organic matter accumulation. Additionally, roots could penetrate the separators and consequently lower their effectiveness.

The impact of the chosen external resistance has been investigated for MFC (Jadhav and Ghangrekar, 2009; Katuri et al., 2011) as well as for CW-MFC systems, where Corbella and Puigagut (2018) found that 220 ohms was the ideal external resistance for their system architecture. The majority of CW-MFC experiments used an external resistance of 1000 ohms (see Table 2.2.1), however, as mentioned above, internal resistances vary greatly from around 100 to up to 4300 ohms. Principally, the potential maximum power is achieved when internal and external resistances are close to each other (Lefebvre et al., 2011). In case the chosen external is higher than the measured internal resistance, the system would actually have the potential of a higher current and power if a lower external resistance

would be chosen. The chosen current also depends on the respective goals, i.e. whether a higher and/or more stable current or potential are more desirable for the respective treatment or performance goals.

Aquatic macrophytic plants play a role in CW-MFC performance as well as nutrient removal. The majority of studies presented in Table 2.3.1 were planted and some investigations on the role of aquatic macrophytic plants were conducted using a variety of species including *Canna indica*, *Carex divisa*, *Cyperus alternifolius*, *Fimbristylis dichotoma*, *Ipomoea aquatic*, *Elodea nuttallii*, *Juncus effuses*, *Juncus gerardii*, *Phragmites australis*, *Scirpus Validus*, *Typha angustifolia*, *Typha latifolia* or *Typha orientalis* (Fang et al., 2013b; Lu et al., 2015; Oon et al., 2017; Rathour et al., 2019; Saz et al., 2018; Villaseñor et al., 2013; J. Wang et al., 2017a; Yakar et al., 2018). As mentioned above, rhizodeposits and exudates of roots can be utilized by anodic bacteria and even serve as the sole organic substrate for the CW-MFC (Lu et al., 2015). Furthermore, the oxygenation of the cathodic section by roots has the potential to enhance the MFC performance (Xu et al., 2016), but could decrease the MFC performance if oxygen is released nearby the anode by increasing its potential. The positive role of plants on organic matter and nutrient removal in CWs is generally accepted (Tanner, 2001). In terms of nutrient removal in the realm of CW-MFC, better removal by planted systems were reported (Oon et al., 2018; Saz et al., 2018; J. Wang et al., 2017a). Saz et al. (2018) also compared different plant species, showing best removal results and current densities with *T. angustifolia*, compared to *Juncus girardii* and *Carex divisa*, supposedly due to a better environment for microorganisms and resulting increase in nutrient removal and current densities. Also higher MFC performance in planted compared to unplanted systems has been reported (Fang et al., 2013a; Oon et al., 2018; J. Wang et al., 2017a). A microbial community structure analysis by Lu et al. (2015) showed that plants significantly increased microbial diversity, however, the planted and unplanted systems' sole carbon sources were rhizodeposits so it would not be surprising if the unplanted microbial community was less diverse due to nutrient limitation. However, also Wang et al. (2017) found that macrophytes increased the relative abundance of EAB when comparing unplanted and planted CW-MFCs fed with synthetic wastewater (WW) (see Table 2.3.1).

**Table 2.3.1.A.** Design and operational conditions of selected CW-MFCs (updated from Corbella, 2017)

Reference	Scale	Planted/ Unplanted	Feeding* and flow	Carbon source	Oxygen source	External resistance ( $\Omega$ )	Internal resistance ( $\Omega$ )
Yadav et al. (2012)	lab	planted	batch	synthetic WW	air-cathode	-	-
Zhao et al. (2013)	lab	planted	batch/ upflow	swine WW	forced aeration	12000	-
S. Liu et al. (2013)	lab	planted	upflow	synthetic WW	air-cathode	1000	156-256
Villaseñor et al. (2013)	meso	planted	HF	synthetic WW	limited - from roots	120	120
Fang et al. (2013a)	lab	planted	upflow	synthetic WW	air-cathode	1000	218-273
Liu et al. (2014)	lab	planted	upflow	synthetic WW	air-cathode	1000	209-582
Liu et al. (2015)	lab	planted	batch	rhizodeposits	air-cathode	1000	-
Oon et al. (2015)	lab	planted	upflow	synthetic WW	forced aeration	1000	820-4300
Doherty et al., (2015c)	lab	planted	upflow- downflow	swine slurry	limited – via downflow	950	500-300
Srivastava et al. (2015)	lab	planted	batch	synthetic WW	air-cathode	-	-
Fang et al. (2015)	lab	planted	upflow	synthetic WW	air-cathode	1000	-
Oon et al. (2016)	lab	planted	upflow	synthetic WW	forced aeration	1000	200-430
Wang et al. (2016a)	lab	planted	batch	synthetic WW	air-cathode	1000	-



**Table 2.3.1.B.** Design and operational conditions of selected CW-MFCs (updated from Corbella, 2017)

Reference	Scale	Planted/ Unplanted	Feeding* and flow	Carbon source	Oxygen source	External resistance ( $\Omega$ )	Internal resistance ( $\Omega$ )
Wang et al. (2016b)	lab	planted	batch	synthetic WW	air-cathode	1000	-
Oon et al. (2017)	lab	planted	upflow	synthetic WW	forced aeration	1000	200-450
L. Xu et al. (2017a)	lab	planted	upflow	synthetic WW	air-cathode	500	130
J. Wang et al. (2017b)	lab	planted	batch	synthetic WW	air-cathode	1000	-
Corbella and Puigagut (2018)	lab	unplanted	batch	real urban	air-cathode	220	-
Y.-L. Oon et al. (2018)	lab	planted	batch/Upflow	synthetic azo-dye WW	forced aeration	1000	150
Saz et al. (2018)	meso	planted	batch/HF	WWTP sludge	air-cathode	1000	-
Xie et al. (2018)	lab	planted	batch/Upflow	synthetic WW	air-cathode	1000	489
F. Xu et al. (2018)	lab	planted	batch/Upflow	synthetic river water	air-cathode	1000	373
Yakar et al. (2018)	lab	planted	batch/Upflow	synthetic WW	limited - from roots <sup>^</sup>	1000	-
Tang et al. (2019)	meso	unplanted	upflow	synthetic WW	forced aeration	12600	196-2000
Rathour et al. (2019)	lab	planted	batch	real azo-dye WW	air-cathode	150	-

\* continuous inflow if not indicated otherwise, i.e. with "batch "

<sup>^</sup> cathode was submerged

In the majority of studies presented in Table 2.3.2 gravel was used as material for filling the main bed volume which is also called packing layer (areas besides from anode and cathode sections). In some studies gravel was mixed with other materials such as sand (Saz et al., 2018) or glass beads (Oon et al., 2018) and others incorporated materials with high a specific surface and porosity such as the light weight aggregate ceramsite (F. Xu et al., 2018), zeolite (clinoptilolite) (Yakar et al., 2018) or reused dewatered alum sludge (Doherty et al., 2015a; Tang et al., 2019; L. Xu et al., 2017a; Zhao et al., 2013). The two latter materials have the additional advantage of being electrically conductive.

**Table 2.3.2.A.** Bed and electrode materials in selected CW-MFCs (updated from Corbella, 2017)

Reference	Main bed material (packing layer)	Anode	Cathode
Yadav et al. (2012)	gravel	-	-
Zhao et al. (2013)	dewatered alum sludge	graphite (plate)	graphite (plate)
S. Liu et al. (2013)	gravel	GAC	GAC+SSM
Villaseñor et al. (2013)	gravel	graphite (rectangular)	graphite (rectangular)
Fang et al. (2013a)	gravel	GAC	GAC+SSM
Liu et al. (2014)	gravel	GAC	GAC+SSM
Liu et al. (2015)	gravel	graphite disk	carbon cloth
Oon et al. (2015)	gravel	CF	CF
Doherty et al., (2015c)	dewatered alum sludge	graphite (granular)	graphite (granular)
Srivastava et al. (2015)	gravel	graphite (granular), GA charcoal	graphite (granular), GA charcoal
Fang et al. (2015)	gravel	GAC+SSM	GAC+SSM
Oon et al. (2016)	gravel	GAC	GAC
Wang et al. (2016a)	gravel	CF, SSM, graphite (rod), foamed nickel	CF, SSM, graphite (rod), foamed nickel

CF carbon felt  
GA granular activated  
GAC granular activated carbon  
SSM stainless steel mesh

**Table 2.3.2.B.** Bed and electrode materials in selected CW-MFCs (updated from Corbella, 2017)

Reference	Main bed material (packing layer)	Anode	Cathode
Wang et al. (2016b)	sand	CF	CF
Oon et al. (2017)	gravel	AC	AC
L. Xu et al. (2017a)	dewatered alum sludge	graphite (gravel) + SSM	graphite (gravel) + SSM
J. Wang et al. (2017b)	sand	CF, SSM, graphite (rod), foamed nickel	CF, SSM, graphite (rod), foamed nickel
Corbella and Puigagut (2018)	gravel	graphite (rod)	CF
Y.-L. Oon et al. (2018)	gravel / glass beads	CF	CF
Saz et al. (2018)	sand / gravel	graphite plate	magnesium plate
Xie et al. (2018)	gravel	graphite plate (perforated)	graphite plate (perforated)
F. Xu et al. (2018)	sand / ceramsite	titanium cylinder filled with sludge and AC	titanium mesh
Yakar et al. (2018)	zeolite	graphite plate	magnesium plate
Tang et al. (2019)	dewatered alum sludge	SSM	CF+SSM
Rathour et al. (2019)	gravel	stainless steel plate	stainless steel plate

AC     activated carbon  
CF     carbon felt  
GAC    granular activated carbon  
SSM    stainless steel mesh

### 2.3.2.3 CW-MFCs electrical performance

Initially the majority of CW-MFC research aimed at maximizing electricity production (S. Liu et al., 2013; Yadav et al., 2012) and also more recent investigations still follow this goal (F. Xu et al., 2018). However, the amount of electricity which can be produced at the current state of the technology is too low to be competitive with other technologies (see chapter 2.2.3). Likewise, due to the high internal resistances, CW-MFCs reported maximum power densities of  $2 \text{ W/m}^3$  (L. Xu et al., 2017b) or  $3.7 \text{ W/m}^2$  (F. Xu et al., 2018), but averages from most systems are again one or more orders of magnitude lower (see Table 2.3.2). In comparison to solar panels with for example  $175 \text{ W/m}^2$  (Panasonic HIT® Photovoltaic Module, 2012) it seems that big-scale electricity production from wastewater by MFC or CW-MFC technology with the nowadays state of the art is not a reasonable goal. In regards to direct utilization of the produced electricity, the technology seems to be only competitive in very specialized niches like biosensing SMFCs in marine sediments (Bond et al., 2002). Therefore the focus of most CW-MFC publications shifted from electricity production to the improvement of general performance and consequentially increased contaminant removal (more details in next Chapter 2.3.2.4), in which power or current density are merely seen as performance indicators.

Apart from the high internal resistance in CW-MFCs, another important factor for the low efficiencies is the use of real urban wastewater which implies that complex organic matter is fed to the system and not easily biodegradable substrate like glucose or acetate which is used in many studies. Therefore the coulombic efficiency (CE) of CW-MFCs were at relatively low values around  $0.01\%$  (Wang et al., 2016b) to  $16.4\%$  (Xie et al., 2018) (see Table 2.3.3). Additionally the voltage, current and therefore power produced in CW-MFCs are highly dependent on the influent wastewater strength and as a consequence can be quite variable. Due to this dependency, CW-MFCs have also been investigated in regards to bioindication purposes (see Chapter 2.3.2.5) (Corbella et al., 2019).

#### 2.3.2.4 CW-MFC contaminant removal

Reported COD removal rates ranged from ca. 50% (J. Wang et al., 2017a, 2016b, 2016a) up to ca. 90-95% in air-cathode systems (Fang et al., 2017, 2016b; Liu et al., 2014; S. Liu et al., 2013; Song et al., 2017; Yakar et al., 2018) and up to nearly 100% when using forced aeration at the cathode (Oon et al., 2017, 2016, 2015) (see Table 2.3.3). The majority of studies which compared the COD removal of CW-MFC mode to control systems (such as similarly built conventional CWs or open-circuit CW-MFC) showed improved COD removal in the range from 4% to 8% (Corbella and Puigagut, 2018; Rathour et al., 2019; Wang et al., 2016b; Xie et al., 2018; F. Xu et al., 2018). However, Srivastava et al. (2015) reported an improvement of 12-20% (compared to open-circuit) and 27-49% (compared to conventional CW). A reason for improvement of COD removal can be seen in the additional organic matter degradation pathway via EAB and the anode, providing a relatively high energy gain for the involved bacteria which are situated in an otherwise mostly anaerobic HF CW bed with low redox and consequently scarce electron acceptors (D.-Y. Huang et al., 2011; Srivastava et al., 2015). Also studies in conventional MFCs showed higher COD removal as compared to open-circuit control systems with the possible explanations that the external load lowered the potential in the anodic compartment and thereby altered the microbial communities and their metabolic activities and/or that different microbial species (including fermentative species) could have utilized the organic matter more effectively by providing more diverse degradation mechanisms (Katuri et al., 2011). Zhang et al. (2015) found indications through CE calculations in wastewater fed MFC systems (comparing closed- and open-circuit), that electrogenic bacteria outcompeted other microbial degradation pathways. Hence the improvement in COD removal could be due to a more competitive and efficient electroactive pathway and a more diverse bacterial community resulting in synergistic effects.

In general, the COD removal in CW-MFC seems to be quite efficient, also when compared to full-scale conventional HF CWs which usually achieved COD removal rates higher than 80% (Dotro et al., 2017). However, due to the anyway relatively high COD removal rates in conventional CWs, the slight improvement exhibited in most studies does not have such a big significance for the CW field. This is why in terms of nutrient removal, the improvement of removal rates of other contaminants such as nitrogen could have a higher relevance.

Only a part of CW-MFC studies investigated nitrogen removal (see Table 2.3.3). Reported values include ammonium removal rates in the range of 68% to 97% (Corbella and Puigagut, 2018; Doherty et al., 2015c; Oon et al., 2017, 2016, 2015; Saz et al., 2018; F. Xu et al., 2018; Yakar et al., 2018; Zhao et al., 2013), as well as one report on a total nitrogen (TN) removal of 75.4% (L. Xu et al., 2017b). When compared to a CW-control system Corbella and Puigagut (2018), Wang et al. (2016a) and L. Xu et al. (2017) reported a 25% higher ammonium, 40.2% higher nitrate and 22.3% higher TN removal, respectively, in the CW-MFC system. Wang et al. (2017b) compared the effect of different electrode materials on nitrate removal (removal of 42.48 - 84.32%) and found that CF and foamed nickel outcompeted graphite rods and SSM possibly due to the higher abundance of EAB in these systems. As for COD removal, the reasons for the improvement could be due to improved conditions for microbial communities. L. Xu et al. (2018) compared microbial communities of a closed-circuit CW-MFC with a CW-control system, showing that (1) diversity and richness were higher in CW-MFC, (2) in the CW-MFC anode compartment the most common microbial functional groups were ammonia oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and anaerobic ammonium oxidation (anammox) bacteria, with NOB and anammox being significantly higher than in the control and (3) in the CW-MFC cathode compartment the microbial functional groups denitrifying bacteria (DNB), dissimilatory nitrate reduction to ammonium (DNRA), and EAB were significantly higher than in the control. In another microbial community analysis in CW-MFC systems, Wang et al. (2016b) found that anodes of closed-circuit MFC as compared to open-circuit systems had a significantly improved richness in EAB, nitrobacteria and DNB. Di Domenico et al. (2015) also found indications that conventional MFC could provide favorable conditions for anammox and Li et al. (2015) observed a higher abundance of anammox bacteria and associated higher nitrogen removal in closed-circuit MFC systems (open-circuit as control). In general, the improved nitrogen removal seems to be related to increased abundance of EAB and other functional groups responsible for nitrogen removal processes.

Around a third of CW-MFC studies presented in Table 2.3.3 also investigated phosphorus removal. Removal rates range from 31% to 94.5% orthophosphate ( $\text{PO}_4^{3-}$ -P) (Corbella and Puigagut, 2018; L. Xu et al., 2017b; Zhao et al., 2013) and 85% to 97% total phosphorus (TP) (Doherty et al., 2015c; Saz et al., 2018; F. Xu et al., 2018; Yakar

et al., 2018). However, as mentioned above, some of the materials used for anodes, cathodes and especially the packing layers (ceramsite, zeolite and dewater alum sludge) had a very high specific surface area and porosity which can lead to high phosphorus sorption, at least for some time, since sorption sites are generally limited. Therefore, more long-term studies would be needed in order to confirm these high phosphorus removal rates.

Out of the presented studies only one reported on sulfate removal with closed-circuit CW-MFC removing 13% less than open-circuit systems (Corbella and Puigagut, 2018). Lovley (2006) described that sulfide abiotically reacts with the electrode to form elemental sulfur which then can be microbially re-oxidized to sulfur and further to sulfate using the anode as electron acceptor. This mechanism could explain the lower sulfur removal rate in closed-circuit MFCs.



**Table 2.3.3.A.** Electrical characteristics and nutrient removal in selected CW-MFCs (updated from Corbella, 2017)

Reference	Power	Voltage	Coulombic Efficiency (CE)	COD removal	Nitrogen removal
Yadav et al. (2012)	16 mW/m <sup>2</sup>	-	-	75%	-
Zhao et al. (2013)	9 mW/m <sup>2</sup>	371 mV (avg)	0.10%	72-77%	77% NH <sub>4</sub> <sup>+</sup> -N
S. Liu et al. (2013)	12 mW/m <sup>2</sup>	640 mV (avg)	-	95%	83% NH <sub>4</sub> <sup>+</sup> -N
Villaseñor et al. (2013)	43 mW/m <sup>2</sup>	1161 mV (max)	0.45%	90-95%	-
Fang et al. (2013a)	302 mW/m <sup>3</sup>	610 mV (max)	-	86%	-
Liu et al. (2014)	45 mW/m <sup>2</sup>	525 mV (max)	8.86%	90%	-
Liu et al. (2015)	18 mW/m <sup>2</sup>	-	-	-	-
Oon et al. (2015)	6.1 mW/m <sup>2</sup>	422 mV (max)	8.86%	100%	91% NH <sub>4</sub> <sup>+</sup> -N
Doherty et al., (2015c)	276 mW/m <sup>3</sup>	434 mV (avg)	0.36%	81%	75% NH <sub>4</sub> <sup>+</sup> -N
Srivastava et al. (2015)	321 mW/m <sup>3</sup>	760 mV (max)	-	81-91%	-
Fang et al. (2015)	852 mW/m <sup>3</sup>	667 mV (max)	1.89%	86%	-
Oon et al. (2016)	93 mW/m <sup>3</sup>	421 mV (max)	1.42%	99%	96% NH <sub>4</sub> <sup>+</sup> -N
Wang et al. (2016a)	-	177 mV (max)	-	52%	-

**Table 2.3.3.B.** Electrical characteristics and nutrient removal in selected CW-MFCs (updated from Corbella, 2017)

Reference	Power	Voltage	Coulombic Efficiency (CE)	COD removal	Nitrogen removal
Wang et al. (2016b)	8.1 mW/m <sup>2</sup>	117 mV (avg)	0.01-0.11‰	44.5%	-
Oon et al. (2017)	185 mW/m <sup>3</sup>	546 mV (avg)	10.28%	99.00%	81% NH <sub>4</sub> <sup>+</sup> -N
L. Xu et al. (2017a)	2000 mW/m <sup>3</sup>	590 mV (max)	1.85%	88.70%	75% TN
J. Wang et al. (2017b)	5.1 mW/m <sup>2</sup>	183 mV (avg)	-	52%	42-84% NO <sub>3</sub> <sup>-</sup> -N
Corbella and Puigagut (2018)	288 and 346 mW/m <sup>3</sup>	102-123 mV (mean)	10-42%	82%	68% NH <sub>4</sub> <sup>+</sup> -N
Y.-L. Oon et al. (2018)	8.7 mW/m <sup>2</sup>	302 mV (avg)	1-3%	41-77%	-
Saz et al. (2018)	5.4-18.1 mW/m <sup>2</sup>	790-1340 mV (avg)	5-10%	85-88%	95-97% NH <sub>4</sub> <sup>+</sup> -N
Xie et al. (2018)	1.5 mW/m <sup>2</sup>	ca. 270 mV (max)	16.40%	68-79	-
F. Xu et al. (2018)	3714 mW/m <sup>2</sup>	266 mV (avg)	-	82	78% NH <sub>4</sub> <sup>+</sup> -N
Yakar et al. (2018)	15.1 mW/m <sup>2</sup>	1410 mV (max)	1.64%	92	93% NH <sub>4</sub> <sup>+</sup> -N
Tang et al. (2019)	8 mW/m <sup>2</sup>	640 mV (max)	0.21-0.36%	92%	97% NH <sub>4</sub> <sup>+</sup> -N
Rathour et al. (2019)	199 mW/m <sup>2</sup>	-	-	70%	-

### 2.3.2.5 CW-MFCs for bioindication

Since microbial fuel cell systems generate electricity from oxidation of organic and inorganic compounds using EAB as catalysts (Logan et al., 2006), it is theoretically possible to link and correlate the produced current with environmental parameters in order to utilize MFCs as biosensors. Biosensors are analytical devices which convert a biological response into a quantifiable and processable signal (Di Lorenzo, 2015). The main advantages of using an MFC as a biosensor include the possibility to monitor systems on-line, in-situ and in real-time without the need of time-consuming analysis in a laboratory and without the negative side-effect of producing chemical waste from the used reactants. However, several issues complicate the use of biosensors for bioindication purposes, including calibration and functional stability over long-term of the biofilm under different conditions in terms of operation and storage as well as technical and commercial competitiveness with already existing technologies (Kissinger, 2005). Consequently, the gap between academic research and commercial application of biosensors is still wide but some MFC-based biosensors are commercially available (Cui et al., 2019).

However, results from MFC bioindication research seem promising, showing linear correlations between organic matter content and produced electric signal with  $R^2$  values above 0.9 (Di Lorenzo et al., 2009; Gonzalez del Campo et al., 2013; Kim et al., 2003; Peixoto et al., 2011b). An important parameter is the range of organic matter concentration in which a linear relationship with the current can be established in different kinds of wastewater, which should be close to critical concentrations e.g. in legislation (Di Lorenzo et al., 2009). Sensitivity of a sensor is another important parameter and is defined as the signal change per unit change of analyte (e.g. COD), hence a sensor with high sensitivity to COD changes would therefore show a large current change per mg COD increase or decrease in the substrate. Also the electric response time of the biosensor is crucial, especially for real-time operation (Liu and Mattiasson, 2002). Reported values vary greatly depending on the systems and substrate used. For example, Kim et al. (2003) used two-chamber MFC and wastewater from a starch processing plant with linear correlations between 0-206 mg BOD/L showing a response time of 30-60 min, and reported a stable operation over 5 years without servicing. Di Lorenzo et al. (2009) used a single-chamber air-cathode MFC and achieved linear correlations in

the range of 50-350 mg BOD/L (using synthetic wastewater) with a response time of 40 min and high reproducibility over a period of 7 months. In another experiment, Di Lorenzo et al. (2014) was able to reduce the response time to less than 3 min by using a small-scale single-chamber (2 cm<sup>3</sup> anodic chamber) air-cathode MFC fed with synthetic wastewater (linear correlation for 3-164 mg BOD/L). However, real urban wastewater is more complex than synthetic wastewater, and many EAB possess limited metabolic versatility (e.g. utilizing only certain fermentation end products), and therefore require previous conversion processes (Kiely et al., 2011b). This limitation can affect the response time, for example showing delays of 10 h for concentrations higher than 78 mg BOD/L (Peixoto et al., 2011b).

Also during experiments with CW-MFCs, which actually did not have the aim of bioindication, a correlation between influent organic matter concentrations and produced electric output could be observed (Oon et al., 2016; Srivastava et al., 2015; Liu et al., 2014). Corbella et al. (2019) investigated lab-scale CW-MFCs fed with real urban wastewater for the use as biosensor and achieved linear relationships ( $R^2$  higher 0.8) after a contact time of more than 10 h with a range from 95 to 190 mg BOD/L. After 20 hours contact time the lower detection limit decreased to 70 and 40 mg BOD/L for the tested gravel and graphite based anodes, respectively (the part on the CW-MFC meso-scale application in their paper will be presented in chapter 4).

### 2.3.3 CWs operated as MECs

#### 2.3.3.1 General information

To date, still relatively few articles were published concerning CWs combined with MECs. In some publications the application of an external power source to the electrodes incorporated in a CW was labelled in different ways, such as electrolysis integrated/augmenting CW, bioelectrochemically-assisted CW, CW incorporating an electrolysis cell or polarized biofilter (Aguirre-Sierra et al., 2016; Gao et al., 2017; Ju et al., 2014a; Srivastava et al., 2018; D. Xu et al., 2017a) (see Table 2.3.4). However, for better readability, the different systems will be all referred to as CW-MEC from here on. In contrast to the majority of conventional MEC technologies, the presented CW-MECs did not aim at the creation of products such as hydrogen or caustic soda, but the improvement of contaminant removal.

#### 2.3.3.2 CW-MEC design and operation strategies

As for CW-MFC systems, also the presented CW-MECs mostly used artificial wastewater (Gao et al., 2018; Ju et al., 2014a; Srivastava et al., 2018; D. Xu et al., 2017a; Zhang et al., 2018). The systems were built in lab- meso- and one even in full-scale, whereas all of them were fed continuously, and besides one tidal flow system all either in a HF or up-flow hydraulic regime. A bit more than half of the systems were planted with aquatic macrophytes like *Canna indica*, *Juncus effusus* or *Cyperus alternifolius* (Gao et al., 2018; Ju et al., 2014b; Srivastava et al., 2018; D. Xu et al., 2017b). As for the CW-MFCs, the anode and cathode materials were carbon and metal based and the main substrate was either gravel only, gravel mixed with bio-ceramic (porous material with high specific surface area), or zeolite only, which has a high specific surface area and porosity as well as high electroconductivity (see Table 2.3.4).

**Table 2.3.4.** Design and operational conditions of selected CW-MECs

Reference	Planted/ Unplanted	Scale	Wastewater	Flow regime	Main bed material (packing layer)	Anode	Cathode
Ju et al (2014a)	planted	lab	synthetic	tidal flow	zeolite	iron and graphite	iron and graphite
Aguirre-Sierra et al. (2016)	unplanted	meso	real urban wastewater	HF	gravel	coke granules	coke granules
Gao et al. (2017)	planted	meso	synthetic	HF	bio-ceramic / gravel	iron plates	iron plates
D. Xu et al. (2017a)	planted	lab	synthetic	up-flow	gravel	GF and GAC	GF and GAC
Gao et al. (2018)	planted	meso	synthetic	HF	bio-ceramic / gravel	iron plates	iron plates
Srivastava et al. (2018)	unplanted	lab	synthetic	up-flow	gravel	granular graphite	granular graphite
P. Zhang et al. (2018)	unplanted	full	WWTP effluent	HF	bio-ceramic / gravel	iron column	iron column

GF    graphite felt  
GAC    granular activated carbon

### 2.3.3.3 CW-MECs electrical performance and contaminant removal

Only Aguirre-Sierra et al. (2016) used a potentiostat to set the anode potential at 0.3 V vs. Ag/AgCl reference electrode, while the rest used either a power supply or solar panels in order to provide additional energy input for the MEC. Hence also the applied current density to the electrodes varied greatly with the lowest value of 100 mA/m<sup>2</sup> by Aguirre-Sierra et al. (2016) and the highest by P. Zhang et al. (2018) being more than 20-fold with 11,500 to 24,500 mA/m<sup>2</sup>.

Only four out of the seven presented CW-MEC studies reported on COD removal, with removal rates ranging from 18% (P. Zhang et al., 2018) up to around 85% (Ju et al., 2014a; Zhou et al., 2018) (see Table 2.3.5). However, Ju et al (2014a) found the same COD removal efficiency when compared to their non-electrolyzed control, and Aguirre-Sierra et al. (2016) found a 5-7% higher removal efficiency compared to a gravel control (unplanted CW) when comparing five different HRTs (0.5, 0.8, 1.7, 3.4 and 4 d). Most presented CW-MEC studies focused on nitrogen or nitrate removal, with ammonium removal rates ranging from 46% to 83%, and nitrate removal rates of 43% to 69% (see Table 2.3.5). Ammonium removal was reported to have increased by only 1% (Ju et al., 2014a) and 4-16% (Aguirre-Sierra et al., 2016) when comparing CW-MEC to their respective control systems. In terms of COD and nitrogen removal, besides a direct influence of EAB as described for CW-MFC systems, electrolysis might have indirectly enhanced aerobic removal pathways in CW-MECs by increasing the DO in the CW-MEC, and subsequently the formed H<sub>2</sub> could further serve as electron donor for nitrate reduction to nitrogen gas, and H<sup>+</sup> could also be involved in autohydrogenotrophic denitrification (Gao et al., 2017).

Only Ju et al. (2014a) reported on sulfur removal and pointed out that no sulfide could be found in the effluent of CW-MEC, whereas 0.71 mg/L sulfide could be found in the control, which reportedly had a positive effect on odor control from sulfide accumulation.

Relatively high phosphorus removal rates in CW-MECs were reported with 66-95% orthophosphate (PO<sub>4</sub><sup>3-</sup>-P) removal (Gao et al., 2018, 2017). Also Ju et al (2014a) reported 85-95% removal, whereas they attributed it to a coagulation of the ferrous iron which formed during

electro-dissolution of the sacrificial metal anode. However, initial sorption effects on the main bed materials zeolite and bio-ceramic probably played a role as well. Again, more long-term studies would be needed in order to discern sorption and (bio)electrochemically effects.

Again, as for CW-MFC, besides direct and indirect bioelectrochemical effects, also the used main bed material likely played a role in adsorption mechanisms in the case of systems using zeolite or the bio-ceramic/gravel mix. The same would apply for most of the electrode materials. Such highly porous, electroconductive materials with high specific surface area are also utilized in a variant of CW-BES which are run in a short-circuit BES mode, meaning that there are no solid-state electrodes or external circuit used, leading to a so called single-piece electrode (the whole bed is filled with electroconductive media and electrically connected) in which electrons supposedly are transmitted along the electroactive media and ions travel with the bulk fluid to perform bioelectrochemical reactions in small anaerobic/anoxic microenvironments (Ramírez-Vargas et al., 2019). Aguirre-Sierra et al. (2016) compared such a short-circuit system (called coke biofilter) in parallel to a CW-MFC, CW-MEC (see Table 2.3.5) and CW-control setup, with the result that the short-circuit setup outperformed all other treatments with a 90-93% COD and 39-97% ammonium removal, respectively (depending on tested HRT). Compared to their CW-MEC this was an increase of 4-11% for COD and 7-30% for ammonium, respectively for short-circuit, whereas for the longest HRT CW-MEC showed a 7% higher ammonium removal. In general the short-circuit single electrode systems are difficult to compare to CW-control, CW-MFC or CW-MEC since the whole bed is filled with highly porous media with a high specific surface area and electroconductivity. Hence, it is difficult to discern between effects by sorption and bioelectrochemical factors. A variant of this short-circuit single electrode BES design is the so-called iron-carbon micro-electrolysis in which iron scraps and biochar are mixed and packed into small stuffing balls, forming numerous micro-scale galvanic cells (Shen et al., 2019; Zheng et al., 2019). These short-circuit BES were not described in more detail here, since they cannot be counted as CW-MFCs and neither as CW-MECs.



**Table 2.3.5.** Electrical characteristics and nutrient removal in selected CW-MECs

Reference	Power source	Current density (mA/m <sup>2</sup> )	COD removal	COD improvement compared to control	Nitrogen removal	Nitrogen improvement compared to control
Ju et al (2014a)	10 V (power supply)	57 to 150	85%	0% <sup>ne</sup>	78% NH <sub>4</sub> <sup>+</sup> -N	1% <sup>ne</sup> NH <sub>4</sub> <sup>+</sup> -N
Aguirre-Sierra et al. (2016)	0.3 V vs. Ag/AgCl at anode (potentiostat)	100	79-89%	5-7% <sup>gr</sup>	20-87% NH <sub>4</sub> <sup>+</sup> -N	4-16% <sup>gr</sup> NH <sub>4</sub> <sup>+</sup> -N
Gao et al. (2017)	0-30 V, 0-5 A (power supply)	700	-	-	68% NH <sub>4</sub> <sup>+</sup> -N	-
D. Xu et al. (2017a)	15 A (power supply)	750*	47-86%	-	79% NO <sub>3</sub> <sup>-</sup> -N	-
Gao et al. (2018)	0-30 V, 0-5 A (power supply)	200 to 800	-	-	43-50% NO <sub>3</sub> <sup>-</sup> -N	17-32% <sup>ne</sup> NO <sub>3</sub> <sup>-</sup> -N
Srivastava et al. (2018)	0.23-0.66 mA (solar panel)	370*	-	-	62-69% NO <sub>3</sub> <sup>-</sup> -N	0-4% <sup>gr</sup> NO <sub>3</sub> <sup>-</sup> -N
P. Zhang et al. (2018)	16-18 V (solar panel)	11500 to 24500	18%	-	46-83% NH <sub>4</sub> <sup>+</sup> -N	-

\* calculated from other parameters in the publication

<sup>gr</sup> gravel only CW-control

<sup>ne</sup> non-electrolyzed CW-MEC control

## 2.4 Summary

In summary the above presented state of the art was composed along the lines of the objective of the thesis and is intended to form the basis for the following presentation and discussion of the carried out investigations. Therefore, besides of the basics on CWs, BES and their combination, more details were presented regarding subjects which were part of the objectives and the respective investigations. Again, the main objective of this work was the improvement and control of wastewater treatment using CW-MFCs and CW-MECs.

In the following chapter, the general design and operation of the utilized experimental systems will be described.

# CHAPTER 3

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## General material and methods

This chapter describes the material and methods which were common to all investigations presented in the following Chapters 4-7. Additional material and methods or modifications used specifically for each investigation are described in the respective chapters.

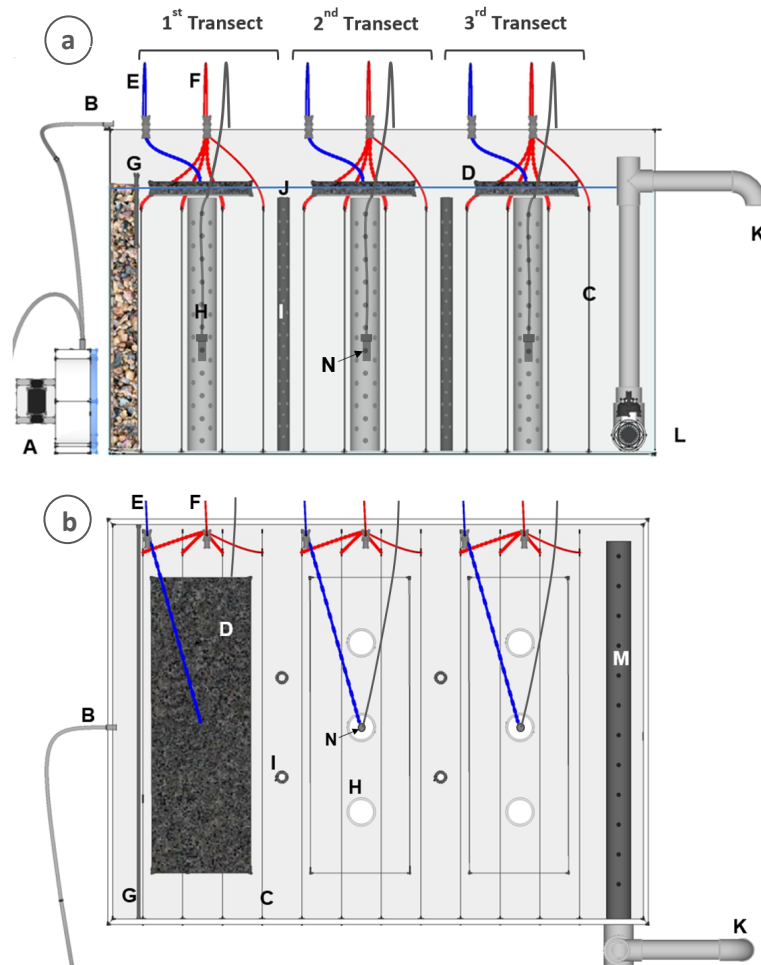
### 3.1 General design

For the purpose of this work, eight unplanted meso-scale horizontal subsurface flow (HF) CW-BES were constructed (see Figures 3.1.1-3). The systems consisted of a PVC reservoir of ca. 0.2 m<sup>2</sup> surface area (55 cm length x 35 cm width) filled up with 4/8 mm granitic riverine gravel. The systems were not planted to avoid an additional influencing parameter and further increase the experimental complexity. Wetted depth was set to be 25 cm (ca. 1 cm below the gravel surface). At the inlet and around the drainage of the outlet 7/14 mm granitic riverine gravel was used. Each CW-BES contained three BES along the flow path, i.e. one in each transect. Each one of the BES consisted of an anode based on four stainless steel meshes (marine grade A316L, mesh width = 4.60 mm, Øwire = 1.00 mm, S/ISO 9044:1999) along the flow path (each one 4 cm away from each other). Each metal mesh covered nearly the whole cross sectional area (0.08 m<sup>2</sup>) of the bed. The three cathodes consisted of a carbon felt (CF) mat (Alfa Aesar, 1.27 cm thick, with a surface of 0.03 m<sup>2</sup>, 99.0% carbon purity) and were placed on the surface of the gravel bed and kept semi-submerged, thus in contact with both liquid media and air (air-cathode).

For the closed-circuit CW-MFC (CW-MFC+) systems, each electrode's anode and cathode were externally connected via a 220 Ω resistance, selected according to results by Corbella and Puigagut (2018). The voltage across the external resistance for each electrode was continuously monitored by means of a datalogger (Campbell Scientific CR1000, AM16/32B Multiplexor). For the open-circuit CW-MFC (CW-MFC-) systems, the anode and cathode were not externally connected and served as a control in order to see the effects of the electrical connection in CW-MFC+. The control conventional CW (CW-control) were introduced in Chapter 5 and contained no anode metal meshes but the cathode carbon felt was left in place in order to not mistake physical filtration effects of the carbon felt with bioelectrochemical effects. This way the two control systems, CW-control and CW-MFC-, should reveal possible effects of the anode material itself on the treatment.

The CW-MEC duplicate was introduced in Chapter 6 and had the same setup as the CW-MFC+ but with an additional reference electrode for each of the three MECs in each transect of a system (see Figure 3.1.1, N). Each MEC was poised at a potential of 0.3 V vs Ag/AgCl at the anode using a potentiostat (nanoelectra NEV 4). The

potential difference between anode and cathode was logged by the potentiostat.



**Figure 3.1.1.** Section- (a) and plan-view (b) of the CW-MFC systems. A: Pump; B: Inflow; C: Anode; D: Cathode; E/F: Anode/Cathode connection to datalogger; G: Inflow barrier to avoid water short-circuiting on surface; H: Gravel core sampling tubes; I: Liquid sampling tubes; J: Water level; K: Standing pipe effluent; L: Drainage; M: Effluent collection tube, N: Reference electrode (at a depth of ca. 15 cm).

Intermediate liquid sampling ports were installed after the first transect and second transect (Figure 3.1.1, I), separating the first, second and third transect of the systems which are basically congruent with the three successive BES along the flow path of the wetland. These sampling ports consisted of two perforated plastic tubes ( $\text{\O} = 1\text{ cm}$ , positioned vertically 5 cm left and right of center). Underneath each cathode three perforated plastic tubes ( $\text{\O} = 3.2\text{ cm}$ , positioned at the center and 8.5 cm left and right of the center) were placed and filled with a plastic mesh “sock” containing the same gravel material as the systems (Figure 3.1.1, H). These socks were removable and were used to take samples for the microbial activity analysis and microbial community analysis.



**Figure 3.1.2** Photograph of one of the CW-MFC+ systems



**Figure 3.1.3** Photograph of the experimental setup with the eight experimental CW-BES (two additional test systems are in the right back)

## 3.2 General operational conditions

All systems received the same primary treated urban wastewater throughout the whole investigation time, except for some breaks and holidays which will be mentioned in the respective chapters. The wastewater was directly collected from a nearby sewer, pretreated by settling (3 h) and stored within an elevated reservoir with a volume of ca. 180 L. From there the primary treated urban wastewater could flow gravimetrically to each system via a tubing distribution system. The inflow rate to each system was regulated with a peristaltic pump (Damova MP-3035-6M) controlled by a variable frequency drive (VFD) (Toshiba VF-nC3S).



# CHAPTER 4

---

## CW-MFCs for bioindication

This chapter is based on the author's contribution to the following article:

Corbella, C., Hartl, M., Fernandez-Gatell, M., Puigagut, J., 2019. MFC-based biosensor for domestic wastewater COD assessment in constructed wetlands. *Sci. Total Environ.* 660, 218–226.  
<https://doi.org/10.1016/j.scitotenv.2018.12.347>

## **Abstract**

Microbial fuel cells (MFCs) are bioelectrochemical systems which enable a bioconversion of chemical energy contained in organic/inorganic compounds into electrical energy. Therefore, the measured MFC potential could be used as a bioindicator for organic matter concentration in wastewater. The resulting biosensor could monitor operational conditions, like organic matter concentration, in natural-based wastewater treatment technologies, such as constructed wetlands (CW). Such an in-situ, online and chemical-free biosensor would be of key importance for plant management optimization. The objective of the present study was to investigate constructed wetland microbial fuel cells (CW-MFCs) as a chemical oxygen demand (COD) assessment tool. Triplicate meso-scale CW-MFCs were periodically fed with real urban wastewater and showed good bio-indication responses between week 3 and 7 of operation (between an accumulated organic loading of ca. 100-200 g COD/m<sup>2</sup>). Especially the majority of increases in organic matter concentrations could be detected, leading the authors to the conclusion that CW-MFCs could be used as an “alarm-tool” for qualitative continuous influent or effluent water quality assessment.

## 4.1 Introduction

Microbial Fuel Cells (MFCs) are bioelectrochemical devices which can generate electrical power by oxidation of organic and inorganic compounds with the aid of electrochemically active bacteria (EAB) as catalyzers at the anode (Logan et al., 2006). The electrical potential and current which is produced by MFCs could therefore potentially be directly dependent and correlated to the incoming concentration of inorganic and organic compounds. Hence, MFCs could not only contribute to contaminant removal from urban wastewater while producing electrical energy, but additionally be used for bioindication purposes, using the electric signal correlated to organic matter contamination (Peixoto et al., 2011b). In conventional MFC systems  $R^2$  values above 0.9 were reported for correlations between MFC signal and COD concentrations (Chang et al., 2004; Di Lorenzo et al., 2009; Gonzalez del Campo et al., 2013; Peixoto et al., 2011b). Constructed wetlands (CWs) offer an ideal environment for the implementation of MFCs, due to the marked redox gradient in the CW filter bed, especially in systems with a horizontal subsurface flow (HF) regime. Also during studies on the combination of CWs and MFCs (CW-MFCs), which originally did not aim on bioindication, a correlation between influent COD and produced electrical signal could be found (Oon et al., 2016; Srivastava et al., 2015; Liu et al., 2014) (see more details in Chapter 2.3.2.5).

Organic matter is one of the parameters which is legally regulated in wastewater treatment processes and concentrations in the effluent discharged to receiving water bodies are generally limited to 125 mg COD/L in the EU (The Council of European Communities, 1991). At the moment the standard methods for the analysis of parameters concerning organic matter content, such as COD, are time-consuming, require a laboratory with trained staff and produce environmentally harmful chemical compounds (Kumlanghan et al., 2007). The electrical signal of a MFC or CW-MFC on the other hand has the potential to provide a continuous in-situ, online and real-time monitoring which enables rapid responses to events occurring in the wastewater treatment systems (Di Lorenzo et al., 2009; Peixoto et al., 2011b).

In nature-based solutions such as CWs and CW-MFCs such a bioindication tool would be of key importance for the assessment and

optimization of operational conditions and consequently for considerations for future designs. However, the use of CW-MFC also requires more expensive materials and more complex design and operation as compared to the relatively low-tech gravel-based conventional CW systems. Corbella et al. (2015) tested different anode materials such as graphite and gravel and showed that gravel with the use of stainless steel mesh (SSM) as current collector, as also used in this study, was suitable for EAB to establish. Therefore, the objective of the present study was to assess the use of CW-MFCs as a suitable wastewater COD biosensor for meso-scale CW-MFC systems fed with real urban wastewater, with the hypothesis that the CW-MFC signal can be correlated to the influent COD concentration.

The hypothesis was that the CW-MFC signal can be correlated to the influent COD content.

## **4.2 Material and methods**

### **4.2.1 Design**

For the purpose of this campaign, three out of the eight meso-scale CW-MFC systems described in more detail in Chapter 3.1 were used (see Figure 3.1.1). Each CW-MFC contained three separate MFCs along the flow path of the system. However, for the purpose of this investigation, only the recorded cell voltage of the first MFC in transect 1 will be considered. At the time of the study all systems were still operated in CW-MFC mode.

### **4.2.2 Operational conditions**

More details on the general operational conditions are presented in Chapter 3.2. During the presented bioindication investigation the 180-L reservoir was re-filled with fresh primary settled domestic wastewater three times a week, every Monday, Wednesday and Friday. Organic matter concentration within the influent reservoir decreased along the two- or three-day-period of storage; therefore, organic matter content entering the systems was highly variable, i.e. constantly decreasing, allowing to effectively track the CW-MFCs electrical response to the organic matter variation, measured as COD (HRT between reservoir and influent of systems was around 2 hours). The CW-MFC systems were operated at a flow rate of 5 L/d, resulting in a theoretical HRT and average OLR of 3.9 days and 4.5 g COD/m<sup>2</sup>·day, respectively.

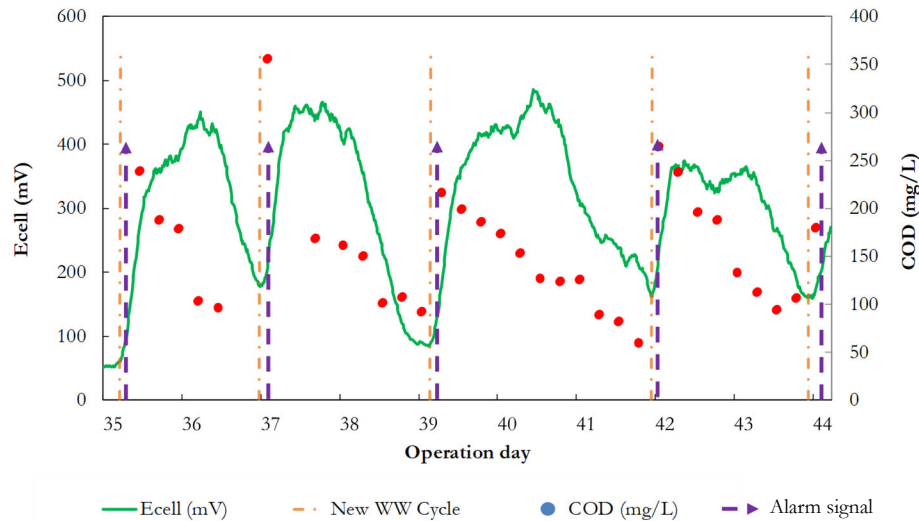
### **4.2.3 Sampling and analysis**

Samples were taken from the influent of each system using an automatic sample taker (SIGMA 900, Standard Portable Sampler). Influent samples were grab samples collected from inlet tubes. The parameter total chemical oxygen demand (COD) was analyzed according to standard methods (APHA-AWWA-WEF, 2005). Statistical

analysis was conducted using Kruskal-Wallis and Shapiro-Wilk tests as well as single-factor analysis of variance (ANOVA).

### 4.3 Results

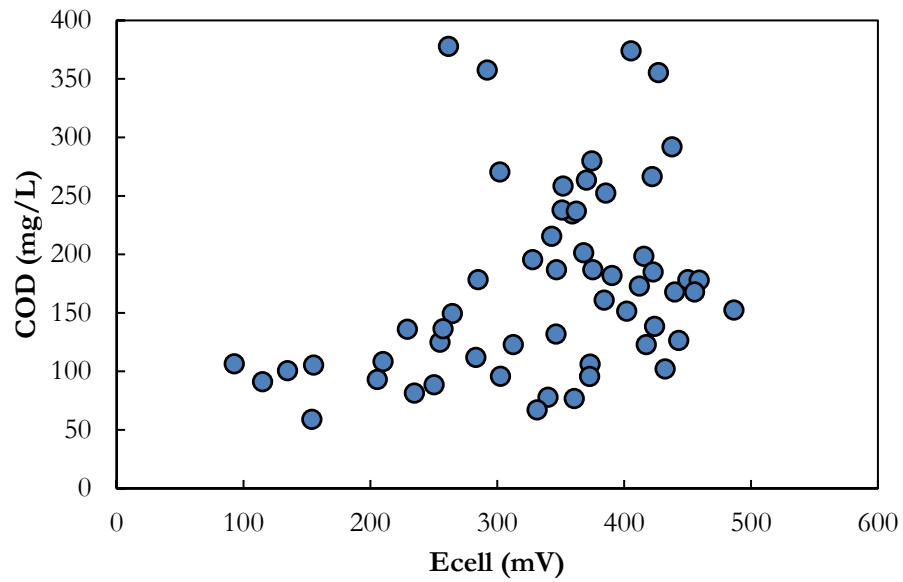
Figure 4.3.1 shows measured cell voltages ( $E_{\text{cell}}$ ) and COD values during a representative time lapse for one of the three meso-scale systems.



**Figure 4.3.1.**  $E_{\text{cell}}$  pattern and COD values over a representative time lapse for one of the experimental systems here considered (the x-axis shows system operation days). Note: times when re-fillings reached the system's influent are shown as vertical orange dot dashed lines; alarm signals are shown as magenta dashed lines with end arrows

The vertical orange dot dashed lines represent times when re-fillings reached the system's influent, resulting in a rapid increase in organic matter concentration, which is in turn represented by the red dots depicting sampled COD values. COD values dropped as low as 60 mg COD/L right before the refilling and leaped up to values as high as 378 mg COD/L. The rapid increase of COD at the beginning of each cycle was immediately followed by a steep increase of  $E_{\text{cell}}$  (up to ca. 400 mV) in most of the cases during the study period. It can also be seen, that  $E_{\text{cell}}$  generally followed the steadily decreasing COD concentration (down to ca. 100 mV), but with a variable delay. Although there is a visual correlation between  $E_{\text{cell}}$  and COD concentrations, it

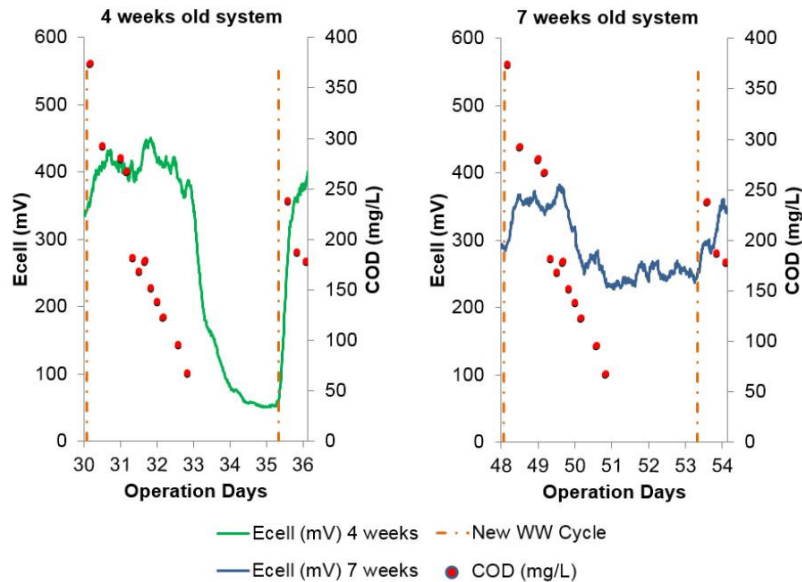
was not possible to find a good statistical correlation between COD and the respective  $E_{\text{cell}}$  values, as shown in Figure 4.3.2.



**Figure 4.3.2.** Correlation between  $E_{\text{cell}}$  and COD concentration for one of the two newer systems (n=57)



Figure 4.3.3 shows  $E_{\text{cell}}$  for one of the newer systems (4 weeks) and the older system (7 weeks) over the same period.



**Figure 4.3.3.**  $E_{\text{cell}}$  pattern and COD values for 4 weeks (left) and 7 weeks old (right) CW-MFC system (the x-axis shows respective system operation days). Note: times when re-fillings reached the system's influent are shown as vertical orange dot dashed lines.

Figure 4.3.3 shows that the bioindication range was decreasing with system age and accumulated organic matter loading. A good bioindication range could be achieved for systems aged ca. 3 weeks (after start-up and biofilm establishment time), for around 3 to 4 weeks (between an accumulated organic loading of ca. 100-200 g COD/m<sup>2</sup>), after that the average  $E_{\text{cell}}$  signal range decreased from ca. 100-350 mV to ca. 250-350 mV. The decreased lower limit of  $E_{\text{cell}}$  potential possibly reflects the effects of accumulated organic matter and endogenous respiration on voltage generation after longer operation time.

In light of these results, CW-MFC could be used as a qualitative tool to track sudden increases of COD at the influent of a wetland. The proposed alarm system to track a rapid influent COD increase is based on the steepness and the duration of a positive slope of  $E_{\text{cell}}$ , as well as

the condition that no alarm signal was raised within less than a Minimum Alarm Interval (MAI). The parameters Minimum Sum of Slopes (MSS), Minimum Slope Limit (MSL) and MAI can be changed in order to calibrate the alarm tool; e.g. by decreasing MSS in order to also capture shorter lasting increases or by decreasing the MSL in order to also raise an alarm for not so steep increases (it will take longer to reach MSS though). The datalogger recording interval (RI) is set to 15 minutes. Equation 4.1 shows the calculation of the slope by subtracting the slope ( $S_i$ ) of the last reading from the present reading. Equation 4.2 sums up  $S_i$  as long as  $S_i$  is positive (i.e. increasing), as soon as it is negative (i.e. decreasing) it is reset to 0. Equation 4.3 calculates the time since the last alarm was raised. Finally, Equation 4.4 describes the conditions needed in order for the alarm system to be triggered; (1)  $S_i$  has to be higher than the MAI, ensuring that the slope is steep enough, (2)  $SS_i$  has to be higher than MSS, ensuring that the slope is increasing over a longer time, and (3)  $AI_i$  has to be higher than MAI, ensuring that the last alarm is longer ago than the MAI. If any of these three conditions is not met, no alarm is raised.

Below is the description of parameters and equations used for the calculation of the alarm signal.

Variable (calibration) parameters included in the “alarm-tool” were set to the following:

$$MSS \text{ (Minimum Sum of Slopes)} = 140 \text{ mV/h}$$

$$MSL \text{ (Minimum Slope Limit)} = 16 \text{ mV/h}$$

$$MAI \text{ (Minimum Alarm Interval)} = 2 \text{ hours}$$

Non-variable parameter included in the “alarm-tool” is the following:

$$RI \text{ (Recording Interval)} = t_i - t_{i-1} = 0.25 \text{ hours}$$

The “alarm-tool” raised an alarm based on the following equations (Eq. 4.1 to Eq. 4.4):

Eq. 4.1

$$S_i (\text{Slope}) = \frac{(E_{cell_i} - E_{cell_{i-1}})}{RI} \quad i \in \mathbb{N}^+$$

Eq. 4.2

$$SS_i (\text{Sum of Slopes}) = \sum_{j=a+1}^i s_j : \begin{cases} s_a \leq 0 & a \in \mathbb{N}^+ \\ s_j > 0, \forall j \in [a+1, i] & j \in \mathbb{N}^+ \end{cases}$$

Eq. 4.3

$$AI_i (\text{Alarm Interval}) = RI \times (i - b) : \begin{cases} Alarm_b = 1 & b \in \mathbb{N}^+ \\ Alarm_c = 0, \forall c \in [b, i] & c \in \mathbb{N}^+ \end{cases}$$

Eq. 4.4

$$A_i (\text{Alarm})(S, SS, AI) = \begin{cases} 0 : (S_i \leq SL \vee SS_i \leq MSS \vee AI_i \leq MAI) \\ 1 : (S_i > SL \wedge SS_i > MSS \wedge AI_i > MAI) \end{cases}$$

The above presented alarm system was able to track most (75-80% across the three systems) of the episodes of rapid COD increase along the study period. Figure 4.3.1 shows the alarm signal at the times it was raised (purple dashed line with arrow at the top), resulting in an alarm response after around 2-4 hours in respect to the freshly refilled wastewater reaching the influent.

## 4.4 Discussion

The bioindication range of the investigated CW-MFC systems decreased over time with the lower limit of  $E_{\text{cell}}$  voltage increasing while the upper  $E_{\text{cell}}$  voltage stayed constant. One important aspect that was observed as well is that the increase in organic matter concentration is followed immediately by the  $E_{\text{cell}}$  potential whereas the decrease in organic matter concentration is only followed by  $E_{\text{cell}}$  after a delay. The decrease in bioindication range as well as the delayed decrease in  $E_{\text{cell}}$  compared to organic matter concentration could both be due to the generation of background level current (unassociated to influent COD levels) due to oxidation of accumulated solids and through endogenous metabolism, as described earlier for lab-scale CW-MFC systems under same conditions (wastewater type, anode and cathode material etc.) (Corbella et al., 2019). Furthermore, the measured parameter COD encompasses soluble and particulate organic as well as inorganic matter. Especially in the case of real urban wastewater, as used in the presented study, the organic/inorganic matter will arrive at the MFC partially in the form of complex compounds, which have to be hydrolyzed before the MFC's EAB are able to oxidize them (Kiely et al., 2011b). Hence, a portion of the measured COD will not contribute to the MFC's  $E_{\text{cell}}$  signal because it is not utilizable during the MFC's contact time. Since the microbial community in real urban wastewater is very diverse (Faulwetter et al., 2009), the EAB also have strong competition for the available substrate, with earlier studies showing that gravel-based lab-scale CW-MFCs have a coulombic efficiency (CE) of 5% only (when inlet concentrations were above 50 mg COD/L) indicating that the vast majority of organic matter was removed by other conventional removal pathways (Corbella et al., 2019). Microbial communities can also change over time depending on environmental conditions (e.g. dissolved oxygen (DO) availability, pH etc.) (Samsó and García, 2014). The resulting microbial diversity and variability would affect as well the MFC bioindication response time, accuracy and functionality. The use of real wastewater with particulate matter poses also the risk of anode clogging and concomitant reduction of the measured MFC signal (Corbella et al., 2016a). In addition, compounds like nitrates or sulfates can act as electron acceptors instead of the MFC anode (Liu and Logan, 2004). Contrarily the MFC could also generate a current through direct anodic oxidation of non-organic compounds, like during direct sulfide oxidation (Lovley, 2006). However, Corbella et

al. (2019) determined that abiotic reactions did not significantly contribute to the MFC signal generation in gravel-based lab-scale CW-MFCs. Investigations by Corbella et al. (2019) indicate that linear correlations between inlet COD and the MFC signal of gravel-based lab-scale CW-MFC systems can be generated, however, due to the abovementioned limitations, the precision, repeatability and operational stability of the systems might be affected.

Some of these limitations could also be observed in the presented meso-scale gravel-based CW-MFC systems, making it difficult to quantify organic matter concentration and water quality. However, the fast and reliable response to increasing organic matter loading in the presented study was utilized for the creation of a dual response “alarm-tool” which showed good bio-indication responses between week 3 and week 7 of operation (between an accumulated organic loading of ca. 100-200 g COD/m<sup>2</sup>). A more long-term investigation would be needed in order to test the alarm-tool functionality in terms of after longer periods of time and at the same time investigate potential maintenance solutions to “reset” the MFC’s bioindication range, especially for the lower COD range.

## 4.5 Conclusions

The triplicate meso-scale CW-MFCs showed good bio-indication responses between week 3 and week 7 of operation (between an accumulated organic loading of ca. 100-200 g COD/m<sup>2</sup>). Especially, increasing organic matter concentration was rapidly followed by an increase in CW-MFC cell potential, leading the authors to the conclusion that CW-MFC could be used as a dual-response “alarm-tool” for qualitative continuous influent water quality assessment. The proposed and developed alarm system is based on the steepness and the duration of a positive slope of  $E_{\text{cell}}$  and was able to track most (75-80% across the three tested systems) of the episodes of rapid COD increase along the study period.

Finally the results were only partly in line with the hypothesis, since the signal could not be entirely correlated to the influent COD content in a quantitative manner. However, the results showed that CW-MFCs could be used as a qualitative “alarm-tool” for continuous influent water quality assessment.

## Acknowledgements

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 676070. This communication reflects only the authors’ view and the Research Executive Agency of the EU is not responsible for any use that may be made of the information it contains.

# CHAPTER 5

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## Contaminant removal and microbial activity in CW-MFCs

This chapter is based on the following article:

Hartl, M., Bedoya-Ríos, D.F., Fernández-Gatell, M., Rousseau, D.P.L., Du Laing, G., Garfí, M., Puigagut, J., 2019. Contaminants removal and bacterial activity enhancement along the flow path of constructed wetland microbial fuel cells. *Sci. Total Environ.* 652, 1195–1208.  
<https://doi.org/10.1016/j.scitotenv.2018.10.234>

## Abstract

Microbial fuel cells implemented in constructed wetlands (CW-MFCs), albeit a relatively new technology still under study, have shown to improve treatment efficiency of urban wastewater. So far the vast majority of CW-MFC systems investigated were designed as lab-scale systems working under rather unrealistic hydraulic conditions using synthetic wastewater. The main objective of this work was to quantify CW-MFCs performance operated under different conditions in a more realistic setup using meso-scale systems with horizontal flow fed with real urban wastewater. Operational conditions tested were organic loading rate ( $4.9 \pm 1.6$ ,  $6.7 \pm 1.4$  and  $13.6 \pm 3.2$  g COD/m<sup>2</sup>·day) and hydraulic regime (continuous vs. intermittent feeding) as well as different electrical connections: CW-control (conventional CW without electrodes), CW-MFC- (open-circuit, external circuit between anode and cathode not connected) and CW-MFC+ (closed-circuit, external circuit connected).

Eight horizontal subsurface flow CWs were operated for about four months. Each wetland consisted of a PVC reservoir of 0.2 m<sup>2</sup> filled with 4/8 mm granitic riverine gravel (wetted depth 25 cm). All wetlands had intermediate sampling points for gravel and interstitial liquid sampling. The CW-MFCs were designed as three MFCs incorporated one after the other along the flow path of the CWs. Anodes consisted of gravel with an incorporated current collector made from stainless steel mesh (SSM), and the cathode consisted of a graphite felt layer. Electrodes of CW-MFC+ systems were connected externally over a 220  $\Omega$  resistance.

Results showed no significant differences between tested organic loading rates, hydraulic regimes or electrical connections, however, on average, systems operated in CW-MFC+ mode under continuous flow outperformed the other experimental conditions. CW-MFC+ compared to conventional CW-control systems showed around 5% and 22% higher COD and ammonium removal, respectively. Correspondingly, overall bacteria activity, as measured by the fluorescein diacetate (FDA) technique, was higher (4% to 34%) in CW-MFC+ systems when compared to CW-control systems.



## 5.1 Introduction

Constructed wetlands (CWs) are engineered systems for water and wastewater treatment, simulating processes occurring in nature (Vymazal, 2011). These natural systems are characterized by their low energy demand, comparative low cost, easy operation and maintenance as well as the possibility to use local materials and labor for their construction. A disadvantage of CWs is their relatively high area demand of ca. 1-10 m<sup>2</sup>/PE (Kadlec and Wallace, 2009).

Microbial Fuel Cells (MFCs) are bioelectrochemical systems that generate current by means of electrochemically active bacteria (EAB) as catalysts (Logan et al., 2006). In a MFC, organic and inorganic substrates are oxidized by bacteria and the electrons are transferred to the anode from where they flow through a conductive material and a resistor to an electron acceptor, such as oxygen, at the cathode (Logan et al., 2006; Rabaey et al., 2007). Compounds oxidized at the anode are mainly simple carbohydrates such as glucose or acetate that can be already present in the environment or obtained from the microbial degradation of complex organic substrates such as organic sediments or wastewater (Min and Logan, 2004; Clare E. Reimers et al., 2001). Therefore, MFCs are able to harvest energy in the form of electricity directly from wastewater (Du et al., 2007; Lefebvre et al., 2011; Min and Logan, 2004).

MFC systems can exploit the naturally occurring redox gradient in horizontal subsurface flow (HF) CWs. The first publication on CWs incorporating MFCs (CW-MFCs) appeared in 2012 and was published by Yadav et al. (2012). Since then publications on the subject per year are increasing, resulting in a rough total of around 79 up until March 2018. So far the vast majority of CW-MFC systems investigated are designed as lab-scale systems working under rather unrealistic hydraulic conditions (up-flow, batch feeding) using synthetic wastewater (Corbella et al., 2016b; Doherty et al., 2015c; Fang et al., 2016a; Liu et al., 2012; Oon et al., 2017; Song et al., 2017; Srivastava et al., 2015; Villaseñor et al., 2013; Y. Wang et al., 2017; F. Xu et al., 2018; L. Xu et al., 2017b; Zhao et al., 2013), which is favorable for fundamental investigations but reflects real conditions only to a limited extent.

As indicated above, the implementation of MFCs in CWs is a relatively new research field, and current available information on this

topic is mostly focused on optimizing treatment efficiency and energy production. Conventional MFCs are able to produce up to  $12 \text{ W}\cdot\text{m}^{-3}$  electricity (Logan and Rabaey, 2012). However, due to high internal resistances the highest reported electrical output from CW-MFCs is  $2 \text{ W}\cdot\text{m}^{-3}$  (L. Xu et al., 2017b), whereas averages for most systems are even a magnitude lower. Systems using wastewater reported electricity production of  $9.4 \text{ mW}\cdot\text{m}^{-2}$  (Zhao et al., 2013) and  $276 \text{ mW}\cdot\text{m}^{-3}$  (Doherty et al., 2015c). In comparison to solar panels with for example  $175 \text{ W}/\text{m}^2$  (Panasonic HIT® Photovoltaic Module, 2012) it seems that electricity production alone from wastewater by MFC or CW-MFC technology is currently not a feasible goal. Besides energy production, CW-MFC systems have also shown to be able to improve the treatment of organic matter and ammonium (see Chapter 2.3.2.4).

The main objective of this work was to quantify and improve the treatment efficiency of urban wastewater with CW-MFCs. The effect of hydraulic regime (continuous/intermittent) and organic loading rate ( $4.9\pm 1.6$ ,  $6.7\pm 1.4$  and  $13.6\pm 3.2 \text{ g COD}/\text{m}^2\cdot\text{day}$ ) on CW-MFCs performance and the effect of CW-MFCs on microbial activity along the flow path of the treatment bed are also discussed. The authors believe that this work will provide a useful insight into the actual net contribution of CW-MFCs on the treatment of urban wastewater. In spite of the lack of plants in the systems, the CW-MFCs used in this research could give additional information on the pollutant removal in larger scale systems under more realistic CWs design and operation conditions; also the here used configuration with three MFCs incorporated one after the other along the flow path of the CWs and the associated measured current along the flow path together with the measured bacterial activity will help to provide a better insight into the bioelectrochemical behavior and nutrient removal of CW-MFCs.

The hypotheses were that CW-MFC+ will outperform control treatments in terms of COD and ammonium removal, that continuous flow and low OLR will benefit contaminant removal, and that bacterial activity can be correlated to the treatment performance.

## 5.2 Material and methods

### 5.2.1 Design

For the purpose of this investigation, the eight meso-scale CW-MFC systems described in more detail in Chapter 3.1.1 were used (see Figure 3.1.1). At the time of the study all systems were still operated in CW-MFC mode. For the CW-MFC- systems, the anode and cathode were not connected (open-circuit). For the conventional HF CW-control (operated from week 12 to week 23), metal meshes were removed from two of the systems that were previously operated under CW-MFC-conditions.

### 5.2.2 Operational conditions

As described in more detail in Chapter 3.1.2, all systems received the same primary treated urban wastewater throughout the whole experimentation period (23 weeks within the period from May until December 2017 excluding breaks of 8 weeks during summer and the first week of December). Wastewater feeding started already 6 weeks before the start of experimentation in order to establish the biofilm in the systems. The wastewater was stored within a reservoir of ca. 180 L that was refilled every weekday in order to keep the organic matter concentration as stable as possible. Sampling and analysis were conducted once a week. This was different from the investigation in Chapter 4, when it was only refilled three times per week for the purpose of the bioindication investigation.

During the first 10 experimentation weeks (from May to July 2017) the effect of hydraulic regime and organic loading rate on the treatment performance of CW-MFC+ and CW-MFC- systems was tested. The compared hydraulic regimes were continuous and intermittent feeding. Continuous flow mode systems received the same flow rate all day long, whereas intermittent flow systems received alternating 4 h of double flow and 4 h of no flow, resulting in the same total flow as continuous flow systems on a daily basis.

Two different hydraulic loading rates were applied, i.e. 26 and 52 mm/day. The higher rate was obtained by doubling the flow rate (and

thereby dividing the HRT in half) resulting in a theoretical HRT and average organic loading rate (OLR) of  $3.9 \pm 0.2$  and  $1.9 \pm 0.1$  days and around  $4.9 \pm 1.6$  and  $13.6 \pm 3.2$  g COD/m<sup>2</sup>·day, during low and high loading periods, respectively (the high OLR is not exactly the double of the low OLR due to natural variations of the urban wastewater used). During experimentation week 1-5 the eight systems were operated under low OLR, and during experimentation week 6-10 with high OLR. The parameter OLR was chosen over HRT for comparison of the periods due to the higher reliability in the calculation of the OLR as opposed to the HRT which is only a theoretical value and could be different to the real HRT in the systems. The other two factors of continuous/intermittent feeding and CW-MFC+/CW-MFC- electrical connection led to duplicates of each combination in the first 10 weeks of experimentation (see Table 5.2.1).

**Table 5.2.1.** Operational conditions during the 23 weeks of experimentation concerning organic loading, hydraulic regime and electrical connection within the systems as well as the resulting individual experimental setups of the eight systems A) 2x continuous flow / CW-MFC+, 2x continuous flow / CW-MFC-, 2x intermittent flow / CW-MFC+ and 2x intermittent flow / CW-MFC-, B) 4x CW-MFC+ and 4x CW-MFC-, and C) 4x CW-MFC+, 2x CW-MFC-, 2x CW-control

Experi- mentation Week <sup>a</sup>	Organic loading rate (g COD/m <sup>2</sup> ·d)	Hydraulic regime	Electrical connection	System setup
1-5	Low OLR1 $4.9 \pm 1.6$	Continuous or Intermittent	CW-MFC+ or CW-MFC-	A
6-10	High OLR $13.6 \pm 3.2$	Continuous or Intermittent	CW-MFC+ or CW-MFC-	A
11	Low OLR2 $6.7 \pm 1.4$	Continuous	CW-MFC+ or CW-MFC-	B
12-23	Low OLR2 $6.7 \pm 1.4$	Continuous	CW-MFC+, CW-MFC- or CW-control	C

<sup>a</sup> only weeks in which experiments were conducted, i.e. excl. 8 weeks during summer and first week of December

Starting from experimentation week 11 (in September 2017, after 6 weeks of summer break during which the systems were fed with water and two weeks of wastewater feeding to restart systems), the treatment efficiency experiments were continued (until end of December 2017, except for the first week of December), this time only with continuous flow and low HLR (ca. 26 mm/day) resulting in a theoretical HRT of  $3.8 \pm 0.3$  days and an average OLR of  $6.7 \pm 1.4$  g COD/m<sup>2</sup>·day. Starting from experimentation week 12, two of the CW-MFC- were converted to conventional HF CWs by removing the SSM anodes, creating a conventional CW-control duplicate without electrodes, and still leaving two CW-MFC- and four CW-MFCs+ for investigation on solely the impact of the different electrical connections for the remaining experimentation weeks 12-23 (see Table 5.2.1).

### **5.2.3 Sampling and analysis**

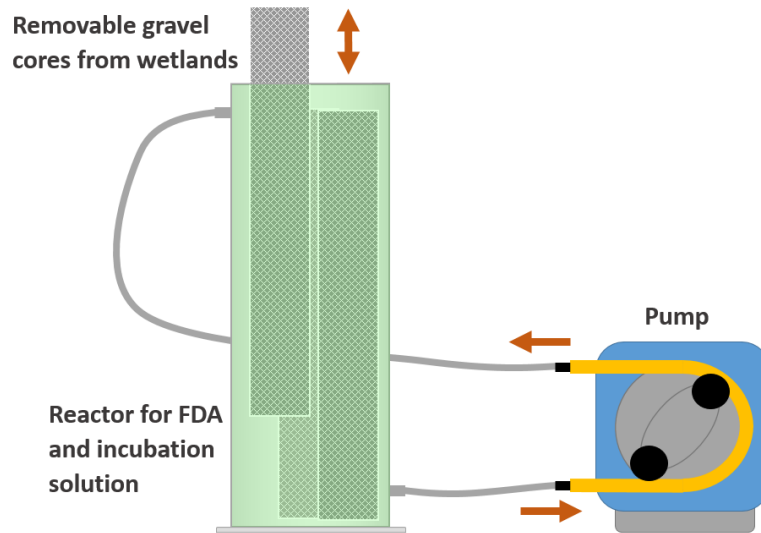
Samples were taken weekly from the influent, the intermediate sampling points placed at 1/3 and 2/3 of the wetland length and the effluent of each system. Influent and effluent samples were grab samples collected from inlet and effluent tubes, respectively. Intermediate samples were 60 mL composite grab samples (four times 15 mL) extracted from the pairs of sampling tubes placed after 1/3 and 2/3 from the inlet by means of a syringe. From each tube, two samples were taken, at 15 and 5 cm depth (i.e., 10 and 20 cm from the bottom of the system). The parameters total chemical oxygen demand (COD), ammonium -N, nitrate -N, nitrite -N, sulfate and orthophosphate -P as well as total suspended solids (TSS) and volatile suspended solids (VSS) were analyzed according to standard methods (APHA-AWWA-WEF, 2005). Physical parameters such as wastewater temperature, dissolved oxygen (DO) concentration (both; EUTECH instruments, EcoScan DO 6) and pH (CRISON pH/mV – meter 506) were measured as well using portable devices.

#### **5.2.4 Statistical analysis**

Contaminant removal efficiencies were calculated on a mass balance basis taking into account the wastewater flow and pollutant concentration. Statistical analyses were conducted using single-factor and two-way analysis of variance (ANOVA) and if necessary post-hoc Tukey HSD and Scheffé multiple comparison tests were performed.

#### **5.2.5 Microbial activity analysis**

Microbial activity was determined by means of the fluorescein diacetate (FDA) hydrolysis, a technique that has shown to correlate well with microbial population and its activity (Adam and Duncan, 2001). The FDA is a colorless compound which can be hydrolyzed by different enzymes releasing fluorescein as an end product, which absorbs strongly at 490 nm. For this procedure, two (out of the four available) CW-MFC+ systems and two CW-control systems were investigated, using the gravel cores contained within the sampling tubes located in each of the transects of the systems (see Figure 3.1.1, H). These gravel cores (three for each transect at a time) were introduced into previously constructed reactors of 10 cm diameter and 28 cm height (see Figure 5.2.1)



**Figure 5.2.1.** Microbial activity analysis setup including a reactor for the FDA and incubation solution in which the removable gravel cores (three per transect) from the wetland systems are submerged. The solution is mixed by means of a peristaltic pump.

At the time the three gravel cores were submerged the reactor already contained a prepared phosphate buffer at pH 7.6 together with 1 mL of 0.4 mM FDA (Acros Organics) resulting in a final concentration of  $8 \cdot 10^{-4}$  mM FDA, following a similar but modified procedure by Jasur-Kruh et al. (2010). This solution was recirculated with a pump and after 50 min a 2 mL sample was taken from the top of the reactor. Fluorescein released was measured using a spectrophotometer (Spectronic GENESYS 8 Thermo Scientific™) at a wavelength of 490 nm and then converted to Fluorescein molar mass via a calibration curve. For the purpose of this study the final Fluorescein molar mass value is then called the microbial activity. Statistical analyses were conducted using Kruskal-Wallis and Shapiro-Wilk tests as well as single-factor ANOVA.

## **5.3 Results and discussion**

### ***5.3.1 Assessment of operational conditions to optimize CW-MFC along the flow path***

#### **5.3.1.1 Overview**

Table 5.3.1 shows an overview for COD, ammonium, nitrate, nitrite and orthophosphate removal results from inlet to outlet, expressed in total specific mass ( $\text{g}/\text{m}^2\cdot\text{d}$ ) for CW-MFC- and CW-MFC+ systems (see SI, Table S5.1 for removal in percentage). Results are further divided into the three different OLR periods (low OLR 1 in first 5 weeks, high OLR in the following 5 weeks and low OLR 2 in the remaining 13 weeks) and different hydraulic regimes (continuous/intermittent) for low OLR 1 and high OLR period and only continuous flow in low OLR 2.



**Table 5.3.1.** COD, ammonium, nitrate, nitrite and orthophosphate average mass removal rate (g/m<sup>2</sup>·d) with standard deviation from inlet to outlet for low OLR 1, high OLR and low OLR 2 as well as intermittent or continuous flow hydraulic regime for CW-MFC- and CW-MFC+ systems

Removal (g/m <sup>2</sup> ·d)		Low OLR 1 (week 1-5)		High OLR (week 6-10)		Low OLR 2 <sup>a</sup> (week 11-23)
		4.9±1.6 g COD/m <sup>2</sup> ·day		13.6±3.2 g COD/m <sup>2</sup> ·day		6.7±1.4 g COD/m <sup>2</sup> ·day
		Intermittent flow	Continuous flow	Intermittent flow	Continuous flow	Continuous flow
<b>COD</b> (n=4/5/11) <sup>b</sup>	<b>CW-MFC-</b>	3.0±1.6	3.0±1.8	8.3±3.5	8.5±3.7	4.6±1.0
	<b>CW-MFC+</b>	2.8±1.7	3.0±1.8	9.6±3.9	9.6±2.9	4.9±1.1
<b>NH<sub>4</sub><sup>+</sup>-N</b> (n=4/5/7) <sup>b</sup>	<b>CW-MFC-</b>	0.2±0.1	0.2±0.1	0.5±0.7	0.6±0.6	0.3±0.2
	<b>CW-MFC+</b>	0.2±0.1	0.3±0.1	0.7±0.5	0.8±0.4	0.5±0.3
<b>NO<sub>3</sub><sup>-</sup>-N</b> (n=4/4/8) <sup>b</sup>	<b>CW-MFC-</b>	-0.009±0.026	-0.013±0.061	0.005±0.014	-0.002±0.018	0.000±0.000
	<b>CW-MFC+</b>	-0.012±0.035	-0.032±0.064	-0.022±0.033	-0.065±0.042	-0.011±0.012
<b>NO<sub>2</sub><sup>-</sup>-N</b> (n=4/4/8) <sup>b</sup>	<b>CW-MFC-</b>	0.023±0.052	0.039±0.078	0.094±0.235	-0.075±0.125	-0.004±0.014
	<b>CW-MFC+</b>	0.028±0.058	0.058±0.080	0.057±0.114	-0.154±0.046	-0.002±0.020
<b>PO<sub>4</sub><sup>3-</sup>-P</b> (n=4/4/8) <sup>b</sup>	<b>CW-MFC-</b>	0.02±0.03	0.03±0.01	0.03±0.04	0.03±0.04	0.01±0.01
	<b>CW-MFC+</b>	0.02±0.02	0.03±0.02	0.02±0.04	0.04±0.06	0.01±0.03

<sup>a</sup> Low OLR 2 results are shown in more detail in section 3.2 on the electrical connection effects

<sup>b</sup> Some experimentation weeks could not be considered due to highly diluted influent or technical analysis problems

With regards to different organic loading periods, only continuously fed systems are discussed and compared for all nutrients, since COD and ammonium treatment, though not being significantly different, were generally higher in continuously fed systems. In addition, continuously fed systems showed a very significant higher current density generation within the first transect (see Figure 5.3.1).

### 5.3.1.2 Hydraulic regime effects

In general, CW-MFC+ and continuously fed systems tended to show higher nutrient removal efficiencies when compared to the rest of operational conditions tested, although no statistically significant differences in COD or ammonium removal were found (for details see SI, Table S5.2). When comparing different hydraulic regimes with the same electrical connection, CW-MFC+ continuous systems had only 2% and 1% higher COD removal than CW-MFC+ intermittent systems during low OLR 1 and high OLR period, respectively. CW-MFC-continuous systems had 2% lower and 4% higher COD removal than CW-MFC- intermittent systems during low OLR 1 and high OLR period, respectively. As expected, the majority of COD was removed within the first transect, since organic matter removal basically follows a first-order degradation (Kadlec and Wallace, 2009).

Ammonium removal rates did not show any significant differences between hydraulic regimes and electrical connections (for details see SI, Table S5.2) but exhibited the same tendency as COD but more pronounced, with continuously fed and CW-MFC+ systems showing higher removal rates. When comparing different hydraulic regimes within the same electrical connection, CW-MFC+ continuous systems showed, on average, 11% and 4% higher ammonium removal than CW-MFC+ intermittent systems during low OLR 1 and high OLR period, respectively. CW-MFC- continuous systems had 6 and 12% higher ammonium removal than CW-MFC- intermittent systems during low OLR 1 and high OLR period, respectively.

Continuously fed systems tended to have a higher nitrate increase throughout all OLR periods, with (an extremely) significant difference ( $F(1, 4)$ ;  $p = 0.0007$ ) only in the high OLR period, probably caused by the shortened HRT (for details see SI, Table S5.2). Continuously fed systems showed higher nitrite removal during low

OLR 1 but also nitrite increase in these systems was higher during high OLR, however, without a significant difference. A significant difference in terms of nitrite removal was only found in terms of electric connection ( $F(1, 4); p = 0.02$ ), for details see SI, Table S5.2. The strong nitrite increase in continuously fed systems in the high OLR period could be a sign of a lack of oxygen and incomplete nitrification. Dissolved oxygen (DO) concentrations in the water column (3 cm and lower below water level) were below the detection limit of the probe along the whole flow path, i.e. at the inflow as well as after first, second and last transect.

An explanation for the slightly higher COD and ammonium removal in CW-MFC+ systems could be that continuous as compared to intermittent flow in HF CWs increases the vertical redox gradient and thereby provided a higher potential to drive MFC reactions (Corbella et al., 2014). The insignificance of differences could be partly due to the relatively high standard deviation, most likely caused by the variation in quality of the used real urban wastewater due to natural causes like rainfall events or dry periods.

Due to the insignificant difference of COD and ammonium removal between hydraulic regimes, the authors decided to continue operation from week 11 onwards with continuous flow only, since this is the regular regime for full-scale HF CWs. In addition, intermittently fed systems showed an extremely significant ( $F(1, 68); p = 3.13e-11$ ) reduction in current density generation within the first transect (see Figure 5.3.1).

Average orthophosphate removal was very similar in the low OLR 1 period and slightly higher in continuously fed systems during high OLR period, however, without a statistically significant difference (for details see SI, Table S5.2). Reason for the difference during high OLR period could be the temporarily (during feeding times) shortened HRT in intermittently fed systems leading to fewer orthophosphate removal through processes like adsorption and precipitation.

### 5.3.1.3 Organic loading effects

Overall, the removal efficiency of COD and ammonium did not depend on the OLR (low period one  $4.9\pm 1.6$ , high  $13.6\pm 3.2$  g COD/m<sup>2</sup>·day and low period two  $6.7\pm 1.4$  g COD/m<sup>2</sup>·day) and the thereby reduced HRT, showing no statistically significant differences (for details see SI, Table S5.3). Total COD and ammonium removal on a mass basis was higher during the high OLR period, due to the higher influent concentrations (see Table 5.3.2). Despite the differing OLRs, removal rates in percentage showed that there were no real differences between OLR periods in COD or ammonium removal (see SI, Table S5.1). In fact the removal efficiencies in percentage were rather increasing a little over time, from around 60% to 70% for COD and from around 25 to 40% for ammonium, probably due to the maturing of the systems. Both average nitrate and nitrite mass in CW-MFC+ systems increased during the high OLR period from in- to outlet. This could be interpreted as an effect of the observed increased ammonium removal through nitrification.

The systems adaptability to fluctuating organic loads illustrates a general asset of CWs; due to the fact that the majority of treatment happens in the first section of HF CWs, the remaining part of the system is able to lower the effects of flow and nutrient concentration peaks to a large degree, given that the systems are not overloaded or clogged (Samsó and García, 2014).

For the selection of the optimal OLR in CW-MFC systems it is important to find a good balance between the provision of sufficient substrate at the anode on the one side and overloading the system and thereby limiting the cathode functionality through growth of heterotrophic bacteria on the other (Doherty et al., 2015c; Freguia et al., 2008; Villaseñor et al., 2013). Capodaglio et al. (2015) tested different OLRs in swine manure fed MFCs and found that lower OLR (volumetric OLR 0.7 kg COD/m<sup>3</sup>·day) advantaged EAB growth and activity over methanogens as compared to higher OLR (volumetric OLR 11.2 kg COD/m<sup>3</sup>·day). The highest OLR chosen in this study (corresponding to 0.06 kg COD/m<sup>3</sup>·day) was governed by the given strength of the available urban wastewater and the highest hydraulic loading possible for continuous operation, given the size of the available feeding tank. Since the two tested OLRs in this study did not show significant differences, it seems they were within the above mentioned

balanced range for the operation of CW-MFC systems, though rather on the very low end compared to MFC studies which used OLRs of a magnitude higher. However, OLRs in the presented study are in the range of conventional HF CW OLRs (Vymazal, 2005). Of course the OLR range for best performance is also dependent on the MFC architecture, e.g. the used anode with gravel and SSM as electron acceptor has to be taken into account as well.

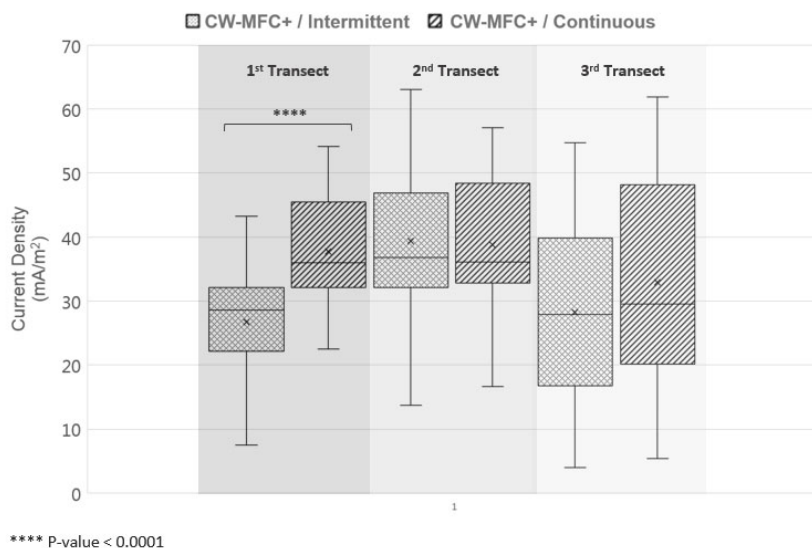
Additionally, by offering a more favorable electron acceptor, MFCs have shown to postpone methane production, for example in experiments using plant MFCs (PMFC) inside rice microcosms (Arends et al., 2014) and in CW-MFCs (Fang et al., 2013a).

With regards to electrical connections, although no significant differences were found within each of the three OLR periods, there was a slight tendency of increased treatment performance for CW-MFC+ systems in high OLR period and low OLR period 2. The authors believe that the absence of any difference among experimental conditions in continuously fed systems for the first experimental period (weeks 1-5) was due to the fact that the systems, and therefore the EAB biofilm, was still immature at the beginning of the experimentation, which is also reflected in the observed current, which was still increasing in all transects at the time (see Figure 5.3.1).

Low OLR 1 and high OLR periods had similar orthophosphate mass removal values although the influent load was doubled in the latter. Also, removal of orthophosphates in the last low OLR period 2 decreased below the levels of low OLR period 1 (see Table 5.3.1). These changes were probably not due to the different organic loading regimes but more likely due to the fact that phosphorus storage in CWs decreases over time due to finite capacity of adsorption sites in the biofilm and media (Kadlec and Wallace, 2009). In any case, the organic loading rate seems to have had no mentionable effect on orthophosphate removal in open- or CW-MFC+ systems.

### 5.3.1.4 Current

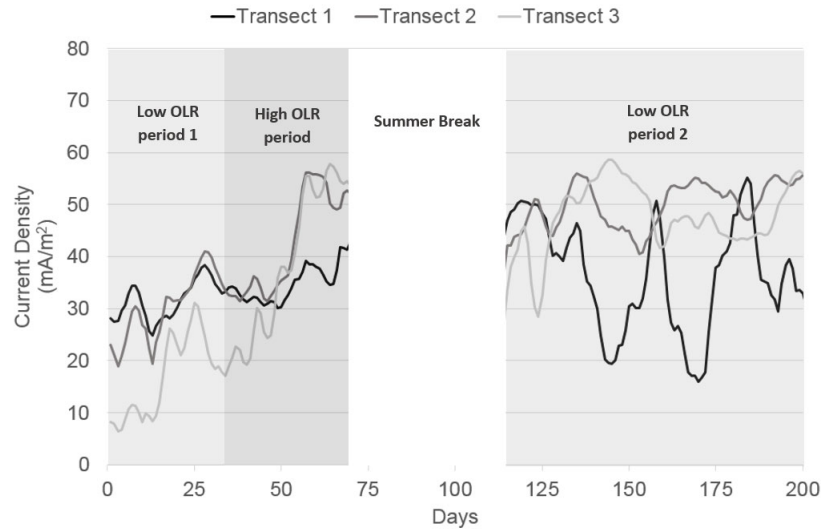
Figure 5.3.1 shows average current densities from the three MFCs corresponding to the three transects along the flow path for the intermittently and continuously fed CW-MFC+ systems.



**Figure 5.3.1.** Current density of intermittently and continuously fed CW-MFC+ systems per electrode and transect along the flow path during the first 10 weeks of experiments

Average current densities (based on the projected anodic surface area) for CW-MFC+/intermittent and CW-MFC+/continuous systems per transect resulted in  $26.8 \pm 9.4$  and  $37.7 \pm 8.1$  mA/m<sup>2</sup> for the first electrode,  $39.4 \pm 10.7$  and  $38.8 \pm 10.2$  mA/m<sup>2</sup> for the second electrode and  $28.2 \pm 9.4$  and  $32.9 \pm 17.1$  mA/m<sup>2</sup> for the third electrode, respectively. Differences among hydraulic regimes were only statistically significant for the first transect ( $p < 0.0001$ ) ( $F(1, 68)$ ;  $p = 3E-11$ ), while differences in second ( $F(1, 68)$ ;  $p = 0.73$ ) and third transect ( $F(1, 68)$ ;  $p = 0.08$ ) were not significant. These results show that the hydraulic regime had an extremely significant effect on the first third of the systems with higher values in continuously fed systems.

With regards to OLR effect, Figure 5.3.2 shows the average current densities per transect of the four CW-MFC+ systems during different OLR periods interrupted by the summer break.



**Figure 5.3.2.** Average current densities from four CW-MFC+ systems for each transect along time

Current densities during low OLR period 1 were  $33 \pm 6$ ,  $32 \pm 9$  and  $16 \pm 9$  mA/m<sup>2</sup> for first, second and third transect, respectively. During the high OLR period current densities increased to  $43 \pm 10$ ,  $45 \pm 11$  and  $43 \pm 13$  mA/m<sup>2</sup> for first, second and third transect, respectively. Finally, during low OLR period 2 current densities amounted to  $31 \pm 13$ ,  $52 \pm 9$  and  $47 \pm 8$  mA/m<sup>2</sup> for first, second and third transect, respectively. Current densities in the first low OLR period were generally lower than in the following high and low OLR period 2. This is probably due to the incomplete maturity of the systems during the first weeks after experimentation start, rather than due to OLR effects, since current densities during the second low OLR period are of similar magnitude than those of the high OLR period.

### **5.3.2 Contaminant removal and microbial activity under different electrical connections**

#### 5.3.2.1 Overview

In this section, contaminant removal efficiency of CW-control, CW-MFC- and CW-MFC+ treatments is addressed from the results obtained during week 12 to 23 of experimentation. During this period, all systems were operated in continuous flow with an average OLR of  $6.7 \pm 1.4$  g COD/m<sup>2</sup>.

Table 5.3.2 summarizes the results of COD, ammonium, nitrate, nitrite and orthophosphate during the last 12 weeks of experimentation for all three electrical connections; CW-control, CW-MFC- and CW-MFC+ systems. The results are shown as average mass at influent, after first transect, after second transect and effluent as well as removal from influent to effluent based on the average mass and percentage.



**Table 5.3.2.** Results for COD, ammonium, nitrate, nitrite and orthophosphate for CW control, CW-MFC- and CW-MFC+ systems during the last 12 experimentation weeks, expressed as average mass at influent, after first transect, after second transect and effluent as well as removal from influent to effluent based on the average mass and percentage

		Influent	1/3	2/3	Effluent	Removal from Influent to Effluent	
						(g/m <sup>2</sup> ·d)	(%)
<b>COD</b> (n=11) <sup>a</sup>	<b>CW-control</b>	6.6±1.5	3.3±1.0	2.5±0.6	2.0±1.1	4.5±1.0	69%
	<b>CW-MFC-</b>	6.4±1.6	3.0±0.9	2.2±0.9	1.8±0.9	4.6±1.0	72%
	<b>CW-MFC+</b>	6.7±1.5	2.9±1.0	2.1±0.9	1.7±0.9	4.9±1.1	74%
<b>NH<sub>4</sub><sup>+</sup>-N</b> (n=7) <sup>a</sup>	<b>CW-control</b>	1.2±0.2	1.1±0.2	0.9±0.2	1.0±0.3	0.3±0.3	19%
	<b>CW-MFC-</b>	1.2±0.1	1.0±0.2	0.9±0.2	0.9±0.2	0.3±0.2	24%
	<b>CW-MFC+</b>	1.3±0.1	1.0±0.1	0.8±0.2	0.7±0.2	0.5±0.3	41%
<b>NO<sub>3</sub><sup>-</sup>-N</b> (n=8) <sup>a</sup>	<b>CW-control</b>	0.002±0.007	0.000±0.000	0.041±0.042	0.002±0.005	0.000±0.009	-2%
	<b>CW-MFC-</b>	0.001±0.004	0.000±0.000	0.031±0.023	0.001±0.004	0.000±0.000	0%
	<b>CW-MFC+</b>	0.000±0.000	0.001±0.003	0.021±0.017	0.011±0.012	-0.011±0.012	NA <sup>b</sup>
<b>NO<sub>2</sub><sup>-</sup>-N</b> (n=8) <sup>a</sup>	<b>CW-control</b>	0.008±0.009	0.003±0.005	0.018±0.026	0.011±0.014	-0.003±0.008	-33%
	<b>CW-MFC-</b>	0.011±0.017	0.014±0.017	0.034±0.017	0.015±0.019	-0.004±0.014	-40%
	<b>CW-MFC+</b>	0.014±0.019	0.013±0.011	0.022±0.026	0.016±0.032	-0.002±0.020	-17%
<b>PO<sub>4</sub><sup>3-</sup>-P</b> (n=8) <sup>a</sup>	<b>CW-control</b>	0.11±0.02	0.11±0.02	0.09±0.02	0.11±0.06	0.00±0.03	1%
	<b>CW-MFC-</b>	0.11±0.02	0.10±0.02	0.09±0.02	0.09±0.02	0.01±0.01	10%
	<b>CW-MFC+</b>	0.10±0.02	0.11±0.02	0.09±0.02	0.09±0.03	0.01±0.03	5%

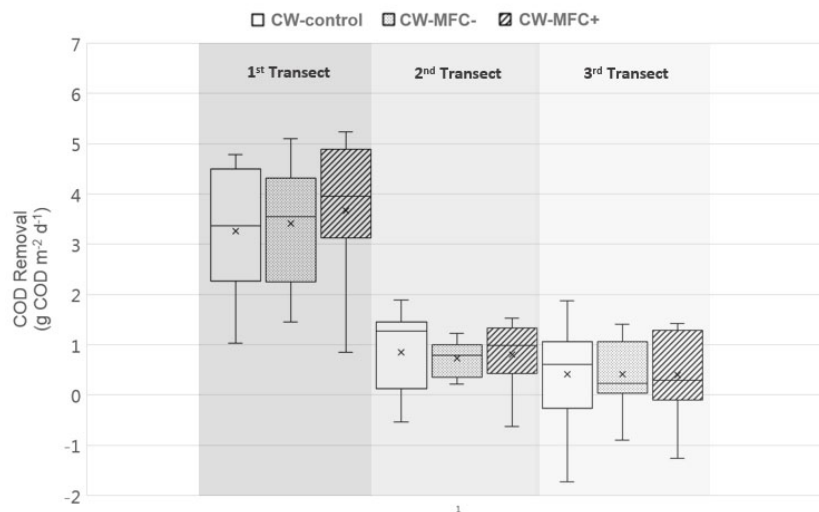
<sup>a</sup> Some experimentation weeks could not be considered due to highly diluted influent or technical analysis problems

<sup>b</sup> Division by zero

### 5.3.2.2 Electrical connection effect

As already previously described, CW-MFC+ systems outperformed CW-MFC- system on average during the first 10 weeks of operation (see Table 5.3.1), however, without significant differences (for details see SI, Table S5.4). COD and ammonium removal from week 11 to 23 showed the same tendency but again without any significant difference. The same is true if compared with a CW-control duplicate (from week 12 to 23) in the way that CW-MFC+ systems outperformed CW-MFC- and CW-control systems as well, however, again without any significant difference. Again, the insignificance of differences, especially in the case of ammonium, could be partly due to the relatively high standard deviation most likely caused by the variation in quality of the used real urban wastewater due to natural causes like rainfall events or dry periods.

Average COD removal on a mass base in the last 12 weeks of experiments (the time when CW-control was tested as well) in CW-MFC+ systems was only 2% higher than in CW-MFC- and 5% higher than in CW-control systems (see Table 5.3.2). Wang et al. (2016b) found higher improvement with 8.3% difference in COD removal comparing closed- to CW-MFC-, however, using a pH control and vertically batch-fed bench-scale systems. Regardless the treatment around 75% of the overall COD mass removal was already removed within the first transect, between 15% and 20% in the second transect and between 5% and 10% in the last (see Figure 5.3.3).

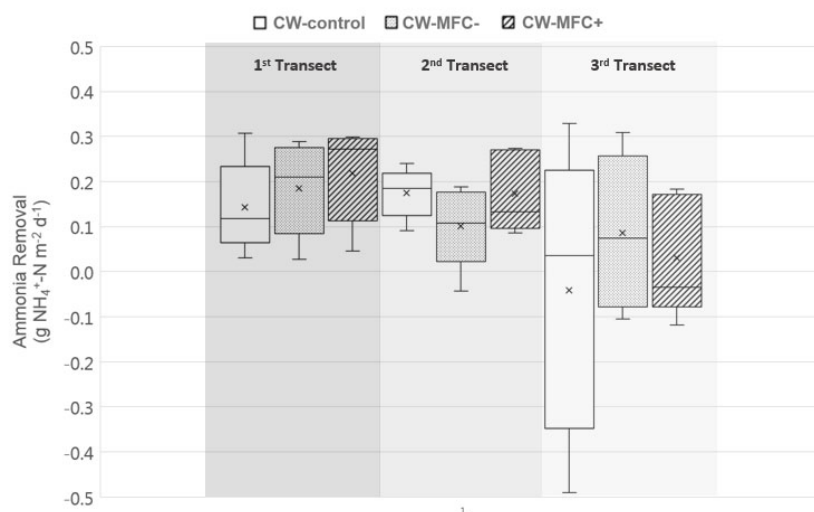


**Figure 5.3.3.** COD removal for each transect for CW-control, CW-MFC- and CW-MFC+ systems (n=11, CW-control duplicate started in week 12 and experimentation week 19 could not be used due to a highly diluted influent)

The overall COD removal of 74% in CW-MFC+ systems is comparable to earlier CW-MFC studies, with 75% (Yadav et al., 2012), 82% (Xu et al., 2018) and 76.5% (Zhao et al., 2013). In this regard, the presented study confirms results of these CW-MFC systems which were less representative for real situations; e.g. all mentioned above were in bench-scale, up-flow hydraulic regime, fed with synthetic or modified wastewater. Yadav et al. (2012) used very fine gravel (2-4 mm), only Xu et al. (2018) used a continuous flow but had a sand media and Zhao et al. (2013) used artificial aeration at the cathode. Some of these factors might influence treatment behavior, long term operation (e.g. clogging due to fine media) and possibly present up-scaling problems (e.g. flow direction, artificial wastewater). In comparison to full-scale HF CW systems the presented COD treatment efficiencies are not outstanding, but authors believe that the reason could be that meso- as well as lab-scale systems often have unfavorable hydraulic conditions due to the smaller scale, resulting in a lower HRT than the calculated theoretical HRT. An additional reason could be the lack of development of plants, which have shown to provide a significant positive wastewater treatment effect in subsurface flow CWs (Tanner, 2001).

Zhang et al. (2015) found indications through CE calculations in wastewater fed MFC systems (comparing closed- and open-circuit), that EAB outcompeted other microbial degradation pathways, while Fang et al. (2013) showed that EAB such as *Geobacter sulfurreducens* and *Betaproteobacteria* inhibited the growth of *Archaea* at the anode. Although the difference in COD removal in the presented study is very low, the more competitive electroactive pathway and potential inhibition of non-electroactive bacteria could have been the reason for the increased COD removal in CW-MFC+ systems.

Average ammonium removal on a mass base in the last 12 weeks in CW-MFC+ systems was 17% higher than in CW-MFC- systems and 22% higher than in CW-control (see Table 5.3.2) but not statistically different (for details see SI, Table S5.4). Average ammonium removal in transects was not as homogeneous across treatments as for COD; in CW-MFC+ systems the majority was removed in the first and second transect and only a small portion in the last, in CW-MFC- systems the majority was removed in the first and the rest in even parts in second and third, and in CW-control basically the whole treatment took place in the first and second transect (see Figure 5.3.4).



**Figure 5.3.4.** Ammonium removal per transect for CW-control, CW-MFC- and CW-MFC+ systems (n=7; CW-control duplicate started in week 12 and five experimentation weeks could not be used due to technical analysis or influent dilution problems due to rainfall)

The high variability in the last transect of CW-control is remarkable and could indicate that it was more unstable than in CW-MFC- or CW-MFC+ systems. Nitrate and nitrite effluent levels were generally very low during the time of electrical connections comparison (only week 11 was unusually high, but probably due to the start-up after summer). Both parameters increased a little in the second transect across all treatments and dropped again in the last (see Table 5.3.2). The only statistically significant difference (F (2, 8); p = 0.03) between electrical connections occurred for nitrate when looking at the removal from inlet to outlet (for details see SI, Table S5.4). Table 5.3.2 shows that the average nitrate level in CW-MFC+ systems was actually very similar after the first transect and even lower after the second transect as compared to CW-control and CW-MFC- systems. Only in the last transect nitrate levels only dropped by nearly half in CW-MFC+ while they went close to the initial influent concentration in the other electrical connections.

The observed average ammonium removal of 41% in CW-MFC+ was rather low compared to preliminary results of Zhao et al.

(2013) with an average of 77%, however, as mentioned above, the system had an artificially aerated cathode. In terms of improvement of efficiency compared to a control, Wang et al. (2016b) reported a 40% improvement of nitrate removal in CW-MFC+s compared to CW-MFC-, however, with a pH control. Most other works on CW-MFCs were rather focused on organic matter and not on nitrogen removal. L. Xu et al. (2018) recently observed an average of 82% total nitrogen removal, however, the systems were continuously up-flow fed bench-scale systems with a tubular shape. Furthermore, L. Xu et al. (2018) did a functional analysis of the microbial community, comparing a CW-MFC+ with a CW-control system, showing that (1) diversity and richness were higher in CW-MFC+, (2) in the CW-MFC+ anode compartment the most common microbial functional groups were ammonia oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and anaerobic ammonium oxidation (anammox) bacteria, with NOB and anammox being significantly higher than in the control and (3) in the CW-MFC cathode compartment the microbial functional groups denitrifying bacteria (DNB), dissimilatory nitrate reduction to ammonium (DNRA), and EAB were significantly higher than in the control. In another microbial community analysis in CW-MFC systems, Wang et al. (2016b) found that anodes of CW-MFC+ as compared to CW-MFC- systems had a significantly improved richness in EAB, *nitrobacteria* and DNB. Corbella et al. (2015) also found that *Geobacter* and methanogenic populations were significantly higher in CW-MFC+ when compared to CW-MFC-.

Of course the microbial community will also be dependent on the used materials for filter media, anode, cathode etc.; Wang et al. (2016a) found a significantly different distribution of microbial communities depending on the used CW-MFC anodes, comparing carbon fiber (CF) felt, graphite rods, foamed nickel and SSM. SSM, the material used in this experiment, and foamed nickel had significantly lower relative abundance of *Proteobacteria* than CF felt and graphite rods, which was related to a lower power production. However, reported voltage outputs by Wang et al. (2016a) using SSM reached averages from ca. 17 to 41 mV, which was by far surpassed in the presented systems with averages of  $304\pm 96$ ,  $462\pm 33$ , and  $457\pm 50$  mV for first, second and third transect, respectively.

The above described enrichment in anammox bacteria was already indicated in earlier research on MFC systems; Di Domenico et al. (2015) observed that MFC mode provides conditions favoring the

cultivation of anammox in the anodic compartment of the anaerobic digestate fed systems used, without inoculating anammox bacteria at any point (only electroactive bacteria *G. sulfurreducens* were inoculated). In another bench-scale MFC experiment, Li et al. (2015), this time using synthetic wastewater, were able to prove higher abundance of anammox bacteria and associated higher nitrogen removal in CW-MFC+ (open-circuit as control). However, these were inoculated with anammox bacteria in advance. Anammox bacteria were detected in conventional HF CW systems without MFC systems as well, however, Coban et al. (2015) could not detect any anammox activity in HF CWs, inferring that the process is of low importance in the nitrogen removal of conventional CW systems.

Another possible ammonium removal pathway could be volatilization due to proton loss at the cathode and associated locally elevated pH, which cannot be excluded since the authors did not have the capability to measure pH on a micro-scale at the cathode, e.g. by using microprobes (Kim et al., 2008).

In MFC systems designed for nitrogen removal, simultaneous nitrification and denitrification (SND) could be accomplished; Virdis et al. (2008) observed that although oxygen was present at the cathode, biofilm stratification at the cathode allowed nitrifying bacteria in the outer layer and putative denitrifying bacteria were found in the inner layers in a micro-anoxic environment. However, large amounts of oxygen around the cathode would inhibit the bioelectrochemical denitrification (Kelly and He, 2014), which is the case for the presented systems, and again there would have been no possibility to measure SND in the presented experimental setup.

Conventional nitrification through supply with oxygen could have only happened at the systems very surface since DO measurements in the influent, effluent and the water column were always below detection limit, and therefore oxygen could have only partly been responsible for ammonium removal, which still could not have explained the differences between treatments. L. Xu et al. (2018) also described how, even in separator-less (e.g. without a membrane between anode and cathode) CW-MFCs, like the ones presented here, unwanted oxygen diffusion to the anode is inhibited by microorganisms which depleted the oxygen before it could reach further down, forming a so-called “microbial separator”. This separator maintained also anaerobic conditions for the anode with just 2 cm distance from the

cathode which showed the highest maximum power density compared to higher distances and systems with a separator. This distance is comparable to the distance between cathode and beginning of the anode (which extends vertically nearly until the bottom) in the presented work.

Orthophosphate removal during the first 10 weeks of operation differed only very slightly between treatments, again with higher rates in CW-MFC+ continuously fed systems with a removal of up to 29% (see Table 5.3.1). Differences were not statistically significant (for details see SI, Table S5.4). Ichihashi and Hirooka (2012) observed phosphate removal of 70-82% in closed-circuit MFC systems, with 4.6–27% in form of precipitation on the cathode, mainly in the form of struvite. Corbella and Puigagut (2018) also found 15 % higher  $\text{PO}_4^{3-}$ -P removal, comparing CW-MFC+ to CW-MFC- systems, and they also found white precipitation on the cathode. However, it was not struvite but mostly Calcite ( $\text{CaCO}_3$ ) and Halite ( $\text{NaCl}$ ). However, maybe the conditions for struvite crystal precipitation were not met, i.e.  $\text{Mg}^{2+}$ ,  $\text{NH}_4^+$ -N, and  $\text{PO}_4^{3-}$ -P should exceed the solubility limit. Struvite solubility decreases with increasing pH (Doyle and Parsons, 2002). In addition, Zhang et al. (2012) found that biological phosphorus uptake, rather than chemical precipitation, can be increased in low current (smaller than 10 A) bioelectrochemical systems which is the case for the study of Corbella and Puigagut (2018) with ca. 1.45 mA and also the presented study with an average of ca. 1.48 mA across all three transects in the first 10 weeks. In any case, in the presented study no white precipitation was found on the cathodes.

Orthophosphate concentrations in the last 12 weeks basically stayed the same along the flow path across all three treatments. As described earlier it seems that adsorption sites already got limited in that period, since removal rates were higher in the first 10 weeks of experiments. In general, phosphorus storage in subsurface flow CWs takes place in plant biomass, bed media or accretion sediments and has a finite capacity (Kadlec and Wallace, 2009).

During the time of electrical connection comparison, from week 12 to 23, average cell voltages in the CW-MFC+ systems for the three transects amounted to  $304 \pm 96$ ,  $462 \pm 33$  and  $457 \pm 50$  V. Average current densities during the electrical connection comparison, from week 11 to 23, were  $31 \pm 15$ ,  $49 \pm 9$  and  $50 \pm 7$  mA/m<sup>2</sup> for transects 1, 2 and 3, respectively. These results are in the range of current densities in



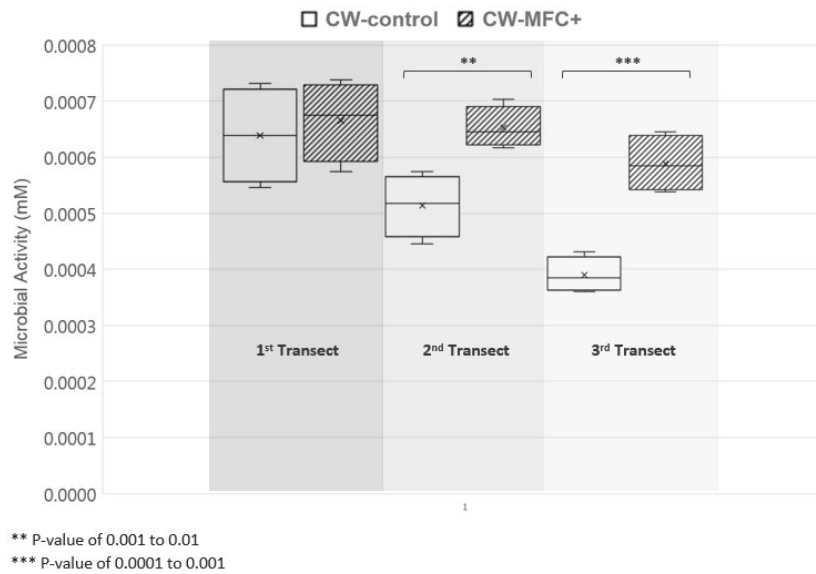
earlier CW-MFC experiments, with averages of 22.3 mA/m<sup>2</sup> by Villaseñor et al. (2013) and 70 mA/m<sup>2</sup> by Yadav et al. (2012). Polarization curves help to electrochemically characterize MFC systems and are shown for a CW-MFC+ replicate in the annex (see SI, Figure S5.1). The resulting maximum power densities and corresponding current densities amounted to 6.7 mW/m<sup>2</sup> and 27.3 mA/m<sup>2</sup> in the first transect, 36.6 mW/m<sup>2</sup> and 92.8 mA/m<sup>2</sup> in the second transect and 35.9 mW/m<sup>2</sup> and 92.8 mA/m<sup>2</sup> in the third transect. The estimated internal resistances derived from the polarization curves (PCs) were around 215 Ω, 100 Ω and 100 Ω for first, second and third transect, respectively. Principally, the potential maximum power is achieved when internal and external resistances are close to each other (Lefebvre et al., 2011). Therefore, it seems that the external resistance of 220 Ω fits very well for the first transect. According to the results, the second and third transect could potentially perform better with a lower external resistance around 100 Ω, however, it was decided to keep the same external resistance for all three transects for this experiment. The lower maximum power density in the first transect could be due to the higher organic loading in the first transect as compared to the second and third, which could a) potentially cause a clogging in the carbon felt cathode, limiting its potential and/or b) as also mentioned above in the discussion on the OLR, it was found that, in MFC systems, lower OLR benefited EAB growth and activity over competing methanogens (Capodaglio et al., 2015).

Coulombic Efficiency (CE) is the proportion of the produced charge to the carbohydrates which are theoretically derived from oxidation of organic and inorganic matter, indicated by the change of COD from transect to transect (Scott, 2016). The CEs over the whole time period in the three consecutive transects ranged from 0% to 8%, -34% to 46% and -89% to 93%, with averages of 1±3%, 10±17% and 2±34%, respectively. Earlier reported CW-MFC CEs range from 0.05-0.06% (Yadav et al., 2012) up to 2.8-3.9% (Liu et al., 2014). However, the authors believe that the parameter CE is not very useful for describing a CW-MFC's electric efficiency, especially if expressed per transect, since not only organic matter from the influent can contribute to the MFC signal but also accumulated organic matter within the gravel bed is a fuel source for MFC (Corbella et al., 2016a). This is probably the reason why the CE could reach high levels in the second and third transect; due to little COD removal and currents similar to the first transect it appears like a high current was produced with only little input.

Therefore, the reported high positive CE values in this paper, especially in the second and third transect, are most likely overestimated. The second and third transect CE even reached negative values due to eventually increasing COD concentrations within the wetland caused by changes in influent wastewater quality.

### 5.3.2.3 Microbial activity

Figure 5.3.5 shows microbial activity, determined through the FDA experiment, along the flow path of the CW-control systems and CW-MFC+ systems (all continuously fed).



**Figure 5.3.5.** Microbial activity along transects for CW-control and CW-MFC+ continuously fed systems

Generally, the activity was highest in the first transect, both in the CW-MFC+ and in CW-control systems (activity analysis was not performed for CW-MFC- systems), and the activity stayed on a higher level in the CW-MFC+ as compared to the CW-control systems. Differences between average microbial activities of CW-MFC+ and CW-control systems were not statistically significant in the first transect

(F (1, 4); p = 0.65), but statistically very significant in the second transect (p < 0.01) (F (1, 4); p = 0.006) and extremely significant in the third transect (p < 0.001) (F (1, 4); p = 0.0006).

The higher microbial activity within the first transect, irrespective of the treatment, is probably due to the higher availability of organic matter as a substrate, favoring the growth of microorganisms (Wu et al., 2014), with a subsequent decrease in microbial activity along the flow path, which has been observed already before in vertical and horizontal flow sequential CW systems (G. He et al., 2014). This decrease in activity is also reflected by the decrease in ammonium and COD removal along the systems flow path. CW-MFC+ showed higher activity than CW-control systems in all three transects. In percentages the microbial activity in CW-MFC+ systems was 4%, 21% and 34% higher than the control in first, second and third transect, respectively. L. Xu et al. (2018) analyzed diversity and richness (activity was not measured) of microbial communities in CW-MFC and CW-control systems and found higher diversity and richness in CW-MFC+ systems. Also Wang et al. (2016b) found higher richness in CW-MFC+ as compared to CW-MFC- systems. Hence, in the presented systems a higher diversity and richness in CW-MFC+s could have contributed to the measured higher activity. Corbella et al. (2015) also found that *Geobacter* and methanogenic populations were significantly higher in CW-MFC+ when compared to CW-MFC-.

As discussed in the section on COD removal comparing electrical connections, EAB in MFCs outcompeted other microbial communities and were also able to inhibit growth of *Archaea* at the anode (Fang et al., 2013a; Zhang et al., 2015). This advantage in competition could be another factor responsible for the increased activity in the studied CW-MFC systems. Also, as mentioned above in the discussion on the OLR, it was found that, in MFC systems, lower OLR benefited EAB growth and activity over competing methanogens (Capodaglio et al., 2015). Therefore, a possible explanation for the varying differences in microbial activity between CW-MFC+ and CW-control systems along the flow path could be that the decreasing OLR from transect to transect is leading from an insignificant difference in the first to a very significant difference in the second and extremely significant difference in the third transect. However, in comparison to the mentioned MFC studies, even the higher OLR at the influent of the presented study is already quite low (around a magnitude lower as in

the MFCs), but in the range of OLRs in conventional HF CWs (Vymazal, 2005). Therefore, the presented results could give an indication that even a further decrease in OLR, from an already relatively low level, still causes a recognizable advantage to the EAB over the methanogenic pathway.

MFCs have also been used for monitoring of microbial activity, in low contaminated environments like groundwater (Tront et al., 2008) or monitoring of anaerobic digestion processes (Liu et al., 2011).

## 5.4 Conclusions

The different tested organic loading rates and hydraulic regimes had no significant effect on treatment efficiency of COD or ammonium in the examined meso-scale horizontal-flow CW-MFC systems, but continuously fed systems showed slightly better treatment performance than intermittently fed systems. In addition, intermittent flow significantly decreased current production in the first transect of CW-MFC+ systems when compared to continuous flow.

In terms of electrical connection, CW-MFC+ systems were able to enhance treatment efficiency in comparison to CW-MFC- and CW-control systems, however, again without significant differences, which might be due to the use of real urban wastewater which varied in strength over time due to natural causes like rainfall events or dry periods.

Microbial activity clearly decreased along the flow path, as did ammonium and especially COD removal. Microbial activity was higher in all three transects in CW-MFC+ mode when compared to control conditions, which could be one of the reasons for the observed enhancement of treatment performance. Differences between CW-MFC+ and control systems were not significant in the first transect but very significant in the second and extremely significant in the third, possibly indicating that the lower organic load along the flow path benefited the activity of EAB over competing non-EAB.

In summary the results were in line with the hypotheses that CW-MFC+ will outperform control treatments in terms of COD and ammonium removal, that continuous flow will benefit contaminant removal, and that bacterial activity can be correlated to the treatment performance. The outcome was not entirely in line with the hypothesis that low OLR will benefit treatment performance, since results showed that the tested OLRs did not affect it.

## **Acknowledgements**

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 676070. This communication reflects only the authors' view and the Research Executive Agency of the EU is not responsible for any use that may be made of the information it contains. Marianna Garfí is grateful to the Spanish Ministry of Economy and Competitiveness (Plan Estatal de Investigación Científica y Técnica y de Innovación 2013-2016, Subprograma Ramón y Cajal (RYC) 2016).

## Supplementary information

**Table S5.1.** COD, ammonium and orthophosphate mass based average removal rate in percentage from inlet to outlet for low OLR 1, high OLR and low OLR 2 as well as intermittent or continuous flow hydraulic regime for CW-MFC- (OC) and CW-MFC+ (CC) CW-MFC systems.

Removal (%)		Low OLR 1		High OLR		Low OLR 2 <sup>a</sup>
		4.9±1.6 g COD/m <sup>2</sup> ·day		13.6±3.2 g COD/m <sup>2</sup> ·day		6.7±1.4 g COD/ m <sup>2</sup> ·day
		Inter- mittent flow	Cont- inuous flow	Inter- mittent flow	Cont- inuous flow	Cont- inuous flow
COD (n=4/5/11) <sup>b</sup>	OC	58%	56%	58%	62%	72%
	CC	56%	58%	68%	69%	74%
NH <sub>4</sub> <sup>+</sup> -N (n=4/5/7) <sup>b</sup>	OC	23%	29%	18%	30%	24%
	CC	27%	38%	35%	39%	41%
NO <sub>3</sub> <sup>-</sup> -N (n=4/4/8) <sup>b</sup>	OC	-95%	-110	44	-24	0%
	CC	-186	-290	-539	NA <sup>c</sup>	NA <sup>c</sup>
NO <sub>2</sub> <sup>-</sup> -N (n=4/4/8) <sup>b</sup>	OC	71%	71%	67%	-78%	-40%
	CC	67%	83%	48%	-314%	-17%
PO <sub>4</sub> <sup>3-</sup> -P (n=4/4/8) <sup>b</sup>	OC	21%	29%	10%	11%	10%
	CC	21%	29%	10%	16%	5%

<sup>a</sup> Low OLR 2 results are shown in more detail in the section 3.2 on the electrical connection effects

<sup>b</sup> Some experimentation weeks could not be considered due to highly diluted influent or technical analysis problems

<sup>c</sup> Division by zero

**Table S5.2.** Two-way ANOVA (with replication) results for the comparison of the factors hydraulic regimes (intermittent vs. continuous) and electric connections (CW-MFC- vs. CW-MFC+) as well as the interaction between the two factors, separated in low OLR 1 and high OLR periods.

Two-way ANOVA			p-value		
			Hydraulic Regime	Electric Connection	Interaction
Low OLR 1	COD	F (1, 4)	0.94	0.93	0.87
	NH <sub>4</sub> <sup>+</sup> -N	F (1, 4)	0.51	0.53	0.98
	NO <sub>3</sub> <sup>-</sup> -N	F (1, 4)	0.67	0.64	0.75
	NO <sub>2</sub> <sup>-</sup> -N	F (1, 4)	0.74	0.52	0.84
	PO <sub>4</sub> <sup>3-</sup> -P	F (1, 4)	0.66	0.85	0.86
High OLR	COD	F (1, 5)	0.45	0.96	0.94
	NH <sub>4</sub> <sup>+</sup> -N	F (1, 5)	0.43	0.71	0.85
	NO <sub>3</sub> <sup>-</sup> -N	F (1, 4)	0.0007 ***	0.03 *	0.10
	NO <sub>2</sub> <sup>-</sup> -N	F (1, 4)	0.44	0.02 *	0.78
	PO <sub>4</sub> <sup>3-</sup> -P	F (1, 4)	0.86	0.62	0.69

\* significant difference (p < 0.05)

\*\* very significant difference (p < 0.01)

\*\*\* extremely significant difference (p < 0.001)



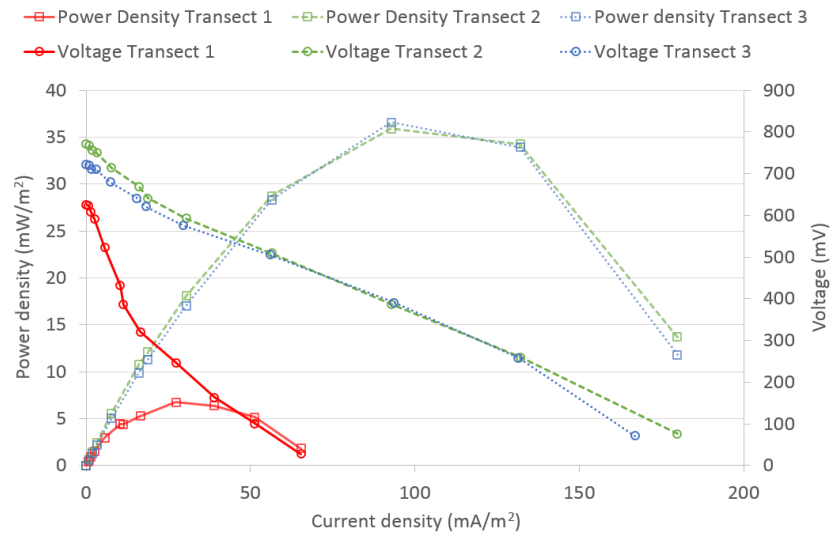
**Table S5.3.** One-factor ANOVA (with replication) results for the comparison of low OLR 1 and high OLR periods (considering only continuously fed CW-MFC+ systems) based on removal percentages ( $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N could not be calculated due to division by zero)

	One-factor ANOVA	p-value
COD	F (1, 4)	0.39
$\text{NH}_4^+$ -N	F (1, 4)	0.84
$\text{PO}_4^{3-}$ -P	F (1, 4)	0.35

**Table S5.4.** One-factor ANOVA (with replication) results for the comparison of the electric connections during the low OLR 2 period, for the total system from inlet to outlet and each of the three transects separately.

One-factor ANOVA		p-value			
		Electric Connection (low OLR 2 period)			
		Inlet-Outlet	Transect 1	Transect 2	Transect 3
COD	F (2, 11)	0.73	0.77	0.91	0.99
$\text{NH}_4^+$ -N	F (2, 7)	0.16	0.55	0.29	0.67
$\text{NO}_3^-$ -N	F (2, 8)	0.03*	0.35	0.38	0.21
$\text{NO}_2^-$ -N	F (2, 8)	0.74	0.33	0.73	0.71
$\text{PO}_4^{3-}$ -P	F (2, 8)	0.84	0.72	0.27	0.14

\* significant difference ( $p < 0.05$ )



**Figure S5.1.** Power density and polarization curves for each transect of one of the CW-MFC+ replicates measured during sampling week 10

# CHAPTER 6

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## **Contaminant removal and microbial community analysis in CW-BES**

This chapter is based on the following article:

Hartl M., Fernández Gatell M., Gagnon V., Weber K.P., Rousseau D.P.L., Du Laing G., Garfí M., Puigagut J. (in preparation)  
Urban wastewater treatment efficiency and microbial community analysis in constructed wetlands operated as bioelectrochemical systems.

## Abstract

The implementation of Microbial Fuel Cells (MFCs) and Microbial Electrolysis Cells (MECs) within constructed wetlands (CWs) was investigated in duplicate for 17 weeks comparing CW-control (conventional CW without MFC or MEC), CW-MFC- (open-circuit MFC), CW-MFC+ (closed-circuit MFC) and CW-MEC systems. All systems were already in operation for almost a year and therefore well established.

Results showed that average ammonium and COD removal was higher in CW-MEC (by 18% and 9%, respectively) and CW-MFC+ (by 16% and 6%, respectively) when compared to CW-control, while CW-MFC- performed similarly to the CW-control. A microbial community analysis showed distinct differences in community composition of CW-MEC anodes and cathodes when compared to all other treatments. The most abundant species was *Sphingobium yanoikuyae* which has not been reported in CW-MEC, or in general in bioelectrochemical systems (BES), such as MFCs or MECs, before. However, the closely related genera *Sphingomonas* and *Sphingopyxis* were reported in other CW-MEC systems. Probably due to the sampling method at the anode, only cathode samples of CW-MFC+ showed a microbial community significantly different from CW-MFC- and CW-control with relative high abundance of the species *Lysinibacillus boronitolerans*, which is closely related to *Lysinibacillus sphaericus*, a species which was also found in other MFC systems and was even identified to be electrochemically active.

## 6.1 Introduction

Constructed wetlands (CWs) for wastewater treatment are a well-established nature based solution around the world (Vymazal, 2011). They offer the benefits of a relatively low cost, low operation and maintenance needs as well as the possibility to use construction materials which are available locally in most parts of the world (García, 2001; Kivaisi, 2001; Puigagut et al., 2007b). Moreover, the treated water can potentially be reused for different applications depending on the effluent quality and prevailing regulations. A disadvantage of CWs is their relative high area demand of ca. 1 to 10 m<sup>2</sup> per person equivalent (PE), depending on the type of wastewater and wetland configuration (Kadlec and Wallace, 2009).

Bioelectrochemical systems (BES) such as Microbial Fuel Cells (MFCs) or Microbial Electrolysis Cells (MECs) are part of a relatively new and fast growing research field (Ramírez-Vargas et al., 2018). Within these BES, electroactive bacteria (EAB) are able to oxidize organic and inorganic substrates and transfer the electrons to an anode from where the electrons flow through a conductive material and a resistor to an electron acceptor, such as oxygen, at the cathode (Logan et al., 2006; Rabaey et al., 2007). EAB utilize a redox-gradient between electrodes (anode and cathode), which naturally occurs in constructed wetland (CW) systems, especially if designed in a continuous horizontal flow (HF) regime.

A MEC is basically a modified MFC, with the main difference that an external power source is supplied to control the potential between anode and cathode and thereby achieve thermodynamically otherwise unfavorable reactions (Rozendal et al., 2006). Another advantage of MECs is that only an additional voltage of 0.2-0.8 V is required for water electrolysis to occur (usually 1.8-3.5 V are required), due to the current produced through the activity of EAB at the anode. MECs are being investigated for a variety of applications, like hydrogen or methane production (Wagner et al., 2009), but are also used for wastewater treatment, either as stand-alone systems (Heidrich et al., 2014) or incorporated in (or hybridized with) other existing systems such as anaerobic digestion (Yu et al., 2018) or membrane technologies (Katari et al., 2014), as well as CWs (Ju et al., 2014a).

CWs operated as MFC or MEC will hereafter be termed CW-MFC and CW-MEC, respectively. In some publications the application

of an external power supply to electrodes incorporated in a CW was labelled in different ways, such as electrolysis integrated/augmenting CW, bioelectrochemically-assisted CW or CW incorporating an electrolysis cell (Gao et al., 2017; Ju et al., 2014a; Srivastava et al., 2018; D. Xu et al., 2017a). However, for better readability, they will be all referred to as CW-MEC in this publication.

Earlier studies of CW-MFC systems mostly used artificial wastewater, which is advantageous for the study of fundamental processes, but less realistic than real urban wastewater (Oon et al., 2018; Saz et al., 2018; Xie et al., 2018; F. Xu et al., 2018; Yakar et al., 2018), which is also the case for CW-MECs, with even less comparable studies available (Gao et al., 2018; Ju et al., 2014a; Srivastava et al., 2018; D. Xu et al., 2017a; Zhang et al., 2018).

At the moment, more promisingly than energy production, MFC, MEC, CW-MFC or CW-MEC systems have shown to be able to improve the removal of several wastewater contaminants. Studies using conventional MFC or CW-MFC systems showed an increase in COD removal of around ca. 5% to 30% (Hartl et al., 2019; Katuri et al., 2011; Srivastava et al., 2015). Apart from COD also ammonium treatment has been improved by MFCs (Kim et al., 2008; Lu et al., 2009) and also CW-MFCs showed an improvement of around 13% to 22% (Corbella and Puigagut, 2018; Hartl et al., 2019).

The main objective of this work was to assess the efficiency of urban wastewater treatment using duplicate systems of conventional CW-control, CW-MFC- (open-circuit MFC), CW-MFC+ (closed-circuit MFC) and CW-MEC systems. CW-control and CW-MFC- are both a control, with the difference that CW-control systems did not have an anode at all, see Chapter 3.1.1. To the best knowledge of the authors this is the first publication to compare these treatments in parallel. In order to be able to interpret the differences in treatment efficiency, a metagenomic analysis of the microbial community was conducted as well. The authors believe that this work will provide a useful insight into the actual net contribution of CW-BESs on the treatment of urban wastewater. In spite of the lack of plants in the systems, the CW-BESs used in this research could give additional information on the pollutant removal in larger scale systems using a more realistic CWs design and operation conditions due to the continuous feeding with real urban wastewater and more realistic horizontal flow hydraulics. In addition, the here used configuration with three MFCs or MECs, respectively,

incorporated one after the other along the flow path of the CWs as well as the associated microbial community analysis for anodes and cathodes could help to provide a better insight into the bioelectrochemical behavior and organic matter and nutrient removal within CW-BESs.

The hypotheses were that CW-MEC and CW-MFC+ will outperform all other treatments due to the involved bioelectrochemical processes. Further hypotheses were that the microbial community in CW-MEC and CW-MFC+ will differ when compared to control systems.

## **6.2 Material and methods**

### **6.2.1 Design**

For the purpose of this investigation, the eight meso-scale CW-BES systems described in more detail in Chapter 3.1.1. were used. Starting from this investigations a duplicate of CW-MFC+ was transformed into CW-MEC systems (see more details in the next Chapter 6.2.2 and Figure 3.1.1), resulting in four treatments, each with a duplicate of CW-MEC, CW-MFC+, CW-MFC- and CW-control systems.

### **6.2.2 Operational conditions**

All systems received the same primary treated urban wastewater throughout the whole experimentation period (17 weeks within the period from 22 February until 21 June 2018 excluding a break during the last week in March). Wastewater feeding started already 10 months earlier so the biofilm in the systems was well established during earlier experiments. The only fundamental change in the setup compared to earlier experiments was that the operation mode of two out of the four earlier CW-MFC systems was changed to CW-MEC by connecting potentiostats to each of the three BES of the two systems two weeks before the first sampling campaign of the presented experiment. The applied hydraulic loading rate was 26 mm/d resulting in a theoretical hydraulic retention time (HRT) of  $3.8 \pm 0.4$  days and an average organic loading rate (OLR) of  $5.3 \pm 1.8$  g COD/m<sup>2</sup>·day (average concentration of  $209 \pm 71$  mg/L COD).

### **6.2.3 Sampling and analysis**

Conventional wastewater parameters were measured for the influent, after the first and second third of the wetland length, and also at the effluent (for more detailed description see Chapter 5.2.3). All samples were analyzed for total suspended solids (TSS), volatile suspended solids (VSS) and total chemical oxygen demand (COD) according to Standard Methods (APHA-AWWA-WEF, 2012); NH<sub>4</sub><sup>+</sup>-N,



according to Solórzano method (Solórzano, 1969);  $\text{NO}_2^-$ -N,  $\text{NO}_3^-$ -N,  $\text{SO}_4^{2-}$ -S and  $\text{PO}_4^{3-}$ -P by ion chromatography (ICS-1000, Dionex Corporation, USA). Physical parameters such as water temperature, dissolved oxygen (DO) concentration, redox and pH were measured using portable devices at the influent, after the first and second transect as well as at the effluent (EcoScan DO 6, ThermoFisher Scientific, USA and CRISON pH/mV – meter 506).

### **6.2.4 Microbial community analysis**

Anode samples consisted of around 25 g of gravel which were taken from the entire depth of the three gravel core sampling tubes of each transect (Figure 3.1.1, H). The gravel was consequently put in sterile 50 ml plastic tubes and frozen at  $-20^\circ\text{C}$ . Non-destructive sampling of the anodic microbial community was difficult in this particular case given the used anode architecture with stainless steel mesh as electron acceptor and surrounding inert gravel. The microbial community samples for the cathode on the other hand were directly scraped off from the carbon felt cathode surface and are therefore representative for the electrode's electroactive microbial community. These scraped off cathode samples consisted of 1-3 g of cathode material from each transect and system (see Figure 3.1.1) which were also put in sterile 50 ml plastic tubes and frozen at  $-20^\circ\text{C}$ . Microbial community extraction was performed by mixing the samples in separate sterile flasks with 100 mL of autoclaved 10 mM phosphate-buffered saline (7.4 pH) followed by 3 hours of shaking on an orbital shaker (100 rpm; Innova 2000 Platform Shaker, New Brunswick Scientific, CT, USA). The extracted microbial solutions, consisting of the supernatant containing the suspension of bacteria, were then filtered through  $0.2\ \mu\text{m}$  filters and frozen at  $-80^\circ\text{C}$ . DNA extraction was performed using the FastDNA™ SPIN Kit for soil (MP Biomedicals, Fisher Scientific, ON, CAN) following the manufacturer's protocol (revision #116560200-201203). The DNA samples were stored at  $-80^\circ\text{C}$  until further analysis. Quantification of DNA was performed using a Qubit® Fluorometer (Invitrogen, ON, CAN) and Qubit® dsDNA BR assay kit. Metagenomic sequencing of 16S ribosomal RNA gene amplicons was performed using a 16S sequencing library prepared under a PCR clean hood for the Illumina MiSeq System (Protocol Part #150442223 Revision B, Illumina Canada, BC, CAN) (Caporaso et al., 2010). Briefly, the 16S V3

and V4 variable region was amplified with PCR using Illumina forward and reverse primers, and samples were barcoded with a unique index in a subsequent PCR. Samples were denatured, diluted to 4 nM, and pooled with a 10% PhiX control spike-in. The pooled sample library was sequenced on an Illumina MiSeq with MiSeq Reagent kit V3 for paired end (2 x 600 bp). Raw reads were de-multiplexed into individual forward and reverse fastq files per sample and were processed using QIIME2 (QIIME.org) (Caporaso et al., 2010) with DADA2 denoising pipeline (Callahan et al., 2016) to dereplicate and detect individual sequence variants (sOTUs) and remove chimeric sequences. Taxonomy was assigned to sOTUs using the Q2 implementation (Bokulich et al., 2018) of a scikit-learn naive Bayes machine-learning classifier (Pedregosa et al., 2011) using the Greengenes database V13\_8 (McDonald et al., 2012).

### **6.2.5 Statistical analysis**

Contaminant removal efficiencies were calculated on a mass balance basis taking into account the wastewater flow and pollutant concentration. Statistical analyses were conducted using single-factor analysis of variance (ANOVA) and if necessary post-hoc Tukey HSD and Scheffé multiple comparison tests were performed.

Analysis of the metagenomic data was done in QIIME2 using principal coordinates analysis (PCoA) of the  $\beta$ -diversity using phylogenetic distances of the microbial community (weighted UniFrac) for the anodes and cathodes in all three transects of all systems (Lozupone and Knight, 2005). Principle Component Analysis (PCA) was done in R (R Core Team, 2014) for the different physical and chemical parameters and all three transects of all systems. Figures were produced using the package ggplot2 (Wickham, 2008). The microbial community was also analyzed using the Shannon-Wiener diversity index.

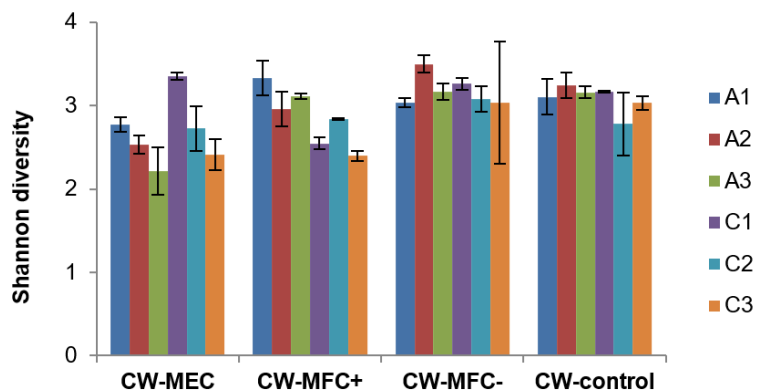
## **6.3 Results and discussion**

### **6.3.1 *Microbial community analysis***

The results of the microbial community analysis are presented and discussed first since they are utilized in the following discussion of contaminant removal results. The goal of the microbial community analysis was to compare the anodic and cathodic microbial communities across the treatments, with the focus on comparing CW-MEC and CW-MFC+ with the two types of control treatments CW-MFC- and CW-control. As mentioned above, the anodic samples could not be taken directly from the electrode surface, therefore the results of the sampled anodic microbial community could give valuable information on the effect of different BES (MFC-, MFC+ and MEC) at a relative far distance from the anode. In general, microbial community analyses are rather indicative in nature and have to be interpreted with care (e.g. a species with relatively low abundance could be over proportionally active and vice versa).

### 6.3.1.1 Richness and evenness of microbial communities

Figure 6.3.1 shows Shannon's diversity index at the genus level for anodes and cathodes in all three transects of all four treatments.

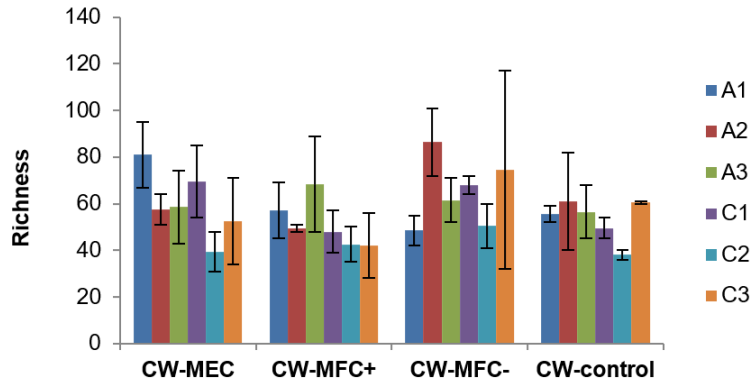


**Figure 6.3.1.** Shannon diversity at the genus level divided into anodes (A1, A2 and A3) and cathodes (C1, C2 and C3) in each transect for each treatment (N=2).

On average across all transects, CW-MEC anodes showed lower average genus diversity ( $2.51 \pm 0.28$ ) than anodes of CW-MFC+ ( $3.13 \pm 0.19$ ), CW-MFC- ( $3.23 \pm 0.24$ ) and CW-controls ( $3.17 \pm 0.07$ ). CW-MEC genus diversity was statistically significantly different in anode 2 (F (3, 2);  $p = 0.4$ , compared to CW-MFC-) and anode 3 (F (3, 2);  $p = 0.03$ , compared to CW-MFC- and CW-control), and very significantly different in cathode 1 (F (3, 2);  $p = 0.0017$ ) compared to all other treatments (see Supplementary information (SI), Table S6.4). In addition, the genus diversity of the anodes and cathodes of the CW-MEC (also partially CW-MFC+) tended to decrease from the inlet to the outlet (see Figure 6.3.1). This might be due to decreasing and limited nutrient availability along the flow path.

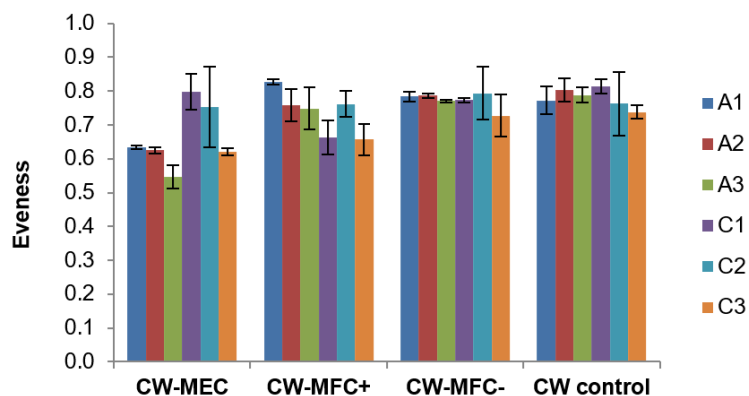
Genus richness (total number of genera) is shown in Figure 6.3.2 and varied between treatments and along system transects, with

lowest genus counts around 40 and the highest in the eighties in CW-MEC transect 1 anode and CW-MFC- transect 2 anode.



**Figure 6.3.2.** Richness of at the genus level divided into anodes (A1, A2 and A3) and cathodes (C1, C2 and C3) in each transect for each treatment (N=2).

The genus evenness is shown in Figure 6.3.3 and was lower in CW-MEC anodes compared to other treatments, indicating that a few genera (or just one genus) of bacteria were dominant. In addition, the evenness of the CW-MEC cathodes tended to be higher than that of the anodes, with decreases from the inlet to the outlet.



**Figure 6.3.3.** Evenness at the genus level divided into anodes (A1, A2 and A3) and cathodes (C1, C2 and C3) in each transect for each treatment (N=2).

When comparing microbial community evenness across treatments, CW-MEC showed statistically significant lower evenness in transect 1 anodes ( $F(3, 2); p = 0.01$ , compared to all other treatments), transect 2 anodes ( $F(3, 2); p = 0.04$ , compared to CW-control) and transect 3 anodes ( $F(3, 2); p = 0.03$ , compared to CW-control and CW-MFC-) (see SI, Table S6.4). When looking at the cathodes of CW-MFC+, CW-MFC- and CW-control, transects 1 and 3 showed lower evenness than CW-MFC- and CW-control, indicating that some genera might have been more dominant, although without statistically significant differences (see SI, Table S6.4).

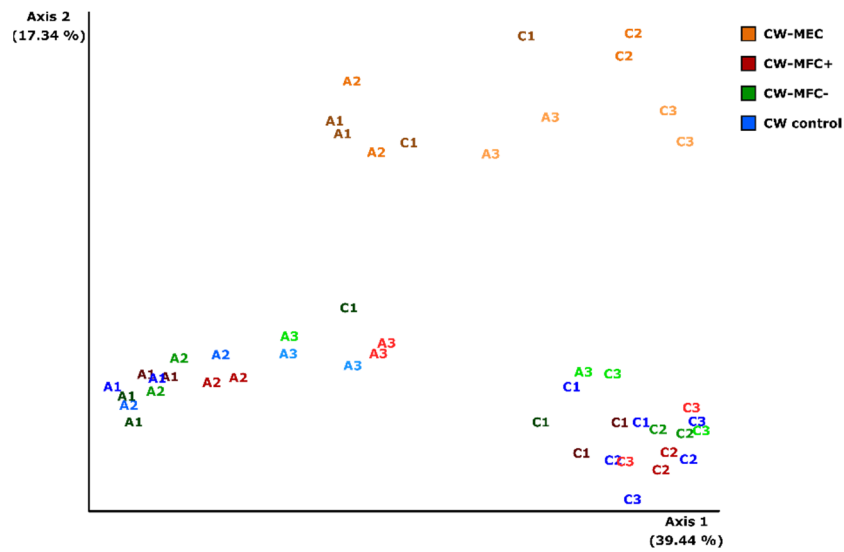
The Shannon's diversity, richness and evenness results showed that the microbial community in CW-MEC anodes was most different from that of other treatments. The lower diversity and evenness suggest that one or a few genus/genera dominated a comparatively homogeneous community. Also other studies found lower microbial diversity in CW-MEC as compared to CW-control (Gao et al., 2018, 2017), while one study found no significant difference (Ju et al., 2014a).

Regarding CW-MFC systems, some studies showed that CW-MFC+ enhance microbial community richness and diversity as

compared to CW-MFC- (Song et al., 2018; F. Xu et al., 2018a), however, experiments lasted only for 4 and 2 months, respectively. In general, microbial communities can change over time, especially in the initial start-up phase, while the cathode and its microbial community are likely to be affected and change over the long-term (T. Li et al., 2016).

### 6.3.1.2 Composition of microbial communities

Differences in operational taxonomic units (OTUs) across all four treatments' anodes and cathodes were analyzed by Weighted UniFrac Principal Coordinates Analysis (PCoA) of the  $\beta$ -diversity using phylogenetic distances shown in Figure 6.3.4.



**Figure 6.3.4.** Principal coordinates analysis (PCoA) of the  $\beta$ -diversity using phylogenetic distances of the microbial community for the anodes (A) and cathodes (C) in all three transects (numbers 1 to 3) of all treatments with CW-MEC in orange, CW-MFC+ in red, CW-MFC- in green and CW-control in blue. The different shades of colors indicate the different duplicate systems.

The distances in Figure 6.3.4 show how the CW-MEC anode and cathode samples are grouped in the upper area of the plot, while CW-MFC+, CW-MFC- and CW-control are grouped in the lower section. Across all treatments the anode samples tend to group on the left hand side and cathode samples rather the right hand side of the plot. Regarding the anode samples the plot visualizes that even at a distance of few millimeters of the anode mesh itself, the CW-MEC samples are distinctly different to the anode samples of all other treatments.

Looking at the phyla level of the different treatments (see Table 6.3.1), CW-MEC results show about twice as much *Proteobacteria* (55% for anodes and 64% for cathodes) compared to CW-MFC+ systems, which were similar to CW-MFC- and CW-control (27-30% for anodes and 31-38% for cathodes). CW-MEC shows a lower proportion of *Firmicutes* in the anodes (14%) compared to the other treatments (21-25%). *Proteobacteria* were also most common in other CW-MEC microbial community studies with relative abundances between 34% and up to 88% (Gao et al., 2017; D. Xu et al., 2017a; Zhang et al., 2018). Comparable CW-MFC+ studies showed as well the highest abundance of the phyla *Proteobacteria* at the anode but with a higher proportion of 44% (Rathour et al., 2019) and 86% compared to a CW-control with only 27% (L. Xu et al., 2018b), which could be again an effect of the too distant sampling points in the present study, and point out the higher impact of the distance on CW-MFC+ as compared to CW-MEC. In general, within the phyla *Proteobacteria* and *Firmicutes*, many electrochemically active species have been described so far (F. Xu et al., 2018).



**Table 6.3.1.A.** Proportion of phyla for each treatment's anodes and cathodes (colors indicate where the respective value falls in the green-yellow-red color range).

**Anodes**

CW-MEC		CW-MFC+		CW-MFC-		CW-control	
Proteobacteria	55%	Proteobacteria	27%	Proteobacteria	30%	Proteobacteria	30%
Firmicutes	14%	Firmicutes	25%	Firmicutes	21%	Firmicutes	23%
Bacteroidetes	7%	Bacteroidetes	10%	Bacteroidetes	9%	Bacteroidetes	11%
Acidobacteria	3%	Planctomycetes	6%	Synergistetes	7%	Chloroflexi	8%
Chloroflexi	3%	Chloroflexi	5%	Chloroflexi	7%	Synergistetes	7%
Planctomycetes	3%	Synergistetes	4%	Euryarchaeota	5%	Euryarchaeota	4%
Synergistetes	3%	Verrucomicrobia	3%	Planctomycetes	3%	Acidobacteria	3%
Actinobacteria	2%	Acidobacteria	3%	Acidobacteria	3%	Actinobacteria	2%
TPD-58	2%	TPD-58	2%	No phylum ID	2%	Spirochaetes	2%
No phylum ID	1%	Actinobacteria	2%	Spirochaetes	2%	Planctomycetes	2%

**Table 6.3.1.B.** Proportion of phyla for each treatment's anodes and cathodes (colors indicate where the respective value falls in the green-yellow-red color range).

**Cathodes**

CW-MEC		CW-MFC+		CW-MFC-		CW-control	
Proteobacteria	64%	Proteobacteria	31%	Proteobacteria	38%	Proteobacteria	35%
Bacteroidetes	8%	Firmicutes	12%	Chloroflexi	11%	Chloroflexi	14%
Actinobacteria	6%	Chloroflexi	10%	Firmicutes	7%	OD1	7%
Firmicutes	5%	Planctomycetes	8%	Acidobacteria	6%	Firmicutes	6%
Nitrospirae	3%	Nitrospirae	8%	Nitrospirae	5%	Chlamydiae	6%
Planctomycetes	2%	Actinobacteria	6%	Chlamydiae	5%	Actinobacteria	5%
Chloroflexi	2%	Chlamydiae	4%	Planctomycetes	5%	Cyanobacteria	4%
Chlamydiae	1%	Cyanobacteria	3%	Actinobacteria	4%	Nitrospirae	4%
Acidobacteria	1%	Acidobacteria	3%	Bacteroidetes	4%	No phylum ID	3%
OP11	1%	OD1	3%	Cyanobacteria	2%	Acidobacteria	3%

The already above described unevenness of the microbial community in CW-MEC anodes can clearly be seen as well on the genus level in Table 6.3.2, with *Sphingobium* representing an average of 27% of genera found in the anodes, with a similar proportion throughout the anodes in the CW-MEC transects (25%, 27% and 29% in transects 1, 2 and 3, respectively). *Sphingobium* was also the most common genus in the CW-MEC cathodes, with an average of 19%, however the proportion of *Sphingobium* at CW-MEC cathodes increased from the inlet to the outlet (8%, 19% and 29% in transects 1, 2 and 3, respectively). The *Sphingobium* genus present in CW-MEC was identified on the species level as *Sphingobium yanoikuyae* (99% sequence-similarity clusters), which is a strictly aerobic, chemo-heterotrophic, gram-negative, rod-shaped bacterium (Takeuchi et al. 2001). This species is mainly known for its capacity to degrade a large variety of mono- and polycyclic aromatic hydrocarbons (PHAs) (Zhao et al. 2015) and is used for bioremediation of environmental pollution (Jin et al. 2016). *Sphingobium yanoikuyae* has not been reported in CW-MEC, or in general in BES, before. Gao et al. (2018) found the closely related genera *Sphingomonas* and *Sphingopyxis* (together with *Sphingobium* part of the family *Sphingomonadaceae*) with a relative abundance of 5% and 2% in their biochar augmented CW-MEC system, while *Sphingomonas* was not among the main 18 genera in their CW-control and *Sphingopyxis* again found with around 2% relative abundance. Both genera, *Sphingomonas* and *Sphingopyxis* were not found in a considerable amount (< 0.2%) in the present study.

To find the aerobic *Sphingobium* genus together with hydrogen oxidizing *Hydrogenophaga* (7.6% in anode and 3.0% in cathode of CW-MEC) in a comparatively high proportion only in CW-MEC, is an indicator for electrolysis taking place in the CW-MEC, creating a favorable environment for them both. Gao et al. (2017) found the genus *Hydrogenophaga* even to be the most abundant in their CW-MEC systems (ca. 25% in CW-MEC and ca. 7% in CW-control) mentioning that this autohydrogenotrophic and denitrifying genus shows the advantage of lower unit cost of electron donor, avoiding organic carbon carryover to the final effluent, and lower biomass yield, which results in lower sludge production (Ergas and Reuss, 2001). In any case, just why the *Sphingobium* and not some other aerobic genus utilized this environment is still unclear and leaves the question open whether the *Sphingobium* might have been directly involved in bioelectrochemical processes and/or indirectly benefitting from them. Other, common

genera which are able to respire oxygen were found in the different treatments cathodes and anodes (e.g. *Acinetobacter*, *Bacillus*, *Brevundimonas* or *Pseudomonas*) but at a much lower relative abundance. Whether the *Sphingobium yanoikuyae* could be counted as an EAB would have to be determined in a separate experiment. In any case, some of the usually found EAB were only present in quite low proportions in the CW-MEC anodic samples (*Arcobacter*: 0.46%; *Desulfobacter*: 0.13%; *Geobacter*: 0.04%; *Shewanella*: 0.00%).

**Table 6.3.2.A.** Top 10 genera present in each of the anodes and cathodes of all four treatments (percent of genus per total OTUs)

CW-MEC				CW-MFC+			
Anodes		Cathodes		Anodes		Cathodes	
Sphingobium	43.0%	Sphingobium	29.0%	Trichococcus	14.9%	Nitrospira	22.2%
Hydrogenophaga	11.9%	Hydrogenophaga	5.3%	Bacillus	13.1%	Lysinibacillus	17.7%
Bacillus	4.0%	Nitrospira	5.1%	Thiobacillus	7.6%	Bacillus	8.8%
Trichococcus	2.9%	Pseudoxanthomonas	4.8%	Acinetobacter	5.3%	Hyphomicrobium	8.2%
Thiobacillus	2.4%	Alishewanella	3.2%	HA73	4.8%	Sulfurimonas	4.1%
Paenisporosarcina	2.3%	Paracoccus	2.9%	Desulfomicrobium	3.2%	Pseudonocardia	3.3%
Pseudomonas	2.1%	Pseudonocardia	2.8%	Desulfomonile	3.1%	Dok59	2.3%
Desulfomonile	1.7%	Thiobacillus	2.5%	WCHB1-05	3.1%	Planctomyces	1.9%
Brevundimonas	1.6%	Sulfurimonas	2.3%	Sulfurimonas	2.9%	Trichococcus	1.8%
HA73	1.5%	Microbacterium	2.2%	Paenisporosarcina	2.6%	Microbacterium	1.7%

**Table 6.3.2.B.** Top 10 genera present in each of the anodes and cathodes of all four treatments (percent of genus per total OTUs)

CW-MFC-				CW-control			
Anodes		Cathodes		Anodes		Cathodes	
Bacillus	9.3%	Nitrospira	17.4%	Trichococcus	14.5%	Nitrospira	12.5%
T78	8.0%	Hyphomicrobium	8.5%	T78	8.9%	Bacillus	12.4%
HA73	6.6%	Thiobacillus	5.2%	Thiobacillus	7.9%	Hyphomicrobium	8.9%
Desulfomicrobium	6.6%	Dok59	4.2%	HA73	6.1%	Paenisporosarcina	4.6%
Methanospirillum	5.1%	Bacillus	3.5%	Desulfomicrobium	5.9%	Aeromonas	3.9%
Desulfomonile	5.1%	Pseudomonas	3.2%	Methanospirillum	5.1%	Crenothrix	3.5%
Thiobacillus	4.4%	Trichococcus	2.6%	Desulfomonile	5.0%	Sulfuritalea	2.7%
Paenisporosarcina	3.7%	Desulfomonile	2.0%	Paenisporosarcina	3.8%	Planctomyces	2.6%
E6	2.9%	Sulfurimonas	2.0%	Sphaerochaeta	2.9%	Ca. Protochlamydia	2.4%
vadinCA02	2.9%	Ca. Rhabdochlamydia	1.8%	vadinCA02	2.8%	Thermomonas	2.3%

Looking at the anode's microbial community results, as mentioned above, sampling points were likely too far away in order to representatively depict the microbial community directly at the anode in the case of CW-MFC+. Hence, the electroactive part of the anodic microbial community of CW-MFC+ was probably not included in a representative manner in the results. In this case, the gained knowledge would be that the analysis showed that CWs operated as CW-MEC show visible (at least indirect) effects and influence on microorganisms and their communities even relatively far away from the anodes while no indirect effects on the wider environment (distance of few millimeters) were found in CW-MFC+ anodes, likely due to the limited range of influence of anodes on the microbial population. However, additional studies on microbial communities at different distances from the electrodes of CW-MEC and CW-MFC systems would need to be conducted in order to confirm this assumption. The used architecture (gravel based anode with stainless steel mesh electron collector with carbon felt air-cathode) could also be partly responsible for the fact that the proportion of common electrogenic genera was lower as compared to conventional BES or CW systems with BES based on more conductive or elaborate materials used. For example, J. Wang et al. (2016) found that different anode materials show a significantly different microbial community distribution with higher relative abundance of *Proteobacteria* in carbon fiber felt and foamed nickel anodes as compared to SSM anodes (as used in this study) or graphite rods.

CW-MFC+ cathode samples neither revealed high abundances of commonly known EAB like *Geobacter*, *Shewanella* or *Arcobacter* (0.00%, 0.06% and 0.61% in CW-MEC cathodes, respectively), even though the CF cathode was directly sampled by carving off parts of the surface. In a comparable CW-MFC study using CF as cathode, the relative abundance of *Geobacter* was 13-16% (Corbella et al., 2015), however, only in the treatment receiving wastewater from a hydrolytic up-flow sludge blanket reactor (HUSB) pre-treatment; *Geobacter* was also absent from the treatment receiving wastewater from a primary settler (similar to the one used in this study). The relatively most abundant genus in the cathodes of CW-MFC+, CW-MFC- and CW-control was an unknown species of the genus *Nitrospira* (8%, 5% and 4%, respectively), while it contributed with a relative abundance of 3% only to the CW-MEC cathode. *Nitrospira* have shown to be able to oxidize nitrite and hydrogen aerobically (Koch et al., 2015). Interestingly, a significant difference in the CW-MFC+ cathodes

compared to other treatments seems to be the relative high abundant species *Lysinibacillus boronitolerans* from the genus *Lysinibacillus*, being second most abundant in CW-MFC+ with ca. 8%. *Lysinibacillus boronitolerans* reached only ca. 1% in CW-MEC and was far below 1% in CW-MFC- and CW-control. A closely related species from the same genus, called *Lysinibacillus sphaericus*, was identified to be electrochemically active and to potentially play an important role in extracellular electron transfer (EET) (H. He et al., 2014; Nandy et al., 2013). In addition, *Lysinibacillus sphaericus* has been shown to be able to nitrify ammonium (Aguirre-Monroy et al., 2019). Again, as for *Sphingobium yanoikuyae*, *Lysinibacillus boronitolerans* has not been reported in MFC or MEC systems or in regards to electrochemical processes before. Whether *Lysinibacillus boronitolerans* could be electrochemically active, like its close relative *Lysinibacillus sphaericus*, would again have to be investigated in a separate future experiment.



### **6.3.2 *Electrical connection effects on contaminant removal***

#### 6.3.2.1 Overview

In this section, contaminant removal efficiency of all four treatments is addressed from the results obtained during the 17 weeks of experimentation. Table 6.3.3 summarizes the results of COD, ammonium, nitrate, nitrite, orthophosphate and sulfate analysis for all four electrical connections.

**Table 6.3.3.A.** Average contaminant loading and removal according to the different treatments

		Influent	1/3	2/3	Effluent	Removal from Influent to Effluent	
		(g/m <sup>2</sup> ·d)				(g/m <sup>2</sup> ·d)	(%)
<b>COD</b> (n=14) <sup>a</sup>	<b>CW</b>	5.3±1.7	2.8±1.0	2.2±1.0	1.9±0.9	3.4±1.5	64±24%
	<b>MFC<sup>-</sup></b>	5.5±1.6	2.9±1.0	2.1±1.1	2.0±1.0	3.5±1.5	63±22%
	<b>MFC<sup>+</sup></b>	5.4±2.1	2.6±1.3	1.8±1.2	1.6±0.9	3.7±1.7	70±18%
	<b>MEC</b>	5.2±1.8	1.9±1.0	1.4±0.7	1.4±0.8	3.8±1.5	73±16%
<b>NH<sub>4</sub><sup>+</sup>-N</b> (n=12) <sup>a</sup>	<b>CW</b>	1.0±0.1	1.1±0.2	0.8±0.1	1.0±0.2	0.0±0.2	2±20%
	<b>MFC<sup>-</sup></b>	1.1±0.1	1.1±0.2	0.9±0.1	1.0±0.2	0.0±0.2	2±23%
	<b>MFC<sup>+</sup></b>	1.1±0.1	1.0±0.2	0.8±0.2	0.9±0.1	0.2±0.2	18±15%
	<b>MEC</b>	1.0±0.1	0.9±0.1	0.8±0.2	0.8±0.1	0.2±0.1	20±12%

<sup>a</sup> Some experimentation weeks could not be considered due to highly diluted influent or technical analysis problems

**Table 6.3.3.B.** Average contaminant loading and removal according to the different treatments

		Influent	1/3	2/3	Effluent	Removal from Influent to Effluent	
		(g/m <sup>2</sup> ·d)				(g/m <sup>2</sup> ·d)	(%)
<b>NO<sub>3</sub><sup>-</sup>-N (n=15)<sup>a</sup></b>	<b>CW</b>	0.000±0.000	0.005±0.012	0.004±0.008	0.000±0.000	0.000±0.000	NA
	<b>MFC<sup>-</sup></b>	0.002±0.005	0.001±0.003	0.009±0.010	0.000±0.000	0.002±0.005	100±DBZ%
	<b>MFC<sup>+</sup></b>	0.001±0.003	0.003±0.006	0.004±0.010	0.000±0.000	0.001±0.004	55±DBZ%
	<b>MEC</b>	0.002±0.005	0.008±0.013	0.014±0.012	0.000±0.000	0.002±0.005	100±DBZ%
<b>NO<sub>2</sub><sup>-</sup>-N (n=15)<sup>a</sup></b>	<b>CW</b>	0.007±0.018	0.009±0.018	0.018±0.022	0.000±0.000	0.007±0.017	100±DBZ%
	<b>MFC<sup>-</sup></b>	0.005±0.012	0.002±0.008	0.025±0.031	0.000±0.000	0.004±0.011	100±DBZ%
	<b>MFC<sup>+</sup></b>	0.016±0.026	0.010±0.019	0.024±0.034	0.000±0.000	0.014±0.025	100±DBZ%
	<b>MEC</b>	0.024±0.028	0.026±0.032	0.038±0.036	0.004±0.014	0.017±0.034	78±DBZ%

<sup>a</sup> Some experimentation weeks could not be considered due to highly diluted influent or technical analysis problems

DBZ - Division by zero

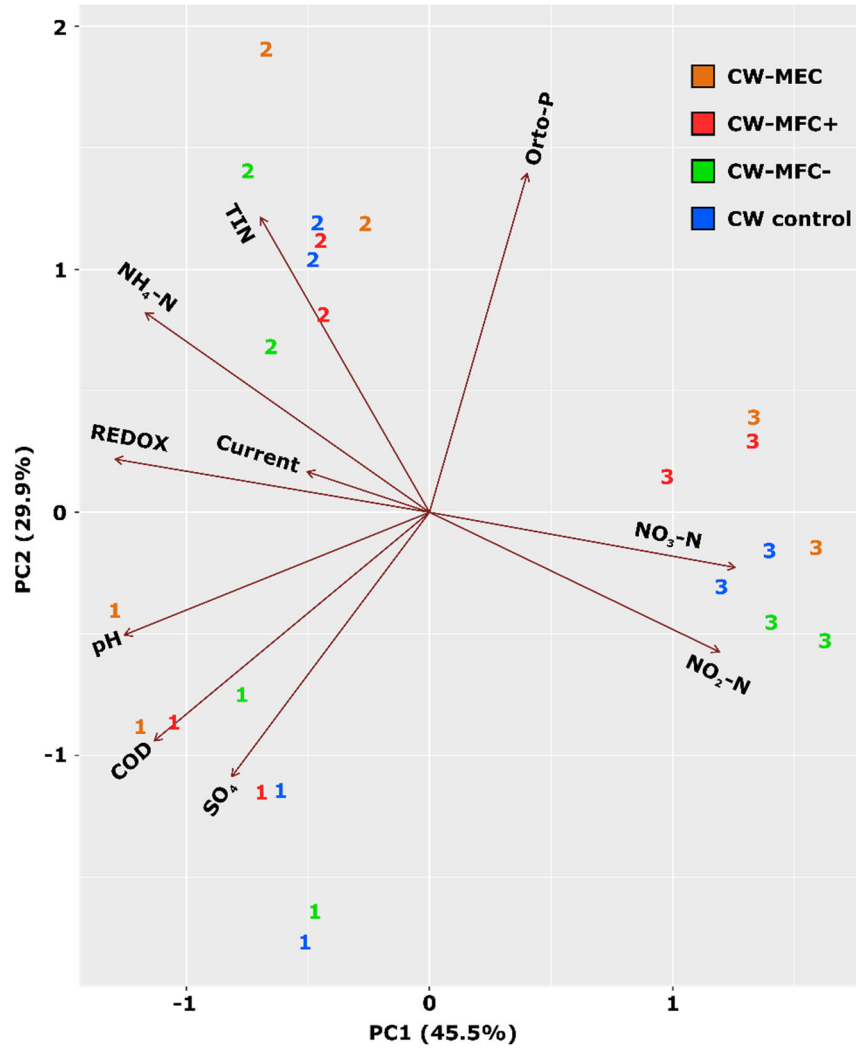
NA – could not be calculated due to low concentrations

**Table 6.3.3.C.** Average contaminant loading and removal according to the different treatments

		Influent	1/3	2/3	Effluent	Removal from Influent to Effluent	
		(g/m <sup>2</sup> ·d)				(g/m <sup>2</sup> ·d)	(%)
<b>SO<sub>4</sub><sup>2-</sup></b> <b>(n=15)<sup>a</sup></b>	<b>CW</b>	2.6±0.4	1.0±0.5	1.2±0.5	1.8±0.8	0.9±0.9	33±38%
	<b>MFC<sup>-</sup></b>	2.7±0.4	0.9±0.3	1.2±0.4	1.6±0.6	1.0±0.8	38±25%
	<b>MFC<sup>+</sup></b>	2.7±0.6	1.3±0.7	1.6±0.5	2.1±0.6	0.6±0.8	22±30%
	<b>MEC</b>	2.6±0.7	2.2±1.1	2.5±0.8	2.8±0.8	-0.2±0.8	-9±31%
<b>PO<sub>4</sub><sup>3-</sup>-P</b> <b>(n=15)<sup>a</sup></b>	<b>CW</b>	0.09±0.04	0.14±0.05	0.14±0.04	0.15±0.05	-0.06±0.05	-62±63%
	<b>MFC<sup>-</sup></b>	0.09±0.04	0.13±0.05	0.13±0.03	0.15±0.05	-0.05±0.05	-57±69%
	<b>MFC<sup>+</sup></b>	0.10±0.05	0.14±0.04	0.14±0.04	0.14±0.03	-0.04±0.05	-46±55%
	<b>MEC</b>	0.09±0.04	0.12±0.03	0.09±0.03	0.09±0.03	0.00±0.04	-4±54%

<sup>a</sup> Some experimentation weeks could not be considered due to highly diluted influent or technical analysis problems

The PCA in Figure 6.3.5 shows how the sampling points group according to their position along the length of the wetland (transect 1, 2 and 3), with the vectors showing where the majority of change or treatment of a certain parameter occurred.



**Figure 6.3.5.** Principle Component Analysis (PCA) of the different pollutant removal and physical/chemical parameters according to the three transects (numbers 1 to 3) and treatments.

Hence, change in pH together with COD and sulfate treatment were highest in transect 1, while ammonium removal is shown to be highest in transect 2. Redox change and current vectors are also pointing primarily towards transect 2, however, with the group of transect 1 samples only a bit further away. The occasional and relatively small changes in nitrate and nitrite are shown to have been most prevalent in transect 3. More details are given in the following subchapters for each parameter.

### 6.3.2.2 Redox and pH

The average values for pH and redox potential are shown in SI Table S6.1 and Table S6.2, respectively. At the influent, average pH (around 7.8) and redox (-118 to -100 mV) values are very similar across all treatments, since the same real urban wastewater was used for all systems. Redox increased after the first and second transect in CW-MFC+ and CW-MEC systems, and again with a very significant difference in CW-MEC (F (3, 15),  $p = 0.004$ , compared to CW-MFC- and CW-control). At the effluent redox across all treatments was positive but without statistically significant differences. The change in redox showed that the CW-MFC+ and especially CW-MEC systems were more aerobic than CW-MFC- and CW-control systems, in the case of CW-MEC most likely due to electrolysis processes producing oxygen and hydrogen. In the case of CW-MFC+ systems it was not clear what led to the increase in redox.

The pH values after the first and second transects are lower in CW-MFC+ and CW-MEC as compared to CW-MFC- and CW-control, and statistically very significantly different in CW-MEC systems (F (3, 14);  $p = 0.0005$ ). At the effluent CW-MEC pH was still significantly different (F (3, 14);  $p = 0.03$ ). The significant difference in CW-MEC pH was likely directly connected to the electrolysis, with a higher amount of hydrogen in solution lowering the pH accordingly. Other studies using CW-MEC also found lower pH in comparison to CW-control systems (Ju et al., 2014a). However, some studies found the opposite effect with CW-MEC producing a more alkaline environment (Gao et al., 2017), possibly due to the interaction with iron ions (iron oxidation leading to reduced redox and higher pH) from the used sacrificial iron anode.

### 6.3.2.3 Organic matter

The average organic loading rate during the 17 experimentation weeks amounted to  $5.3 \pm 1.8$  g COD/m<sup>2</sup>·day. The measured average organic matter removal from inlet to outlet expressed as COD was highest in CW-MEC and nearly as high in CW-MFC+ mode, with an increase of 6-10% as compared to CW-MFC- and CW-control (see Table 6.3.3), however without a significant difference (see SI, Table S6.3). The insignificance of differences could be partly due to the relatively high standard deviation most likely caused by the variation in influent quality due to natural causes like rainfall events or dry periods affecting the used real urban wastewater.

Generally, the majority of organic matter is removed in the first section of a CW; values in Table 6.3.3 show that the difference in COD reduction between treatments was even more pronounced in the first transect than from in- to outlet, however, again without significant difference between systems (see SI, Table S6.3). Interestingly, in CW-MEC mode 88% of the total inlet to outlet removal was already accomplished after the first transect, whereas all other three treatments accomplished ca. 74% of the overall treatment within the first transect. This could potentially lead to a reduction of required CW area for COD treatment in CW-MEC systems or give an indication for the best positioning of CW-MEC and CW-MFC within a system. In transect 2 and 3 of CW-MEC, COD removal was lower compared to other treatments, probably because the organic matter load after transect 1 was already lower and less easily biologically degradable organic matter was left. As a result, the differences between CW-MEC and other treatments were partly equalized at the effluent of the systems.

Average COD removal in the presented CW-MEC systems amounted to  $73 \pm 16\%$  and were 10% and 9% higher than CW-MFC- and CW-control, respectively. There are very few publications on contaminant removal from urban wastewater using CW-MEC systems and none to the knowledge of the authors that compares CW-MEC and CW-MFC systems in parallel. In addition to that, most of other studies focused on nitrate removal. The four publications reporting on COD removal in CW-MECs showed varying results between 18% and 89% possibly due to the use of synthetic wastewater (easily biodegradable COD), different media (e.g. zeolite or coke granules) (Aguirre-Sierra et al., 2016; Ju et al., 2014a) and/or higher achieved current densities (D. Xu et al., 2017a; Zhang et al., 2018). The studies which also compared



their removal to control systems showed no difference, in the case of Ju et al. (2014a), and 5-7% higher removal in the CW-MEC system by Aguirre-Sierra et al. (2016), which was the only other CW-MEC study which also used a potentiostat, as well applying a potential of 0.3 V vs. Ag/AgCl at the anode.

Average COD removal in the presented CW-MFC+ systems amounted to 70±18% and was 7% and 6% higher than CW-MFC- and CW-control, respectively. These results are similar to earlier publications with the same CW-MFC architecture using urban wastewater (Corbella and Puigagut, 2018; Hartl et al., 2019). Also other publications using solid-state electrodes but different wastewater sources produced quite similar results (Oon et al., 2018; Rathour et al., 2019; Xie et al., 2018; F. Xu et al., 2018). Higher removal was achieved by Yakar et al. (2018) with 92.1% COD removal, however, using zeolite (clinoptilolite) which is a highly porous medium. Other CW-BES used very different systems, e.g. without solid state electrodes but highly porous as well as electro-conductive media (single electrode short-circuit BES) or iron-carbon micro-electrolysis in CWs (Ramírez-Vargas et al., 2019; Shen et al., 2019; Zheng et al., 2019), which are difficult to compare to the presented setup and therefore not considered in the discussion.

The positive effect of CW-MFC+ on COD removal as compared to CW-MFC- and CW-control in the presented study could be due to direct effects of the BES, like EAB outcompeting other degradation pathways, such as anaerobic degradation (Corbella and Puigagut, 2018; Zhang et al., 2015). EAB might also be inhibiting growth of *Archaea* at the anode (Fang et al., 2013a). Also in the presented microbial community analysis, low to no *Archaea* were found in the anodic sections of CW-MEC and CW-MFC+ (0.6% and 0.0%, respectively) as compared to CW-MFC- and control (4.8% and 4.3%, respectively). Additionally, in terms of microbial community, an increase in microbial activity, determined by means of fluorescein diacetate (FDA) hydrolysis, was observed in CW-MFC+ in an earlier study (Hartl et al., 2019). This increased activity could have led to an improved biodegradation and at least partly explain the improved COD removal in CW-MFC+ (and possibly CW-MEC) as compared to CW-MFC- and CW-control.

Wang et al. (2019) analyzed organics and nutrient removal in CW-MFC in a multifactorial experiment, concluding that the most

influential factors for COD removal were - more related to bioelectrochemical intensification - the DO concentration in the cathode zone (2.0 mg O<sub>2</sub>/L) as well as external resistance ( $\leq 500 \Omega$ ; 220  $\Omega$  were used in the present study). Substrate conductivity on the other hand was found to be not a significant factor for COD removal. Therefore, besides a direct influence of EAB, at least in the case of CW-MEC, the microbial community analysis pointed towards an indirect effect of the BES, changing the environment through electrolysis and the subsequent release of oxygen (at the anode) and hydrogen (at the cathode) into the system. This can be deduced from the redox results and high relative abundance of aerobic and hydrogen oxidizing bacteria, which would also explain the lower abundance of *Archaea* (majority are anaerobic and methanogenic) in CW-MEC and CW-MFC+. The increased redox, or DO as mentioned above by Wang et al. (2019) as one of the most influential parameters for COD removal in CW-MFC, could then be assumed to be the main factor for the increased COD removal in CW-MEC of the presented study. A disadvantage of the electrolysis in CW-MEC process could be an increase in CH<sub>4</sub> emission as measured by Ju et al. (2014). However, this assumption would need to be addressed in future research focusing on CW-MEC's methane and gas emissions in general.

#### 6.3.2.4 Nitrogen

Average ammonium removal on a mass base from inlet to outlet was significantly higher in CW-MEC (F (3, 12); p = 0.02) and CW-MFC+ (F (3, 12); p = 0.01) systems, as compared to CW-MFC- and CW-control (see Table 6.3.3). The differences between treatments were very clear also when looking at ammonium removal per transect, with significant differences in transect 1 and 3 (see SI, Table S6.3). Ammonium removal per transect was not as homogeneous as for COD, with CW-MEC and CW-MFC+ removing around a half of the total removal within transect 1 and 2, while CW-MFC- and CW-control basically only removed ammonium in transect 2.

All measured outlet NO<sub>2</sub>-N and NO<sub>3</sub>-N concentrations were below detection except for nitrite in the CW-MEC mode, with a specific mass of 0.004 g NO<sub>2</sub>-N/m<sup>2</sup>·day (see Table 6.3.3), which could be due to the oxygen release through hydrolysis at the CW-MEC anodes, but

probably rather due to comparatively higher nitrite influent concentrations in CW-MEC (see Table 6.3.3). The reasons for the higher nitrite concentrations in CW-MEC as compared to other treatments could not be explained, especially since influent concentrations of other parameters like ammonium and nitrate were similar across all treatments. The only significant difference between treatments was found for nitrate removal in transect 3 ( $F(3, 15)$ ;  $p = 0.02$ ) (see SI, Table S6.3) but on a very low concentration level. Generally, nitrate and nitrite levels within the systems were very low.

Out of the few studies on nutrient removal in CW-MEC systems, the majority focused on nitrate removal from nitrate rich wastewater, with nitrate removal rates from 4% to 32%. Ammonium removal rates in CW-MEC reached from 20 to 87% (Aguirre-Sierra et al., 2016; Gao et al., 2017; Ju et al., 2014a; Zhang et al., 2018). Generally, it is noteworthy that all but two comparable CW-MEC studies showed a higher current density than the presented study. It has been suggested that current increase improves biofilm formation by enhancing cell-to-cell signaling and extracellular polymeric substance (EPS) release (Huang et al., 2013). On the contrary, Ju et al. (2014a) found a negative correlation between achieved current intensity (from 5700 mA/m<sup>2</sup> to 15000 mA/m<sup>2</sup>) and nitrate removal and also Srivastava et al. (2018) observed that increasing the current only benefited nitrate removal up to a certain point (in their case above the tested 370 mA/m<sup>2</sup>) after which the current inhibited the removal. This study's current density is well below this point with an average of 99 mA/m<sup>2</sup>.

Average NH<sub>4</sub><sup>+</sup>-N removal in the presented CW-MFC+ systems amounted to only 18±15%. This is lower than recent literature on CW-MFC hybrids, however, as mentioned already, systems performed better in past experiments. The improvement in CW-MFC+ is still very pronounced with 16% higher removal than CW-MFC- and CW-control.

Yakar et al. (2018) achieved 93.2% NH<sub>4</sub><sup>+</sup>-N removal using up-flow CW-MFC with zeolite (clinoptilolite) as substrate and synthetic wastewater, and assumed that the zeolite system performed best due to the high porosity providing a more aerobic micro-environment favoring nitrification; also enhanced adsorption and cation-exchange sorption as well as better plant development in the media might play a role. Xu et al. (2018) investigated the treatment of synthetically simulated polluted river water using up-flow CW-MFC reactors and achieved on average 78% ammonium removal as compared to a higher

removal of 84% in CW-control, however, the study period was only 50 days. Saz et al. (2018) achieved 88%  $\text{NH}_4^+\text{-N}$  removal in the unplanted mesocosm, while the different planted systems achieved even 95 to 97% removal. Corbella and Puigagut (2013) reported 68% ammonium removal with an improvement of 10% as compared to CW-MFC-.

Generally, plants had a strong effect on nitrogen removal results, independent from electrical connection effects (Oon et al., 2018; Ramírez-Vargas et al., 2019; Saz et al., 2018). The presented systems were unplanted, however, the very low ammonium removal in the presented study must have had other reasons as well, probably the system aging and potential partial cathode clogging. The cathode maturation (clogging through algae growth, biomass accumulation etc.) may also have led to a decline of oxygen transfer rates and consequently lower performance. While the growth of EAB on the anode will increase the generated current and related transformation of nutrients, the development and maturing biofilm on cathodes can decrease the air-cathode's (as used in this study) ability to transfer oxygen and thereby current and nutrient removal might decrease (Kiely et al., 2011a; Rossi et al., 2018; Zhang et al., 2017). Anyway, the improvement in ammonium removal of CW-MEC and CW-MFC+ was still very pronounced, with 18% and 16%, respectively, higher removal efficiency as compared to CW-MFC- and CW-control, similar to comparable lab-scale systems by (Corbella and Puigagut, 2018) and earlier experiments using this setup (Hartl et al., 2019).

In conventional subsurface flow CWs the main nitrogen removal mechanisms are nitrification (requiring carbon and oxygen) and denitrification (requiring anoxic conditions and high amounts of carbon) (García et al., 2010). Earlier microbial community analysis found genera indicating that more unusual nitrogen removal pathways like anaerobic ammonium oxidation (anammox) or dissimilatory nitrate reduction to ammonium (DNRA) are more prevalent in BES (J. Wang et al., 2016a; L. Xu et al., 2018b). In the present study, no indication for these unusual pathways could be found, however, the higher nitrification and denitrification capacity in CW-MEC could be explained by the effects of electrolysis of water, producing oxygen and  $\text{H}^+$  at the anode and  $\text{H}_2$  at the cathode. As described for COD removal, electrolysis might have indirectly enhanced nitrification by increasing the DO (here measured as increased redox) in the CW-MEC, and subsequently the formed  $\text{H}_2$  would further serve as electron donor for

nitrate reduction to nitrogen gas, and  $H^+$  could also be involved in autohydrogenotrophic denitrification (Gao et al., 2017). A disadvantage of these processes might be the release of other nitrous gases such as  $NO_x$  and  $N_2O$ , depending on pH, which could not be easily controlled in CW systems (Mousavi et al., 2012), however, demanding future research into the matter. Accordingly, the present study found a higher abundance of aerobic genera in CW-MEC as compared to CW-MFC- and CW-control, as well as a genus that oxidizes hydrogen (*Hydrogenophaga*, unknown species), with 8% in the anode and the 4% in cathode exclusively in CW-MEC systems. This higher abundance of *Hydrogenophaga* (chemoorganotroph and facultative hydrogen autotroph), feeding on the hydrogen produced at the CW-MEC electrodes, could have led to higher denitrification rates in CW-MEC as other species in this genera (*Pseudoflava* and *Taeniospiralis*) are known for anaerobic nitrate respiration with denitrification. Another genus with a possible impact on denitrification found in higher relative abundance in CW-MEC as compared to other treatments is *Pseudomonas* (ca. 1% in anode and 0.6% in cathode of CW-MEC, lower in all other treatments).

In the case of CW-MFC+ systems, *Lysinibacillus boronitolerans* was found with a relatively high abundance (8%) at the cathode while a closely related species from the same genus, called *Lysinibacillus sphaericus*, was found to be electrochemically active and able to nitrify ammonium (Aguirre-Monroy et al., 2019; H. He et al., 2014; Nandy et al., 2013). Hence, in the case of CW-MFC+, part of the reason for higher ammonium removal could be the relatively high abundance of *Lysinibacillus boronitolerans*. In addition, Kim et al. (2008) observed high relative abundance of ammonia oxidizing bacteria (AOB) in a single-chamber air-cathode MFC. However, in the presented systems, the maturing cathodes could have even inhibited these AOB.

#### 6.3.2.5 Sulfate

Average sulfate mass removal from inlet to outlet and particularly in transect 1 was extremely significantly different between treatments ( $F(3, 15)$ ;  $p = 0.0004$ ), see also SI, Table S6.3). As can be seen in Table 6.3.3, specific sulfate mass in the first transect decreased in all four treatments but to a larger extent in CW-control and CW-MFC- systems than in CW-MFC+ and especially when compared to CW-MEC

mode systems. In the course of transect 2 and 3, average sulfate mass increased to a similar extent in all treatments.

Very few other studies on CW-MEC or CW-MFC reported on sulfate removal in the systems. The ones which do so however, report a similar pattern of lower sulfate removal in CW-BES systems; Ju et al. (2014a) reported that no sulfide (product of sulfate reducing bacteria (SRB)) could be found in CW-BES, with a positive effect on odor control from sulfide accumulation, whereas 0.71 mg/L sulfide could be found in the control (sulfate results were unfortunately not published). Also Corbella and Puigagut (2018) found 13% higher sulfate removal in control systems as compared to CW-MFC+ systems. Lovley (2006) described that sulfide abiotically reacts with the electrode to form elemental sulfur which then can be microbially re-oxidized to sulfate and further to sulfate using the anode as electron acceptor. This re-oxidation has been described to be facilitated by the *Desulfobulbus* species, however, in this study's microbial community analysis this species amounted to only 0.1% of all species at the CW-MEC anode and even below 0.1% at all other treatments' anodes. In general, the conducted microbial community analysis showed that genera known to reduce sulfur (such as *Desulfomonile* and *Desulfomicrobium*) were found to a higher degree at anodes of CW-MFC+, and especially CW-MFC- and CW-control as compared to CW-MEC (see Table 6.3.2), probably also due to an alteration of the environment caused by the BES (e.g. increase of DO in the systems).

### 6.3.2.6 Phosphorus

Average orthophosphate mass removal from inlet to outlet was significantly different between the treatments ( $F(3, 15)$ ;  $p = 0.02$ , see also SI, Table S6.3). As shown in Table 6.3.3, the mass from inlet to outlet increased on average in CW-control, CW-MFC- and CW-MFC+, whereas the average mass stayed the same in CW-MEC mode systems. The increase could be due to release of orthophosphate that has been adsorbed during the preceding 10 months of operation. Looking at the three transects in detail, Table 6.3.3 also shows that orthophosphate mass increased in all four treatments in transect 1 and stayed the same until the outlet in the case of CW-control, CW-MFC- and CW-MFC+. However, in the case of CW-MEC the average

measured mass decreased again in transect 2 and stayed on that level until the outlet leading to the question on what difference in processes or conditions led once more to the decrease of orthophosphate in transect 2 of CW-MEC systems.

Studies using CW-MEC further showed that the in-situ formation of ferric ions from a sacrificial anode may cause precipitation, adsorption and flocculation of phosphorus (Gao et al., 2017; Ju et al., 2014a; Zhang et al., 2018). Although, the applied voltage or resulting current densities were mostly substantially higher with 700, 57-150 and 11500-24000 mA/m<sup>2</sup>, respectively, as compared to 99 mA/m<sup>2</sup> in the present study, the process could have happened on a smaller scale and have been responsible for the different behavior of CW-MEC in transect 2. This would show that not only the presence of an anode (as in CW-MFC-) or possibly as well a closed-circuit (as in CW-MFC+) are enough for increased orthophosphate removal, but that a current has to be applied (as in CW-MEC).

Gao et al. (2017) found that orthophosphate removal in CW-MEC was around 68% to 97% while removal in the CW-control system stayed below 45% over the study time period of 20 days. In a follow up pilot-scale investigation with a similar setup orthophosphate removal was between 66% and 97%, increasing with the current density applied (Gao et al., 2018). In the most recent study Gao et al. (2019) included this system in a combination of HF and surface flow system on a pilot scale, resulting in a total phosphorus (TP) removal of 53%.

Ju et al. (2014a) observed orthophosphate removal in their CW-MEC exceeding 95% during a 210 day experiment and found that the lower the intensity the lower the phosphorus removal (opposite effect as described above for nitrate in those systems). The already described full-scale CW-MEC reported on by Zhang et al. (2018) achieved an average TP removal of around 37% and the total amount of phosphorus accumulating organisms (PAOs) decreased throughout the study of 300 days of operation. Xu et al. (2018) investigated the treatment of synthetically simulated polluted river water using up-flow CW-MFC reactors and achieved on average 95% TP removal as compared to a removal of 94% in CW-control, however, the study period was only 50 days.

Additionally, the long-term effects in some of these systems are not known yet. The systems of the presented study showed higher initial orthophosphate removal rates in the first 10 weeks during an earlier

experiment (Hartl et al., 2019), but were already 40 weeks in operation at the time of the start of this study. Phosphorus storage in subsurface flow CWs is generally known to have a finite capacity (Kadlec and Wallace, 2009).

### 6.3.2.7 Electrical behavior

Table 6.3.4 shows average and maximum potentials and current densities for CW-MFC+ treatments in all 3 transects.

**Table 6.3.4.** Average, standard deviation and maximum for potential and current density of CW-MFC+

Transect	Potential (mV)		Current Density (mA/m <sup>2</sup> )	
	Avg. ± SD	Maximum	Avg. ± SD	Maximum
1	379±77	579	41±8	63
2	394±62	585	43±7	63
3	357±74	541	39±8	59

The average current density of all three transects together was therefore 41 mA/m<sup>2</sup> for CW-MFC+, which is in the higher range of comparable studies, which showed values of 175, 19.8, 8.5 and 6.1 mA/m<sup>2</sup>, respectively (Corbella and Puigagut, 2018; Saz et al., 2018; Xie et al., 2018; Yakar et al., 2018). Interestingly, as already observed in an earlier study using these systems (Hartl et al., 2019), transect 2 in CW-MFC+ has a higher average and maximum potential and current, indicating that transect 1's advantage of higher organic matter concentration might be exceeded by negative effects like resulting cathode clogging and/or the assumption that MFCs potentially perform better under lower OLR which could benefit EAB's growth and activity as compared to competing methanogens (Capodaglio et al., 2015).

The coulombic efficiency (CE) is the proportion of the produced current to the carbohydrates which are theoretically derived from



oxidation, indicated by the change of COD from transect to transect (Scott, 2016). The resulting average CE values were  $2.2 \pm 3.0\%$ ,  $8.1 \pm 8.7\%$  and  $34.4 \pm 3.9\%$ , for transects 1, 2 and 3, respectively. Note that CE could have a negative value in case that COD concentrations were increasing from the influent to the end of transect 1 or from one transect to the other. Generally, it can be assumed that only the CE value measured in transect 1 gives an usable indication, if at all, since not only organic matter from the influent can contribute to the MFC signal but also accumulated organic matter within the gravel bed is a fuel source for MFC (Corbella et al., 2016a). Therefore, especially CEs in transect 2 and 3 were much higher than in comparable CW-MFCs due to the fact that very little COD was removed but still a comparable current as in transect 1 was produced. Other CW-MFC studies showed CEs of CW-MFCs from  $0.01\%$  (Wang et al., 2016b) up to  $16.4\%$  (Xie et al., 2018).

A polarization curve (PC) analysis (see SI, Figure S6.1) of one of the CW-MFC+ duplicates showed that the maximum power densities of  $36$ ,  $17$  and  $23 \text{ mW/m}^2$  in transect 1, 2 and 3, respectively, were achieved at current densities of  $99$ ,  $64$  and  $76 \text{ mA/m}^2$ , respectively.

The estimated internal resistances derived from the PC analysis were around  $83 \Omega$ ,  $94 \Omega$  and  $89 \Omega$  for first, second and third transect, respectively. Principally, the potential maximum power is achieved when internal and external resistances are close to each other (Lefebvre et al., 2011). However, for the current experiment and its primary goal contaminant removal the lower external resistances could have been beneficial, since lower external resistances increase the generated current and studies have also shown that consequently organic matter removal was increased (Aelterman et al., 2008; Gil et al., 2003; Katuri et al., 2011).

Table 6.3.5 shows the poised potential and the resulting average current applied to each transect in CW-MEC systems.

**Table 6.3.5.** Poised potential (at Anode vs. Ag/AgCl reference electrode) and resulting average current applied also expressed in current density per electrode surface area and anodic compartment volume in CW-MEC

Transect	Poised Potential (V)	Current	Current Density per Area	Current Density per Volume
		(mA)	(mA/m <sup>2</sup> )	(mA/m <sup>3</sup> )
		Avg. ± SD	Avg. ± SD	Avg. ± SD
1	0.3	19±11	447±269	2031±1221
2	0.3	18±9	427±201	1939±912
3	0.3	8±5	178±106	809±483

The poised potential of 0.3 V vs. Ag/AgCl reference electrode at the anode was chosen on the basis of experiences showing that such a potential benefits the growth of EAB genera such as *Geobacter* in mixed bacterial cultures (Fricke et al., 2008; Liu et al., 2008). As pointed out already in the above sections, the resulting current to CW-MEC systems was much lower than in most other CW-MEC publications, showing values of 700, 57-150 and 11500-24000 mA/m<sup>2</sup>, respectively (Gao et al., 2017; Ju et al., 2014a; Zhang et al., 2018).

## 6.4 Conclusions

CW-MEC and CW-MFC+ systems were able to enhance COD and ammonium removal in comparison to CW-MFC- and CW-control systems and also showed differences in the removal of other contaminants and the metagenomic of microbial communities.

- COD; Average COD removal was improved by 9% and 6% in CW-MEC and CW-MFC+ systems, respectively, however, without a statistically significant difference. Reasons for the increase could be direct effects of the BES such as an increased microbial activity, EAB outcompeting other removal pathways and synergies between EAB and other microbial communities. In the case of CW-MEC also indirect effects through an electrolysis induced increase of redox and subsequent aerobic degradation likely had an effect.
- $\text{NH}_4^+\text{-N}$ ; Average ammonium removal was increased by even 18% and 16% in CW-MEC and CW-MFC+, respectively, in this case showing a statistically significant difference. As for COD, BES could have affected the treatment directly through higher microbial activity and EAB advantages over other microbial communities and synergies with them. In the case of CW-MEC, electrolysis potentially led to two additional effects; 1) higher DO led to increased nitrification and/or 2) hydrogen was used for anaerobic nitrate respiration with denitrification. Future research should focus on the impact of BES and especially CW-MEC mode on the nitrogen cycling within such systems in order to ascertain the detailed mechanism causing the increased ammonium and nitrogen removal.
- $\text{SO}_4^{2-}$ ; Sulfate removal was lower in CW-MFC+ and especially CW-MEC as compared to CW-MFC- and CW-control, possibly due to an abiotic reaction of sulfide with electrodes to form elemental sulfur which then can be microbially re-oxidized to sulfur and further to sulfate using the anode as electron acceptor.
- $\text{PO}_4^{3-}\text{-P}$ ; Ortho-phosphate removal was higher in CW-MEC, potentially be due to in-situ formation of ferric ions from the sacrificial anode, which may have caused precipitation, adsorption and flocculation of phosphorus.

- The mostly insignificant differences between CW-control and CW-MFC- systems show that the anode material alone (SS mesh) apparently does not have an influence on the contaminant removal or microbial community.
- Microbial community analysis; the microbial community analysis showed significant differences in the CW-MEC anode and cathode samples, with the most abundant species *Sphingobium yanoikuyae* which has not been reported in CW-MEC, or in general in BES, before. However, the closely related genera *Sphingomonas* and *Sphingopyxis* (same family of *Sphingomonadaceae*) were reported in other CW-MEC system. The effect of CW-MFC+ anodes seems to be limited to a distance of less than a few millimeters from the anode SSM, since no noteworthy differences could be seen between sampled microbial communities of CW-MFC+, CW-MFC- and CW-control at the anodes. Probably due to this sampling method at the anode, only directly carved off cathode samples of CW-MFC+ showed a microbial community significantly different from CW-MFC- and CW-control with relative high abundance of the species *Lysinibacillus boronitolerans*. A closely related species from the same genus, called *Lysinibacillus sphaericus*, was also found in other MFC systems and even identified to be electrochemically active and able to nitrify ammonium. Both species, *Sphingobium yanoikuyae* and *Lysinibacillus boronitolerans* were described for the first time in BES and would need to be tested for electrochemical activity in separate experiments.

In summary the results were in line with the hypotheses that CW-MEC and CW-MFC+ will outperform all other treatments due to the involved bioelectrochemical processes. Furthermore the results were in line with the hypotheses that the microbial community will differ in CW-MEC and CW-MFC+ as compared to control systems. Whereas, the difference in microbial community could only be confirmed for cathodes of CW-MFC+ since anode samples were likely taken from too far away from the electrode.

## **Acknowledgements**

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 676070. This communication reflects only the authors' view and the Research Executive Agency of the EU is not responsible for any use that may be made of the information it contains. Marianna Garfí is grateful to the Spanish Ministry of Economy and Competitiveness (Plan Estatal de Investigación Científica y Técnica y de Innovación 2013-2016, Subprograma Ramón y Cajal (RYC) 2016).

## Supplementary information

**Table S6.1.** Results for pH for CW-control, CW-MFC, CW-MFC+ and CW-MEC systems at the influent, after first transect, after second transect and effluent as well as overall average (dF = 3, n = 14).

pH (-)	Influent	1/3	2/3	Effluent	AVG
<b>CW-control</b>	7.78±0.28	7.69±0.24	7.73±0.24	8.17±0.32	7.85±0.27
<b>CW-MFC-</b>	7.77±0.28	7.67±0.27	7.69±0.29	8.09±0.37	7.81±0.30
<b>CW-MFC+</b>	7.77±0.25	7.48±0.25	7.49±0.29	8.01±0.25	7.69±0.26
<b>CW-MEC</b>	7.80±0.35	6.93±0.34 <sup>**</sup> , <sup>a</sup>	7.22±0.49 <sup>**</sup> , <sup>b</sup>	7.84±0.30 <sup>*c</sup>	7.45±0.37 <sup>**</sup> , <sup>b</sup>

\* significant difference (p < 0.05)

\*\* Very significant difference (p < 0.01)

<sup>a</sup> for CW-MEC compared to all other treatments

<sup>b</sup> for CW-MEC compared to CW-MFC- and CW-control

<sup>c</sup> for CW-MEC compared to CW-control

**Table S6.2.** Results for redox for CW-control, CW-MFC-, CW-MFC+ and CW-MEC at the influent, after first transect, after second transect and effluent as well as overall average (dF = 3, n = 14).

		In-fluent	1/3	2/3	Effluent	Average
redox (mV)	CW-control	-103±57	-105±57	-112±61	176±102	-36±69
	CW-MFC-	-103±59	-106±59	-129±71	143±93	-49±70
	CW-MFC+	-118±65	-94±56	-90±56	210±110	-23±72
	CW-MEC	-100±58	35±47*	44±46*	262±136	60±72*

\* Very significant difference ( $p < 0.01$ ), for CW-MEC compared to all other treatments

**Table S6.3.** One-factor ANOVA (with replication) results for the comparison of the electric connections during the experimental period, for the total system from inlet to outlet and each of the three transects separately.

	One-factor ANOVA	p-value Comparing Electric Connections			
		Inlet-Outlet	Transect 1	Transect 2	Transect 3
COD	F (3, 14)	0.88	0.62	0.47	0.96
NH <sub>4</sub> <sup>+</sup> -N	F (3, 12)	0.02*	0.01*	0.10	0.01*
NO <sub>3</sub> <sup>-</sup> -N	F (3, 15)	0.47	0.27	0.26	0.02*
NO <sub>2</sub> <sup>-</sup> -N	F (3, 15)	0.32	0.81	0.58	0.65
SO <sub>4</sub> <sup>2-</sup>	F (3, 15)	0.0004**	5E-05**	0.82	0.44
PO <sub>4</sub> <sup>3-</sup> -P	F (3, 15)	0.02*	0.89	0.13	0.64

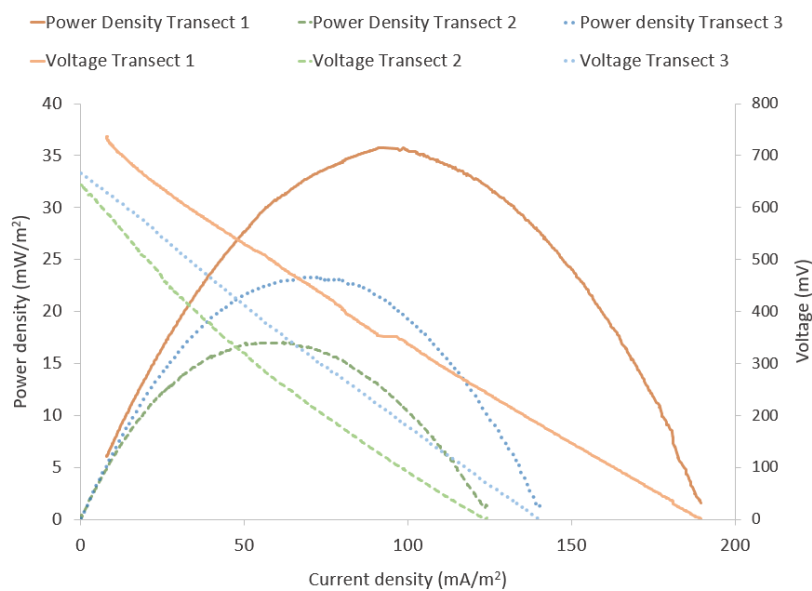
\* significant difference ( $p < 0.05$ ).

\*\* Extremely significant difference ( $p < 0.001$ ).

**Table S6.4.** One-factor ANOVA (with replication) results for the comparison of the electric connections during the experimental period, for the total system from inlet to outlet and each of the three transects separately.

One-factor ANOVA		p-value Shannon's diversity			p-value Evenness		
		1	2	3	1	2	3
<b>Per transect</b>							
<b>Anode</b>	<b>F (3, 2)</b>	0.24	0.04 <sup>*,b</sup>	0.03 <sup>*,c</sup>	0.01 <sup>*,d</sup>	0.04 <sup>*,a</sup>	0.03 <sup>*,c</sup>
<b>Cathode</b>	<b>F (3, 2)</b>	0.0017 <sup>**<sub>,d</sub></sup>	0.75	0.51	0.15	0.99	0.26

\* significant difference ( $p < 0.05$ ).  
 \*\* Very significant difference ( $p < 0.01$ )  
<sup>a</sup> CW-MEC compared to CW-control  
<sup>b</sup> CW-MEC compared to CW-MFC-  
<sup>c</sup> CW-MEC compared to CW-MFC- and CW-control  
<sup>d</sup> CW-MEC compared to all other treatments



**Figure S6.1.** Power density and polarization curves for each transect of one of the CW-MFC+ replicates



# CHAPTER 7

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## Organic micropollutant removal in CW-BES

This chapter is based on the following article:

Hartl M., García-Galán M. J., Matamoros V., Fernández Gatell M., Rousseau D.P.L., Du Laing G., Garfí M., Puigagut J. (submitted January 2020) Constructed wetlands operated as bioelectrochemical systems for the removal of organic micropollutants. *Science of the Total Environment*.

## Abstract

The removal of organic micropollutants (OMPs) has been investigated in constructed wetlands (CWs) operated as bioelectrochemical systems (BES). The operation of CWs as BES (CW-BES), either in the form of microbial fuel cells (MFC) or microbial electrolysis cells (MEC), has only been investigated in recent years. The presented experiment used CW meso-scale systems applying a realistic horizontal flow regime and continuous feeding of real urban wastewater spiked with four OMPs (pharmaceuticals), namely carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBU) and naproxen (NPX). The study evaluated the removal efficiency of conventional CW systems (CW-control) as well as CW systems operated as closed-circuit MFC (CW-MFC+) and MEC (CW-MEC). Higher removal rates were obtained for three out of the four compounds (CBZ, DCF and NPX) with an increase of 10-17% in CW-MEC and 5% in CW-MFC systems, compared to the CW-control. However, no statistically significant differences were found. IBU removal was similar amongst treatments.

## 7.1 Introduction

Organic micropollutants (OMPs) encompass a large variety of active compounds, ranging from pharmaceuticals and personal care products to plasticizers, surfactants, anticorrosives or nanomaterials (Thomaidis et al., 2012) (see Chapter 2.1.3.4 for more details). In the following, the four OMPs, all of them pharmaceuticals, investigated in this study will be described in more detail.

CBZ, an anticonvulsant and sedative drug also used to treat epilepsy and bipolar disorder, is one of the most frequently detected pharmaceuticals in basically all kind of environments at concentrations reaching the  $\mu\text{g L}^{-1}$  level (Hai et al., 2018; Tran et al., 2018). CBZ is resilient to degradation in CAS treatment systems, showing a low tendency to sorption in sewage sludge and to photodegradation (Andreozzi et al., 2003; Calisto et al., 2010; Hai et al., 2011). Furthermore, it is not eliminated from sewage sludge by anaerobic digestion (Carballa et al., 2007). Even tertiary treatments such as advanced oxidation processes (UV/chlorination) have resulted in incomplete removal of CBZ (W. Wang et al., 2016; Zhou et al., 2016). Its ubiquity and resilience has led to consider it as a potential marker of anthropogenic contamination in water (Hai et al., 2018). The anti-inflammatories DCF, IBU and NPX also occur in comparatively high concentrations in WWTP effluents (Gros et al., 2012, 2010; Mamo et al., 2018). Furthermore, IBU and NPX belong to the most detected NSAIDs in WWTP influents worldwide due to their widespread use as over-the-counter pharmaceuticals (i.e. sold directly to a consumer without a prescription from a healthcare professional). For instance, IBU is one of the most consumed anti-inflammatories in Europe, with estimated yearly consumptions of 250 tons in Spain alone (Ortiz de García et al., 2013). Since DCF is an NSAID which often requires a prescription, it is found to a lesser extent in WWTP influent compared to IBU and NPX. Still, it is one of the most widely prescribed anti-inflammatories and shows lower and more variable removal rates (7-75%) than IBU and NPX in WWTPs (40-100% and 40-98%, respectively) (García-Galán et al., 2016; Gros et al., 2012; Mamo et al., 2018). Similarly to CBZ (which rarely exceeds 10% removal in conventional WWTPs (Joss et al., 2005; Zhang et al., 2014)), DCF is usually classified as a recalcitrant compound (Hai et al., 2018; Osorio et al., 2016; Zhang et al., 2008). Due to the resulting high concentrations in WWTPs discharges, this NSAIDs may pose a risk to

aquatic ecosystems and different non-target species (Huerta et al., 2016; Ruhí et al., 2016). For instance, DCF has been found in larvae of caddisflies and leeches at concentrations up to 183 ng g<sup>-1</sup> (Huerta et al., 2015), and it was responsible for the drastic decline of vulture populations in Pakistan which fed on cattle carcasses treated with this drug (Oaks et al., 2004). Considering these data, the need and search for alternative and more efficient treatments is evident. In particular, nature-based, low-cost treatment systems such as microalgae-based systems or constructed wetlands (CWs) are currently being intensively investigated and, so far, showed promising results regarding OMPs removal (Ávila et al., 2014b, 2014a; García-Galán et al., 2018; Matamoros et al., 2015). Specifically, CWs are well-established systems for wastewater treatment and have been successfully applied in different climate zones worldwide (Langergraber and Haberl, 2001; Molle et al., 2005). The removal efficiency of OMPs in CWs varies with design, operation and type of CW (e.g. surface, subsurface vertical/horizontal flow) employed. In the case of subsurface horizontal flow (HF) CWs, the removal of OMPs ranges from poor to very efficient, depending on characteristics such as bed depth, media size, loading frequency or potential clogging (Ávila et al., 2014b; Matamoros and Bayona, 2006). Various CW intensification strategies have been developed over the last decades and were also tested for the treatment of OMPs, with promising results especially for biodegradable OMPs, but further research is still needed (Ávila et al., 2014b; Nivala et al., 2019b; Zhang et al., 2014).

A relatively recent development in the field of wastewater treatment is based on coupling CWs with bioelectrochemical systems (BES) such as Microbial Fuel Cells (MFCs) (Villaseñor et al., 2013; Yadav et al., 2012) and Microbial Electrolysis Cells (MECs) (Ju et al., 2014a) called CW-MFC and CW-MEC, respectively, from here on (see more details in Chapter 2.3). Earlier studies of CW-BES or BES systems for OMP removal used artificial wastewater, which is advantageous for the study of fundamental processes, but less realistic than real urban wastewater (Li et al., 2019; Pun et al., 2019; Wang et al., 2015).

For instance, Li et al. (2019) investigated bisphenol A and IBU removal in lab-scale CW-MFC systems fed with synthetic wastewater, obtaining removals of 82-96%, a 9% higher removal than in their control CW. Also Pun et al. (2019) reported high removal rates up to 99% for

11 different OMPs, (including CBZ (99%) and NPX (95%)) in a lab-scale HF CW with an integrated BES. However, their configuration works in short-circuit mode (no solid-state electrodes) using a highly porous as well as electro-conductive medium (graphitized coke), which makes it difficult to distinguish the proportion of conventional contaminant and OMP removal effect related to the CW operated as BES from that related to the improved physico-chemical factors like sorption. Wang et al. (2015) investigated the removal of a variety of OMPs using conventional MFCs in single-chamber setup (60% CBZ, 4-8% DCF, 18-20% IBU and 12-19% NPX removal) as well as double-chamber setup (ca. 20% and 70% CBZ, 23% and 45% DCF, 40% and 87% IBU, and 40% and 84% NPX removal, in the anode and cathode chamber, respectively) using synthetic wastewater. Regarding other OMPs, apart from the ones addressed in this study, studies using CW-MFCs were published on sulfamethoxazole with 38-50% removal (Li et al., 2018) and >99% removal (S. Zhang et al., 2016b), one on sulfadiazine removing >99% (Song et al., 2018), one on tetracycline removing >99% (S. Zhang et al., 2016a), and one on phenanthrene and anthracene with removal ranging from 88.5% to 96.4% (J. Wang et al., 2019).

The present experiment used CW meso-scale systems which, despite being unplanted, were intended to give additional information on OMP removal in larger scale CW-BES systems with a more realistic horizontal flow, continuous feeding of real urban wastewater and realistic spiking concentration levels of OMPs. Additionally, to the best knowledge of the authors this is the first publication on OMP removal in CW-MEC, and consequently also the first one to compare OMP removal efficiency of CW-MFC and CW-MEC side by side. To this end, duplicate systems with conventional CW (CW-control), closed-circuit CW-MFC (CW-MFC) as well as CW-MEC (CW-MEC) configuration have been used.

The hypothesis was that CW-MEC and CW-MFC+ will improve organic micropollutants removal as compared to the CW-control system.

## **7.2 Material and methods**

### **7.2.1 Design**

For the purpose of this investigation, six out of the eight meso-scale CW-MFC systems described in more detail in Chapter 3.1.1. were used. Tested treatments included CW-control, CW-MFC+ and CW-MEC (see Figure 3.1.1).

### **7.2.2 Operational conditions**

The experimental CWs were mature at the time this work was conducted. They had been operated under similar conditions for eighteen months before the current experiment was carried out. During the experiment, the systems were fed with fresh pre-settled urban wastewater every weekday. Influent wastewater was spiked with the target OMPs at a final concentration of 4 µg/L for 4 weeks. Samples for OMP analyses were taken after one week of the start of daily OMP dosing (which represents a bit less than two times the nominal HRT in order to ensure that the OMPs had reached the outlet of the CW during sampling).

Further details on pre-treatment are given in Chapter 3.1.2. The average hydraulic loading rate (HLR) applied during the experiment was 28 mm/d, resulting in a nominal HRT of 3.6±0.3 days and an average organic loading rate (OLR) of 8.7±2.5 g COD/m<sup>2</sup>·day.

### **7.2.3 Sampling and analysis**

#### **7.2.3.1 Water quality parameters**

Eight sampling campaigns for the characterization of conventional wastewater quality parameters were conducted during 12 weeks. These campaigns were conducted already 3 weeks before OMP sampling started, and continued during the OMP sampling period, whereas conventional wastewater samples were taken just before the OMP dosing on weekdays. Conventional wastewater parameters were

measured for the influent, after the first and second third of the wetland length, and as also at the effluent (for more details see Chapter 5.2.3). All samples were analyzed for total suspended solids (TSS), volatile suspended solids (VSS) and total chemical oxygen demand (COD) according to Standard Methods (APHA-AWWA-WEF, 2012);  $\text{NH}_4^+\text{-N}$ , according to Solórzano method (Solórzano, 1969);  $\text{NO}_2^-\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ ,  $\text{SO}_4^{2-}\text{-S}$  and  $\text{PO}_4^{3-}\text{-P}$  by ion chromatography (ICS-1000, Dionex Corporation, USA). Physical parameters such as water temperature, dissolved oxygen (DO) concentration and pH were measured directly in the influent, using portable devices after the first and second transect, as well as in the effluent (EcoScan DO 6, ThermoFisher Scientific, USA and CRISON pH/mV – meter 506, Spain, respectively).

### 7.2.3.2 OMP analysis

High purity standards (>99%) of the parent compounds and the isotopically labelled compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA). Detailed information on their physical and chemical characteristics is given in Table S7.1 of the Supplementary Information (SI). Standard solutions of the mixtures of the four compounds were made at the appropriate concentrations and used to dope the influent wastewater. Five OMP sampling campaigns were conducted during 3 weeks. Grab samples were taken from the system influent and effluent sampling points (see Figure 3.1.1, points B and K, respectively). All water samples were filtered and processed using a methodology adapted from the one published by Matamoros and Bayona (2006). Briefly, 50 mL of influent and 100 mL of effluent samples were filtered (0.7  $\mu\text{m}$  Whatman™ glass microfiber filters GF/F), acidified to pH 2-3 with HCl (0.02M) and spiked with a mixture of surrogate standards to a final concentration of 50 ng L<sup>-1</sup> (atrazine-d<sub>5</sub>, mecoprop-d<sub>3</sub>, tonalide-d<sub>3</sub>, and dihydrocarbamazepine). Solid phase extraction was then performed, using 200 mg Strata™-X polymeric cartridges from Phenomenex (Torrance, CA, US), previously conditioned with 3 mL of hexane, 3 mL of ethyl acetate, 5 mL of MeOH and 5 mL of acidified milli-Q water. Elution was performed with 10 mL of hexane/ethyl acetate (1:1, v:v). The eluted extract was evaporated under a gentle nitrogen stream to a volume of 100  $\mu\text{L}$ , and triphenylamine was added as an internal standard (20 ng). Finally, vials

were reconstituted to 300  $\mu$ L and analyzed by GC-MS/MS as described by Matamoros et al. (2017).

#### **7.2.4 Statistical analysis**

Statistical analysis were conducted using single-factor analysis of variance (ANOVA) and if necessary post-hoc Tukey HSD and Scheffé multiple comparison tests were performed.



## 7.3 Results and discussion

### 7.3.1 Electrical behavior

Table 7.3.1 shows average and maximum measured cell voltages ( $E_{\text{cell}}$ ) and consequent current densities for CW-MFC treatments in all 3 transects.

**Table 7.3.1.** Average, standard deviation and maximum for  $E_{\text{cell}}$  and current density of closed-circuit CW-MFC systems. Note: The surface area of each electrode was used for current density calculations.

Transect	$E_{\text{cell}}$ (mV)		Current Density per Area (mA/m <sup>2</sup> )		Current Density per Volume (mA/m <sup>3</sup> )	
	Avg ± SD	Max	Avg ± SD	Max	Avg ± SD	Max
	1	372±119	552	40±13	60	183±59
2	378±81	577	41±9	62	186±41	282
3	372±128	711	40±14	77	183±64	350

Average current densities for CW-MFC (all transects considered) resulted in 40 mA/m<sup>2</sup>. Differences in current density between transects were not statistically significant. A polarization curve (PC) analysis (see SI, Figure S7.1) showed that maximum power densities of 30, 11 and 24 mW/m<sup>2</sup> in transect 1, 2 and 3 of CW-MFC mode, respectively, were achieved at current densities of 79, 35 and 66 mA/m<sup>2</sup>, respectively, which is higher than that described by Saz et al. (2018) (ca. 20 mA/m<sup>2</sup>) under comparable conditions.

The estimated internal resistances derived from the PC analysis were around 108 Ω, 220 Ω and 124 Ω for first, second and third transect, respectively. Principally, the potential maximum power is achieved when internal and external resistances are close to each other (Lefebvre et al., 2011). Coincidentally, the external and internal

resistance were exactly the same in transect 2. However, for the current experiment and its primary goal contaminant removal the lower external resistances in transects 1 and 3 could have been beneficial, since lower external resistances increase the generated current and studies have also shown that consequently organic matter removal was increased (Aelterman et al., 2008; Gil et al., 2003; Katuri et al., 2011).

The coulombic efficiency (CE) is the proportion of the produced current to the carbohydrates which are theoretically derived from oxidation, indicated by the change of COD from transect to transect (Scott, 2016). The resulting average CE values amounted to  $1.4\pm 2.4\%$ ,  $9.5\pm 7.6\%$  and  $-29.4\pm 4.6\%$ , for transects 1, 2 and 3, respectively. Note that CE can have a negative value when COD concentrations were increasing from the influent to the end of transect 1 or from one transect to the other. Generally, it can be assumed that only the CE value measured in transect 1 gave a useful indication since not only organic matter from the influent can contribute to the MFC signal but also accumulated organic matter within the gravel bed is a fuel source for MFC (Corbella et al., 2016a). Therefore CE in transect 2 could be higher than CE in transect 1, and CE in transect 3 was even negative on average. Comparable CW-MFC studies produced CEs from 0.01% (Wang et al., 2016b) up to 16.4% (Xie et al., 2018).

Table 7.3.2 shows the poised potential and the resulting achieved average current for each transect in CW-MEC systems.

**Table 7.3.2.** Poised potential (at Anode vs. Ag/AgCl reference electrode) and resulting average current applied also expressed in current density per surface area and volume in CW-MEC (MEC)

Transect	Poised Potential (V)	Current (mA)	Current Density per Area (mA/m <sup>2</sup> )	Current Density per Volume (mA/m <sup>3</sup> )
		Avg. ± SD	Avg. ± SD	Avg. ± SD
1	0.3	23±11	535±263	2434±1197
2	0.3	10±5	223±112	1015±510
3	0.3	5±3	120±74	545±334

The poised potential of 0.3 V vs. Ag/AgCl reference electrode at the anode, was chosen on the basis of experiences showing that poised potential around this value benefits the growth of electroactive bacteria (EAB) genera such as *Geobacter* in mixed bacterial cultures (Fricke et al., 2008; Liu et al., 2008). The average current density in CW-MEC was more than double in transect 1 compared to transect 2, and that in transect 2 was again roughly double of that in transect 3, assumingly because the organic matter concentration was decreasing along the flow path through the systems.

The CW-MEC current densities in all three transects were low when compared to other similarly built CW-MEC systems which showed values ranging from 200 to 24500 mA/m<sup>2</sup> (Gao et al., 2017; Srivastava et al., 2018; Xu et al., 2017; Zhang et al., 2018).

### 7.3.2 Removal efficiency of conventional wastewater parameters

Results on the removal of conventional contaminants in all three treatments (CW-control, CW-MFC+ and CW-MEC systems) are summarized in Table 7.3.3. All results were obtained during 8 weeks of intensive sampling (5 weeks before the OMP sampling campaigns and the three weeks during the OMP sampling campaign). Data is shown

as average mass loading rate at the system inlet (influent), after the first and second transects and effluent, as well as mass removal rate from influent to effluent based on the average mass and percentage. During this period, all systems received continuous flow with an average OLR of  $8.7 \pm 2.5$  g COD/m<sup>2</sup>·day.

**Table 7.3.3.** Results for conventional contaminants in all treatments during the 8 sampling weeks, expressed as average mass loading rate at influent, after first transect, after second transect and effluent as well as removal from influent to effluent based on the average mass removal rate and percentage.

		In-fluent	1/3	2/3	Effluent	Removal from Influent to Effluent	
						(g/m <sup>2</sup> ·d)	(%)
COD (n=8)	CW-control	8.6±2.6	4.4±1.9	3.7±2.2	3.7±1.6	4.9±1.4	57
	CW-MFC+	8.9±2.4	4.4±2.3	3.8±2.3	4.0±1.5	4.9±0.5	55
	CW-MEC	8.7±2.5	3.9±2.3	2.5±1.4	2.6±1.0	6.1±0.8	70
NH <sub>4</sub> <sup>+</sup> -N (n=7) <sup>a</sup>	CW-control	1.2±0.4	1.0±0.4	0.9±0.3	1.1±0.3	0.1±0.2	10
	CW-MFC+	1.3±0.4	1.0±0.3	0.8±0.2	1.0±0.3	0.3±0.2	24
	CW-MEC	1.2±0.4	0.9±0.3	0.7±0.2	0.9±0.2	0.3±0.3	28
SO <sub>4</sub> <sup>2-</sup> (n=6) <sup>a</sup>	CW-control	2.0±1.3	0.5±0.5	0.4±0.3	0.8±0.6	1.1±0.9	58
	CW-MFC+	2.1±1.4	0.6±0.4	0.6±0.4	1.1±0.9	1.0±0.3	48
	CW-MEC	2.2±1.4	0.8±0.7	1.0±0.8	1.1±0.9	1.1±0.8	51
PO <sub>4</sub> <sup>3-</sup> -P (n=6) <sup>a</sup>	CW-control	0.09±0.05	0.09±0.06	0.09±0.05	0.09±0.05	0.00±0.03	2
	CW-MFC+	0.09±0.05	0.09±0.06	0.08±0.05	0.08±0.05	0.01±0.03	7
	CW-MEC	0.09±0.05	0.07±0.06	0.06±0.05	0.08±0.05	0.01±0.04	7

<sup>a</sup> Some experimentation weeks could not be considered due to highly diluted influent or technical analysis problems

Except for pH (see more details below), no statistically significant differences were found for general wastewater quality parameters (see SI, Table S7.2 and S7.3). On average, CW-MEC showed higher COD and  $\text{NH}_4^+\text{-N}$  removal than the CW-control, with an increase of 13% and 18%, respectively. CW-MFC+ removed 2% less COD than CW-control, but 18% more  $\text{NH}_4^+\text{-N}$  on average, which is slightly higher than previous results measured on the same systems (Hartl et al., 2019). The improvement in  $\text{NH}_4^+\text{-N}$  removal is similar to that obtained in other studies on CW-MFC+ (Corbella and Puigagut, 2018) and CW-MEC (Gao et al., 2018). However,  $\text{NH}_4^+\text{-N}$  removal was generally low, and decreased towards the end of the study period, which was also observed for COD, although to a lesser extent. It is assumed that aging and possible partial clogging of the carbon felt (CF) cathodes might have limited the COD removal performance of CW-MFC+ systems. This partial clogging could have also negatively affected ammonia oxidizing bacteria (AOB) in the cathode biofilm, which were described to enhance nitrification in a single-chamber air-cathode MFC by Kim et al. (2008). In the case of  $\text{NH}_4^+\text{-N}$ , it could also be assumed that due to the system aging and the accompanying development and establishment of the microbial communities, the systems turned more anaerobic, which in turn would have lowered the nitrification rates. However,  $\text{NH}_4^+\text{-N}$  was still removed to a greater extent in CW-MFC+ and CW-MEC systems. At least for CW-MEC, this could be due to electrolysis happening at the anode, releasing oxygen and hydrogen, which increased aerobic and hydrogen consuming processes. The increased DO, identified by Wang et al. (2019) as one of the most influential parameters for COD removal in CW-MFC, could then be assumed to be the main factor for the increased COD removal in CW-MEC of the presented study. The bulk DO as measured by the portable meter in the systems' sampling tubes was always below the detection limit. However, the electrolysis might be effective on a much smaller scale and thus not be reflected in the bulk DO. The higher denitrification capacity in CW-MEC, and possibly to some degree in CW-MFC+ could be explained by electrolysis induced  $\text{H}_2$  serving as electron donor for nitrate reduction to nitrogen gas, and  $\text{H}^+$  could also be involved in autohydrogenotrophic denitrification (Gao et al., 2017). Additionally, in an earlier study, an increase in microbial activity was observed (Hartl et al., 2019), which could have led to an overall improved biodegradation. For both COD and  $\text{NH}_4^+\text{-N}$  removal, the lack of plants in the meso-scale systems could have had an effect as well

on overall treatment efficiencies, since the presence of plants has shown to improve treatment efficiency in HF CWs (Tanner, 2001).  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N were generally below the limit of detection. A recent study showed that planted CW-MFC+ systems show higher power density and contaminant removal, however, dead plant parts in turn also reduced the power production (Yang et al., 2019).

Average  $\text{SO}_4^{2-}$ -S removal was lower in CW-MFC+ and CW-MEC systems than in CW-control, especially after the second transect. This was also observed by Corbella and Puigagut (2018) who found 13% higher  $\text{SO}_4^{2-}$ -S removal in control systems than in CW-MFC+ systems, likely due to the re-oxidation of sulfides to sulfur and further to sulfate using the MFC anode as electron acceptor (Lovley et al., 2006).

Average  $\text{PO}_4^{3-}$ -P removal was higher in CW-MEC and CW-MFC+ as compared to CW-control, and again most distinct after the second transect. These results show that the third transect might have had kind of an equalizing effect when comparing  $\text{PO}_4^{3-}$ -P as well as  $\text{SO}_4^{2-}$ -S reduction between treatments. Generally,  $\text{PO}_4^{3-}$ -P removal efficiency was lower when compared to current literature regarding CW-MFC (Corbella and Puigagut, 2018; Saz et al., 2018; Xu et al., 2018; Yakar et al., 2018) or CW-MEC (Gao et al., 2018; Ju et al., 2014; Zhang et al., 2018) systems. However, many studies were conducted only over a short time and it is generally known that phosphorus storage in subsurface flow CWs has a finite capacity and therefore removal by sorption normally decreases over time (Kadlec and Wallace, 2009), as could have been the case in this study as the wetlands were operated for over 18 months.

The average values for pH measurements at each sampling point are shown in SI, Table S7.2. The results for influent and average pH values of all sampling points were statistically not significantly different across treatments. However, after the first transect, CW-MEC systems showed a lower pH than other treatments on average, being significantly different ( $F(2, 3)$ ;  $p = 0.0008$ ) from CW-MFC+ as well as CW-control. After the second transect, pH values of all three treatments were significantly different from each other ( $F(2, 3)$ ;  $p = 1e-06$ ), with CW-MEC showing the lowest pH, followed by a higher pH in CW-MFC+ and the highest in CW-control (meaning the smallest change since the influent inlet in the system). pH values at the effluent were generally higher than in the previous two transects within the systems, and the

difference between treatments was again only statistically different in the CW-MEC systems (F (2, 3); p = 0.0029).

Changes in pH within the system might affect the activity of bacteria, and influence the charge state as well as hydrophobicity of certain OMPs (Wang et al., 2015). While the measured pH in solution showed some significant differences between treatments, the changes seemed not big enough to alter the charge state and hydrophobicity of the investigated OMPs significantly, especially in the case of CBZ with its high pK<sub>a</sub> of 13.9 (see SI, Table S7.1). However, as for DO, pH at the micro-scale, e.g. near the cathode or anode, might have changed more drastically, and could have created micro-environments in which charge state and/or hydrophobicity were influenced. Unfortunately, it was not possible to measure these changes in pH on a micro-scale with the presented setup.

### **7.3.3 Removal efficiency of organic micropollutants**

Table 7.3.4 shows the removal of the four target OMPs for all three treatments (see also SI, Figure S7.2 for box- and whisker plots).



**Table 7.3.4.A.** Results for OMPs carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBU) and naproxen (NPX) in CW-control, closed-circuit CW-MFC+ and CW-MEC systems during the 5 sampling campaigns, expressed as average background, influent and effluent concentration, average mass loading rate at influent and effluent as well as removal from influent to effluent based on the average mass removal rate and percentage. (Concentration variability in the influent concentrations is due to the background concentration of the urban wastewater for each of the compounds).

OMP (n=5)	Back-ground	Influent		Treatment	Effluent		Removal	
	(µg/L)	(µg/L)	(µg/m <sup>2</sup> ·d)		(µg/L)	(µg/m <sup>2</sup> ·d)	(µg/m <sup>2</sup> ·d)	(%)
CBZ	3.5±2.2	5.3±2.2	149±61	CW-control	4.6±1.4	123±41	26	17%
				CW-MFC+	4.3±1.0	116±26	33	22%
				CW-MEC	3.7±0,8	99±24	50	34%
DCF	0.6±0.3	4.2±1.9	137±56	CW-control	2.7±1.4	73±17	65	47%
				CW-MFC+	2.2±1.0	65±20	72	52%
				CW-MEC	2.2±0,8	59±16	79	57%

**Table 7.3.4.B.** Results for OMPs carbamazepine (CBZ), diclofenac (DCF), ibuprofen (IBU) and naproxen (NPX) in CW-control, closed-circuit CW-MFC+ and CW-MEC systems during the 5 sampling campaigns, expressed as average background, influent and effluent concentration, average mass loading rate at influent and effluent as well as removal from influent to effluent based on the average mass removal rate and percentage. (Concentration variability in the influent concentrations is due to the background concentration of the urban wastewater for each of the compounds).

OMP (n=5)	Back-ground	Influent		Treatment	Effluent		Removal	
	(µg/L)	(µg/L)	(µg/m <sup>2</sup> ·d)		(µg/L)	(µg/m <sup>2</sup> ·d)	(µg/m <sup>2</sup> ·d)	(%)
IBU	12.6±3.6	18.6±8.8	523±202	CW-control	12.0±2.0	321±53	202	39%
				CW-MFC+	12.6±1.7	341±40	182	35%
				CW-MEC	12.0±2.2	320±52	202	39%
NPX	3.8±0.7	10.2±1.4	273±29	CW-control	7.6±2.4	203±62	70	25%
				CW-MFC+	7.1±2.0	191±50	82	30%
				CW-MEC	6.1±1.5	163±37	109	40%

Average OMP removals were slightly higher in CW-MEC (by 10-17%) and CW-MFC+ (by 5%) as compared to the CW-control for CBZ, DCF and NPX. However, these differences were not statistically significant for any of the three compounds. Regarding IBU, the average removal was similar in all treatments, with CW-control and CW-MEC exhibiting the same average removal rates and a 4% lower removal in CW-MFC.

### 7.3.3.1 Carbamazepine

Average CBZ removal was higher in CW-MEC and CW-MFC+ as compared to CW-control with values of 34%, 22% and 17%, respectively (see Table 7.3.4 and SI, Figure S7.2a). The CW-control system removal of 17% is in accordance with results of previous studies on treatment capacity in conventional HF CW systems (not operated as BES), reporting removals of 13% (Nivala et al., 2019b) and 21% (Matamoros et al., 2017). These results show that CBZ can be removed to a certain degree in HF CWs (supposedly due to anaerobic processes), however, CBZ is not biodegradable in aerobic conditions and therefore VF CWs show lower removal rates (Hai et al., 2011; Jekel et al., 2015; König et al., 2016; Nivala et al., 2019b).

The only other study looking at CBZ removal in CWs operated as BES resulted in removal of more than 99% from synthetic wastewater (Pun et al., 2019). However, this system was operated in short-circuit and used a bed of highly porous and electroconductive media (graphitized coke), in which anodic and cathodic processes were uncontrolled (comparable to a CW-MFC but without solid state electrodes or external connection). Their own sorption experiments showed that ca. 30% of the compound was removed solely by abiotic sorption onto the highly porous media. Also in conventional MFC and MEC (poised potential of -0.4 V vs Ag/AgCl at the anode) systems, Werner et al. (2015) identified hydrophobic sorption as the dominant mechanism for CBZ removal, attributing the removal (>80%) mainly to the large anode areas provided by the graphite fiber brushes (material with high sorption propensity) and the attached biofilm. However, graphite has a high sorption propensity as well, unlike the used gravel in the presented study. Although CBZ can actually not be considered hydrophobic (log D of 2.77, see SI, Table S7.1), it is less polar than the

other three tested OMPs, and therefore the contribution of sorption to CBZ removal is potentially higher than in the three other tested OMPs.

Considering the low CBZ removal in conventional CAS (rarely exceeds 10% (Joss et al., 2005; Zhang et al., 2014)), the results obtained for CW-MFC+ and especially in the case of CW-MEC describe a real improvement. The reason for increased removal in the presented CW-MFC+ and CW-MEC systems compared to CW-control could be manifold. Electrosorption and hydrophobic sorption could have played a role with CW-MFC+ and CW-MEC offering additional sorption sites at the electrodes, and biofilm, and thereby improving the removal. However, these sorption sites are finite and longer term investigations using BES incorporated in CWs for CBZ removal are suggested. An effect of pH changes (see SI, 2) on hydrophobicity and charge in the different treatments is unlikely in the case of CBZ due to the high  $pK_a$  of 13.9 (see SI, Table S7.1). However, an increase in microbial activity observed in CW-MFC+ in an earlier study (Hartl et al., 2019) could have led to an improved biodegradation and at least partly explain the improved removal in CW-MFC+ and possibly CW-MEC as compared to the CW-control. Although no microbial activity studies in CW-MEC are known to the authors it could be assumed that it is affected in a similar way as in CW-MFC. Further investigation of the microbial communities, especially of CW-MEC, are suggested.

### 7.3.3.2 Diclofenac

Average DCF removal was higher in CW-MEC and CW-MFC+ as compared to CW-control with values of 57%, 52% and 47%, respectively (see Table 7.3.4 and SI, Figure S7.2b). DCF removal of 47% in CW-control was higher than in other publications on conventional HF CW systems, reporting 25% (Nivala et al., 2019) and  $19 \pm 21\%$  removal (Matamoros et al., 2017). There are no publications yet on DCF removal by CW-MFC+ or CW-MEC systems. DCF removal rates in the presented CW-MFC+ were high even when compared to conventional MFC systems fed by synthetic wastewater, which reached only 4-8% in a single-chamber closed-circuit MFC and up to ca. 23% and 45% in the anode and cathode chamber of a double chamber MFC, respectively (Wang et al., 2015). De Gusseme et al. (2012) applied biogenic Pd nanoparticles as a biocatalyst to a conventional MEC

(voltage of -0.8 V applied to the circuit) for the catalytic dechlorination of DCF (from synthetic wastewater with 1 mg/L DCF) and achieved full removal while no significant removal was achieved without the use of the nanoparticles. In conventional CW systems (not operated as BES), vertical flow (VF) CW systems are more efficient removing DCF through aerobic processes, with performances ranging from 50-70% (Ávila et al., 2014a, 2014b; Matamoros et al., 2007; Nivala et al., 2019b), while the removal in HF CWs is lower and thought to happen through anaerobic degradation (Ávila et al., 2010). The biological removal of DCF is not fully understood and results are usually very variable (Zhang et al., 2008). Although the log  $K_{ow}$  of DCF is high with 4.26, it gets deprotonated and becomes highly hydrophilic in the pH range of 6.6 to 7.6 of the presented systems, with a log D of 1.70 to 1.04 (see SI, Table S7.1), resulting in a low sorption propensity. DCF is also recalcitrant (though not as strongly as CBZ), therefore removal rates in conventional WWTPs are also relatively low and variable with elimination values in the range of 7-75% (Zhang et al., 2014). Given the charge and sorption characteristics of DCF, conventional sorption and pH effects seem unlikely to have influenced the DCF removal to a great extent. However, electrosorption at the electrode with opposite charge (i.e. at the positive charged cathode, since DCF has a negative charge, see SI, Table S7.1) could have contributed to the DCF removal (Kong et al., 2013; Yang et al., 2015). CW-MFC+ systems have been shown to enhance microbial activity (Hartl et al., 2019). Hence, DCF removal was possibly enhanced through an increase in microbial activity in CWs operated as BES, which could have led to the observed DCF removal improvement in CW-MFC+ and CW-MEC as compared to the CW-control. Another factor could be the potential electrolysis of water in CW-MEC, producing oxygen and  $H^+$  at the anode and  $H_2$  at the cathode. The produced oxygen could have increased the aerobic biodegradation of DCF in CW-MEC and thereby explain the enhancement in removal as compared to CW-MFC+.

### 7.3.3.3 Ibuprofen

IBU removal was not very different across treatments with 39% removal in CW-control and CW-MEC, and 35% in CW-MFC+ systems (see Table 7.3.4 and SI, Figure S7.2c). Anyway, the here reported removal rates were comparable to those found in HF CW systems (28%) (Matamoros et al., 2017; Nivala et al., 2019b). To the knowledge of the authors, there are no publications yet on IBU removal by CW-MEC systems and just one other publication which currently addresses IBU removal using a CW-MFC; Li et al. (2019) reported IBU removal rates of 82-96% from synthetic wastewater in a CW-MFC, which was 9% higher than their open-circuit control, with 63-79% of the removal happening in the anodic section.

Removal rates in conventional MFC systems reached values of 18-20% in single-chamber closed-circuit systems, and up to ca. 40% and 87% in anode and cathode chambers of a double-chamber MFC, respectively (synthetic wastewater was used) (Wang et al., 2015).

In general, IBU is highly hydrophilic and therefore sorption is low, with a log D of 1.16 to 2.10 in the measured pH range (see SI, Table S7.1). Aerobic conditions favor its biodegradation (Monsalvo et al., 2014; Quintana et al., 2005), hence VF CWs show removal rates above 88% (Ávila et al., 2010; Nivala et al., 2019b; Vystavna et al., 2017). This is probably also why plants – known to provide oxygen to the systems via their roots (Kadlec and Wallace, 2009) – improved IBU removal in HF CWs (Y. Li et al., 2016). Removal rates in conventional WWTPs are usually high (41-100% ) due to these aerobic removal mechanisms (Zhang et al., 2014). In general, the authors suggest to confirm the obtained results of all OMPs in planted CWs operated as BES.

In summary, IBU removal was not improved through CW-MFC+ or CW-MEC, although other studies on CW-MFC+ or conventional MFC were able to achieve that in comparison to control systems. In terms of charge, sorption propensity and biodegradability, IBU has similar characteristics as DCF and NPX, therefore other factors seem to have been responsible for the lack of difference between treatments. Further investigation should be carried out to confirm and possibly explain the results reported here.

### 7.3.3.4 Naproxen

Average NPX removal was higher in CW-MEC and CW-MFC+ as compared to CW-control with values of 40%, 30% and 25%, respectively (see Table 7.3.4 and SI, Figure S7.2d). The 25% NPX removal in the CW-control was lower than in comparable HF CW systems showing 32% (Nivala et al., 2019b) and 66% removal (Matamoros et al., 2017). The short-circuit CW-BES by Pun et al. (2019) removed more than 95% of NPX from synthetic wastewater; only a fraction (13.1-18.5% according to abiotic sorption tests) of that was retained within the material and therefore unrelated to biological activity of bacteria. Removal rates in conventional MFC systems operated by Wang et al. (2015) reached ca. 12-19% in single-chamber closed-circuit systems and up to ca. 40% and 84% in the anode and cathode of double-chamber MFC, respectively (all using synthetic wastewater).

Sorption of NPX is low, with a log D of 0.61 to -0.18 in the pH range of 6.6-7.6 (see SI, Table S7.1). Generally, NPX is mainly removed by biodegradation, and preferably under aerobic conditions (Kahl et al., 2017), hence VF CWs show high removal rates above 88% (Ávila et al., 2010; Nivala et al., 2019b; Vystavna et al., 2017). Again as for IBU, removal rates in conventional WWTPs are relatively high and in the range of 40-98% (Zhang et al., 2014). As for DCF, NPX removal differences across treatments were unlikely influenced by differences in charge or sorption but possibly enhanced by electrosorption in CWs operated as BES. Again, also an increase in microbial activity could have led to the observed NPX removal improvement in CW-MFC+ and CW-MEC as compared to the CW-control. A potential increase in oxygen through electrolysis at the anode could explain the enhanced IBU removal in CW-MES as compared to CW-MFC.

According to Cecconet et al. (2017), BES are theoretically more efficient in removing OMPs which are hydrophobic and positively charged. The former due to the better adsorption onto charged electrodes and the latter due to the better interaction with the negatively charged biofilm. The four OMPs presented in this study are all hydrophilic at neutral pH (see SI, Table S7.1) and show low removal in WWTPs. Furthermore, the four investigated OMPs are negatively charged (DCF, IBU and NPX), or neutrally charged (CBZ) under the pH range of the systems (SI, Table S7.1). Therefore, it could be stated that the presented CW-MFC+ and CW-MEC were even able to improve the removal of theoretically resilient OMPs such as CBZ, DCF and NPX.

However, electrosorption to the positively charged cathode could have even improved the adsorption of the negatively charged OMPs, DCF, IBU and NPX in CW-MFC+ and CW-MEC. These OMPs are present in the form of charged ions or polar molecules and could therefore have been adsorbed after migrating to the system's electrode with opposite charge (Kong et al., 2013; Yang et al., 2015). Apart from that, MFCs seem to offer a beneficial environment for the growth of non-electroactive bacteria and increasing the metabolic rate of anaerobic bacteria due to the artificial presence of an insoluble electron acceptor, i.e. an anode (Fang et al., 2013a). Additionally, CW-MFC+ mode has shown to increase microbial activity (Hartl et al., 2019) and EAB seem to outperform other microbial communities (Zhang et al., 2015). Some studies claim that CWs operated as MFC enhance microbial community richness and diversity (Song et al., 2018; F. Xu et al., 2018a), however, experiments lasted only for 4 and 2 months, respectively, and microbial communities are known to change over time and might expose different behaviors especially in the initial start-up phase. Another important factor to consider, apart from charge, sorption effects and direct impact on microbial communities, is the biodegradability of the compound (Wang et al., 2015). The BES itself might have influenced environmental conditions, especially on a micro-scale (e.g. at the electrodes or adjacent pore spaces) changing factors like pH and DO, which in turn could have indirectly affected microbial communities and their degradation of OMPs in the systems. Unfortunately, as mentioned above it was not possible to measure these parameters on such a small scale in the present study. However, the microbial community analysis in Chapter 6 and similar studies reported electrolysis in the CW-MEC systems (Gao et al., 2017) which would cause oxygen and hydrogen to be released and consequentially increase aerobic and hydrogen consuming microbial processes. The increase in aerobic processes could therefore explain at least partly the higher removal of NPX and DCF in CW-MEC and possibly the improved treatment compared to CW-MFC.



## 7.4 Conclusions

The investigation of meso-scale CWs operated as BES (CW-BES) resulted in the following conclusions:

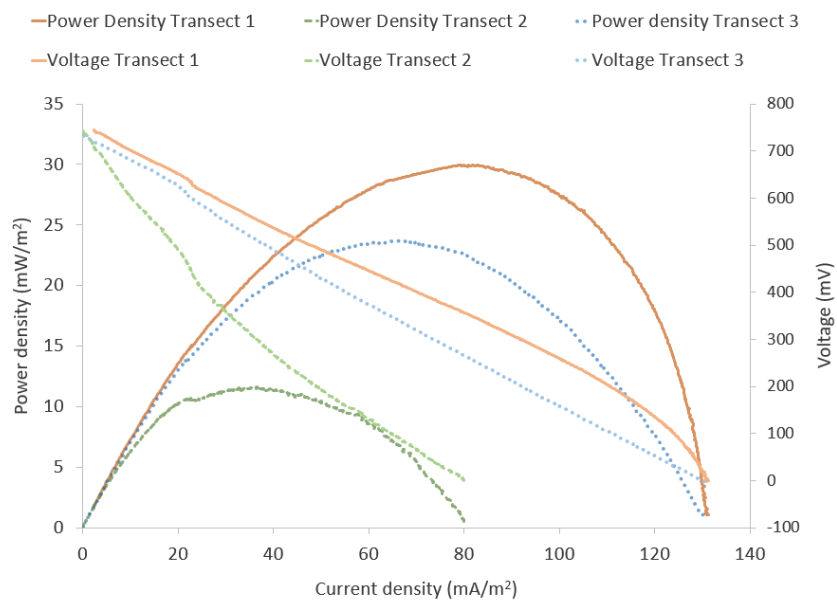
- The treatment performance for three out of four investigated OMPs (CBZ, DCF and NPX) was improved, with removal efficiencies 10-17% higher in CW-MEC and 5% higher in CW-MFC+ systems than those obtained in the CW-control systems. However, in all three cases no statistically significant differences were found.
- Average IBU removal rates showed no relevant differences when comparing treatments.
- The improved removal of CBZ, DCF and NPX in CW-BES could be due to direct effects of BES, such as increased microbial activity, or, in the case of CW-MEC, indirect effects through an electrolysis induced increase of DO and subsequent aerobic degradation, at least in the case of DCF and NPX. Hydrophobic (and electro-) sorption might have played an additional role in the removal of CBZ, and electrosorption effects in the case of DCF and NPX.
- In terms of OMP removal, CWs operated as BES could provide an additional benefit for the removal of the most recalcitrant compounds such as CBZ and DCF, due to their limited biodegradability and removal in other biological systems.
- However, further research should be carried out in order to discern the underlying mechanisms leading to the OMP removal improvement and also to use this information to refine and upgrade the design and operation of CW-BES systems, also including detailed effects of vegetation.
- The increased removal of conventional wastewater parameters COD and  $\text{NH}_4^+\text{-N}$  could be due to direct effects of the BES such as an increased microbial activity as well as indirect effects in the case of CW-MEC, through an electrolysis induced increase of DO and subsequent aerobic degradation; and in the case of  $\text{NH}_4^+\text{-N}$ , hydrogen resulting from the electrolysis might have additionally enabled autohydrogenotrophic denitrification.

In summary the results could not confirm the hypothesis that CW-MEC and CW-MFC+ will improve organic micropollutants removal as compared to the CW-control system, since the differences in removal were not statistically significantly different. However, the results indicated a higher OMP removal in CW-MEC and CW-MFC+ in the case of three out of four OMPs.

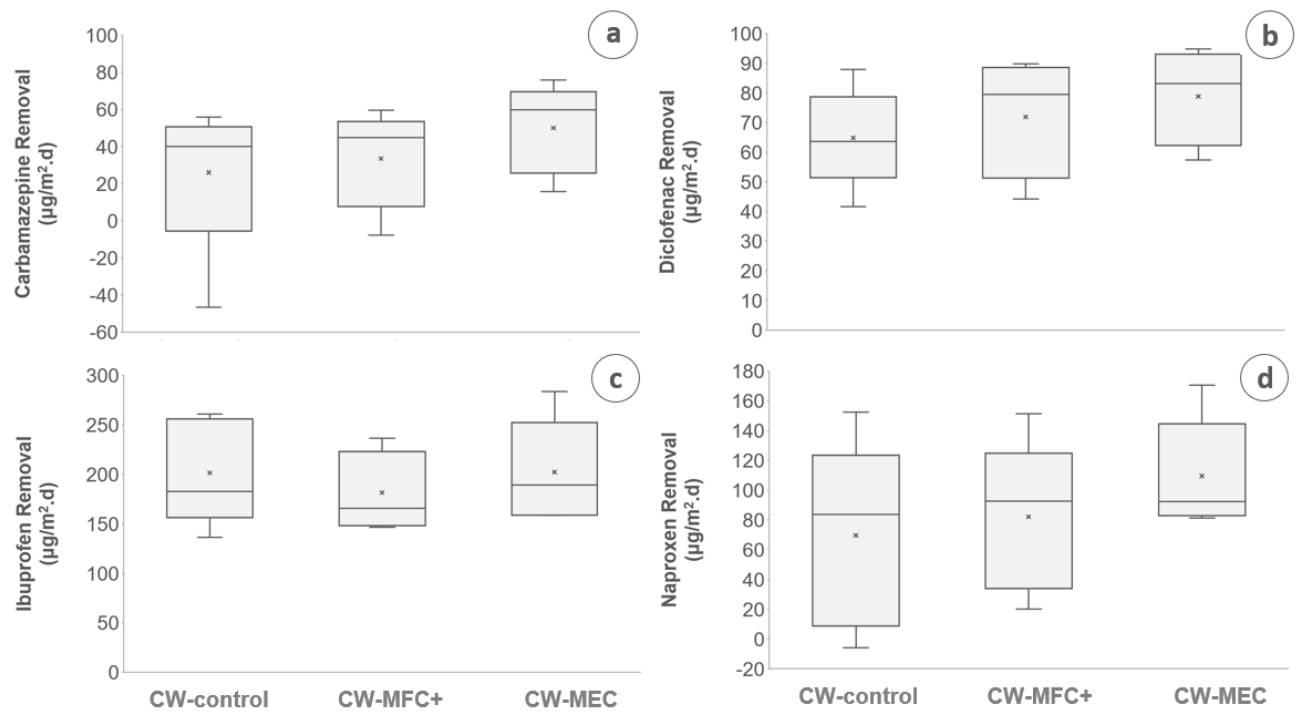
## **Acknowledgements**

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 676070. This communication reflects only the authors' view and the Research Executive Agency of the EU is not responsible for any use that may be made of the information it contains. M.J. García-Galán would like to thank the Spanish Ministry of Economy and Competitiveness for her Juan de la Cierva research grant (IJCI-2017-34601). Marianna Garfí is grateful to the Spanish Ministry of Economy and Competitiveness (Plan Estatal de Investigación Científica y Técnica y de Innovación 2013-2016, Subprograma Ramón y Cajal (RYC) 2016).

## Supplementary information

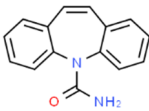
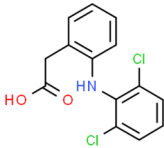


**Figure S7.1.** Power density and polarization curves for each transect of one of the closed-circuit CW-MFC+ replicates measured during sampling week 4.



**Figure S7.2.** Specific removal from influent to effluent for all four OMPs (a; CBZ, b; DCF, c; IBU and d; NPX) comparing all treatments (n=5). The box- and whisker plots show the minimum and maximum (lower and upper whiskers), first and third quartile (lower and upper end of box), median (horizontal line in box) and average (marked as an "x") values.

**Table S7.1.A.** Chemical structure and characteristics of the selected OMPs used in this study and their respective hydrophobicity and charge states estimated from the compound's Log D and pK<sub>a</sub>, respectively (relative to the experimental pH of 7 – 7.5). Log K<sub>ow</sub> describes the octanol-water partition coefficient which is a compound's measure of the ratio of concentrations in octanol and water (Schwarzenbach et al., 2003). Log D is the partition coefficient for a compound at a specified pH

Compound	Structure <sup>a</sup>	Classification	Log K <sub>ow</sub>	Log D (pH 6.6-7.6) <sup>c</sup>	Hydro-phobicity	pK <sub>a</sub> <sup>b</sup>	Charge state
Carbamazepine		Anticonvulsant	2.45 <sup>b</sup>	2.77	hydrophilic	13.90	neutral
Diclofenac		Anti-inflammatory	4.51 <sup>d</sup>	1.70 to 1.04	hydrophilic	4.15	negative

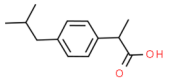
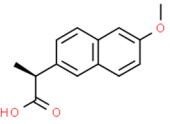
<sup>a</sup> chemspider.com

<sup>b</sup> <https://pubchem.ncbi.nlm.nih.gov>

<sup>c</sup> chemicalize.com (data has been obtained from the empirical model)

<sup>d</sup> Avdeef et al. (1998)

**Table S7.1.B.** Chemical structure and characteristics of the selected OMPs used in this study and their respective hydrophobicity and charge states estimated from the compound's Log D and pK<sub>a</sub>, respectively (relative to the experimental pH of 7 – 7.5). Log K<sub>ow</sub> describes the octanol-water partition coefficient which is a compound's measure of the ratio of concentrations in octanol and water (Schwarzenbach et al., 2003). Log D is the partition coefficient for a compound at a specified pH

Compound	Structure <sup>a</sup>	Classification	Log K <sub>ow</sub>	Log D (pH 6.6-7.6) <sup>c</sup>	Hydro-phobicity	pK <sub>a</sub> <sup>b</sup>	Charge state
Ibuprofen		Anti-inflammatory	3.97 <sup>b</sup>	2.10 to 1.16	hydrophilic	5.30	negative
Naproxen		Anti-inflammatory	3.18 <sup>b</sup>	0.61 to -0.18	hydrophilic	4.15	negative

<sup>a</sup> chemspider.com

<sup>b</sup> <https://pubchem.ncbi.nlm.nih.gov>

<sup>c</sup> chemicalize.com (data has been obtained from the empirical model)

**Table S7.2.** Results for pH for CW-control, CW-MFC and CW-MEC systems during the OMP spiking and sampling weeks at the influent, after first transect, after second transect and effluent as well as overall average.

pH (-)	In-fluent	1/3	2/3	Effluent	Average
<b>CW-control</b>	7.50±0.00	7.35±0.05	7.35±0.00*	7.70±0.01	7.48±0.02
<b>CW-MFC+</b>	7.45±0.05	7.09±0.02	7.05±0.07*	7.66±0.07	7.32±0.05
<b>CW-MEC</b>	7.54±0.07	6.69±0.09*	6.60±0.05*	7.15±0.03*	7.00±0.06

\* very significant difference ( $p < 0.01$ )

**Table S7.3.** One-factor ANOVA (with replication) results for the comparison of conventional wastewater parameters between the electric connections during the sampling period, for the total system from inlet to outlet and each of the three transects separately (statistically significant different if p-value < 0.05).

One-factor ANOVA	p-value			
	Comparing Electric Connections			
F (2, 8)	Inlet-Outlet	Transect 1	Transect 2	Transect 3
<b>COD</b>	0.37	0.84	0.42	0.97
<b>NH<sub>4</sub><sup>+</sup>-N</b>	0.20	0.21	0.93	0.99
<b>SO<sub>4</sub><sup>2-</sup></b>	0.97	0.98	0.16	0.36
<b>PO<sub>4</sub><sup>3-</sup>-P</b>	0.96	0.76	0.57	0.20





## CHAPTER 8

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# General discussion

The objective of this thesis was the improvement and control of wastewater treatment using CW-BES. This chapter discusses the already in detail presented findings of the executed experiments in a wider context, describes in how far the objectives could be met, presents gained insights as well as encountered problems, and attempts to give indications on how to apply this knowledge in further research or even realize it in practice. The following sub-chapters are structured like the thesis itself insofar that it is starting with the use of CW-MFC for bioindication (Chapter 4) and continues with CW-BES for contaminant removal (i.e. Chapters 5-7). However, the Chapters 5-7 are discussed in a summarized fashion across all three investigations where appropriate, like in the case of conventional contaminant removal which was investigated in all three experiments so these results are discussed all together.

## 8.1 CW-MFC for bioindication

The investigation on the use of CW-MFC for bioindication purposes was one of the main objectives of this thesis. The current which is produced by MFC is a consequence of oxidation of organic and inorganic compounds catalyzed by EAB at the anode. As a consequence it has been shown to be possible to correlate the MFC or CW-MFC signal with the influent COD concentration in earlier investigations (Corbella et al., 2019; Di Lorenzo, 2015). Therefore, the hypothesis was that the CW-MFC signal is directly dependent on the influent COD content and can therefore be correlated and consequently used as a COD assessment tool.

The results of the experiment in Chapter 4 showed that the triplicate CW-MFC biosensors performed quite well as an COD assessment tool between the third and the seventh week of operation (between an accumulated organic loading of ca. 100-200 g COD/m<sup>2</sup>). Due to the fact that the majority (75-80%) of sudden increases in COD during this time could be indicated within 2-4 h, but COD decreases not, it was suggested to use the systems as an “alarm tool” for sudden COD increases due to contamination events.

Several challenges have been encountered which were partly as well already described in literature. In the presented meso-scale CW-MFC systems, a satisfactory statistical linear correlation (i.e. R<sup>2</sup> values above 0.8 or 0.9 even) could not be achieved, which was one of the reasons to rather suggest the use as a qualitative alarm tool instead of a quantitative COD assessment tool. However, research on conventional MFC (Di Lorenzo et al., 2009; Gonzalez del Campo et al., 2013; Kim et al., 2003; Peixoto et al., 2011b) as well as lab-scale CW-MFC (Corbella et al., 2019; Liu et al., 2014; Oon et al., 2016; Srivastava et al., 2015) systems achieved high or at least satisfactory correlations between COD and the resulting MFC electric signal.

An important factor is the use of real urban wastewater. Wastewater is more complex than synthetic wastewater (e.g. glucose or acetate). These complex compounds require previous conversion processes, such as hydrolysis of long-chain organics to simple carbohydrates, in order to be utilizable by the EAB and consequently contribute to the MFC signal via EET (Kiely et al., 2011b). This delay influences the biosensors response time to a concentration increase in

the influent, since the complex portion of the wastewater has to be converted before it will affect the signal. This delay also affects the accuracy of the sensor since simple carbohydrates resulting from longer lasting conversion processes will increase the signal at a later time, when the influent concentrations are possibly actually decreasing again. Thereby also the response time of the signal in regard to decreasing influent concentrations is delayed. The response time to decreasing concentrations was also affected by other effects, such as utilization of accumulated organic matter as well as endogenous respiration of microorganisms. These effects cause a signal by EAB which is independent from the actual influent organic matter concentration, and also reduce the bioindication range of the CW-MFC (i.e. the signal does not go below a certain lower limit anymore). However, all biosensors for wastewater will have to deal with these issues as a consequence of its complexity.

A more controllable factor in regards to bioindication of COD in real urban wastewater is the scale of the system. The used CW-MFC systems were built on a meso-scale and at the same time designed for maximum nutrient removal. Therefore the aim during construction was to electrochemically connect as much bed volume as possible in the three consecutive anodes (see 3.1.1). However, this was not ideal for biosensor application, since the whole system had a theoretical HRT of a bit less than 4 d, so each transect's CW-MFC compartment had an HRT of ca. 1.3 d (effective anodic chamber volume of 3.8 L) which also means that the wastewater flowing through the first transect's anode (and its signal used for the experiment) had potentially produced a signal over this period of time, increasing the response time to decreasing COD concentrations, as well as the baseline signal described above, which negatively affected the correlation, caused the lower bioindication limit to increase and thereby decrease the range. In follow-up experiments by students, the much smaller gravel sampling cores (see Figure 3.1.1, H) of two additional similarly built "test CW-MFC" systems were turned into anodes and were utilized for bioindication experiments producing more promising bioindication results (Barbero et al., 2019). Also conventional MFC systems used for COD or BOD bioindication were relatively small with an anodic chamber volume of 2-100 ml, showing response times between 3 min and 1 h (Di Lorenzo, 2015). The response rate of the presented CW-MFC to increasing influent COD was between 2 and 4 hours, however, due to the above described reasons the response time for decreasing

COD concentrations was much longer (1-2 days). Hence, the question arises why to use CW-MFCs at all and not just MFCs placed within a CW? MFC biosensors could certainly be used before or after the CW-MFC, and possibly as well within the filter bed. However, effects on hydraulics such as short-circuiting need to be considered and also the question arises whether conditions in a conventional MFC are less representative of the conditions in a CW than a CW-MFC with a granular medium inside (e.g. biofilm formation, filter bed structure etc.).

Concerning the upper limit of bioindication, the presented CW-MFC systems were electrochemically limited due to a too small cathode area and consequently too small cathode to anode surface area ratio. Previous experiments within the research group using a similar CW-MFC architecture resulted in an ideal cathode to anode surface ratio of 4:1 (Corbella et al., 2015). Due to physical design constraints the ratio in the presented systems was only around 1:1. As a result the MFC signal could not raise above a certain limit even when COD supply would have allowed for that, affecting the upper bioindication limit and thereby decreasing its range. This circumstance was also indicated visually in the capped peaks of the  $E_{\text{cell}}$  signal (see Figure 4.3.1).

In summary, contrary to the hypothesis, the CW-MFC could not be used to quantitatively correlate the produced  $E_{\text{cell}}$  signal with COD influent concentrations. However, it was shown to be possible to utilize the produced signal as a binary qualitative alarm tool, since increasing influent COD concentrations were reflected in a sharp signal increase within a few hours of response time. However, in general, it seems to be better to implement smaller MFCs or CW-MFCs within CWs in order to improve the response time and sensitivity of the sensor. Nevertheless, it should still be big enough to reflect the processes occurring in a CW system e.g. as a perforated tubular device with a diameter big enough to provide space for an anode, and a sufficient gravel (or other granular electrode media) surface area for biofilm growth. The resulting small anodic surface area would then also make it easier to implement a sufficiently big cathode with a ratio to the anode of 4:1. Such CW-MFC units could be spatially distributed like a grid over the whole bed surface area as well as in different depths. This CW-MFC biosensor network could consequently provide an in-situ online monitoring of an array of signal data points which could give important indications on organic loading, toxic substances in the

influent as well as wetland hydraulics such as dead zones, short-circuits, or clogging. Hence, further research using small-scale CW-MFC units within CWs is suggested.

## 8.2 CW-BES for contaminant removal

The improvement of contaminant removal in the investigated CW-BES was one of the main objectives of this thesis. HF CWs offer a suitable environment for the implementation of BES, due to the marked redox gradient from the aerobic bed surface to the anaerobic bottom of the bed. This natural redox gradient is utilized by placing the anode in the anaerobic and the cathode in the aerobic section in order to create a potential difference which drives the bioelectrochemical reactions. HF CWs have a relatively large surface area requirement per person equivalent of around 3-10 m<sup>2</sup>/PE (Hoffmann et al., 2011). Unlike other CW-MFC studies the maximization of electricity production using CW-MFC was not a primary goal, however, the improvement of the electrochemical performance affects the contaminant removal. The majority of earlier CW-BES studies used artificial wastewater and lab-scale reactors (often up-flow and batch fed), which is advantageous for the study of fundamental processes, but reflects real conditions to a limited extent only. The research in this work was conducted using real urban wastewater fed to meso-scale HF CW-BES with duplicates of systems for each tested treatment. The general hypothesis was that CW-BES, such as CW-MEC and CW-MFC+ will outperform the control treatments CW-MFC- and CW-control.

### 8.2.1 Hydraulic regime

Two different hydraulic regimes, with intermittent and continuous feeding of wastewater, were tested over a period of 10 weeks in order to assess the best conditions for CW-MFC performance and contaminant removal (see Chapter 5). The hypothesis was that continuous flow will outperform intermittent flow. Contaminant removal results showed no statistically significant differences, however, continuously fed systems performed slightly better in terms of COD and ammonium removal. More importantly, in the first transect, the continuously fed CW-MFC+ produced an extremely statistically significantly higher current density than the intermittently fed CW-MFC+ duplicate.

A potential reason for this behavior could be that the continuous feeding ensured a lower redox potential in the CW-MFC anode compartment while the intermittent feeding facilitated a higher redox potential at the anode. In accordance with that, experiments by Corbella et al. (2014) showed that higher redox gradients were found in continuously fed HF CWs when compared to intermittently fed systems (401 V vs. 362 V, respectively), additionally intermittently fed systems exhibited higher daily variations of redox potential. A higher redox gradient allows for a higher  $E_{\text{cell}}$  and consequently a higher current in the CW-MFC. Another, or additional possibility for the lower current in intermittently fed CW-MFC could be that the intermittent regime caused an organic overloading of the cathode, consequently dropping the redox potential and limiting its oxygen supply and functionality (see more details in next section).

Finally, in line with the hypothesis, it was decided to use a continuous flow regime for all remaining experiments, due to the extremely significant increase of CW-MFC performance, together with the slight increase of contaminant removal.

### **8.2.2 Organic loading rate**

Parallel to the investigations on the hydraulic regime, also the effects of different organic loading rates on CW-MFC performance were investigated ( $4.9 \pm 1.6$  and  $13.6 \pm 3.2$  g COD/m<sup>2</sup>·day) (see Chapter 5). The hypothesis was that low OLR will result in better contaminant removal results. The percental removal efficiency did not differ with a statistical significance when comparing the different applied OLRs. The removal efficiency even increased with higher tested OLRs, from 60% to 70% for COD and from around 25 to 40% for ammonium. However, this was probably rather because the OLR was increased over time and therefore an effect of the still maturing biofilm and microbial communities in the systems was observed. For the electrical performance of an CW-MFC it is important to find a good balance between high enough OLR in order to supply the EAB with sufficient organic and inorganic matter on the one hand, and overloading the system and consequently limiting the cathode's functionality through a lowered oxygen supply and growth of heterotrophic bacteria on the other hand (Doherty et al., 2015c; Freguia et al., 2008; Villaseñor et al.,

2013). There was no significant difference in terms of produced current by the CW-MFCs in the different OLR periods, although they were increasing, however, again probably rather due to the still maturing biofilm. Even during a low OLR, the provided real urban wastewater resulted in average  $E_{\text{cell}}$  voltage averages of  $304\pm 96$ ,  $462\pm 33$ , and  $457\pm 50$  mV (31 to 50 mA/m<sup>2</sup>) for first, second and third transect, respectively, which is comparable with other CW-MFC systems (see Table 3.3.3) .

Finally, the hypothesis that low OLR would benefit contaminant removal could not be confirmed. However, at the end anyway an OLR closer to the lower tested OLR was chosen for the remaining experiments (i.e.  $6.7\pm 1.4$  g COD/m<sup>2</sup>·day for the 13 experimentation weeks following the operational condition experiments). First of all it provided a sufficient amount of organic and inorganic matter for the CW-MFCs to perform well. A further reason was that the wastewater used was real urban wastewater, so the OLR could only be increased by increasing the flow rate and thereby decreasing the HRT (below the aimed at 4 d), which was not possible for a longer term due to operational constraints in regards to the maximum volume of wastewater that could be stored and delivered to the systems per day.



### 8.2.3 Microbial community characterization

The results of the two microbial community characterization techniques which were performed in the course of the investigations are presented before the general discussion of the contaminant removal, due to the fact that the microbial community characteristics were assumed to have had an impact on most of the contaminants investigated.

#### 8.2.3.1 Microbial community analysis

An assessment of the microbial community structure was performed based on the characterization of PCR amplified DNA segments (see Chapter 6). The results showed statistically significant differences in microbial composition of CW-MEC anodes and cathodes when compared to all other treatments as well as CW-MFC+ cathodes when compared to all other cathodes. The hypothesis was that the communities in CW-MEC and CW-MFC+ will differ from CW-control communities.

The differences in microbial communities were likely an important reason for differences in contaminant removals found in the different treatments, since microbially mediated processes govern the majority of relevant contaminant conversion and removal processes (Faulwetter et al., 2009). Particularly revealing was that the two most common genera in CW-MEC anodes and cathodes were the aerobic *Sphingobium* genus followed by the hydrogen oxidizing *Hydrogenophaga* genus. Both were not present in a relevant proportion in any other treatment, which was a clear indication that electrolysis took place in the CW-MEC systems. CW-MEC require only an additional applied voltage of 0.2-0.8 V between the electrodes in order to overcome the thermodynamic barrier for water electrolysis to occur whereas usually 1.8-3.5 V are required. This is due to the already supplied electrons from oxidation of (in)organic matter catalyzed by EAB at the anode (Lu and Ren, 2016). Therefore it seems that the poised potential of only 0.3 V vs. Ag/AgCl at the anode was sufficient for electrolysis to take place. This poised potential resulted in an average measured current density of  $447 \pm 269$ ,  $427 \pm 201$  and  $178 \pm 106$  mA/m<sup>2</sup> in the three transects, respectively, which is

comparable to other studies applying such a small potential or power source (see Table 3.3.5). As a consequence, the electrolysis in CW-MEC seemed to have changed the environment in the systems dramatically by increasing the DO (indicated by redox measurements) and hydrogen concentrations noticeably, parameters which generally affect contaminant removal. This change in environment was also apparent when looking at most the abundant genera in CW-MEC, the aerobic *Sphingobium* and the hydrogen oxidizing *Hydrogenophaga*. (see a detailed discussion in Chapter 8.2.4). The species found in the *Sphingobium* genus is called *Sphingobium yanoikuyae* and has not been reported in CW-MEC, or in general in any BES so far. However, the closely related genera *Sphingomonas* and *Sphingopyxis* were reported in other CW-MEC systems. In general, CW-MEC anodes showed the lowest genus diversity, while anodes of all other treatments showed similar values (significantly different in transect 2 and 3). Looking at the cathode's genus diversity, CW-MEC cathode in transect one was very statistically significantly lower than in all other treatments. Also the evenness was statistically significantly lower in all three CW-MEC anodes as compared to all other treatments. The lower diversity and evenness are clear indications for few genera or just one genus dominating, which was the case with *Sphingobium yanoikuyae* in CW-MEC cathodes and especially in the anodes. Also other studies found lower microbial diversity in CW-MEC as compared to CW control (Gao et al., 2018, 2017), while one study found no significant difference (Ju et al., 2014a). CW-MEC produce a very stable and specific environment, therefore it seems reasonable that one, probably specialized, genus is able to dominate it.

Probably due to the sampling method at the anode, only cathode samples of CW-MFC+ showed a microbial community significantly different from CW-MFC- and CW-control with relative high abundance of the species *Lysinibacillus boronitolerans*, which is closely related to *Lysinibacillus sphaericus*, a species which was also found in other MFC systems and has been identified to be capable of EET, i.e. a EAB. Regarding CW-MFC systems, some studies showed that CW-MFC+ enhance microbial community richness and diversity as compared to CW-MFC- (Song et al., 2018; F. Xu et al., 2018a), however, experiments lasted only for 4 and 2 months, respectively. In general, microbial communities can change over time, especially in the initial start-up phase, while the cathode and its microbial community

are likely to be affected and change over the long term (T. Li et al., 2016).

The indifference in microbial community composition in anode samples when comparing CW-MFC+ to CW-control was probably due to the design of the gravel sampling cores (see Figure 3.1.1, H). The sampling points were probably too far away (a few mm) from the stainless steel mesh anode. A maximum distance of ca. 15 Å between EAB and the electrode surface is allowed for DEET via physical contact, whereas the mechanisms and maximum distances for DEET via pili or nanowires are still under investigation (Kracke et al., 2015; Malvankar et al., 2015). Maximum distances in regards to MEET via redox mediators are still being investigated as well. However, reported maximum anodic biofilm thickness ranged from around 14 to 26 µm in pure culture and 50 µm in mixed culture closed-circuit MFCs (Read et al., 2010), or up to 80 µm in a so-called tower morphology of a pure culture of *Shewanella oneidensis* (Mclean et al., 2010).

Hence, the gained knowledge from the likely too distant sampling point could be that CW-MFC+ seemed to have had no significant direct or indirect effects on the microbial communities situated a few mm away, whereas CW-MEC mode did seem to have had an effect on the microbial community even at a relatively high distance to the anode. In any case, additional studies on microbial communities at different distances from the electrodes of CW-MEC and CW-MFC systems would be needed in order to confirm this assumption. For future experiments using such gravel core sampling tubes it is therefore suggested to also include an anode within the sampling tubes in order to be able to also sample the anode itself and the electrochemically active part of the biofilm.

As already mentioned, so far the two species found in the study's CW-MEC electrodes, *Sphingobium yanoikuyae* and the CW-MFC+ cathode, *Lysinibacillus boronitolerans*, have not been identified as EAB yet. However, especially in the case of *Lysinibacillus boronitolerans* due to the close relative being an EAB, the possibility seems likely. In the case of *Sphingobium yanoikuyae*, the interesting part is that there is very few literature about it but closely related genera were reported in CW-MECs. Both species have not been described in BES or CW-BES before. So far Koch and Harnisch (2016a) reported 94 confirmed bacteria species to be capable of EET (see Chapter 2.2.2) and also stated that the identification of bacteria to be capable

of EET is not straightforward as there were no clear standards yet. In any case it would be of great interest to test these two species and to see whether they are capable of EET and could therefore be counted as newly identified EAB.

Finally, the results were in line with the hypothesis that microbial communities differ from CW-control in the case of CW-MEC anodes and cathodes, but as already mentioned only for CW-MFC+ cathodes due to the described sampling issues.

### 8.2.3.2 Microbial activity analysis

An FDA analysis showed an increased microbial activity of 4%, 21% and 34% in CW-MFC+ transects 1, 2 and 3, respectively, when compared to CW-control (statistically very and extremely significant in transects 2 and 3, respectively) (see Chapter 5). The hypothesis was that bacterial activity is correlated to contaminant removal rates. The increase in activity difference along the flow path could indicate that the decreasing OLR along the transects benefited the activity of EAB over competing non-electrogenic bacteria. The higher microbial activity could be also linked to higher diversity and richness found in CW-MFC+ microbial communities (J. Wang et al., 2016a; F. Xu et al., 2018), as also mentioned in the previous chapter. Furthermore, EAB also outcompeted other microbial communities such as methanogenic bacteria (Capodaglio et al., 2015), which could also be an indicator for their higher microbial activity. However, in the end it is difficult to ascertain which exact reasons could have led to the increased bacterial activity and future research would be needed. In summary, the results were in line with the hypothesis, showing increased bacterial activity with increased contaminant removal rates (see more details on contaminant removal in the chapter below).

## 8.2.4 Conventional contaminant removal

Conventional contaminants for wastewater characterization such as COD, ammonium, orthophosphate and sulfate were measured in CW-MEC and CW-MFC+ as well as the control systems CW-MFC- and CW-control throughout at least two out of the three treatment performance investigations (see Tables 8.2.1 to 8.2.4). Due to changes in the system setup, CW-MEC were only investigated in Chapters 6 and 7, and CW-MFC- only in chapters 5 and 6. CW-MFC+ and CW-control were investigated throughout all three investigations. At the time of the start of the respective experiments in Chapters, 5, 6 and 7, the systems were in operation for 4 months, 10 months and 18 months, respectively. The durations of the three campaigns were 13, 12 and 17 weeks, respectively. The average OLRs during the three campaigns amounted to  $6.7 \pm 1.4$  g COD/m<sup>2</sup>·day,  $5.3 \pm 1.8$  g COD/m<sup>2</sup>·day and  $8.7 \pm 2.5$  g COD/m<sup>2</sup>·day, respectively. The higher OLR in the last investigation was the result of more concentrated wastewater due to technical issues with the wastewater intake from the sewer.

### 8.2.4.1 COD

Table 8.2.1 shows an overview of average COD removal over the course of the three investigations presented in Chapters 5-7.

**Table 8.2.1.** Overview of average COD removal in terms of specific mass (g/m<sup>2</sup>·d) and percentage (%) from influent to effluent, according to the different treatments over the course of the three investigations

Chapter		COD Removal			
		CW-MEC	CW-MFC+	CW-MFC-	CW-control
5	n=11	-	4.9±1.1 (74%)	4.6±1.0 (72%)	4.5±1.0 (69%)
6	n=14	3.8±1.5 (73%)	3.7±1.7 (70%)	3.5±1.5 (63%)	3.4±1.5 (64%)
7	n=8	6.1±0.8 (70%)	4.9±0.5 (55%)	-	4.9±1.4 (57%)

CW-MFC+ performed better than control systems in the first two investigations and a bit worse than the remaining CW-control in the third investigation. It is noteworthy that removal in percentage was generally decreasing over time in all four treatments. Although CW-MEC COD removal also showed a little drop of 3% points as well from the investigation in Chapter 6 to Chapter 7, the CW-MEC systems seemed to have been more stable than all other treatments, since CW-MFC+ and CW-control both showed a comparatively big drop of 15% and 7% points in the same time period. As a consequence CW-control even removed slightly more COD than CW-MFC+ in the last experiment. Part of the decrease in removal in percentage was likely due to the higher OLR in Chapter 7, however, the relative performance decrease of CW-MFC+ in comparison to CW-control could have happened due to cathode fouling or clogging (see more detail in Chapter 8.4.2), which could have been worsened by the higher OLR. However, even the latter higher OLR was still within the range of maximum OLRs used for design of HF CWs, which is around 4-13 g BOD<sub>5</sub>/m<sup>2</sup>·day (Dotro et al., 2017) (around 6-20 g COD/m<sup>2</sup>·day when applying a COD/BOD<sub>5</sub> ratio of 3/2 as seen in test BOD<sub>5</sub> measurements of the used influent wastewater). A reason for the decreased removal in response to the higher, but still relatively low OLR, could have been that the meso-scale systems probably had a much lower hydraulic retention time (HRT) than the calculated and aimed for theoretical HRT of 4 days. Unpublished tracer test results have shown that the real HRT could have been rather around 2 d. Further reasons for the general drop in performance of CW-control and CW-MFC+ could have been aging or hydraulic clogging, which would be surprising already after 18 months, also due to the relatively moderate OLR chosen (see also Chapter 8.2.2). Potentially the lack of aquatic macrophytic plants in the systems played a role as well, since plants are generally known to improve treatment performance (see Chapter 2.1.4). Plants had a strong effect on contaminant removal results, and especially nitrogen, independent from electrical connection effects in other CW-BES (Y.-L. Oon et al., 2018; Saz et al., 2018).

### **CW-MFC+**

The COD removal in CW-MFC+ was still higher compared to CW-MFC- and CW-control during the first two investigations, with

increases of plus 2-7%. The COD removal of 74% and 70% during the first two experiments was comparable to other CW-MFC studies (see Table 3.3.3). Many studies with clearly higher removal rates of up to nearly 100% often used artificial aeration and/or highly porous material with a high specific surface area such as ceramsite, zeolite or dewatered alum sludge in the bed's main layer (see Table 3.3.2), whereas the two latter are also electroconductive materials. Most CW-MFC studies which included control systems for comparison in their investigation showed similar COD removal increases in the range from 4% to 8% (Corbella and Puigagut, 2018; Rathour et al., 2019; Wang et al., 2016b; Xie et al., 2018; F. Xu et al., 2018), only Srivastava et al. (2015) reported an improvement of 12-20% (compared to CW-MFC-) and 27-49% (compared to conventional CW-control) (see Table 3.3.3). The increased performance of COD removal in CW-MFC+ compared to control systems during the first two investigations could have occurred due to several reasons. Direct effects in which CW-MFC could have increased COD removal could include EAB outcompeting other degradation pathways, such as anaerobic degradation (Corbella and Puigagut, 2018; Zhang et al., 2015). Additionally, EAB have shown to be able to inhibit the growth of *Archaea* at the anode (Fang et al., 2013a). Also in the presented microbial community analysis, low to no *Archaea* were found in the anodic sections of CW-MFC+ (0.6%) (the same is true for CW-MEC with 0.0%) as compared to CW-MFC- and control (4.8% and 4.3%, respectively). Additionally, an increase in microbial activity was observed in CW-MFC+ compared to CW-control (see Chapter 5 and Chapter 8.2.3.2), which could have led to an improved biodegradation and at least partly explain the improved COD removal in CW-MFC+. Other structural community analyses have shown higher diversity and richness in CW-MFC+ microbial communities (J. Wang et al., 2016a; F. Xu et al., 2018). Generally, the EAB pathway potentially offered an additional organic matter removal pathway while providing a relatively high energy gain for the involved bacteria (D.-Y. Huang et al., 2011; Srivastava et al., 2015). There are also indications from conventional MFC studies that MFC caused the potential in the anodic compartment to drop and thereby enabled different microbial species to utilize organic matter more efficiently (Katuri et al., 2011). In summary, the increased COD removal in CW-MFC+ could be due to direct and indirect effects of a more competitive and efficient EAB pathway which also creates synergies with a variety of bacterial communities.

## CW-MEC

CW-MEC removed 73% and 70% COD during the two last investigations and outperformed all other treatments, with increases of 9-10% and 13%, respectively as compared to the control systems. Only a part of the few available CW-MEC studies also investigated COD removal, while most focused on nitrogen. The available data showed removal rates ranging from 18 to 85% (see Table 3.3.5). In regards to improvement compared to control, Ju et al (2014a) found the same COD removal efficiency (compared to a non-electrolyzed control), and Aguirre-Sierra et al. (2016) found a 5-7% higher removal efficiency (compared to a CW-control when testing different HLR). The reasons for the increased COD removal, could include the same direct and indirect effects as already listed above for CW-MFC+ but additionally electrolysis of water seemed to have taken place in CW-MEC according to the results of the microbial community analysis and measurements of increased redox levels (see Chapter 6 and Chapter 8.2.3.1). Electrolysis of water was also observed by Gao et al. (2017) in their CW-MEC. In the presented study it caused the aerobic *Sphingobium* and the hydrogen oxidizing *Hydrogenophaga* genus to be the most abundant genera in CW-systems (see Chapter 6 and Chapter 8.2.3.1). The former is strictly aerobic and therefore added an aerobic treatment pathway for organic matter removal. The latter is a chemoorganotroph and facultative H<sub>2</sub> autotroph, and could therefore have contributed to the COD removal via the chemoorganotroph pathway at least during periods when H<sub>2</sub> was not sufficiently supplied.

In general, COD removal rates in conventional HF CWs are relatively high as well with >80% (Dotro et al., 2017). Therefore, the slight improvement in COD removal would probably not justify the incorporation of a BES. However, CW-BES could make a difference worth the additional investment for other contaminants such as nitrogen or organic micropollutants (see next Chapter 8.2.4.2 and Chapter 8.2.5).

In general the results were in line with the hypothesis that CW-MEC and CW-MFC+ increase COD removal as compared to control systems, however, as discussed except for the last investigations whereas operational issues were the likely reason for that.



### 8.2.4.2 Nitrogen

Table 8.2.2 shows an overview of average ammonium removal over the course of the three investigations presented in Chapters 5-7. The average ammonium loading rates during the three investigations amounted to  $1.2 \pm 0.2$  g  $\text{NH}_4^+\text{-N}/\text{m}^2\cdot\text{day}$ ,  $1.1 \pm 0.1$  g  $\text{NH}_4^+\text{-N}/\text{m}^2\cdot\text{day}$  and  $1.2 \pm 0.4$  g  $\text{NH}_4^+\text{-N}/\text{m}^2\cdot\text{day}$ , respectively.

**Table 8.2.2.** Overview of average ammonium removal in terms of specific mass ( $\text{g}/\text{m}^2\cdot\text{d}$ ) and percentage (%) from influent to effluent, according to the different treatments over the course of the three investigations

Chapter		Ammonium Removal			
		CW-MEC	CW-MFC+	CW-MFC-	CW-control
5	n=7	-	$0.5 \pm 0.3$ (41%)	$0.3 \pm 0.2$ (24%)	$0.3 \pm 0.3$ (19%)
6	n=12	$0.2 \pm 0.1$ (20%)	$0.2 \pm 0.2$ (18%)	$0.0 \pm 0.2$ (2%)	$0.0 \pm 0.2$ (2%)
7	n=7	$0.3 \pm 0.3$ (28%)	$0.3 \pm 0.2$ (24%)	-	$0.1 \pm 0.2$ (10%)

Average ammonium removal rates were decreasing as well after the first investigation, however, contrary to COD, the ammonium removal rates increased again in the third investigation, but could not reach the level of the first anymore. While COD removal was not statically significantly different across treatments in any investigation, ammonium removal was statistically significantly higher in CW-MEC and CW-MFC+ during the second investigation in Chapter 6. However, standard deviations were high in all three investigations. As for COD, the possible reasons for the increased removal could be manifold, from system aging, to the lack of plants. In any case, for ammonium the differences between CW-MEC and CW-MFC+ as compared to control systems endured despite generally decreasing removal efficiencies. The inhibition of ammonium removal due to bioelectrochemical reasons as a consequence of cathode clogging (see more details in Chapter 8.4.2) seemed less likely as for COD, since the improvement in CW-MFC+ as compared to control was still more pronounced even at lower total ammonium removal rates. Possibly cathode fouling or clogging was occurring but other nitrogen removal pathways were able to establish and/or take over (see below).

**CW-MFC+**

The average ammonium removal in CW-MFC+ systems was constantly higher than that of control systems, i.e. 15-16% when compared to CW-MFC- and 14-22% when compared to CW-control. The above reported CW-MFC+ average ammonium removal rates of 18-41% were relatively low when compared to other CW-MFC publications which showed ammonium removal rates of 68% to 97%, or TN removal of 75.4% (see Table 3.3.3). As described for COD, in some studies highly porous media used might have had an effect as well (see Table 3.3.2). However, the increase compared to CW-control systems is comparable to other studies by Corbella and Puigagut (2018), Wang et al. (2016a) and L. Xu et al. (2017) who reported a 25% higher ammonium, 40.2% higher nitrate and 22.3% higher TN removal, respectively, in the CW-MFC systems.

The improvement of ammonium removal in CW-MFC+ as compared to CW-MFC- and CW-control could again have a multitude of reasons. First of all, again the same reasons as already stated above for COD removal through CW-MFC+, such as direct effects of EAB like higher measured bacterial activity, EAB being more competitive and offering an additional treatment pathway, as well as indirect effects such as the change of the environment in the system and synergies between EAB and non-electrochemically active bacteria. Microbial community analysis results from earlier studies in MFC and CW-MFC indicated that unusual nitrogen removal pathways like anaerobic ammonium oxidation (anammox) or dissimilatory nitrate reduction to ammonium (DNRA) were more prevalent in the systems (Di Domenico et al., 2015b; J. Wang et al., 2016a; L. Xu et al., 2018b). Also an increase in ammonia oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) at the anode and an increase of denitrifying bacteria (DNB) and EAB at the cathode were found (Kim et al., 2008; L. Xu et al., 2018b). In the case of denitrification it has been shown to be possible that instead of oxygen, nitrate was used as a TEA at the cathode (Jia et al., 2008) (see also Figure 2.2.1). In the present study, no indication for a significant enhancement of these pathways could be found, however, unfortunately the CW-MFC+ anode samples were probably not representative of the electrochemically active biofilm (see Chapter 6 and Chapter 8.2.3.1). What could be seen clearly was that *Lysinibacillus boronitolerans* showed a relatively high abundance of 8% at the cathode, whereas the closely related species *Lysinibacillus*

*sphaericus*, was found to be electrochemically active and able to nitrify ammonium (Aguirre-Monroy et al., 2019; H. He et al., 2014; Nandy et al., 2013) (see Chapter 6). In case *Lysinibacillus boronitolerans* has similar capabilities as its close relative, its high abundance could have led at least partly to the higher ammonium removal in CW-MFC+ as compared to the control systems. This species is suggested to be investigated in more detail in future research. In general the improved nitrogen removal in CW-MFC+ seems to be related to increased abundance of EAB and other functional groups responsible for a variety of nitrogen removal processes and pathways.

### **CW-MEC**

CW-MEC removed 20% and 28% ammonium on average during the two investigations and, as for COD, outperformed all other treatments. The increases amounted to 18% in comparison to both, CW-MFC- and CW-control. CW-MFC+ ammonium removal was only 2-4% lower compared to CW-MEC. As for CW-MFC+ the CW-MEC ammonium removal rates were pretty low when compared to other CW-MECs, with removal of ammonium ranging from 46% to 83%, and nitrate removal rates of 43% to 69% (see Table 3.3.5). However, when compared to control systems, increases in other publications ranged from only 1% (Ju et al., 2014a) to 4-16% (Aguirre-Sierra et al., 2016). In this respect the presented systems performed better than other studies. Besides a direct influence of EAB (as already described for CW-MFC systems and), electrolysis appeared to have taken place and had a big influence on the system's environment. The investigations in Chapter 6 showed that the aerobic *Sphingobium* and the hydrogen oxidizing *Hydrogenophaga* genus were most abundant genera in CW-MEC anodes and cathodes (see Chapter 6 and Chapter 8.2.3.1). Whereas, usually, HF CW systems are anoxic/anaerobic in the anode zone. Hence electrolysis indirectly affected the treatment by increasing the DO (confirmed via redox measurements) and consequently promoted aerobic removal pathways. Gao et al. (2017) described similar processes in their CW-MEC, and pointed out that the formed H<sub>2</sub> could further serve as electron donor for nitrate reduction to nitrogen gas, and H<sup>+</sup> could also be involved in autohydrogenotrophic denitrification. The higher abundance of *Hydrogenophaga* (chemoorganotroph and facultative hydrogen autotroph) could have led as well to higher denitrification rates in CW-MEC, since other

species in this genus, such as *Pseudoflava* and *Taeniospiralis*, are known for anaerobic nitrate respiration with denitrification. *Pseudomonas* was another genus with a possible impact on denitrification which was found in higher relative abundance in CW-MEC as compared to other treatments (ca. 1% in anode and 0.6% in cathode of CW-MEC, lower in all other treatments). In summary, apart from the direct effects of EAB, the CW-MEC seemed to have had indirect effects by offering an increased variety of environments and consequently of microbially mediated nitrogen conversion and removal pathways which could explain the higher ammonium removal rates as compared to all other treatments.

In general the results were in line with the hypothesis that CW-MEC and CW-MFC+ are able to increase ammonium removal as compared to control systems. However, results showed a relatively high variability and statistically significant differences could only be found during the investigation in Chapter 6.

### 8.2.4.3 Phosphorus

Table 8.2.3 shows an overview of average orthophosphate removal over the course of the three investigations presented in Chapters 5-7.

**Table 8.2.3.** Overview of average orthophosphate removal in terms of specific mass ( $\text{g}/\text{m}^2\cdot\text{d}$ ) and percentage (%) from influent to effluent, according to the different treatments over the course of the three investigations.

Chapter		Orthophosphate Removal			
		CW-MEC	CW-MFC+	CW-MFC-	CW-control
5	n=8	-	0.01±0.03 (5%)	0.01±0.01 (10%)	0.00±0.03 (1%)
6	n=15	0.00±0.04 (-4%)	-0.04±0.05 (-46%)	-0.05±0.05 (-57%)	-0.06±0.05 (-69%)
7	n=6	0.01±0.04 (7%)	0.01±0.03 (7%)	-	0.00±0.03 (2%)

While orthophosphate was still removed on average at least to some extent during the first investigation it seemed like a release would have taken place during the second investigation, whereas the release was lower in CW-MEC and CW-MFC+ as compared to CW-MFC- and CW-control. The third investigation was more similar to the first again, with a small removal in all treatments, whereas the removal was higher by 5% in CW-MFC+ and CW-MEC.

#### CW-MFC+

Phosphorus removal rates in other CW-MFCs was higher and ranged from 31% to 94.5% orthophosphate ( $\text{PO}_4^{3-}\text{-P}$ ) and 85% to 97% total phosphorus (TP) removal (see Table 3.3.3). However, some of the materials used for anodes, cathodes and especially the packing layers (ceramsite, zeolite and dewater alum sludge) had a very high specific surface area and porosity. The sorption in these materials could be the reason for the high phosphorus removal. If so the effect would only last for a certain time, since sorption sites are generally

limited also in highly porous material. Therefore more long-term studies of CW-MFC+ phosphorus removal behaviors would be needed.

### **CW-MEC**

Also CW-MEC publications showed higher phosphorus removal rates in CW-MECs with 66-95%  $\text{PO}_4^{3-}$ -P removal. Ju et al (2014a) attributed the high removal rates to coagulation of the ferrous iron which formed during electro-dissolution of the sacrificial metal anode. However, as for CW-MFC+, initial sorption effects on the main bed materials zeolite and bio-ceramic probably played a role as well. Again, more long-term studies would be needed in order to be able to differentiate between the limited sorption and (bio)electrochemical effects.

### 8.2.4.4 Sulfate

Table 8.2.4 shows an overview of average sulfate removal over the course of the three investigations presented in Chapters 6-7.

**Table 8.2.4.** Overview of average sulfate removal in terms of specific mass ( $\text{g}/\text{m}^2\cdot\text{d}$ ) and percentage (%) from influent to effluent, according to the different treatments over the course of the two last investigations.

Chapter		Sulfate Removal			
		CW-MEC	CW-MFC+	CW-MFC-	CW-control
5	-	-	-	-	-
6	n=15	$-0.2\pm 0.8$ (-9%)	$0.6\pm 0.8$ (22%)	$1.0\pm 0.8$ (38%)	$0.9\pm 0.9$ (33%)
7	n=6	$1.1\pm 0.8$ (51%)	$1.0\pm 0.3$ (48%)	-	$1.1\pm 0.9$ (58%)

Sulfate removal was generally lower in CW-MFC+ and CW-MEC when compared to the control systems. However, the differences were only statistically significantly different in the second investigation in Chapter 6, and standard deviations were relatively high

Corbella and Puigagut (2018) as well reported that CW-MFC+ removed 13% less sulfate than CW-MFC- control systems. The reason for lower sulfate removal in CW-BES could be that sulfide abiotically reacted with the electrode to form elemental sulfur which then can be microbially re-oxidized to sulfur and further to sulfate using the anode as electron acceptor (Lovley, 2006). This mechanism could explain the lower sulfur removal rate in CW-MFC+.

The reaction of sulfide with the electrode might also be an explanation for lower sulfide content found in the CW-MEC systems of Ju et al. (2014a) as compared to their the control. As a consequence CW-MEC systems reportedly emitted less odor from sulfide accumulation.

### **8.2.5 Organic micropollutant removal**

The treatment performance of four organic micropollutants (OMPs) was investigated in Chapter 7. The hypothesis was that CW-MEC and CW-MFC+ are able to increase OMP removal. The tested OMPs included three non-steroidal anti-inflammatory drugs (NSAIDs), namely diclofenac (DCF), ibuprofen (IBU) and naproxen (NPX), and a psychiatric drug, carbamazepine (CBZ). For CBZ, DCF and NPX the treatment could be improved by 10-17% in CW-MEC and by 5% CW-MFC. However, in all three cases no statistically significant differences were found. IBU removal was similar amongst treatments. To some degree, the reasons for the improved removal rates in CW-BES potentially coincide with those for conventional contaminant removal, such as increased microbial activity and synergies between EAB and non-electrochemically active bacteria or changed environmental conditions in the case of CW-MEC. However, OMPs in general, and therefore also the presented OMPs specifically, show individual characteristics which likely had an influence and will be discussed in more detail in the following sub-sections addressing each compound.

#### **8.2.5.1 Carbamazepine**

CBZ removal rates were 34%, 22% and 17%, for CW-MEC, CW-MFC+ and CW-control, respectively (see Table 7.3.4 and SI, Figure S7.2a). There are no CW-BES which are really comparable to the presented systems that treated CBZ to date. Reasons for the improved treatment might include electrosorption and hydrophobic sorption offering additional sorption sites at the electrodes and biofilm. CBZ can actually not be considered as hydrophobic (log D of 2.77, see SI, Table S7.1), but it is less polar than the other three tested OMPs, and therefore the contribution of sorption to CBZ removal is potentially higher. However, even these sorption sites are finite and longer term investigations using BES incorporated in CWs for CBZ removal would be needed. Again the increase in microbial activity (see Chapter 5 and Chapter 8.2.3.2) could have played a role in the higher removal as compared to CW-control. The impact of the already described indirect effects of electrolysis (increase in aerobic and hydrogen oxidizing genera, see Chapter 8.2.3.1) would have rather constituted a disadvantage because CBZ has been shown to be rather



biodegradable in dominantly anaerobic conditions in HF CWs, but not biodegradable in aerobic conditions in VF CWs (Hai et al., 2011; Jekel et al., 2015; König et al., 2016; Nivala et al., 2019b). Removal rates in HF CW were for example 13% (Nivala et al., 2019) and 21% (Matamoros et al., 2017). In any case, the observed removal of the recalcitrant CBZ was promising in CW-MFC+ and especially in CW-MEC systems. Especially, when considering that removal rates of CBZ in conventional activated sludge (CAS) WWTPs rarely exceed 10% (Joss et al., 2005; Zhang et al., 2014). Further investigation of the precise involved removal processes and microbial communities is suggested.

### 8.2.5.2 Diclofenac

DCF removal rates were 57%, 52% and 47%, for CW-MEC, CW-MFC+ and CW-control, respectively (see Table 7.3.4 and SI, Figure S7.2b). There are no CW-BES studies on DCF removal so far but removal rates were high even when compared to conventional single- and double-chamber MFCs (4-45%) (Wang et al., 2015). De Gusseme et al. (2012) achieved full removal in a MEC when applying biogenic Pd nanoparticles but no significant removal was achieved without the nanoparticles. The biological removal of DCF is not fully understood and results are usually very variable (Zhang et al., 2008). Generally it has a low sorption propensity and can be considered a recalcitrant, although not as strongly as CBZ. Looking at the reasons for the improved removal in CW-BES, again electrosorption might have played a role whereas hydrophobic sorption probably was not a factor due to the low sorption propensity. Increased microbial activity could have improved the removal in CW-BES. In the case of DCF the more aerobic environment created in CW-MEC could have improved its removal, since DCF showed higher treatment by aerobic processes in VF CW with 50-70% (Ávila et al., 2014a, 2014b; Matamoros et al., 2007; Nivala et al., 2019b). Given the fact that the treatment in WWTP varied greatly between 7 and 75% (Zhang et al., 2014), the presented systems performed comparatively well. However, the more aerobic VF CWs are able to perform with higher removal rates without the need for a BES. In any case, it would be interesting to investigate in more detail the responsible mechanisms and processes leading to the increased DCF removal in HF CW-BES.

### 8.2.5.3 Ibuprofen

IBU removal rates were 39%, 35% and 39%, for CW-MEC, CW-MFC+ and CW-control, respectively (see Table 7.3.4 and SI, Figure S7.2c). No other CW-MEC investigated IBU removal. One other publication reported on removal in CW-MFC with removal rates between 82 and 96%, with 9% improvement compared to CW-MFC- (Li et al., 2019). In general, the characteristics of IBU are not too different from NPX (or DCF, except that DCF is considered recalcitrant), with a low sorption propensity (see SI, Table S7.1) and higher removal in aerobic conditions (Monsalvo et al., 2014; Quintana et al., 2005), hence VF CWs showed removal rates above 88% (Ávila et al., 2010; Nivala et al., 2019b; Vystavna et al., 2017). Therefore, unknown factors must have led to the indifference in removal results across the tested treatments which again would call for more research.

### 8.2.5.4 Naproxen

NPX removal rates were 40%, 30% and 25%, for CW-MEC, CW-MFC+ and CW-control, respectively (see Table 7.3.4 and SI, Figure S2d). As for CBZ, no CW-BES which are really comparable to the presented systems treated NPX. MFC systems by Wang et al. (2015) reached ca. 12-19% in single-chamber closed-circuit systems and up to ca. 40% and 84% in the anode and cathode of double-chamber MFC, respectively. As for DCF and IBU, sorption of NPX is low and aerobic biodegradation pathways are more efficient than anoxic/anaerobic ones. Therefore VF CWs showed high removal rates above 88% (Ávila et al., 2010; Nivala et al., 2019b; Vystavna et al., 2017). Removal rates in conventional WWTPs are relatively high and in the range of 40-98% (Zhang et al., 2014). As for DCF, the increased removal in CW-BES was unlikely due to hydrophilic sorption but possibly due to electrosorption effects. Also the increase of redox and DO caused by electrolysis of water in CW-MEC could have likely led to increased removal rates.

### 8.2.5.5 Potential of CW-BES for OMP removal

Looking at the OMP removal results in CW-BES, the technology seems more promising for some and less for other OMPs.

For OMPs which generally show higher removal in aerobic systems and treatment pathways, such as DCF, IBU and NPX it might make more sense to use an (artificially aerated) VF CW or other technologies with dominantly aerobic conditions and processes. CW-BES based on dominantly anaerobic HF CWs might have an inherent disadvantage especially when operated in CW-MFC+ mode, even though the study showed that in case of some OMPs, this potential disadvantage could be overcome partly or maybe even entirely via other removal pathways. Especially in the case of CW-MEC mode it could be interesting to try to hybridize or integrate it with a VF CW, since MEC are more independent from the environmental and redox conditions. It could be possible that some of the OMPs and/or their metabolites which react better to aerobic treatment pathways, could additionally benefit from additional removal pathways offered by BES, fostering removal mechanisms such as electrosorption, EAB removal pathways, synergies between EAB and non-electrochemically active microbial community as well as other direct and indirect BES effects.

However, CW-BES showed advantages compared to other technologies in the case of recalcitrant OMPs such as CBZ (and to some degree also DCF) since CBZ is not well removed in other treatment systems and basically not at all via aerobic removal pathways.

In general, according to Cecconet et al. (2017), BES should be theoretically more efficient in the removal of OMPs which are hydrophobic and positively charged. The former due to better adsorption onto charged electrodes and the latter due to better interaction with the negatively charged biofilm. However, the four OMPs presented in this study are all hydrophilic at neutral pH (see SI, Table S7.1). Furthermore, the four investigated OMPs are negatively charged (DCF, IBU and NPX), or neutrally charged (CBZ) under the pH range of the systems (see SI, Table S7.1). Therefore, it seemed like the presented CW-MFC+ and CW-MEC were even able to improve the removal of theoretically quite resilient OMPs such as CBZ, DCF and NPX. However, one could assume that hydrophobic OMPs would generally be removed more easily in a variety of treatment technologies

or even in the sewage system already, due to the higher chances of hydrophobic compounds to be removed by sorption. Hence, the real potential, or a so-called niche, of CW-BES for OMP removal could be its use for the treatment of hydrophilic and positively charged OMPs. Additionally, as described above, very recalcitrant compounds like CBZ seem to be worth future investigations in regards to treatment by CW-BES.

In summary, results could not confirm the hypothesis that CW-MEC and CW-MFC+ will improve organic micropollutants removal as compared to the CW-control system, since the differences in removal were not statistically significantly different across treatments. However, the results showed a higher OMP removal in CW-MEC and CW-MFC+ as compared to CW-control in the case of three out of four OMPs.

### 8.3 CW-BES electrical performance

CW-MFC+ and CW-MEC cannot really be compared directly with each other in terms of electrical performance since the systems operate quite differently, i.e. the former with a resistor (or load) between electrodes producing electrical power, and the latter is supplied with an additional power source. Hence, the electrical performance is presented separately for CW-MFC+ and CW-MEC in the following.

#### 8.3.1 CW-MFC electrical performance

Table 8.3.1 shows the evolution of the electrical performance of CW-MFC+ systems over the course of the three treatment performance investigations. At the time of the start of the respective experiments in Chapters, 5, 6 and 7, the systems were in operation for 4 months, 10 months and 18 months, respectively. The three campaigns were 13, 12 and 17 weeks long, respectively.

**Table 8.3.1.** Overview of CW-MFC+ electrical performance parameters during the three investigations

CW-MFC+						
	Average Voltage (mV)			Average Current density (mA/m <sup>2</sup> )		
	1	2	3	1	2	3
Ch. 5	281±123	481±80	435±70	31±13	52±9	47±8
Ch. 6	379±77	394±62	357±74	41±8	43±7	39±8
Ch. 7	372±119	378±81	372±128	40±13	41±9	40±14

	Maximum power density (mW/m <sup>2</sup> )			Internal resistance (Ω)		
	1	2	3	1	2	3
Ch. 5	7	37	36	215	100	100
Ch. 6	36	17	23	83	94	89
Ch. 7	30	11	24	108	220	124

During the first investigation in Chapter 5, the systems were probably still establishing and the biofilms maturing. Interestingly, the

first transect was showing lower values in terms of voltage, current density and maximum power density as transect 2 and 3 during this period. The higher standard deviations and Figure 5.3.2 also showed well how the voltage in transect 1 fluctuated during this period, which could have been a response to changing influent wastewater strength (dry periods, rainfall events etc.). Transect 2 and 3 showed similar fluctuations but to a much lesser degree, possibly because the effect was already buffered after the wastewater flowed through the preceding transect(s). Hence, the fluctuations in CW-MFC+ transect 1 during the first investigation could be a remnant of the already diminishing bioindication capacity described in Chapter 4 and Chapter 8.1. Contrarily, during the latter two investigations the maximum power density in transect 1 was more than double that of transect 2 and nearly double of that in transect 3, while average voltages and current densities in all transects were quite similar. A potential reason could be the higher nutrient availability during the Polarization Curve (PC) analysis which were conducted in order to attain the maximum power density. The highest reported maximum power densities in other CW-MFC studies were  $2 \text{ W/m}^3$  (L. Xu et al., 2017b) and  $3.7 \text{ W/m}^2$  (F. Xu et al., 2018), however the value in the majority of other studies was one or more magnitudes lower, in the range of the presented values (see Table 3.3.3).

The PC curves allowed also for the calculation of internal resistances. Principally, the potential maximum power is achieved when internal and external resistances are close to each other (Lefebvre et al., 2011). Therefore, it seemed that the external resistance of  $220 \Omega$ , suggested by Corbella and Puigagut (2018) for a similar CW-MFC system architecture, was a relatively good fit, especially in transect 1 during the first investigation and transect 2 during the third. Otherwise a lower external resistance could have potentially led to a better performance, since lower external resistances increase the generated current and studies have also shown that consequently organic matter removal was increased (Aelterman et al., 2008; Gil et al., 2003; Katuri et al., 2011). However, for the sake of consistency and comparability between the investigations and transects, it was decided to keep the same external resistance. Internal resistances in other CW-MFC studies were mostly higher with values going up to  $4300 \Omega$  (see Table 3.3.3).

Generally the increase in current or power density for electricity production was not a goal of the presented studies. On the one hand because electricity production is still a few orders of magnitude lower than that of other electrical energy sources such as photovoltaics, and on the other hand because the contaminant removal was the focus of the conducted investigations. In any case, the electrical performance parameters are still useful since they describe influencing factors and are indicators for the contaminant removal performance in CW-MFC.

### 8.3.2 CW-MEC electrical performance

Table 8.3.2 shows the evolution of the electrical performance of CW-MEC systems during the two last investigations which also included CW-MEC systems. The CW-MECs in all three transects of both duplicate systems, each received a poised potential of 0.3 V vs. Ag/AgCl reference electrode at the anode. This potential was chosen on the basis of experiences showing that poised potential around this value benefit the growth of EAB (Fricke et al., 2008; Liu et al., 2008).

**Table 8.3.2.** Overview of CW-MEC electrical performance parameters during the two last investigations

Transect	CW-MEC					
	Average current (mA)			Average Current density (mA/m <sup>2</sup> )		
	1	2	3	1	2	3
Ch. 6	19±11	18±9	8±5	447±269	427±201	178±106
Ch. 7	23±11	10±5	5±3	535±263	223±112	120±74

The average current and current densities were generally decreasing along the flow path of the system and the three transects.

These current densities were on the lower end of the spectrum in comparison to other CW-MEC, showing values ranging from 200 to 24500 mA/m<sup>2</sup> (see Table 3.3.5). This was probably a consequence of the low power applied via the potentiostat, while most other studies applied power via a standard DC power supply or a solar panel. Only one other study by Aguirre-Sierra et al. (2016) also used a potentiostat which was also poised at the same potential, resulting in a current

density of  $100 \text{ mA/m}^2$ , which was relatively similar to the lower reported current density in the two investigations of this study.

In general it is questionable in how far the CW-MEC which use a power supply or a solar panel are comparable to studies using a potentiostat. When using a potentiostat the anode potential is controlled at a certain value with the help of a reference electrode, which makes it possible to control the conditions and consequent processes within the system relatively precisely. If a certain current or voltage is applied between the electrodes via a power supply or a solar panel such precise adjustments at the respective electrodes are not possible. Especially in studies in which very high amounts of electricity were supplied a further question arises about how far bioelectrochemical processes are still determining performance and treatment, and not simply electrochemically processes. In the end, the use of potentiostats probably provides advantages for investigations in which exact conditions should be tested, like a certain set anode potential, whereas for applications in full scale system a power source from a solar panel could be more attractive due to operational and maintenance considerations. CW-MEC maintenance and operational consideration will be discussed in more detailed in the Chapter 8.4.2.



## 8.4 CW-BES challenges

In this chapter the challenges in regards to CW-BES for wastewater treatment and control are discussed in a bigger context, based on experiences and results from the presented work as well as other publications. This should provide an as honest as possible perspective on the above described potentials and possible improvements through CW-BES for wastewater treatment and control.

### 8.4.1 *Economic and environmental considerations*

Generally, CW-BES for wastewater treatment will have to prove that the advantages in contaminant removal are worth the additional investment in order to establish themselves in practice. In regards to CW-MFC systems, Corbella et al. (2017) conducted a life cycle assessment (LCA), comparing a conventional CW, a CW-MFC with a gravel anode and a CW-MFC with a graphite anode. Their results showed that the graphite-based CW-MFC would be the most environmentally friendly since it would reduce the system footprint by 20%, however, the system was 1.5 times more expensive than the conventional CW, therefore the investment would be only worth it if higher treatment goals would be required and materials for the MFC would become cheaper.

The material costs of CW-BES are higher than for conventional CWs, mainly due to the materials needed for anodes and cathodes as well as main bed materials in the case of systems which fill the whole volume with electroconductive material, such as coke granules or zeolite (see Tables 3.3.2 and 3.3.4). The carbon felt used in this study as a cathode material was useful for experimental purposes, but due to very high cost (up to 800 €/m<sup>2</sup> for high quality) and operational issues (see Chapter 8.4.2) it does not seem reasonable to apply it in full-scale systems. Therefore it would be important to find synergies with other sectors in which materials suitable for electrode utilization in CW-BES arise as a waste product and could be recycled. Potential sectors include the metal processing, petroleum or coal industry, which produce waste materials in granular form such as crushed graphite electrodes (which are originally used for corrosion protection and need to be exchanged frequently), petroleum coke (pet coke – an oil refining

by-product), or anthracite. The fact that materials that would otherwise be a waste product would be recycled and reused for wastewater treatment is a positive aspect. In general, these materials must meet several criteria such as high conductivity (low resistivity), biocompatibility (non-toxic and chemically stable, to allow for biofilm formation and not contaminate the water), or other structural properties. Even suitable waste materials are still very expensive (up to 700-800 €/ton for granular crushed electrodes or pet coke) in Europe when compared to gravel appropriate for CW beds (ca. 20 €/ton). However, prices could decrease when purchased in higher amounts but would likely vary as well depending on how distant the closest industry producing such materials would be to the CW-BES construction site. In some areas around the world the distance could be too far to be reasonable to transport these relatively heavy materials. Hence, future research on CW-BES should also focus on minimizing the volume of anode and cathode compartments while still meeting required treatment goals (see more details in Chapter 8.5.1).

Another factor are the materials used as current collector, such as stainless steel mesh (SSM). SSM used for CW-BES has to be very resistant to aggressive environments such as seawater, or wastewater in this case. The material used in this study as electrode current collector was SAE 316L stainless steel (often referred to as marine grade) and its alloying constituents, apart from iron, include chromium, nickel and molybdenum. Of course the impacts of the mining, production and disposal of such materials has to be considered (e.g. in a LCA).

As already mentioned above, also the here used potentiostat for CW-MEC operation was mostly used for experimental setups so far, whereas most studies utilized a solar panel or standard power supply. There are potentiostats for several hundred Euros on the market (e.g. nanoelectra, Spain), and also reference electrodes are available for less than 100 €, however, they have unfortunately also led to operational issues as discussed in more detail in the following chapter.

#### **8.4.2 Operational considerations**

Some operational challenges have to be considered as well when utilizing CW-BES. As for other CW intensification strategies, also

CW-BES complicate the operation and maintenance of the systems to a certain extent as compared to a conventional CW design. Again, as for the economic considerations, these have to be weighed up against the benefits of CW-BES and future investigations and developments will hopefully be able to find solutions to them.

The maturation of the air-cathode in CW-MFC systems might have led to a decline of its bioelectrochemical capacity, due to decreased oxygen mass transfer and consequently lower reaction rates. While the growth of EAB on the anode potentially increases the generated current and related transformation of nutrients, the development and maturing biofilm on cathodes can decrease the air-cathode's (as used in this study) ability to transfer oxygen and thereby current, and as a consequence nutrient removal might decrease (Kiely et al., 2011a; Rossi et al., 2018; Zhang et al., 2017). This phenomenon is also called cathode clogging or fouling, which might become an issue particularly after long-term operation. Especially when exposed to sunlight, also algae might grow on the surface, at first maybe even increasing the cell potential by providing oxygen, however after a certain point the algae, together with organic matter accumulation and microbial biofilm growth, would inhibit the oxygen supply to the cathode and consequently reduce the cell potential and resulting CW-MFC performance. Hence, the border between advantages of a bio-cathode and disadvantages of biofouling seems to be thin line. Air-cathode fouling was also observed in an additional planted CW-MFC+ duplicate which was provided with UV-light but not utilized for the presented investigations. Also Arends et al. (2014) observed this effect in their PMFCs. Some studies have also observed a strong dependence of air-cathode performance on the circadian rhythm (day-night cycle), due to the variation of oxygen mass transfer (Arends et al., 2014; Strik et al., 2010; Ter Heijne et al., 2010). A remedy for fouling of carbon felt itself was possible with ethylenediamine or nitric acid which according to Zhu et al. (2011) led to power density increases of 25% and 58%, respectively, which was attributed to changing the surface attributes of the felt. However, judging from own experiences the carbon felt was losing its structure and not performing so well anymore after several washing cycles and tended to dissolve. A possible solution to overcome these limitations would be the control of the anode potential with a CW-MEC as used in the above described two latter investigations. However, full-scale CW-BES applications will likely not use carbon felt cathodes, due to the above described issues with

clogging, as well as root penetration. Furthermore, water level variation caused by evapotranspiration has been shown to be a main influencing factor on CW-MFC performance (Corbella et al., 2015), for which the usually relatively thin (2-3 cm) CF would not be suitable. In how far cathode excessive biofilm formation or fouling could also be an issue for granular media would have to be investigated in future research. One study has shown that the surface area decrease of AC granules was 21% lower in CW-MFC+ as compared to the decrease in a CW control, indicating that CW-MFC+ was more clogging resistant (Yang et al., 2018). The alleviation of the clogging was assumed to be caused by the micro-electric field in the CW-MFC+, in a way that protons shifting from anode to cathode could have removed the mainly negatively charged suspended solids and bacteria from the pores. However, Corbella (2017) found that solids accumulation in the anodic chamber might have led to a limitation of the tested graphite based anode CW-MFC, to an extent that the performance did not differ to the CW-MFC treatment with a gravel based anode.

However, also operational considerations specifically for CW-MEC systems emerged from the experience during the two last investigations. First of all, obviously the reference electrodes need regular maintenance including cleaning of the porous frit and the refilling or exchange of the electrolyte. This is especially important if they are continuously submerged in the filter bed and exposed to wastewater and biofilm growth. Hence the reference electrode needs to be maintained and calibrated regularly using another reliable reference electrode. The MEC mode furthermore led to the dissolution of metal, e.g. the current collector wires made from stainless steel at the cathodes. As a consequence these collector wires had to be exchanged frequently. This phenomena possibly also led to some processes related to contaminant removal. For example as mentioned in chapter 8.2.4.3, about increased phosphorus removal in CW-MEC, which Ju et al (2014a) attributed to coagulation of the ferrous iron which formed during electro-dissolution of the sacrificial metal electrodes.

## 8.5 Technical and practical recommendations for CW-BES

Some technical and practical recommendations for the use of CW-BES can be deduced and concluded on the basis of the above described results, discussion, identified challenges and considerations as well as the wealth of experience from other studies.

### 8.5.1 *Materials*

#### 8.5.1.1 Electrodes

As described in Chapter 8.4, the CF felt used in this study as cathode material was useful for experimental conditions but will not be for full-scale implementation due to factors such as water level variation, physical destruction or replacement through root growth, clogging or bio-fouling, as well as the high price. A gravel and SSM based anode, as used in this study, has been shown to perform similarly to a graphite and SSM based anode after some time of operation, due to solids accumulation (Corbella, 2017), but an LCA has also shown that gravel based anodes did not perform as well in economic terms since the treatment benefits could not justify the additional investment (Corbella et al., 2017).

In general, the material used for the electrodes should be electroconductive (low resistivity), bio-compatible, provide a high specific surface area, ideally a waste-product from another industry or process, and of course inexpensive. Materials used in other CW-BES studies for anodes and cathodes apart from CF included other carbon based materials such as graphite, GAC, or a waste product called pet coke, as well as metals in the shape of plates, rods, columns or meshes, or a combination of both (see Tables 2.3.2 and 2.3.4). Granular materials also require a current collector, which was mostly made from SSM, as also used in this study. As described in Chapter 8.4 and Chapter 2.3.2.2 a very resistant SSM material is necessary due to the harsh environment in wastewater treatment systems which consequently leads to a higher environmental impact. Furthermore,

CF, certain metals and other modified materials such as platinum coated cathodes are more expensive than a granular media (Li and Sheng, 2012; Srikanth et al., 2011). The choice of the electrode material will also depend on the local availability of materials and the electrical connection (CW-MFC+ or CW-MEC, see more details in Chapter 8.5.4) and the treatment purpose.

In summary, granular electroconductive materials such as pet coke or crushed electrodes seem to be the most promising electrode materials at the moment, especially if they could be purchased relatively inexpensively from industries which regard them as a by-product or waste. Generally, porous media would be prone to clogging in a wastewater treatment context due to the high amount of suspended solids, however, this could potentially be alleviated to some extent due to the already mentioned effect of the micro-electric field and a stream of protons alleviating clogging in the pores (Yang et al., 2018). Apart from bioelectrochemical effects, the granular highly porous materials used in other studies also seemed to have potential positive effects on contaminant removal besides bioelectrochemical effects, such as sorption (see Chapter 8.2.4.3) and could possibly also be beneficial for electro-sorption (assumed to have played a role in OMP removal in Chapter 7 and Chapter 8.2.5). Last but not least the reuse of a waste material would also be in line with the paradigm shift towards a circular economy.

### 8.5.1.2 Main bed material

The main bed material of the CW-BES bed, i.e. all the filling media besides the materials used for cathodes and anodes, has been mainly gravel in the CW-BES studies so far, but there are also several ones using highly porous material, such as ceramsite or bio-ceramic as well as electrically conductive materials such as zeolite or dewatered alum sludge, a by-product from drinking water treatment works (see Tables 2.3.2 and 2.3.4). The arguments for and against other main bed materials than the conventional gravel follow the same lines as for the electrode materials in which costs and availability have to be weighed up with the benefits in terms of treatment efficiency. In general, gravel has been proven to be a reliable bed filling material over decades of CW research and application and has prevailed in full-scale

application against many contenders, for example when looking back at investigated materials for improved phosphorus removal, for which also natural and man-made products, as well as industrial by-products have been tested extensively (Ballantine and Tanner, 2010; Vohla et al., 2011). The future will show whether gravel will also persist against electroconductive and porous material with high specific surface area, however, until then the recommendation would be to use the additional investment in CW-BES rather for electrode materials and current collectors, and continue to use suitable gravel as a main bed filling material.

### **8.5.2 Design**

Assuming that granular electroconductive media such as pet coke is used, the ratio of electrode volume (and the resulting electrode surface area) to the volume of conventional material will be in a relationship of tension between economic and treatment efficiency considerations. The bulk volume of electrode material should be as low as possible due to the relatively high price of materials when compared to that of gravel (see Chapter 8.4.1), and as high as possible in order to connect and create as much electrode volume and consequently electrode surface area as possible and needed for the targeted wastewater treatment improvement that would justify the additional investment for the CW-BES. However, with attention to keeping an appropriate distance and redox gradient between cathode and anode (see more on electrode spacing below in Chapter 8.5.2.1). Also, assuming that not the whole CW bed will be operated as a CW-BES, the positioning of the CW-BES within the CW bed will be discussed below in Chapter 8.5.2.2.

#### **8.5.2.1 Electrode spacing and use of separators**

Generally, the distance between anode and cathode should be as small as possible in order to reduce ohmic resistance, but be high enough in order to maintain a sufficient redox potential gradient. A possible solution to meet both prerequisites, at least in lab- to pilot-scale sized systems, were the use of glass wool separators which

prohibited oxygen from penetrating lower anaerobic compartments, as used in the presented studies and elsewhere (Yadav et al., 2012; Zhao et al., 2013) (see more details in Chapter 2.3.2.2). However, Doherty et al. (2015c) mentioned that glass wool layers might promote long-term clogging problems due to organic matter accumulation. Additionally, roots could penetrate the separators and consequently lower their effectiveness. Also, research has shown that glasswool separators decreased CW-MFC electrical performance and that the biofilm underneath the cathode apparently formed a “microbial separator” which consumed the atmospheric oxygen before it could diffuse down into lower anoxic/anaerobic zones of the CW bed (L. Xu et al., 2018a). Therefore, it seems recommendable for full-scale applications to not use any separator but to rather keep enough space between the electrodes and make use of the formed “microbial separator” to keep the anode section as anaerobic as possible.

Regarding electrode spacing, CW-MFC studies comparing different distances found highest voltage output using spacings of 5 cm (compared to 2 and 10 cm) (Xu et al., 2018), 10 cm (when compared to 12.5, 15, 17.5 and 20 cm) (Corbella et al., 2016b), 13 cm (compared to 8 and 18 cm) (Xie et al., 2018) and up to 15 cm (however only compared to higher distances of 30 and 45 cm). The presented study used a spacing of 1 cm only, and achieved relatively high average voltages of around 350-400 mV (see Chapter 8.3.1). However, a glass wool separator was used. The optimal spacing would also be dependent on other factors such as system scale, architecture (e.g. cathode aeration), operation (e.g. flow direction), wastewater characteristics and materials used. Although the above presented comparative studies found distances between 5 and 15 cm to be performing best, it could be recommendable to perform some tests before going for a full-scale implementation, in order to consider the respective architecture, materials, wastewater type and operational mode used in a smaller scale system which however should be representative in scale in terms of vertical redox distribution, i.e. system depth and vertical electrode spacing.

Since CW-MEC systems are only dependent on the naturally occurring redox potential gradient to a limited extent, the electrode spacing is not as important, although of course the electrodes should be distant enough to avoid a short-circuit. In this regards it is anyway



recommended to have a power/electrolysis source which is protected against short-circuiting in order to avoid damage.

### 8.5.2.2 CW-BES positioning

Generally, from a spatial perspective, the majority of organic matter removal occurs in the first section of a HF CW, partly due to physical filtration and sedimentation effects but to a large degree also due to microbially mediated and other processes (see Chapter 2.1.3). This pattern was also very pronounced in this study's contaminant removal, especially in the case of MEC mode, where a proportion of 88% of the total COD mass removal in the systems already occurred within the first transect, as compared to 74% in CW-MFC+, CW-MFC- and CW-control (see Chapter 6.3.2.3 and Table 6.3.3). In accordance with that the microbial activity investigation in Chapter 5 showed as well highest activity in the first transect with decreasing values in transect 2 and 3 (see Figure 5.3.5). In regards to ammonium, the investigations in Chapter 5 and 6 showed that the removal patterns were not as homogenous, but the majority was removed in more or less similar big proportions within the first and second transect. In general the treatment was less variable and more stable in CW-MFC+ and especially CW-MEC as compared to the control systems (see Chapter 5, Figure 5.3.4 and Chapter 6.3.2.4).

The variation of redox on a spatial scale is likely the most important factor for the positioning of CW-MFC+ systems. Redox potential is generally increasing from the inlet to the outlet of a HF CW system. Vertically the redox is decreasing from the top to the bottom (García et al., 2003). Therefore also the redox potential gradient is highest near to the influent, and consequently the maximum voltages and BES performance potentially highest.

Given the above stated, CW-BES should be placed at the beginning, near the influent of a HF CW bed, due to the results presented in the investigations and especially due to the higher redox potential gradient, which is particularly relevant for CW-MFC+, while CW-MEC is less dependent on the naturally occurring gradient (see more details 8.5.4).

However, there are also indications that a CW-MFC+ placed more towards the end of the CW bed could be beneficial. Although microbial activity was decreasing in the CW-MFC+ transects, the statistically significant differences when compared to CW control were found in transect 2 (very significant) and transect 3 (extremely significant) (see Chapter 8.2.3.2 and Figure 5.3.5). Also a study by Capodaglio et al. (2015) tested different OLRs in swine manure fed MFCs and found that lower OLR (volumetric OLR 0.7 kg COD/m<sup>3</sup>·day) promoted EAB growth and activity over methanogens as compared to higher OLR (volumetric OLR 11.2 kg COD/m<sup>3</sup>·day). Hence, given that there is still enough substrate available, the positioning more towards the center or end of the bed could be advantageous for EAB. In addition, improvement of ammonium removal in CWs seems to be a higher priority than COD since COD removal is already quite good with >80% in HF CWs (Dotro et al., 2017), and the investigations in Chapter 5 and 6 showed that nitrogen was removed to a great extent as well in the second transect of CW-MEC and CW-MFC+ systems.

In summary, CW-MFC+ seem to best positioned at the beginning, i.e. near the influent of a HF CW in order to utilize the maximum redox potential gradient. In terms of ammonium removal enhancement it could be worth investigating whether a placement more towards the center or end of the bed could be beneficial.

Consequently a possibility could be to place a CW-MFC+ at the beginning of the bed, and afterwards a CW-MEC, which could be even powered by the electricity produced in the CW-MFC+. Thereby, nitrification and denitrification pathways of both CW-MFC+ and CW-MEC could be utilized (more details on these pathways see Chapter 8.2.4.2). There are already successful studies in which conventional MFCs power conventional MECs (Sun et al., 2009; Wang et al., 2011, 2013; Zhang and Angelidaki, 2012b), consequently also research using CW-MFC+ and CW-MEC in series or other combinations would be recommended.

### 8.5.2.3 Modular CW-BES

In conventional MFC or MEC studies, modular systems have been used in the past, especially for upscaling. Often they were stacked, in series or parallel, in order to increase the overall potential or current (Feng et al., 2014; Gil-Carrera et al., 2013). While this could also be an option in CW-BES, the idea of a modular BES within a CW could also be interesting in terms of operation and maintenance. First of all, in the case of bioindication using CW-MFC, results indicated that it would be beneficial to use smaller CW-MFC units, as already mentioned in Chapter 8.1. These could be inserted in a CW bed in perforated tubes similar to those used in this study's setup (see Figure 3.1.1, H). This way the bioindication could be improved and it would be easier to maintain the systems since the anodes and cathodes are removable. In a similar fashion it could be interesting to build removable modular CW-MFC+ and CW-MEC units for wastewater treatment purposes, which can be removed and maintained, or exchanged, which could solve some of the operational problems mentioned in Chapter 8.4.2. This would certainly pose several challenges in terms of design, construction or hydraulics, and of course operation due to potentially quite big modules, but could be worth further investigations.

### 8.5.2.4 Plants and CW-BES

As for conventional CWs, also the vast majority of CW-MFCs reported increased treatment efficiency and/or improved electrical performance in the case of CW-MFC+ when systems were planted (see Chapters 2.3.2.2). Although, the majority of CW-MEC was planted as well, the plant effects there are supposedly less pronounced since the performance and treatment is not as dependent on and influenced by environmental conditions such as redox because the processes are controlled by applying a power source. However, more research in the effects of plants on CW-MEC is needed and at least the multitude of auxiliary advantages (see Chapter 2.1.4) should be considered, hence the recommendation to plant systems. Regarding the types of plants, a variety of common macrophytic aquatic plants were tested. As an example, Saz et al. (2018) also compared different plant species,

showing best removal results and current densities with *T. angustifolia*, compared to *Juncus girardii* and *Carex divisa*, supposedly due to a better environment for microorganisms and resulting increase in nutrient removal and current densities.

### **8.5.3 Operation**

As described in Chapter 8.2.1, a continuous feeding showed to be beneficial for the electrical performance of CW-MFC+, most likely due to the increased redox potential gradient in the systems. In terms of OLR the investigations carried out in this work did not show differences in treatment or CW-MFC+ performance, but there are indications that MFC generally perform better in low loaded systems (see chapter 8.2.2).

It could be assumed that CW-MECs are even more resilient or indifferent towards different operation strategies and higher OLRs. An indication for that was the more stable contaminant removal and lower standard deviations as compared to other treatments throughout the investigations in Chapters 5-7. Also CW-MFC+ seemed to be more stable in this sense, when compared to control systems, but not to the extent of CW-MECs. Further research is recommended in order to test the boundaries of CW-MEC systems in terms of operational parameters such as applied hydraulic and organic loading.

### **8.5.4 CW-MFC+ or CW-MEC?**

As mentioned above, the advantages of CW-MEC, were a higher average contaminant removal when compared to all other treatments with lower standard deviations indicating a more stable performance (see Chapters 8.2.4 and 8.2.5), most likely because the CW-MEC is not as dependent on environmental conditions like redox to such a great extent or operational conditions such as cathode clogging and/or higher OLR as shown in Chapter 8.2.4. Additionally the investigations have shown that CW-MEC introduce a variety of additional removal pathways via electrolysis promoted aerobic and hydrogen oxidizing genera (see Chapter 8.2.3.1).

An advantage of CW-MFC+ in comparison to CW-MEC is that it does not require an energy supply but is even producing a small electrical energy from the oxidation of (in)organic matter at the anode. Furthermore, as compared to CW-MEC, it is less complex in operation and maintenance and could be favorable for contaminant removal requiring anaerobic treatment processes.

Hence, as mentioned above in Chapter 8.5.2.3 the best option could be not one or the other but combining the advantages of both systems in one treatment system, where the CW-MFC+ part could supply the energy needed for the CW-MEC. A perspective for such a combination as a niche application in metal containing wastewater will be described in the next Chapter 8.6. However, in terms of power source, a cheap and reliable photovoltaic system, i.e. a solar panel, could be even a better and easier solution for the power supply of CW-MEC, as shown in a couple of studies already (see Chapter 2.3.3).

Finally, the best choice will depend again on the requirements and respective conditions, e.g. removal pathways of targeted contaminants or local availability of materials.

## 8.6 Future research and perspective of CW-BES technology

Since CW-BES are still a relatively recent development, their potential is very likely not exhausted yet, which is also reflected by the numerous and increasing publications on the topic over the last years accompanied by first pilot- and full-scale applications. Hence, the knowledge and experiences with these systems are increasing too, but at the same time more questions are arising and also limitations have been encountered.

In terms of use of CW-MFC for bioindication, future investigations are suggested to focus on prolonging and improving the bioindication capacities of CW-MFCs, preferentially utilizing smaller and multiple CW-MFC units within a CW bed or a reactor in order to receive data from not just one but an array of monitoring points distributed over the whole area and in different depths. Such networks could give valuable information on contaminant load and removal as well as operational observations in terms of hydraulics (e.g. dead zones or short-circuiting) and potential clogging within CWs and other wastewater treatment systems.

Regarding CW-BES for the improvement of treatment performance, several crucial points would need to be addressed and require more future research. Among them are the above mentioned search for appropriate and more affordable electrode materials, investigating the determining factors for the design in terms of size and volume of the anodes and cathodes and their spacing in full-scale systems, the effect of placement of CW-BES within the filter bed on the removal of different contaminants, investigations on effects of more different plants, potential advantages and synergies of CW-MFC+ and CW-MEC in combination or in a modular design, testing the boundaries of different CW-BES in regards to OLR and HLR, and the implementation of further LCAs looking into the net benefit of different CW-BES configurations and also to identify their main weaknesses.

In terms of competition with other successful CW intensification strategies like artificial aeration, or other biological and/or technical wastewater treatment systems in general it could be also essential to find niches for CW-MFC+, CW-MEC and other CW-

BES configurations in which these systems offer an added benefit which other technologies cannot provide. The continuous research on BES on a more fundamental level could provide the basics for that. For example the constantly discovered bacteria capable of EET and in general the more and more deeper understanding of the involved mechanisms and interactions with other microbial communities could enable future research to aim for more tailor-made solutions, e.g. targeting the removal of certain compounds by providing or creating the right conditions for certain EAB, processes, removal pathways and whole bacterial communities.

There are also indications from conventional MFC studies that MFC caused the potential in the anodic compartment to drop and thereby enabled different microbial species to utilize organic matter more efficiently (Katuri et al., 2011).

A concrete example for such a niche application could be the retainment, accumulation and controlled concentrated release of metals from metal containing wastewater in a continuously fed CW-BES. The metal-rich wastewater would be fed to the CW-BES which would be operated as an HF CW-MFC+ for the majority of time and thereby provide anoxic/anaerobic conditions in the lower zones of the filter bed. The anode of the CW-MFC could even cause the redox potential to further drop as shown by conventional MFC studies (Katuri et al., 2011). These conditions would enable sulfide formation and consequent co-precipitation of the metals with sulfide (e.g.  $\text{Fe}^{2+}$  and  $\text{S}^{2-}$  to  $\text{FeS}$ ). This way the metals would be removed from the relatively highly diluted wastewater, and precipitated and accumulated over time in the filter bed. Now, this very same CW-BES could be switched from CW-MFC+ mode to CW-MEC mode for a relatively short period of time, in which the anode is poised with a positive potential causing electrolysis induced oxygen release to occur in the otherwise anaerobic anodic volume. Consequently the accumulated metals would be dissolved again in the liquid phase and could be flushed out of the system in a much higher concentration as they arrived initially at the influent. This flushed out effluent water with relative low dilution and relatively high metal concentrations would then be collected separately. An advantage of this short-term operation of the CW-BES as CW-MEC would be that the operational issues concerning the maintenance of the reference electrode (in the case of using a potentiostat) and the dissolving metal connections mentioned in

Chapter 8.4.2 would be less severe. Of course this change of environment to aerobic conditions could also be achieved by an artificial aeration system, however, the switch from negative to positive charge of the anode as a consequence of turning the CW-MFC+ into a CW-MEC could potentially even further enhance the release of likewise positively charged metal ions, bringing the formerly electrically adsorbed metals in solution as well. In any case, both strategies could be tested in combination and separately. An initial lab-scale experiment by the author showed promising results but could not be published or included into the thesis, due to time limitations. Theoretically, the different metals (e.g. Co, Cu, Fe or Zn) contained in the “concentrate” collected at the effluent could then be recovered, e.g. through selective sorbent materials, and contribute as well to the described paradigm shift towards a circular economy in the introduction of this thesis.



## CHAPTER 9

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# Conclusions

In conclusion, the conducted research using meso-scale CW-BES systems fed with real urban wastewater showed promising results in regards to the optimization of wastewater treatment and control.

In terms of control, the bioindication investigation in **Chapter 4** has shown that CW-MFC were able to indicate COD increases well (75-80% of increases within 2-4 h), but decreasing COD was only reflected in the signal after a delay of up to a day. Hence the system was proposed to be used as an alarm tool for rapidly increasing COD concentrations. Therefore wastewater treatment facilities could use this technology to monitor and identify pollution events within a few hours on-site and online, without the need for lengthy, costly and environmentally harmful water sampling and analysis campaigns. However, contrary to the hypothesis it was not possible to correlate the CW-MFC signal at all times to the varying COD concentration in the influent and use it as a quantitative COD assessment tool. Future investigations are suggested to focus on prolonging and improving the

bioindication capacities of CW-MFCs, preferentially utilizing smaller and multiple CW-MFC units within a CW bed.

The investigations in **Chapters 5-7**, on the optimization of wastewater treatment performance using CW-BES with different electrical connections, have led to several conclusions. Initial investigations on operational conditions showed that, 1) in line with the hypothesis, continuous wastewater feeding outperformed an intermittent feeding regime in terms of current production in the first transect of CW-MFCs, and 2) contrary to the hypothesis that a lower OLR benefits treatment performance, the different tested OLRs caused no statistically significant differences in current production or contaminant removal. Hence, the remaining investigations used a continuous feeding regime and kept a relatively low OLR due to operational constraints. These investigations showed the following contaminant removal results in comparison to the used control treatments;

- COD removal was on average up to 13% and up to 7% higher in CW-MEC and CW-MFC+, respectively.
- Ammonium removal rates were on average up to 18% and up to 22% higher in CW-MEC and CW-MFC+, respectively.
- Phosphorus removal was variable but had the tendency of higher removal in CW-MEC and CW-MFC+.
- Sulfate was generally removed less in CW-MEC and CW-MFC+.
- The removal rates of the organic micropollutants carbamazepine (CBZ), diclofenac (DCF) and naproxen (NPX) were improved on average by 10-17% in CW-MEC and by 5% in CW-MFC. Ibuprofen (IBU) removal was similar amongst treatments.

Hence, the results were generally in line with the hypotheses that CW-MEC and CW-MFC+ improve contaminant removal of conventional contaminants, as well as organic micropollutants in the case of CBZ, DCF and NPX. However, statistically significant differences could only confirm the hypothesis in the case of ammonium in Chapter 6. Generally, the total removal rates of conventional

contaminants decreased over time. The main reason for this decrease was probably cathode maturation and clogging, which was also observed by other studies with similar conditions. CW-MEC was less affected, likely because its performance was less dependent on the cathode potential. However, the improvement of CW-MEC and CW-MFC+ as shown above was still pronounced, especially in the case of ammonium.

The two microbial community characteristics investigations in CW-BES could provide partly an explanation for the improved contaminant removal in CW-BES. Microbial activity was shown to be higher by 4-34% in CW-MFC+ as compared to CW-control in **Chapter 5**. Results were therefore in line with the initially posed hypothesis. Furthermore, the microbial community analysis conducted in **Chapter 6** revealed that in line with the hypothesis, microbial communities in CW-MEC cathodes and anodes were statistically significantly different compared to other treatments. The main reason was attributed to electrolysis taking place in the systems, causing increased oxygen and hydrogen concentrations. Consequently, the genera with the highest relative abundance in CW-MEC were aerobic *Sphingobium* and hydrogen oxidizing *Hydrogenophaga*, which were both not present in a relevant amount in any other treatment. The present species in the *Sphingobium* genus was *Sphingobium yanoikuyae* and has not been reported in any BES so far. However, closely related genera were reported in other CW-MEC systems. Probably due to a sampling issue at CW-MFC+ anodes, only the cathodes showed a statistically significantly different microbial community to the other treatments in line with the hypothesis. Interestingly, CW-MFC+ cathodes showed a high abundance of the species *Lysinibacillus boronitolerans*, a close relative of *Lysinibacillus sphaericus*, which was identified to be EAB. Both, *Sphingobium yanoikuyae* and especially *Lysinibacillus boronitolerans* are therefore potentially candidates for newly identified EAB and suggested to be investigated in more detail.

Besides the higher microbial activity, the differences in microbial communities and the direct and indirect effects of electrolysis in CW-MEC, additional reasons for the improved contaminant removal found in comparable BES and CW-BES studies include synergies between EAB and other microbial communities, and especially in the case of organic micropollutants electrosorption could have played an additional role.

All in all the investigated CW-BES systems showed that wastewater treatment could be improved even on a meso-scale and when fed with complex real urban wastewater, since the majority of earlier studies were performed using lab-scale systems fed with synthetic wastewater, which is advantageous for the study of fundamental processes, but reflects real conditions to a limited extent only. The observed increase in efficiency would theoretically translate into a lower surface area requirement for these nature-based solutions and therefore alleviates one of the main disadvantages of conventional CW systems. The next step for the technology is now to be tested in pilot-scale and if successful, in a further step to be applied in full-scale. In order for that to succeed several challenges have to be addressed, regarding economic and environmental factors such as required materials, especially in the case of electrodes, as well as operation and maintenance because of the more complex system components and design as compared to conventional CWs. Ideally, niches for the use of CW-MEC and CW-MFC+ would be found, in which the systems are able to offer unique traits which favors their use over that of other nature-based or technical solutions.

Finally, also potential explanations on why these systems were improving the treatment could be given by the help of microbial activity and microbial community characterization techniques. Last but not least the presented investigations and results also succeeded in leading to a multitude of further questions and challenges which can be addressed and hopefully be solved by future research.

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## Acknowledgements

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This PhD project was a truly great journey, with many highs, and also a few lows, and I want to thank everyone from the bottom of my heart who was involved and supported its realization. It would not have been possible without you.

First of all, I would like to say a big thank you to my main supervisor at UPC Barcelona, Dr. Jaume Puigagut, for his continuous support and for sharing his comprehensive knowledge. I really enjoyed our long talks, and I am very glad that again and again he was able to reassure me, remembering me that the force is with me, when most needed. I could not have wished for a better mentor.

Many thanks to my co-supervisor at UPC Barcelona, Dr. Marianna (Anna) Garfí, for her guidance, wisdom and support, in particular when realizing our technical and educational outreach project in Peru, and also when navigating through the bewildering bureaucracy associated with a joint PhD programme.

My sincere thanks go to my main supervisor at Ghent University, Prof. Dr. ir. Diederik Rousseau, for his continuous advice and providing thorough feedback at all times. I am especially thankful for his support and guidance for the outreach and dissemination project in Peru, and sharing his great knowledge when co-writing a follow-up project proposal.

I would also like to thank my co-supervisor at Ghent University, Prof. Dr. ir. Gijs du Laing, for his constant support and sharing his enormous knowledge during my stay at Ghent University. I am particularly thankful for his dedication as coordinator of the SuPER-W European joint doctorate programme and innovative training network.

Of course the project would not have been possible in the first place without a financial basis, therefore I am deeply thankful and honored to have received generous funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 676070. In this regard I would also like to thank the Global Minds Fund from the Federal Government of Belgium, and the *Convocatòria Ajuts* 2018 funding by the *Centre de Cooperació per al Desenvolupament* (CCD), UPC for their generous support of the outreach and dissemination project in Jaén, Peru.

My sincere gratitude goes to the external reviewers of this thesis Prof. Dr. Yaqian Zhao and Prof. Dr. ir. Stijn Van Hulle. The latter is also part of my thesis defense jury, together with Prof. Dr. Xavier Sanchez-Vila, Ass.-Prof. Dr. Sebastià Puig Broch, Dr. Marc Viñas and Dr. Clara Corbella Vidal, to whom I extend my sincere gratitude as well.

Special thanks go to all the collaborating research institutes and magnificent co-authors who greatly enriched this project's publications and research, including Dr. Kela P. Weber and Dr. Vincent Gagnon (both Royal Military College of Canada), Dr. Victor Matamoros (CSIC, Barcelona), Dr. María Jesús García-Galán (GEMMA, UPC), Diego F. Bedoya-Ríos (Pontificia Universidad Javeriana, Colombia) and of course my predecessor at GEMMA, Dr. Clara Corbella who accomplished groundbreaking work in the field of CW-MFCs.

Furthermore I am very grateful to my home institution, the Group of Environmental Engineering and Microbiology (GEMMA) at UPC Barcelona for providing me a second home. Thanks to Joan, Ivet, Chus, Enrica, and Ruben for their perpetual support, for the long hours in the lab with Javi, the sewer excursions with Eduardo, and the immense help of Eva with all the bureaucracy. It was always great fun with my PhD colleagues Dulce, Larissa, Laura, Marta, David, Diego, Clara, Maria, Alessandro, Estel, Toni, Vojtech, Simone and Lucas. During my time there I was immensely lucky to work with many motivated and brilliant Bachelor and Master students, which were an integral part of this project. So thanks to Fabio, Ivan, Cristina, Alberto, Carlos, Katerina, Joaquin, Antoine, Ramon, Vladimir and also Felipe. I want to especially thank Marta Fernández-Gatell for her great devotion to the project and her continued commitment and support, even after successfully finishing her Master degree and becoming a PhD colleague, as well as Manuel Barbero del Río for his long-term support of the project and especially his remarkable technical skills and hands-on approach which greatly benefitted this work.

I am also very thankful to the Ecochem research group at Ghent University for their warm welcome and support during my secondment stay and investigation there. It was a great experience, with fantastic people, and I especially enjoyed all the social activities of the group, including infamous football tournaments, board game nights and pub quizzes, always accompanied with delicious Belgian beer. Also my sincere gratitude for the support during my experiments by



several brilliant minds from UGent's Center for Microbial Ecology and Technology (CMET).

In regards to the outreach and dissemination project in Peru, special thanks go to Aura Vidal Carrasco and Laura Lucio (NGO Engineers without Borders Catalunya), Mirtha Isabel Villanueva Cotrina (NGO GRUFIDES, Cajamarca, Peru), the Peruvian regional government initiative UGEL, the extremely hospitable family Don Sinclair and the impressive and brave women and man of the *Committees monitoreo y Vigilancia Ambiental* (CVA) who are literally fighting for their environment and freedom.

Many thanks also to my internship company Amphos 21, for their input, guidance and providing me with a great work environment.

One of the greatest aspects of the SuPER-W PhD programme and training network was meeting the PhDs from the other projects twice a year. A big thanks to all the involved professors, coordinators and partners, and especially the program manager Dr. ir. Han Vervaeren. It was an amazing group with fantastic people and passionate discussions. Many of them have become dear friends and continue to be a great source of joy and support. Luckily one of them, Larissa Terumi Arashiro, shared my home and host university, proving to be a great friend and extremely helpful colleague throughout the whole journey.

Last but not least I want to thank my family and friends, for always being there for me. You are the biggest joy and bedrock of my life. I dedicate this work to my wonderful niece and all future generations to come, hoping to have made a small contribution to a change in our mindsets and actions, for a world in which the environment does not need protection from humans anymore.

*“We are faced with an absolute choice: We can build the sort of cities we are building, continue to accumulate resources and power to run around like blowflies in cars, and be killed before long. Or we can live easily on the earth. It's possible for us to construct biological systems that work, it's well within our capacity.”*

Bill Mollison, 1928-2016



# Curriculum Vitae

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## Personal Information

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Name	Marco Hartl
Date & place of birth	7th of June 1987, Schladming, Austria
Citizenship	Austria
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E-Mail	<a href="mailto:hartlmarco@gmail.com">hartlmarco@gmail.com</a>

## Academic background

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<p><b>PhD</b> 10/2016 – present</p>	<p><b>Universitat Politècnica de Catalunya. BarcelonaTech, Spain (UPC) &amp; Ghent University, Belgium (UGent)</b> European joint doctorate programme “Sustainable Product, Energy and Resource Recovery from Wastewater” (SuPER-W). Innovative Training Networks (ITN) - Marie Skłodowska-Curie Actions. Thesis: <i>“Constructed wetlands operated as bioelectrochemical systems for improvement and control of wastewater treatment”</i></p>
<p><b>MSc</b> 10/2011 – 03/2014</p>	<p><b>University of Natural Resources and Life Sciences, Vienna (BOKU) &amp; Lincoln University, New Zealand (LU)</b> International Joint Master (whole program in English): Natural Resources Management and Ecological Engineering (NARMEE) Thesis: „Performance of a two-stage constructed wetland in the alpine region of Austria under peak loads”</p>
<p><b>BSc</b> 10/2007 – 07/2011</p>	<p><b>University of Natural Resources and Life Sciences, Vienna (BOKU)</b> Bachelor program: Environment and Bio-Resources Management Thesis: <i>“Pathways and data availability of selected water-relevant substances”</i> (in German)</p>

**Selected Professional Experience**

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03/2016 – 08/2016	<p><b>BIOTOP Landscape Design</b> Ecological Engineer, full-time, Vienna Consultation of international partners on ecological, hydraulic and technical matters regarding BIOTOP (chlorine- and chemical-free) swimming ponds and natural pools, as well as;</p> <ul style="list-style-type: none"><li>• Project planning and drawing (AutoCAD 2D)</li><li>• Analysis of water and substrate samples with following interpretation</li><li>• R&amp;D, e.g. column filtration tests for water quality improvement</li></ul>
11/2014 – 02/2016 <b>Centre</b>	<p><b>BAUER Constructed Wetland Competence</b> Wetland Engineer, full-time, Sultanate of Oman Project and research coordinator investigating effects of polymer (HPAM) contaminated produced water on five different wetland plant species in a pilot-scale surface flow treatment wetland system. In general:</p> <ul style="list-style-type: none"><li>• Supervision of final construction works at the research facility</li><li>• Coordination and planning of operation and monitoring</li><li>• Sampling, laboratory analysis and data preparation as well as reporting to the BAUER management and the project's client</li><li>• General environmental monitoring at the Nimr water treatment plant's large-scale surface flow constructed wetland (area of 350ha)</li></ul>

**Languages**

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German	Native language
English	Fluent
Spanish	Good command

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**List of Publications**

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**Articles published in indexed journals**

**Hartl M.**, Bedoya-Ríos D.F., Fernández Gatell M., Rousseau D.P.L., Du Laing G., Garfí M., Puigagut J. (2019) Contaminants removal and bacterial activity enhancement along the flow path of constructed wetland microbial fuel cells. *Science of the Total Environment*. <https://doi.org/10.1016/j.scitotenv.2018.10.234>.

Corbella C., **Hartl M.**, Fernández Gatell M., Puigagut J. (2019) Novel membraneless MFC-based biosensor for domestic wastewater COD assessment in constructed wetlands. *Science of the Total Environment*. <https://doi.org/10.1016/j.scitotenv.2018.12.347>

**Articles submitted or in preparation for indexed journal**

**Hartl M.**, García-Galán M. J., Matamoros V., Fernández Gatell M., Rousseau D.P.L., Du Laing G., Garfí M., Puigagut J. (submitted) Constructed wetlands operated as bioelectrochemical systems for the removal of organic micropollutants. *Science of the Total Environment*.

**Hartl M.**, Fernández Gatell M., Gagnon V., Weber K.P., Rousseau D.P.L., Du Laing G., Garfí M., Puigagut J. (in preparation) Urban wastewater treatment efficiency and microbial community analysis in constructed wetlands operated as bioelectrochemical systems.

**Other publications**

**Hartl M.**, Hogan J., Ioannidou V. (2018) Treatment of effluents from meat, vegetable and soft drinks processing using Constructed Wetlands. In: A.I. Stefanakis, editor, *Constructed Wetlands for industrial wastewater treatment*. John Wiley & Sons, Inc. ISBN: 978-1-119-26834-5

**Internet publication without peer review process**

**Hartl M.** (2014) Performance of a two-stage constructed wetland in the alpine region of Austria under peak loads. Master thesis. *University of Natural Resources and Life Sciences (BOKU), Vienna*.

## Contributions to conferences

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### Oral presentations (presenter is underlined)

Hartl M., García M.J., Matamoros V. Fernández-Gatell M., Rousseau D.P.L., Du Laing G., Garfí M., Puigagut J. (2019) Emerging contaminants removal in constructed wetlands operated as bio-electrochemical systems. *The 8th International Symposium for Wetland Pollutant Dynamics and Control (WETPOL) 2019*. 17-21 Juni 2019, Aarhus, Denmark.

Barbero, M., Garfí, M., Rousseau, D.P., Du Laing, G., Díez-Montero, R., Hartl, M., Puigagut, J. (2019). Novel technique for the hydraulic characterization of horizontal subsurface-flow constructed wetlands based on the electrical signal by microbial fuel cells. 8th International Symposium for Wetland Pollutant Dynamics and Control, June 17-21. Aarhus, Denmark.

Fernández-Gatell M., Hartl M., M., Puigagut J. (2018) Bacterial activity and biomass assessment in constructed wetlands based on the electrical signal of microbial fuel cells. *The 8th International Symposium for Wetland Pollutant Dynamics and Control (WETPOL) 2019*. 17-21 Juni 2019, Aarhus, Denmark.

Arashiro L., Hartl M. (both first author and presenter together), Gonzalez L.L., Ceron Chafía P., Ramírez Morales J.E., Kehrein P., Van Hulle S.W.H., Garfí M., Rousseau D.P.L. (2019) Educational Outreach On Water And Sanitation In Rural Communities In Jaén Peru. *9th International Young Water Professionals Conference*. 23-27 June 2019, Toronto, Canada.

Hartl M., Fernández-Gatell M., Rousseau D.P.L., Du Laing G., Garfí M., Puigagut J. (2018) Constructed wetlands operated as bioelectrochemical systems for treatment of urban wastewater. *16th IWA International Conference on Wetland Systems for Pollution Control*. 1-4 October 2018, Valencia, Spain.

Bedoya-Rios, D., Fernandez, M., Hartl, M., Rousseau, D., Du Laing, G., Garfí, M. and Puigagut, J. (2018) Bio-electrochemical Systems Implemented In Constructed Wetlands Enhance Heterotrophic Bacterial Activity. *16th IWA International Conference on Wetland Systems for Pollution Control*. 1-4 October 2018, Valencia, Spain.

Hartl M., Muccioli F., Genovese I., Garfí M., Puigagut J. (2017) Real-time water quality assessment with constructed wetland microbial fuel cells. *The 7th International Symposium for Wetland Pollutant Dynamics and Control (WETPOL) 2017*. 21-25 August 2017, Big Sky, USA.

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### Poster presentations

Hartl M., Garfí M., Puigagut J., Rousseau D.P.L., Du Laing G. (2019)  
(Bio-)electrochemical technologies for resource recovery and plant anagement in wastewater treatment wetland systems. 3rd IWA Resource Recovery Conference. 8-12 September 2019, Venice, Italy.

Hartl M., Muccioli F., Genovese I., Garfí M., Puigagut J. (2017)  
Preliminary results on organic matter and ammonia removal enhancement with constructed wetland microbial fuel cells. *The 7th International Symposium for Wetland Pollutant Dynamics and Control (WETPOL) 2017*. 21-25 August 2017, Big Sky, USA.

### Participation in research projects

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03/2016 – 02/2020	<p><b>Sustainable Product, Energy and Resource Recovery from Wastewater (SuPER-W) (676070)</b> Funded by the European Commission H2020-MSCA-ITN-2015 (European Joint Doctorates)</p>
01/2018 – 12/2018	<p><b>Exploring the performance of the wastewater treatment system and its optimization using environmental technologies in the city of Jaén, Peru: Technical and Educational approaches</b> Funded by the Federal Government of Belgium (through VLIR-UOS)</p>
05/2018 – 02/2020	<p><b>Improving sustainable sanitation and energy access in rural areas of Peru and Colombia: constructed wetlands and small-scale digesters. (CCD-2018U003)</b> Funded by CCD-UPC</p>

### Tutoring experience

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- 3 Master students (1 year thesis; Environmental Engineering, UPC)
- 3 Bachelor students (6 month thesis; Biosystems Engineering or Civil Engineering, UPC)
- 7 Visiting students (>3 months visit)

**Teaching experience**

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03/2019	<b>Chemical analysis - Ion selective electrodes.</b> Practical Exercises. Ghent University, Belgium
02/2018	<b>Hydraulics - Filtration in granular media.</b> Practical Exercises. UPC Barcelona, Spain

**Workshop organization**

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10/2017	<b>Workshop on Modelling Tools.</b> UPC Barcelona, Spain
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