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TUM School of Life Sciences

# **Benchmark and strategy development for microbial fuel cells in industrial wastewater treatment**

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### Conference contributions

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Beer, I.; Brunschweiler, S.; Glas, K.; Elsner, M.; Ivleva, N.P. Characterization of Biofilms Used in Microbial Fuel Cells with Raman- and Scanning Electron Microscopy-based Techniques. **2021**. Wasser, Gesellschaft deutscher Chemiker, online.

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Brunschweiler, S.; Kranich, V.; Hofmann, T.; Glas, K. Einsatz von mikrobiellen Brennstoffzellen zur Abwasserbehandlung. **2021**. VLB-Jahrestagung, online.

## Abstract (English)

The chemical energy hidden in wastewater can be extracted, turning an energy-intensive treatment process into an energy-independent one. However, conventional aerobic treatment is very energy intensive, and anaerobic treatment requires a post-treatment step to meet stringent discharge requirements. Therefore, Microbial Fuel Cells (MFCs) have attracted a lot of attention because of their ability to extract electrical energy directly from wastewater during the treatment process. Since combustion losses can be avoided, theoretically the greatest energy value can be obtained from the organic load. However, most MFC research is currently still taking place on a laboratory scale in the treatment of synthetic or municipal wastewater. Commercialization of MFC technology will require large-scale plants, for which a suitable MFC design and operation concepts must first be identified, since neither configuration nor operating system has been clearly established yet. In addition, no specific concepts and application fields currently exist for the treatment of industrial wastewater.

Consequently, with regard to MFCs in industrial wastewater treatment, the aim of this work is to develop a benchmark that serves for modeling the required overall efficiency of MFCs and to identify the most relevant key factors in order to derive enhancement strategies. By providing an overview of current MFCs in industrial wastewater treatment and developing a benchmark, the targets for long-term operation of MFCs can be established allowing critical factors for design and operation to be identified. The resulting enhancement strategies were validated and the overall evaluation with the developed benchmark allowed an assessment regarding the commercialization potential.

Compared to the first MFC design (MFC 1.0), the enhanced MFC design (MFC 2.0) increased the power density by a factor of up to 11 and extended the long-term stability to one year by increasing the specific cathode surface area and reducing the electrode spacing in conjunction with avoiding fiber clogging on the anode side. In addition to using beneficial brewery wastewater with high content of easily degradable organic acids and high conductivity, the performance of the MFC was further stabilized and improved by changing the operating mode to continuous operation and reducing the hydraulic retention time to 6 h, resulting in a mean organic removal rate of  $6.5 \pm 1.9 \text{ kg}/(\text{m}^3 \cdot \text{d})$ . Although the overall energy efficiency is low compared to anaerobic treatment, the enormous wastewater treatment potential forms the basis for MFCs to become an alternative to conventional treatment technologies if self-sufficient treatment is targeted. Due to the wide range of operating conditions and the modularity of stack systems, MFCs can become a promising option especially for industrial wastewater treatment.

# Abstract (Deutsch)

Aus einer energieintensiven Abwasserreinigung kann ein energieunabhängiger Prozess werden, wenn die im Abwasser verborgene chemische Energie extrahiert wird. Allerdings ist die konventionelle aerobe Behandlung sehr energieintensiv und die anaerobe Behandlung erfordert einen Nachbehandlungsschritt, um die strengen Einleitungsanforderungen zu erfüllen. Daher haben mikrobielle Brennstoffzellen (MBZs) viel Aufmerksamkeit auf sich gezogen, da sie in der Lage sind, während des Reinigungsprozesses direkt elektrische Energie aus dem Abwasser zu gewinnen. Damit kann theoretisch der größte Energiewert aus der organischen Fracht gewonnen werden, da Verbrennungsverluste vermieden werden können. Jedoch finden die meisten MBZ-Forschungen derzeit noch im Labormaßstab mit der Aufbereitung von synthetischem oder kommunalem Abwasser statt. Für die Kommerzialisierung der MBZ-Technologie sind Großanlagen erforderlich, für die zunächst ein geeignetes MBZ-Design und ein geeignetes Betriebskonzept ermittelt werden muss, da sich bisher weder eine Konfiguration noch Betriebssystem eindeutig durchgesetzt hat. Außerdem gibt es noch keine konkreten Konzepte und Anwendungsbereiche für die Behandlung von Industrieabwasser.

Ziel dieser Arbeit ist es daher, im Hinblick auf MBZs in der industriellen Abwasserreinigung eine Benchmark als Basis zur Modellierung der erforderlichen Gesamteffizienz von MBZs zu entwickeln und die wichtigsten Schlüsselfaktoren zu identifizieren, um daraus Verbesserungsstrategien abzuleiten. Durch die Zusammenstellung von aktuellen MBZ-Studien in der industriellen Abwasserreinigung und die Entwicklung einer Benchmark können die Ziele für den langfristigen Betrieb von MFCs zur Identifizierung von kritischen Faktoren für Design und Betrieb, festgelegt werden. Die daraus resultierenden Verbesserungsstrategien wurden validiert und die Gesamtbewertung mit der entwickelten Benchmark ermöglichte eine Einschätzung des Kommerzialisierungspotenzials.

Im Vergleich zum ersten MBZ-Design (MBZ 1.0) erhöhte verbessertem MBZ-Design (MBZ 2.0) die Leistungsdichte um einen Faktor von bis zu 11 und verlängerte die Langzeitstabilität auf ein Jahr, indem die spezifische Kathodenoberfläche vergrößert und der Elektrodenabstand verringert wurde, während gleichzeitig das Verstopfen der Bürstfasern anodenseitig vermieden werden konnte. Zusätzlich zur Verwendung von vorteilhaftem Brauereiabwasser mit hohem Gehalt an leicht abbaubaren organischen Säuren und hoher Leitfähigkeit, wurde die Leistung der MBZ weiter stabilisiert und verbessert, indem die Betriebsart auf Dauerbetrieb umgestellt und die hydraulische Verweilzeit auf 6 h reduziert wurde. Als Ergebnis wurde eine durchschnittliche organischen Entfernungsrates von  $6.5 \pm 1.9 \text{ kg}/(\text{m}^3 \cdot \text{d})$  erzielt. Obwohl die Gesamtenergieeffizienz im Vergleich zur Benchmark der anaeroben Behandlung gering ist, bildet das enorme Abwasserbehandlungspotenzial die Grundvoraussetzung dafür, dass MBZs eine Alternative zu konventionellen Behandlungstechnologien werden können, sofern eine autarke Aufbereitung angestrebt wird. Aufgrund des breiten Spektrums an Betriebsbedingungen und der Modularität von MBZ-Stack-Systemen können MBZs eine vielversprechende Option insbesondere für die industrielle Abwasserreinigung werden.



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# Abbreviations and Symbols

## Abbreviations

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Parameter	Description
AC	Alternating current
<i>BOD</i> <sub>5</sub>	Biochemical oxygen demand
COD	Chemical oxygen demand
CPE	Constant phase element
DC	Direct current
DC-MFC	Dual-chamber microbial fuel cell
DOC	Dissolved organic carbon
EAM	Electroactive microorganisms
ECM	Electrical circuit model
EET	Extracellular electron transfer
EIS	Electrochemical impedance spectroscopy
EMF	Electromotive force
GDL	Gas diffusion layer
LSV	Linear sweep voltammetry
MCA	Membrane cathode assembly
MET	Microbial electrochemical technology
MFC	Microbial fuel cell
MPP	Maximum power point
NER	Normalized energy recovery
OCV	Open circuit voltage
PEM	Proton exchange membrane
SC-MFC	Single-chamber microbial fuel cell
SHE	Standard hydrogen electrode
SWOT	Strengths, weaknesses, opportunities and threats
TEA	Terminal electron acceptor
TIN	Total inorganic nitrogen
TKN	Total Kjeldahl nitrogen
TOC	Total organic carbon
TSS	Total suspended solids
VCR	Varying circuit resistance

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## Symbols and model parameter

Parameter	Unit	Description
$\eta_{act}$	-	activation losses
$\eta_{conc}$	-	concentration losses
$\eta_{MFC}$	%	overall efficiency of MFCs
$\eta_{ohmic}$	-	ohmic losses
$A_{cat/an}$	$m^2$	surface area of the anode or cathode
b	-	number of electrons
CE	%	coulombic efficiency
$COD_0$	$g/L$	COD concentration in the beginning
$COD_t$	$g/L$	COD concentration after time t
$\Delta G$	$kJ/mol$	standard Gibbs free energy
$E_{emf}$	V	electromotive force
$E_{MFC}$	V	voltage of MFC
$\Delta E^0$	V	standard cell voltage
$\Delta E_{anode}$	V	anode potential
$\Delta E_{cathode}$	V	cathode potential
F	$C/mol$	Faraday's constant (95485.33)
$\Delta COD$	%	COD treatment efficiency
HRT	$h$	hydraulic retention time
I	A	produced current
$L_{cable}$	-	inductance attributed to cable connections
M	$g/mol$	molar mass
n	-	stoichiometry factor of redox reaction
$NER_{kgCOD}$	$kWh/kgCOD$	normalized energy recovery normalized to the degraded COD concentration
$NER_{vol}$	$kWh/m^3$	normalized energy recovery normalized to treated volume
OLR	$kg/(m^3 \cdot d)$	organic loading rate
ORR	$kg/(m^3 \cdot d)$	organic removal rate
P	kW	electrical power output
$P_{cat/an}$	$W/m^2$	power density normalized to the anode or cathode surface area
$P_{vol}$	$W/m^3$	power density normalized to the volume
$R_{conc}$	$\Omega$	resistance due to concentration losses
$R_{ct}$	$\Omega$	charge transfer resistance
$R_{ext}$	$\Omega$	external resistance
$R_{int}$	$\Omega$	internal resistance
$R_{ohmic}$	$\Omega$	ohmic resistance
VE	%	voltage efficiency
$V_{MFC}$	L	liquid volume of the MFC
$Z_{W,an}$	-	Warburg element assigned to the anode

# 1 Introduction

Renewable energy sources, efficient industrial processes and the recovery of energy and chemicals from waste are currently important topics in research and industry (Molognoni et al. 2017). In order to enhance the self-sufficiency of wastewater treatment processes, the hidden chemical energy in wastewater can be extracted so that an energy-consuming process can be converted into an energy-independent process (Gude 2016). In particular, industrial wastewater, which is characterized by a high organic content with Chemical Oxygen Demand (COD) concentrations of 1 to 200 g/L, offers a high energy potential (Hamza et al. 2016). Anaerobic treatment of wastewater can already generate energy in the form of biogas, which shows that self-sufficient wastewater treatment with biological treatment technologies is technically possible (Stiefel (2017) p.156 ff). In addition to the energy aspects of wastewater treatment, reuse of wastewater is already possible alongside end-of-pipe solutions. Even the economic viability of resource recovery could already be demonstrated in the food and beverage sector, saving fresh water and discharge costs (Verhuelsdonk et al. 2021).

However, conventional biological wastewater treatment technologies are limited in several aspects, as aerobic treatment systems are energy-intensive and anaerobic treatment systems do not sufficiently exploit the energy potential of wastewater (He et al. 2017). In addition, the stringent regulatory discharge requirements cannot be met solely by anaerobic treatment, making post-treatment unavoidable (He et al. 2017).

Microbial fuel cells (MFCs) have attracted considerable attention due to their ability to generate electrical energy directly from wastewater during the treatment process (Saratale et al. 2017; Chen et al. 2019). A comparison of the calculated net energy output from wastewater by different options (methane, hydrogen, ethanol, electricity) indicates the potential for MFCs to capture the greatest energy value from COD because combustion losses can be avoided (Rittmann 2006). Together with the advantages of lower sludge production compared to aerobic treatment or insensitivity to the operating environment compared to anaerobic treatment, direct conversion of substrate energy to electricity makes further development of MFC technology attractive (He et al. 2017). In addition, the combination with anaerobic and aerobic treatment and the possibility of wastewater reuse can provide further application options. In particular, the highest performance of MFCs can be expected under fixed conditions, making industrial wastewater (e.g., from the food and beverage industry) with constant composition technologically interesting (Koch et al. 2019). From an economic point of view, the company's own wastewater treatment plant can save discharge and freshwater costs.

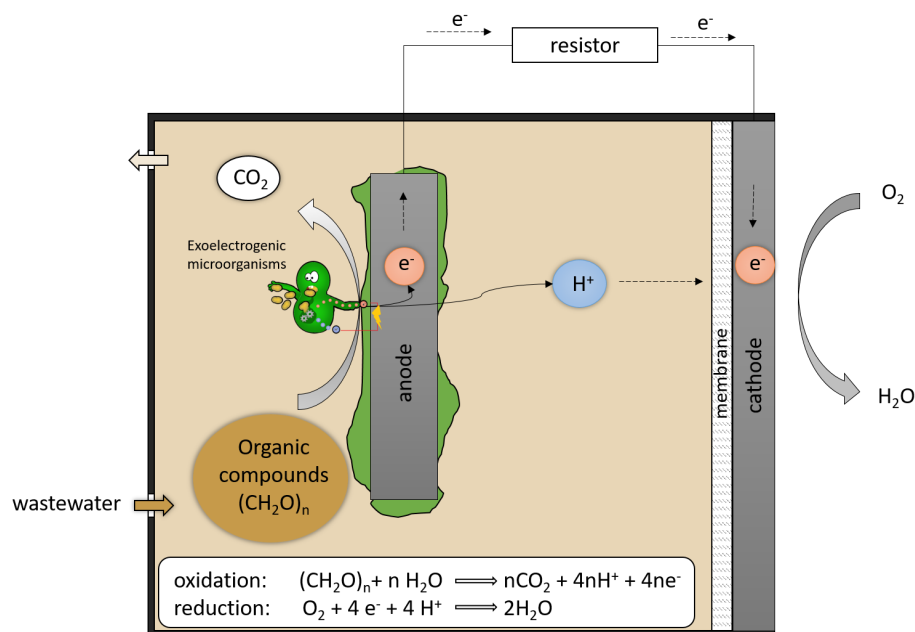
Nevertheless, most MFC research currently takes place at the laboratory scale, often treating synthetic or municipal wastewater. Commercialization of MFC technology requires large-scale systems, in which influence parameters in design, components, and operation are critical. In addition, key components in industrial wastewater must be identified to maximize MFC performance in terms of energy and treatment efficiency. Therefore, based

on the principles of MFC technology, an overview of potential MFC designs, materials, and process management systems are provided subsequently. The electrochemical characterization, described in the following section, allows a classification in the MFC research field and the definition and key figures must be calculated to evaluate the application potential within conventional wastewater treatment technology.

## 1.1 Principles of Microbial Fuel Cells

In general, microbial electrochemical technologies (MET) are gaining attention due to their dual function of wastewater treatment and energy recovery from wastewater (Chen et al. 2019). METs use microorganisms that biologically oxidize organic compounds and transfer the generated electrons to the anode (Logan et al. 2006). These microorganisms can transfer electrons outside of cell membranes as part of their respiratory process, which is called extracellular electron transfer (EET), and therefore are often termed exoelectrogenic bacteria or electroactive microorganisms (EAM) (Logan 2009a; Chen et al. 2019). Microbial fuel cells (MFCs) are one of various METs (e.g., microbial electrolysis cells, microbial electrosynthesis) that utilize this interaction between microorganisms and an electrode (Schröder et al. 2015).

In MFCs for wastewater treatment, oxidation of organic compounds commonly takes place in the anode chamber, which is combined with oxygen reduction at the air cathode, as schematically shown in **Figure 1.1**. Through the proton exchange membrane (PEM) and through an external circuit, the released protons ( $H^+$ ) and electrons ( $e^-$ ) of the oxidation process can reach the cathode, enabling the reduction of oxygen to water at the cathode.



**Figure 1.1:** Schematic representation of a single-chamber microbial fuel cell (adapted from Brunschweiler et al. (2020a))

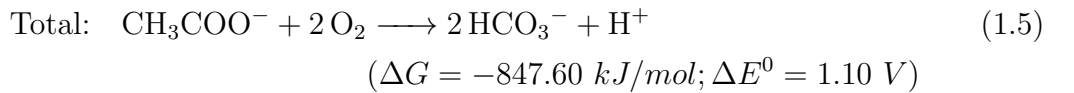
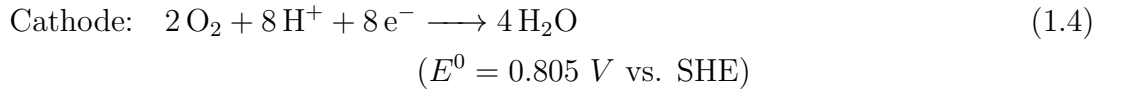
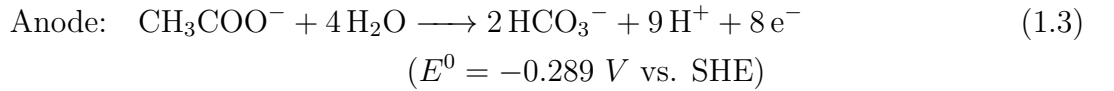
The electron transport between the anaerobic anode and the aerobic cathode is determined by the electromotive force ( $E_{emf}$ ) or standard cell voltage ( $\Delta E^0$ ) of the system, which depends on the redox potential of the involved couples (Rozendal et al. 2008).  $\Delta E^0$  can be calculated using the Gibbs free energy ( $\Delta G$ ), which measures the maximum amount of useful work that can be obtained from a reaction of a thermodynamic system (Gude 2016). In **Equation 1.1**,  $n$  represents the stoichiometry factors of the redox reaction and  $F$  represents the Faraday's constant (95,485.33 C/mol).

$$\Delta E^0 = -\frac{\Delta G}{n \cdot F} \quad (1.1)$$

The capability of electricity generation is determined by the  $\Delta E^0$  of the overall reaction, which can be calculated by the difference of the anode and cathode potential (**Eq. 1.2**) (Gude 2016):

$$\Delta E_{total}^0 = \Delta E_{cathode} - \Delta E_{anode} \quad (1.2)$$

Thus, during energy generation in MFCs, electrons produced by EAMs through the oxidation of a substrate (e.g., acetate) at low potential flow through the electron transport chain to the final electron acceptor (e.g., oxygen) at higher potential (Logan et al. 2006). For the acetate-oxygen couple, the  $\Delta E^0$  and  $\Delta G$  can be calculated with (**Equations 1.3-1.5**) (Rozendal et al. 2008).



The Gibbs free energy  $\Delta G$  of the reaction in MFCs is negative, resulting in a positive  $\Delta E^0$ , which indicates electricity can be generated from the reaction (Rozendal et al. 2008). Thus, a high difference between redox potentials leads to a high theoretical potential. Wastewater consists of a variety of organic compounds that can be oxidized and determine the anodic potential. In addition, the reduction of oxygen on the cathode side is most suitable when an air cathode with passive oxygen diffusion is chosen because of its high oxidation potential and availability, as well as low energy requirements. However, heavy metal ions, nitrate and sulfate can also act as terminal electron acceptors (TEA) and can even be removed in MFCs (Chen et al. 2019).

The EET mechanisms of EAMs during oxidative substrate degradation at the anode can be divided into three currently known pathways. Direct EET can occur (1) via outer membrane cytochromes or membrane-bound redox enzymes or (2) via generated conducting pili or pilus-like structures. Some EAMs can also secrete (3) redox-active molecules

(e.g., phenazines, flavins, and quinones) through which electrons can be transferred to an external acceptor, termed indirect EET. (Marsili and Zhang 2010; Koch and Harnisch 2016; Kumar et al. 2017; Winaikij et al. 2018)

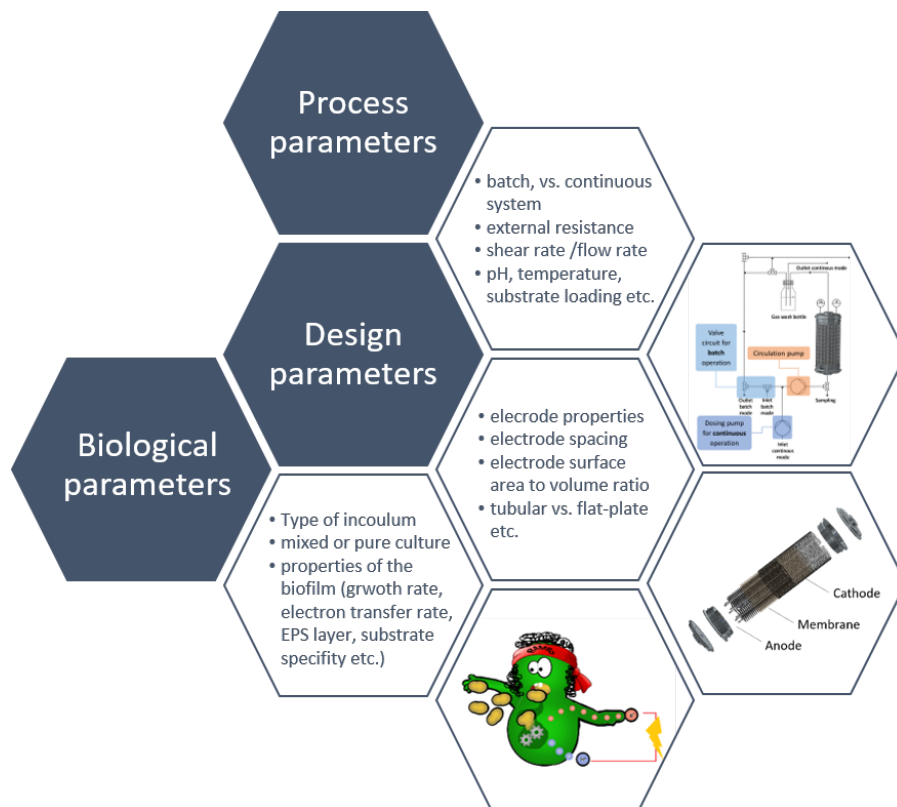
Currently, the EET mechanism is described in detail mainly for the exoelectrogenic species of either *Geobacterceae* or *Shewanellaceae* (Koch and Harnisch 2016). Species of both families have the capability of direct EET, although indirect EET is the predominant electrode interaction for most electroactive *Shewanellaceae* (Koch and Harnisch 2016). For wastewater treatment with MFCs, mixed microbial communities are usually used instead of pure cultures because they are adaptable to nutrients, resistant to stress, and reported high power output (Gezginci and Uysal 2016). In addition, inoculation with specific microorganisms is not beneficial in the long term because the microorganisms are displaced by those present in the wastewater (Madjarov et al. 2016). It has been previously reported that *Geobacter*-dominated biofilms have already been generated when using different wastewater sources for the inoculum of MFCs (Gimkiewicz and Harnisch 2013; Riedl et al. 2017). However, according to a literature survey by Koch and Harnisch (2016), 94 species have already been designated as electroactive that do not relate to one specific ecological niche. In general, not just a single genus determines the performance of MFC, rather the diversity and complexity of the microbial communities is critical, although *Geobacter spp.* may define the performance in terms of coulombic efficiency (CE) (Koch et al. 2019). Moreover, according to Koch et al. (2019) less diverse and more stable microbial communities in MFCs would perform the highest during reproducible conditions, such as in specific industrial wastewater.



## 1.2 State of the art

The number of publications on the topic of microbial fuel cell continues to grow exponentially (1301 publications in 2020, 529 publications in 2010, Scopus - November 2021). On the one hand, this shows the great interest in this technology, but on the other hand also the multitude of different aspects that have to be considered. Limiting the search to microbial fuel cell in wastewater treatment also shows an exponential increase (445 publications in 2020, 141 publications in 2010, Scopus - November 2021), suggesting that about one-third of MFC studies focus the technology on the wastewater sector.

Furthermore, in the field of MFCs for wastewater treatment, a large number of different influencing factors must be considered. One possible differentiation is the categorization into the three main parameters: Process, Design and Biology (Borole et al. 2011; Saratale et al. 2017; Gadkari et al. 2018), which is depicted in **Figure 1.2**. The main focus of this thesis is on MFC design and operating conditions that affect MFC performance. Therefore, the next step is to review the state of the art on these aspects, including design, components, and operation of MFCs. In addition, the main MFC characterization tools are summarized in order to evaluate the effects of the different influencing parameters.

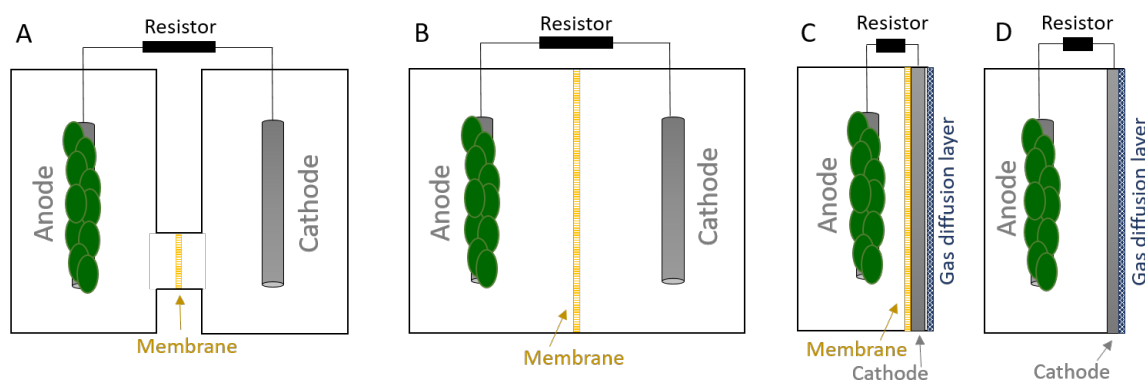


**Figure 1.2:** Important parameters influencing the MFC performance

### 1.2.1 MFC Design

Although a variety of MFC designs exist at laboratory scale, MFCs always consist of an anode and a cathode, separated in most cases by a membrane. MFC designs typically distinguish between single-chamber (SC) and dual-chamber (DC) MFCs, which can be cubic, cylindrical, H-cell, or plate and tube-shaped (Janicek et al. 2014; Cheng and Logan 2011; Zuo et al. 2007).

DC-MFCs consist of an anode and a cathode chamber separated by a membrane and are often used in H-form (**Figure 1.3A**) or in cubic form (**Figure 1.3B**). Due to the physical separation of the chambers, DC-MFCs are ideal for basic parameter testing for new substrates, inoculum or electrode materials (Sun et al. 2014). Low-cost H-type MFCs are easy to handle and stable in operation, but the small membrane area and large electrode spacing result in low performance (Oh and Logan 2006; Ahn et al. 2014), which can be improved by cubic shaped DC-MFCs. However, oxygen must be dissolved as TEA in the cathode chamber of DC-MFCs, which requires an energy input that negatively affects the energy balance of MFCs (Wang et al. 2017). The use of other TEAs such as metal ions, nitrate, or sulfate would result in lower power generation than MFCs with oxygen reduction due to lower standard potential (Lee et al. 2018), but microbial cathodes open new perspectives for DC-MFCs in wastewater treatment by simultaneously removing other nutrients (e.g., nitrogen (Liang et al. 2013)). SC-MFCs with an air cathode (**Figures 1.3C and 1.3D**) are widely used and promising for wastewater treatment (Chen et al. 2019), as typically higher power densities can be achieved due to the use of membrane cathode assemblies (MCA) with improved cathode reaction and shorter electrode spacing (Sun et al. 2014). In addition, removal of the membrane and use of cathodes with gas diffusion layer (GDL) lead to higher power densities due to further reduction in internal resistance, but oxygen diffusion into the anaerobic anodic chamber could lead to lower CE and bioelectrocatalytic activity (Liu and Logan 2004; Ahn et al. 2014). Furthermore, SC-MFCs have high potential for scale-up due to simpler design and cost savings (Goswami and Mishra 2017).



**Figure 1.3:** Schematic representation of different MFC designs: **A** H-shaped DC-MFC; **B** Cube-shaped DC-MFC; **C** SC-MFC; **D** membraneless SC-MFC (adapted from Sun et al. (2014))

Therefore, SC-MFCs in tubular or flat plate design are mainly used for scale-up (**Table 1.1**) (Janicek et al. 2014; Logan et al. 2015). Most tubular MFCs consist of a tubular anode surrounded by a separator for electrically isolating the anode from the cathode (Janicek et al. 2014). Few studies have reported on tubular designs without a separator (e.g., Cheng and Logan (2011)). Rectangular anode chambers with a separator between the anode and cathode can be termed flat plate MFCs (Janicek et al. 2014). In this context, flat-plate MFCs could also consist of bipolar plates, as known from conventional hydrogen fuel cells (e.g., Dekker et al. (2009)). Although there are advantages and disadvantages of tubular and flat MFCs (**Table 1.1**), the main criteria of both designs are the reduction of electrode spacing and the large cathode surface area per volume (Janicek et al. 2014; Logan et al. 2015). However, for both designs already pilot scale studies treating wastewater exist, more comparable studies are needed for drawing a conclusion in design. Pilot-scale MFCs for wastewater treatment already exist for both designs, but further comparable studies are needed for a full evaluation.

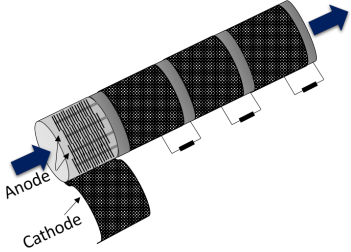
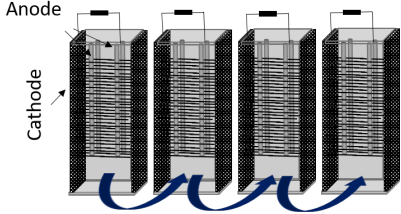
Stacking individual MFCs in series or parallel seems to be a promising choice to improve the overall system performance due to the electrochemical limitations of individual MFCs. Thus, multiple individual MFCs can be electrically connected in series to increase voltage or in parallel to improve current. Similarly, hydraulic connection in parallel or series can be applied to both tubular and flat plate MFCs. In addition, each individual MFC can consist of multiple electrodes and be assembled into stackable modules.

### 1.2.2 MFC Components

The MFC design must not only consider scalability, but the choice of individual components in particular is critical, as the position of the electrodes and the surface area affect the performance of the MFC. Therefore, several studies focus on electrode material or structure, surface modifications of electrodes, separators and their spatial arrangement (Krieg et al. 2014). A variety of carbon and metal materials and their modification have already been explored for the application as anode or cathode in MFCs. The main challenge in this context is the production of low-cost electrode materials to improve the commercialization potential of large-scale MFCs for wastewater treatment (Wei et al. 2011).

Suitable **anode materials** not only need to be cost effective and scalable, but also meet the requirements of high electrical conductivity, biocompatibility, chemical and mechanical stability and durability, and most importantly provide a large specific and electroactive surface area for biofilm attachment (Kumar et al. 2013; Guo et al. 2015). These criteria are not fully met by either carbon-based or metal-based materials, so a combination of them and surface modification strategies could lead to attractive electrode materials (Guo et al. 2015). Depending on the objective of an MFC study, planar (e.g., carbon plate, carbon rod, stainless steel mesh) or three-dimensional electrodes (e.g., foam, felt, granular activated carbon, carbon fiber brush) can be selected. Plane electrodes with defined surface area and uniform surface properties are more suitable for basic research on microbe-electrode interactions, 3D electrodes provide a large specific surface area for

**Table 1.1:** Advantages and disadvantages of tubular and flat-plate scale-up designs (according to Rismani-Yazdi et al. (2008); Janicek et al. (2014); Dutta and Kundu (2018)) and pilot-scale examples

Scale-up design	Tubular	Flat-plate/rectangular				
Sketch						
Advantages	<ul style="list-style-type: none"> <li>• Tubular cathodes provide a promising architecture for scale-up</li> <li>• Maintaining optimal cross-sectional dimensions and surface-to-volume ratio during scale-up</li> <li>• Minimal dead space, providing favorable flow conditions</li> </ul>	<ul style="list-style-type: none"> <li>• Minimization of electrode spacing leads to increased ion diffusion rates and low internal resistance</li> <li>• Possible integration into existing basins</li> <li>• Easily stackable bipolar electrodes</li> </ul>				
Disadvantages	<ul style="list-style-type: none"> <li>• Often large electrode spacing</li> <li>• Difficult to integrate directly into existing basins</li> </ul>	<ul style="list-style-type: none"> <li>• Often non-optimal flow conditions</li> <li>• Close electrode spacing can limit the growth of exoelectrogenic microorganisms, potentially leading to clogging</li> </ul>				
Examples of pilot-scale MFCs for wastewater treatment	MFC stack system	Type	Reference	MFC stack system	Type	Reference
	40 x 0.25 L	SC	Zhuang et al. (2012)	5 x 18 L	SC	Dong et al. (2015)
	96 x 2 L	SC	Ge and He (2016)	50 x 20 L	DC	Liang et al. (2018)
	2 x 10 L	SC	Lu et al. (2017)	1 x 255 L	SC	Hiegemann et al. (2019)
				12 x 8 L	SC	Babanova et al. (2020)
				2 x 50 L	SC	Goto and Yoshida (2019)

electroactive biofilms, which makes them more beneficial for improving MFC performance (Wei et al. 2011; Zhou et al. 2011; Guo et al. 2015). For wastewater treatment applications, packed (e.g., granular activated carbon) and brush structures are ideal because of the large surface area for bacterial attachment, with slightly better avoidance of clogging by brush electrodes due to high porosity (Wei et al. 2011).

In particular, MFCs with carbon fiber brush anodes already achieved higher power densities than MFCs with other anode materials (e.g., graphite granules, carbon cloth) due to their large surface area (Lanas and Logan 2013). Their ease of fabrication and good scalability make them a promising anode material (Logan et al. 2007), although compared to planar electrodes pressed against a separator, their use results in larger electrode spacing and clogging of the fibers could reduce performance (Janicek et al. 2014; Brunschweiler et al. 2020b). Therefore, carbon fiber brush anodes have already been used in several MFC studies for wastewater treatment not only at laboratory scale (Hays et al. 2011; Lanas et al. 2014; Brunschweiler et al. 2020b; Littfinski et al. 2021) but also at pilot scale (Dong et al. 2015; Feng et al. 2014; Hiegemann et al. 2019).

In general, the anode materials can also be used as cathode current collectors (Santoro et al. 2017). On the **cathode side**, a distinction can be made between the reduction of oxygen by abiotic catalysts or the reduction of other ions by microorganisms. The following physically defined properties, which directly affect performance, and indirect characteristics, which take into account economic aspects, are decisive for the choice of cathodes: longevity, selectivity, thermodynamic performance, kinetic performance, price difference and costs for catalyst, periphery and process (Harnisch and Schröder 2010). Therefore, MFCs with oxygen reduction reaction on the cathode side mainly use an air cathode with platinum- or carbon-based catalyst (e.g., activated carbon). Thus, for MFC with oxygen reduction reaction on the cathode side, mainly MFCs with an air cathode with platinum-based or carbon-based (e.g., activated carbon) catalyst are used. Activated carbon with stainless steel mesh as the current collector is a widely used combination due to its low cost and satisfactory performance (Logan 2009b; Santoro et al. 2017; Wang et al. 2017). Biocatalysts used to reduce pollutants such as nitrates and their low cost are potential advantages, making them beneficial for some future applications, even if lower power densities are expected (Rismani-Yazdi et al. 2008).

The purpose of **separators or membranes** in MFCs is the physical separation of anode and cathode compartments so that crossover processes, e.g., oxygen diffusion into the anaerobic anode compartment, can be limited and short circuits can be prevented, but simultaneously ion transfer between the anode and cathode compartments can occur (Krieg et al. 2014; Hernández-Fernández et al. 2015). Therefore, a separator or membrane in an MFC should ideally meet the following requirements: low cost, low biofouling, low oxygen and substrate transfer, long-term stability, higher proton transfer (hydrogen ions) than other cations, and high rejection of anions (Dizge et al. 2019; Abdallah et al. 2019). The most commonly used material type is the proton exchange membrane (e.g., Nafion) due to its high proton conductivity, but due to its high price and the fact that specificity

is not mandatory for proton conduction in MFCs, a variety of lower cost cation and anion exchange membranes (CEM and AEM) have already been tested and membrane-less MFCs came into focus (Krieg et al. 2014; Santoro et al. 2017). In addition, various other membranes (e.g., composite, porous, ceramic, ultra- or nanofiltration membranes) and materials (e.g., J-cloth, nylon fibers, glass fibers, and ceramics) have already been investigated in METs (Santoro et al. 2017; Dizge et al. 2019). To date, no clear evidence exists for BES regarding whether AEM or CEM are generally preferable, as the results seem to depend more on the specific BES conditions (Krieg et al. 2014). By directly comparing Nafion, AEM, CEM, and UFM, Kim et al. (2007) demonstrated that many membrane types, including membranes with negatively charged species transfer, could be used, even MFCs with AEM produced the highest power density and CE in one configuration. However, it has already been shown that the membrane type also affects the dynamics of the microbial community in an MFC, especially the archaeal diversity (Sotres et al. 2014). In addition, the commercialization of MFCs focuses on material costs, so several membrane-less MFCs have been successfully operated (e.g., Dong et al. (2015); Hiegemann et al. (2019)) after Liu and Logan (2004) reported an increase in energy production after membrane removal (Abdallah et al. 2019). The effects of electrode spacing versus membrane resistance on internal resistance can be shown when directly comparing membrane-less MFCs, where the electrode spacing must be larger due to crossover processes, and MFCs with membrane cathode assemblies (MCA), where the combination of membrane and cathode can reduce the electrode spacing. The larger electrode spacing in membrane-less MFCs has a more negative effect on power density than the additional membrane resistance in MFCs with MCA due to the higher internal resistance (Zhang et al. 2014; Ahn et al. 2014). Moreover, although the CE is reduced in membrane-less MFCs because of the probable oxygen transfer, the similar COD removal can take place in shorter hydraulic retention time (HRT) (Ahn et al. 2014). Thus, the overall performance of the MFC is affected by membrane selection, with the main issues for sustainable operation being fouling, scaling, durability, low selectivity, and low ion exchange capacity (Dizge et al. 2019). The main problem of fouling of the membrane cannot be solved by using membrane-less MFCs either, since inorganic deposits on the cathode side also affect the power density (Hiegemann et al. 2019). However, costs can of course be reduced by using membrane-less MFCs.

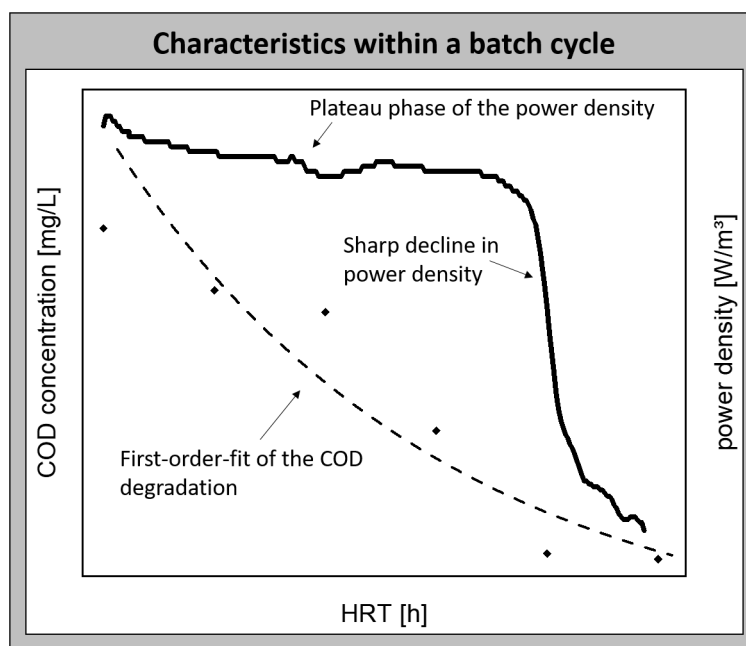
In summary, the selection and combination of MFC components limits the MFC design options. Currently, there is no single option for either the anode, cathode, or membrane, so their combination is critical depending on the MFC application. The development of even lower cost materials is essential to push the commercialization of MFCs.

### 1.2.3 MFC Operation mode

In addition to the distinction between SC- and DC-MFC, the operation mode can be distinguished between (fed-)batch or continuous operation. Fed-batch MFC systems, in contrast to continuous systems, are fed with wastewater discontinuously. Most laboratory

systems are operated in batch mode, but continuous wastewater treatment is essential for commercialization.

MFC performances may differ depending on the operating mode because the important factors affecting COD removal and power densities - organic loading rate (OLR) and flow rate, and associated HRT and shear stress - differ for the two operating modes. Together with the pH value and the temperatures, the effects of these influence factors on MFC performance have already been described in principle (Borole et al. 2011; Oliveira et al. 2013; Goswami and Mishra 2017): High OLRs result in high power density and high COD removal, but CE is reduced by the concurrent presence of methanogens. High flow rates also increase power density, but negatively impact COD removal and CE due to lower HRT. High shear rates result in thicker and denser biofilms that enhance electron transfer. Although there is a complex interplay of these influencing factors, a general relationship between power density and COD concentration within a batch or a few cycles of feed can be schematically illustrated (**Figure 1.4**). Characteristic is the combination of an exponential COD degradation within a batch cycle and a plateau phase of the power density, which decreases sharply over time.



**Figure 1.4:** Representative correlation of COD degradation efficiency and power densities within a batch cycle.

For municipal wastewater treatment, Kletke et al. (2019) determined a maximum TOC elimination rate of 50 % to achieve a constant power density, since a sharp drop in performance was observed at TOC concentrations below 50 – 75 mg/L. However, due to the discontinuously increased loading, which promotes the growth of non-electroactive microorganisms, a negative effect on CE has already been shown in fed-batch operation compared to continuous operation (Borole et al. 2009). More stable power densities and more stable COD concentrations in the effluent can be expected with continuous operation, but HRT and shear rate need to be considered.

Higher performance (Huang and Logan 2008; Ahn and Logan 2010), similar but more stable performance (Zuo et al. 2007), and lower performance (Lanas et al. 2014; Wu et al. 2016) have already been reported for continuous mode compared to fed-batch mode, so no clear trend can currently be identified. For example, in the study of Wu et al. (2016) the lower performance was attributed to the lower flow rate in continuous mode, which likely resulted in thicker biofilms and poorer mass transfer. The same contrasts were observed in the evaluation of COD removal efficiencies and CE. Higher COD removal was observed in fed-batch operation because of longer HRT and not due to the mode of operation compared to continuous operation (Ahn and Logan 2010; Lanas et al. 2014). The differences in CE and COD removal may also be the result of variations in microbial dynamics in MFCs in fed-batch and continuous operation (Pannell et al. 2016). In addition, both higher CEs (Pannell et al. 2016) and lower CEs (Lanas et al. 2014) have been reported for continuous operation compared to fed-batch MFCs. In summary, the contrary effects on power density and COD removal become apparent when evaluating the modes of operation. However, the differences in MFC performance when comparing fed-batch and continuous operation can be attributed to the influencing factors of OLR, flow rate, HRT, and shear rate. For a direct comparison of operation modes, these factors must be identical in order to analyze the effects of discontinuous and continuous operation. For this purpose, an MFC system was designed in section 4.2.



## 1.3 Characterization of MFCs

A variety of surface, analytical, spectroscopic, biochemical, and electrochemical characterization techniques are possible for the study of MFCs (Sekar and Ramasamy 2013). In this work, electrochemical evaluation and wastewater treatment performance were deployed. Although there are a variety of different MFC designs, components, and different operating conditions, key parameters can be calculated for classification of different MFC systems.

### 1.3.1 Electrochemical characterization

Information on the electrochemical properties of a single electrode or the whole MFC can be get by the use of electrochemical methods (e.g., Polarization techniques (Linear sweep voltammetry (LSV), varying circuit resistance (VCR)), Electrochemical impedance spectroscopy (EIS)), which are already used for chemical fuel cells (Zhi et al. 2014). Polarization techniques measure the change in electrode potential or MFC voltage from its equilibrium state due to a current flow (Logan et al. 2006). These changes occur due to the three characteristic overpotentials (activation, ohmic and diffusion) in electrochemical cells based on their relationship between voltage/potential and current (Zhi et al. 2014). The overpotentials can be the source of the internal resistances of an MFC and are divided into three characteristic zones in which different processes are dominant (**Figure 1.5**) (Logan et al. 2006; Chen et al. 2019; Varanasi et al. 2018):

#### Zone (1): Activation losses $\eta_{act}$ :

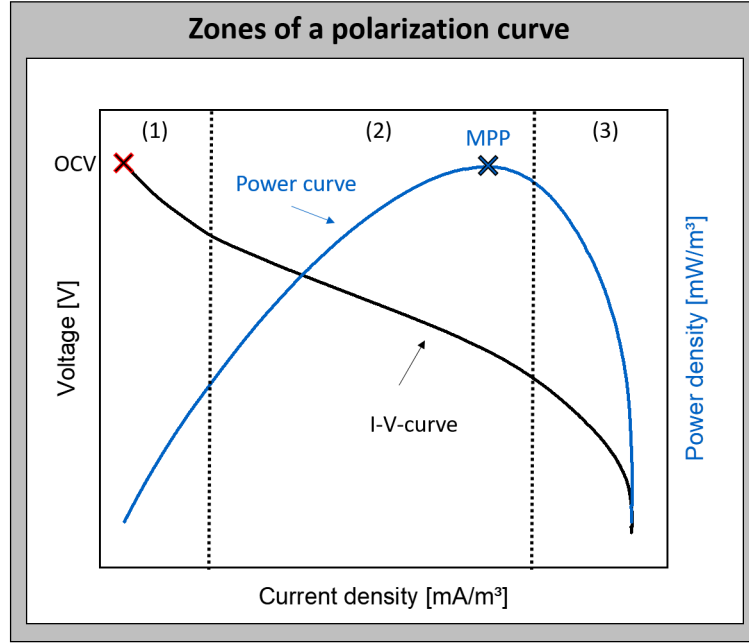
- a strong decline of the operating voltage after open circuit voltage (OCV)
- non-linear behaving resistance
- represent the energy required for activating the oxidation/reduction reactions at the electrode surface

#### Zone (2): Ohmic losses $\eta_{ohmic}$ :

- a steady decline in the voltage with an increase in the current
- linear behaving resistances
- represent the sum of resistances of electrodes, membrane, electrolyte, connections, current collectors

#### Zone (3): Concentration losses $\eta_{conc}$ :

- a rapid decline in voltage at higher currents
- non-linear rate limiting resistances
- represent the diffusion rate of the substrate or reactants and the rate of microbial metabolism



**Figure 1.5:** Polarization and power curve. (1), (2) and (3) correspond respectively to the zones in which activation, ohmic and concentration polarization losses are dominant.

In addition to the main losses, OCV and maximum power point (MPP) can be determined from the polarization curves. The OCV the maximum voltage available, which occurs at zero current (Logan et al. 2006). Theoretically, the OCV should approach the thermodynamic  $E_{emf}$  of about 1.1 V. In practice, however, the OCV is much lower (maximum 0.8 V) due to various potential losses (Logan et al. 2006). Consequently, the operational voltage output  $E_{MFC}$  can be calculated by subtracting the voltage losses according to **Equation 1.6** (Rismani-Yazdi et al. 2008).

$$E_{MFC} = E_{emf} - (\eta_{act} + \eta_{ohmic} + \eta_{conc})_{cathode} - (\eta_{act} + \eta_{ohmic} + \eta_{conc})_{anode} \quad (1.6)$$

The power curve determined from the current-voltage curve (I-V-curve) usually has a parabolic shape with a MPP (**Figure 1.5**). The MPP is reached when a balanced distribution of energy between external and internal resistances ( $R_{int} = R_{ext}$ ) and microbial metabolism per substrate turnover is achieved (Chen et al. 2019). In general, the power output  $P$  can be calculated with **Equation 1.7**, whereby the current  $I$  is determined by **Equation 1.8**. For reaching the MPP, adjustment of the power is commonly known for DC voltage source and can be mathematically reached, if the first deviation of the power output ( $\frac{dP}{dR_{ext}}$ ) is zero, where the derivation is described in the following **Equations 1.9** (Stiny (2018) p.140 f).

$$P = E_{MFC} \cdot I = I^2 \cdot R_{ext} \quad (1.7)$$

$$I = \frac{E_{MFC}}{R_{int} + R_{ext}} \quad (1.8)$$

$$\frac{dP}{dR_{ext}} = \frac{(R_{int} + R_{ext})^2 - [R_{ext} \cdot (2 \cdot R_{int} + 2 \cdot R_{ext})]}{(R_{int} + R_{ext})^4} = 0 \quad (1.9)$$

$$\Rightarrow \text{Numerator has to be zero:}$$

$$R_{int}^2 + 2R_{int}R_{ext} + R_{ext}^2 - 2R_{int}R_{ext} - 2R_{ext}^2 = 0$$

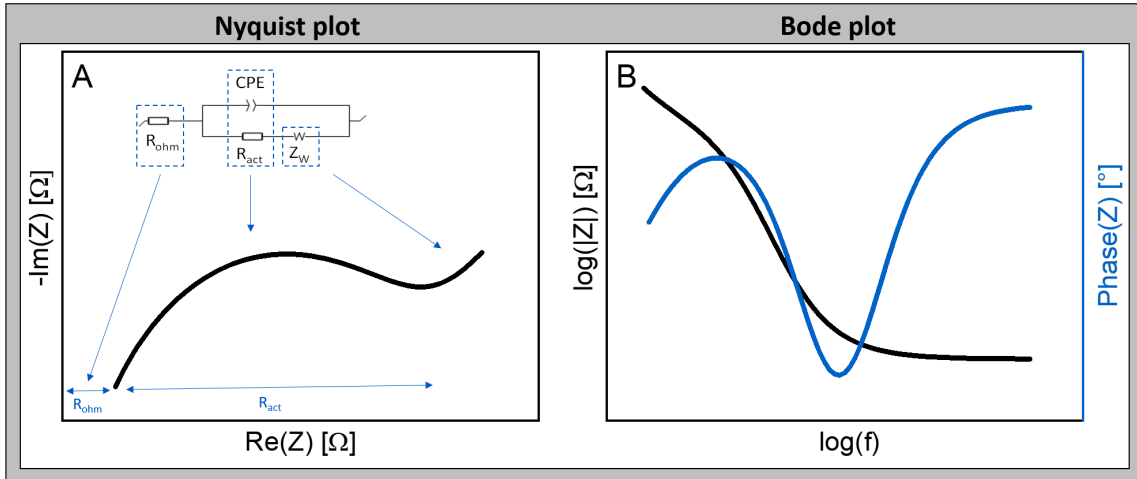
$$\Rightarrow R_{int}^2 - R_{ext}^2 = 0 \quad \Rightarrow R_{int} = R_{ext}$$

Internal resistance limits the achievable MPP of an MFC, making internal resistance a key parameter for improving MFC performance. Therefore, determining the total internal resistance and dividing it into the three main losses is essential to continuously adjust the MFC system.

Polarization curves can be generated for the entire MFC or independently for anode and cathode using a reference electrode. Either LSV is performed with a potentiostat at low sampling rates (about 1 mV/s) or a resistor box that varies the external resistance (VCR) at stable conditions can be used to record polarization curves of MFCs (Logan et al. 2006). Due to the presence of microorganisms, the duration of both measurement procedures in the form of stabilization periods for each resistance step (VCR) or in the form of low scan rates (LSV) is critical for reproducibility (Sánchez et al. 2020). The value of the internal resistance ( $R_{int}$ ) of the MFC can be calculated from the slope of the linear part of the polarization curve (Logan et al. 2006).

With polarization curves, the underlying mechanisms are often difficult to analyze due to overlapping factors for potential drop, so additional EIS measurements help to distinguish the different contributors to the total internal resistance (Kashyap et al. 2014). Polarization techniques are based on direct current (DC) measurements that have the limitations imposed by electrode polarization, which changes the concentration of electroactive species around the electrode due to charge attraction and diffusion limitations (Sánchez et al. 2020). Impedance is the frequency-dependent resistance of an element to current flow and can therefore be measured by applying sinusoidal alternating current (AC) excitation waves at various frequencies and measuring the resulting current response. The results of an impedance measurement is commonly represented in a Nyquist or Bode plot (**Figure 1.6**). In the Nyquist plot, the x-axis represents the real part (ohmic) of the impedance and the y-axis the imaginary part (capacitive, inductive) of the impedance. The applied frequency can be seen in the Bode plot, in which the absolute value of impedance and the phase angle are plotted over the logarithmic frequency (Sekar and Ramasamy 2013).

For detailed impedance analysis, an electrical circuit model (ECM) is used in which electrical components such as resistors, inductors and capacitors simulate the physical, chemical and biological processes taking place in the MET device (He and Mansfeld 2009; Sánchez et al. 2020). Ohmic losses are typically represented by a resistor, activation losses are often described as a parallel connection of ohmic resistor and capacitor (Faradaic processes), and concentration losses could be represented by a Warburg element (Yoho et al. 2015). Several different ECMs have already been proposed based on the MET design for single electrodes and whole electrochemical cells (Sekar and Ramasamy 2013; Sánchez et al. 2020). The impedance values of the individual components of the circuit can be quantified by fitting suitable ECMs representing the electrochemical structure. The ohmic resistance ( $R_{ohmic}$ ) and the charge transfer resistances of anode and cathode ( $R_{ct}$ ) corre-



**Figure 1.6:** **A** Nyquist plot with corresponding EIS and **B** Bode plot simulated with EC-LAB<sup>®</sup>.

spond to the intersection of the curve with the x-axis of the Nyquist plot (Sánchez et al. 2020). For a complete evaluation of the internal resistance,  $R_{conc}$  can be calculated using **Equation 1.10** (Wei et al. 2013b; Wu et al. 2019), with  $R_{int}$  determined by LSV measurement.

$$R_{int} = R_{ohmic} + R_{act} + R_{conc} \quad (1.10)$$

Models for whole electrochemical cells are suitable for determining the limiting factors of electrochemical systems, including the impedance of the elements present between the electrodes. Thus, targeted strategies can be developed to specifically reduce the internal resistance in order to increase the performance of MFCs.

MFCs for wastewater treatment are not only evaluated by electrochemical characterization, which has an impact on their power output. Moreover, to become a possible alternative for wastewater treatment, the possibilities and limitations of wastewater treatment efficiency must also be characterized.

### 1.3.2 Wastewater treatment performance

In general, industrial wastewater differs from municipal wastewater mainly in its higher COD concentrations (e.g., brewery wastewater), but can be usually treated with the same biological treatment systems (anaerobic and/or aerobic). In particular, industrial wastewater with a high organic load is treated biologically with anaerobic or aerobic treatment or a combination thereof and also in combination with chemical-physical treatment, depending on the concentration, temperature and requirements (Rosenwinkel et al. (2019) p.149). As an example of highly polluted organic wastewater, the composition of brewery wastewater is shown in **Table 1.2**:

**Table 1.2:** Brewery wastewater composition (Glas 2009) as an example for highly polluted industrial wastewater

Parameter	Units	Mean	Minimum	Maximum
<i>COD</i>	mg/L	2628	933	5515
<i>DOC</i>	mg/L	651	190	1620
<i>BOD</i> <sub>5</sub>	mg/L	1668	600	3671
<i>TIN</i> <sup>a</sup>	mg/L	12.5	4.2	29.6
<i>TKN</i> <sup>a</sup>	mg/L	58.2	26.6	126.8
<i>NH</i> <sub>4</sub> – <i>N</i>	mg/L	2.8	0.3	21.1
<i>NO</i> <sub>2</sub> – <i>N</i>	mg/L	1.3	0.1	20.7
<i>NO</i> <sub>3</sub> – <i>N</i>	mg/L	12.3	0.9	26.2
<i>Ortho</i> – <i>P</i>	mg/L	10.6	0.8	41
<i>Total P</i>	mg/L	11.8	1	33.6

<sup>a</sup>total inorganic nitrogen (TIN), total Kjeldahl nitrogen (TKN)

In addition to defined carbon and nitrogen sources, MFCs are capable of removing multiple contaminants such as heavy metals, sulfates, phenol, and pyridine derivatives (Mathuriya 2013; He et al. 2017; Brunschweiler et al. 2021b). In terms of wastewater treatment, MFC studies focus on the removal of organics by oxidation in the anode chamber (Pant et al. 2010; Pandey et al. 2016). Therefore, the evaluation of treatment efficiency mainly refers to the efficiency of organic degradation. In order to describe the organic matter loaded into the MFC per unit volume and time, the organic loading rate (OLR;  $kg/(m^3 \cdot d)$ ) can be calculated with the COD concentration in the beginning ( $COD_0$ ; g/L) and the HRT (h) using **Equation 1.11** (Abdallah et al. 2019):

$$OLR = \frac{COD_0}{HRT} \quad (1.11)$$

As described in Section 1.2.3, OLR can affect power output and treatment efficiency. *COD*, Biochemical oxygen demand (*BOD*<sub>5</sub>), total organic carbon (*TOC*), or dissolved organic carbon (*DOC*) can be determined to evaluate overall treatment efficiency. Mainly, *COD* removal is measured for wastewater treatment efficiency (**Equation 1.12**) as it is required for Coulomb efficiency and energy recovery. However, measurement of the *TOC* or *DOC* concentration can also be performed and a factor for calculating *COD* can be determined (e.g., the *COD:DOC* ratio for brewery wastewater is approximately 3-4 Glas (2009)). Thus, the following equation can be used to describe the percentage *COD* or *DOC* treatment efficiency ( $COD_{eff.}$ ; %):

$$COD_{eff.} = \frac{COD_0 - COD_t}{COD_0} \cdot 100 \quad (1.12)$$

Hereby,  $COD_t$  (g/L) represents the *COD* concentration after time *t* in the process. The percentage *COD* removal efficiency does not consider the HRT, meaning the average time the influent spends in the anodic compartment of the MFC, which is important for evaluation compared to conventional treatment technologies. Therefore, the organic removal

rate (ORR;  $kg/(m^3 \cdot d)$ ) of an MFC can be calculated with the degraded COD concentration ( $\Delta COD$ ; g/L) using **Equation 1.13**.

$$ORR = \frac{\Delta COD}{HRT} \quad (1.13)$$

In conclusion, to combine the treatment efficiency with the power output and gives information about the possible electrons produced in relation to the total COD concentration, several key figures can be calculated for possible classification of MFC results, which are described in the next section. In addition, to combine treatment efficiency with power output and provide information on electrons potentially generated relative to total COD removal, several key figures are used to estimate MFC results.

### 1.3.3 Calculation of key parameters

The calculation of the following key parameters enables the assessment of the MFC performance in relation to MFC studies and for classification in conventional wastewater treatment. The performance of MFCs are generally evaluated in terms of power density, related to the anode ( $P_{an}$ ;  $W/m^2$ ) or cathode surface area ( $P_{cat}$ ;  $W/m^2$ ) or treated volume ( $P_{vol}$ ;  $W/m^3$ ) (Logan et al. 2006; Varanasi et al. 2018). Using **Equations 1.14 and 1.15** allow the power densities to be classified regardless of the design.

$$P_{cat/an} = \frac{E_{MFC} \cdot I}{A_{cat/an}} \quad (1.14)$$

$$P_{vol} = \frac{E_{MFC} \cdot I}{V_{MFC}} \quad (1.15)$$

$P_{cat/an}$	Power density normalised to the anode or cathode surface area ( $W/m^2$ )
$E_{MFC}$	Voltage of the MFC (V)
$A_{cat./an}$	Surface area of the anode or cathode ( $m^2$ )
$P_{vol}$	Power density normalised to the volume ( $W/m^3$ )
$I$	Current (A)
$V_{MFC}$	Liquid volume of the MFC (L)

Normalised energy recovery (NER) combines the power output with the wastewater treatment efficiency. The power generated by the MFC system is normalized to either the degraded COD concentration ( $NER_{kgCOD}$ ;  $kWh/kgCOD$ ) or to the treated wastewater volume ( $NER_{vol}$ ;  $kWh/m^3$ ) (Ge et al. 2013).

$$NER_{vol} = \frac{P \cdot t}{V_{MFC}} \quad (1.16)$$

$$NER_{kgCOD} = \frac{P \cdot t}{V_{MFC} \cdot \Delta COD} \quad (1.17)$$

$$(1.18)$$

- $P$  Mean electrical power output within treatment time ( $kW$ )  
 $V_{MFC}$  Liquid volume of the MFC ( $m^3$ )  
 $t$  Treatment time (HRT)( $h$ )

Additional to the power parameters, the overall efficiency ( $\eta_{MFC}$ ; %) of MFCs can be represented by the product of two types of efficiency: Coulombic efficiency (CE; %) and voltage efficiency (VE; %) (Hamelers et al. 2010; Sleutels et al. 2016). VE is the ratio between the generated voltage ( $E_{MFC}$ ) and electromotive force  $E_{emf}$  (Logan et al. 2006; Varanasi and Das 2017) and CE describes the fraction of the degraded substrate effectively converted into electrons (Logan et al. 2006). **Equation 1.21** for an SC-MFC with oxygen reduction at the cathode was used to calculate the CE.

$$\eta_{MFC} = CE \cdot VE \quad (1.19)$$

$$VE = \frac{E_{MFC}}{E_{emf}} \quad (1.20)$$

$$CE = \frac{M \cdot \int_{t_0}^t I(t) dt}{F \cdot n \cdot V_{MFC} \cdot \Delta COD} \quad (1.21)$$

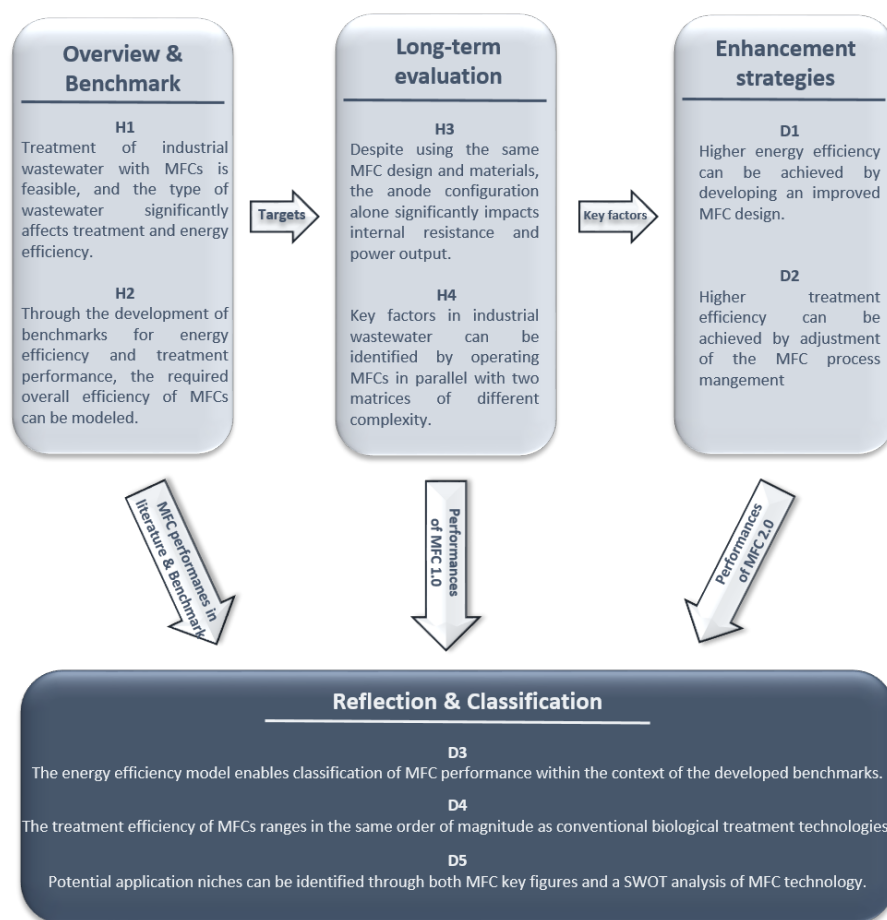
- $M$  Molar mass of oxygen ( $32 \text{ g/mol}$ )  
 $I$  Produced current ( $A$ ) integrated over time  $t$  ( $s$ )  
 $F$  Faraday's constant ( $96485 \text{ C/mol}$ )  
 $b$  Number of electrons exchanged per molecule of oxygen (4)  
 $V_{MFC}$  Liquid volume of the MFC ( $L$ )  
 $\Delta COD$  Degraded concentration of the COD ( $g/L$ )

## 2 Objective of this thesis

In the previous section, it was demonstrated that there are several aspects to be considered when treating wastewater with MFCs. So far, no design, material or mode of operation has clearly prevailed, as there are both strengths and weaknesses in each case. In addition, most MFC configurations have been investigated using municipal wastewater, thus there are no specific concepts yet regarding the configuration and potential applications in industrial wastewater treatment.

Therefore, with regard to MFCs in industrial wastewater treatment, the objective of this work is to develop a benchmark as a basis for modeling the required overall efficiency of MFCs and to identify the most important key factors in order to derive enhancement strategies.

In order to achieve the main objectives, this work clarifies the hypotheses (H1 - H4) in **Figure 2.1**. On this basis, enhancement strategies can be applied (D1 & D2) and the results reflected and classified (D3 - D5).



**Figure 2.1:** Visualization of the relationships between the hypotheses and the discussion topics.



## 3 Results

The results were published in individual publications, which are presented in an overview in **Table 3.1**. The publications are summarized in the following sections, highlighting the individual contributions of the author of this thesis, Sarah Brunschweiler. Full copies of the publications can be found in **Appendix A.1 - A.4**.

**Table 3.1:** Overview of the four publications including title, major objectives, methods and data evaluation and main statements

Publication no.	1	2	3	4
Publication title	Industrial wastewater treatment with simultaneous energy recovery using microbial fuel cells - a review	Microbial fuel cells for brewery wastewater treatment – efficiency requirements and treatment performance	The effect of clogging on the long-term stability of different carbon fiber brushes in microbial fuel cells for brewery wastewater treatment	Key factors of brewery wastewater influencing treatment efficiency and power output of microbial fuel cells
Major objectives	Classification of MFCs for the treatment of various industrial wastewaters and evaluation of their potential applications in competition with conventional wastewater treatment.	Development of scenario-based benchmarks to model the required overall efficiencies of MFCs.	Evaluation of the influence of the anode configuration in long-term MFC operation.	Identification of key factors in brewery wastewater treated with MFCs in long-term operation.
Methods and data evaluation	Literature review on MFC treatment of different industrial wastewaters at laboratory and pilot scale	Complete analysis of conventional wastewater treatment plants at two breweries for direct and indirect discharges.	Parallel operation of MFCs with two different anode configuration for 180 days. <ul style="list-style-type: none"> <li>• Measurement of power densities, DOC concentrations, pH and conductivity</li> <li>• Electrochemical analysis by EIS and LSV</li> <li>• FTIR and SEM analysis of the carbon fiber brushes</li> </ul>	Parallel treatment of beer wort and brewery wastewater with MFCs for 180 days. <ul style="list-style-type: none"> <li>• Measurement of DOC and anion (organic acids, nitrate, nitrite, sulfate, phosphate) concentrations</li> <li>• Analysis of generated gas volume and composition</li> <li>• Measurement of power densities, pH, redox potential and conductivity</li> <li>• Electrochemical analysis by LSV</li> </ul>
Main statements	<ul style="list-style-type: none"> <li>• The type of industrial wastewater is not crucial.</li> <li>• Self-sufficient treatment has already been approved.</li> <li>• Removal of nitrogen, sulfate and TSS is possible.</li> <li>• Modularity and wide range of application are the main advantages of the MFC.</li> </ul>	<ul style="list-style-type: none"> <li>• Energy efficiency: MFCs are an attractive option if self-sufficient treatment is provided.</li> <li>• COD treatment efficiency: MFCs can meet the benchmark requirements for all scenarios.</li> <li>• Compact MFC systems with direct power generation could become an interesting option for small breweries.</li> </ul>	<ul style="list-style-type: none"> <li>• Long-term stability and biocompatibility of carbon fiber brushes for more than 180 days.</li> <li>• Fiber clogging was reduced by using 5 cm diameter brushes.</li> <li>• Power densities of MFCs with two 5 cm diameter brushes were continuously more than doubled compared to MFCs with one 10 cm diameter brush, due to a 58% reduction in internal resistance.</li> </ul>	<ul style="list-style-type: none"> <li>• The MFC efficiency strongly depends on the composition of the wastewater: a high proportion of easily degradable organic acids and a high conductivity improve the MFC performance.</li> <li>• The process conditions and not the inoculum mainly determine the microbial composition of the biofilm.</li> <li>• Inhibition of competing methanogens is essential.</li> </ul>

### 3.1 Industrial wastewater treatment with simultaneous energy recovery using microbial fuel cells - a review

The research article “*Industrial wastewater treatment with simultaneous energy recovery using microbial fuel cells - a review*” was published in “BrewingScience” in 2020 (doi: 10.23763/BrSc20-15brunschweiger). Sarah Brunschweiger developed the concept, collected the literature data, wrote the manuscript and revised the publication according to the comments of the reviewers.

Most MFC studies currently focus on the treatment of simple substrates, synthetic or municipal wastewater, thus limited research exists on the treatment of industrial wastewater (e.g., brewery, dairy, paper, swine) using MFCs. Higher COD concentrations in industrial wastewater represent the main difference between municipal and industrial wastewater. Savings in discharge fees could make MFC treatment of industrial wastewater more attractive than treatment of municipal wastewater if the required treatment performance can be achieved. Therefore, a review of MFC studies for the treatment of different types of industrial wastewater enabled a focused assessment of their application potential. In this context, the questions of whether MFCs can be used for industrial wastewater in general and whether they can compete with conventional anaerobic-aerobic wastewater treatment plants were of critical relevance. An overview of power densities and COD removal rates could be provided, although a direct comparison of MFC studies is not possible due to numerous influencing parameters (type of inoculum, electrode material, configuration (single or dual chamber), and operating conditions), which may lead to discrepancies in key factors. Nevertheless, the distinction between laboratory and pilot scale indicated trends in key factors for evaluating MFCs from a commercialization perspective. In addition to the direct comparison of MFCs with conventional anaerobic or aerobic treatment, combination possibilities were also identified.

The feasibility of simultaneous power generation and treatment of industrial wastewater has been demonstrated in several MFC laboratory tests and even in some pilot-scale studies. The type of wastewater was not decisive. Median COD removal rates of 81 % and 80 % and median power densities of  $3.5 \text{ W/m}^3$  ( $171 \text{ mW/m}^2$ ) and  $1.0 \text{ W/m}^3$  ( $1.6 \text{ mW/m}^2$ ) were determined at laboratory and pilot scale, respectively. In addition, the removal of nitrogen, total suspended solids (TSS) and sulphates with MFCs and self-sufficient industrial wastewater treatment could already been demonstrated. Modularity and wide range of operation conditions were identified as the main advantages of MFCs, making them an alternative or complement to conventional industrial wastewater treatment. Both low and high COD concentrations can be treated with MFCs, with no aeration required as with aerobic treatment, and small-scale use could become a potential niche over anaerobic treatment. The major challenges of efficient scaling-up and reducing investment costs can be mitigated by the combination options with conventional biological treatment. In conclusion, this review provided a basis for evaluating MFCs in industrial wastewater treatment and further analysis of industrial wastewater treatment plants in terms of energy recovery and treatment performance will allow calculation of a realistic benchmark.

## 3.2 Microbial fuel cells for brewery wastewater treatment - efficiency requirements and treatment performance

The research article “*Microbial fuel cells for brewery wastewater treatment – efficiency requirements and treatment performance*” was published in “BrewingScience” in 2021 (doi: 10.23763/BrSc21-02brunschweiger). Sarah Brunschweiger developed the concept, interpreted the data and contributed to the manuscript and revised the publication according to the comments of the reviewers.

The application potential of MFCs in industrial wastewater treatment has already been demonstrated and the opportunities offered in combination with conventional biological wastewater treatment technologies have already been identified. In the case of municipal wastewater treatment plants, concepts already exist for integrating MFCs into conventional treatment plants. However, there are currently no detailed energy- and wastewater-related benchmarks quantifying the requirements for industrial wastewater treatment with MFCs. Hence, for a possible commercialization of the MFC technology, benchmarks for direct and indirect discharges were defined by analyzing two brewery wastewater treatment plants. The purpose of this work was to clarify the energy recovery and treatment performance requirements that MFCs must meet in order to compete with the state of the art. In addition to the distinction the discharge quality, the wastewater treatment plants were divided into sections and subsections in order to allocate energy consumption, energy gain and COD removal efficiency to the individual treatment steps. All energy consumers were considered and the potential electrical energy gain in the form of biogas was calculated. Based on the data obtained from these investigations, benchmarks for MFCs were determined for various application scenarios and achievable energy recoveries were calculated using the developed energy efficiency model.

Due to the negative energy balance of the energy-intensive aerobic process (Scenario 1A and 1B), MFCs with self-sufficient treatment are an attractive replacement option. However, it is questionable whether the discharge conditions can be achieved with MFCs alone, since the two treatment technologies differ in terms of the treatment process (anaerobic vs. aerobic). In contrast, to achieve an energy gain comparable to the more similar treatment technology of anaerobic treatment, MFCs require an overall energy efficiency of at least 18 % for direct discharge (Scenario 2B) or 23 % for indirect discharge (Scenario 2A). A current rather realistic efficiency of 4.5 % is sufficient to replace the complete biological wastewater treatment in case of direct discharge (scenario 4B) by several stacked MFCs. In terms of COD removal performance, MFCs can meet the benchmark requirements for all scenarios examined in this study, even though a longer HRT is currently required for its assurance. In summary, the energy efficiency of MFCs needs to be increased, but the necessary COD treatment performance can already be achieved. At this stage, however, MFCs may already be attractive as pre-treatment step prior to aerobic treatment to reduce energy costs because they are compact, stackable, and can generate electricity directly.

### 3.3 The effect of clogging on the long-term stability of different carbon fiber brushes in microbial fuel cells for brewery wastewater treatment

The research article “*The effect of clogging on the long-term stability of different carbon fiber brushes in microbial fuel cells for brewery wastewater treatment*” was published in “Bioresource Technology Reports” in 2020 (Doi: 10.1016/j.biteb.2020.100420). Sarah Brunschweiler developed the concept, mainly conducted the laboratory work, collected and interpreted the data, wrote the manuscript and revised the publication according to the comments of the reviewers.

For the commercialization of MFC technology in wastewater treatment, scale up is mandatory, which is currently still associated with various material and design-related challenges. In general, carbonaceous electrodes largely fulfill the requirements for anode materials such as high electrical conductivity, biocompatibility, chemical and mechanical stability, durability and large surface area. Due to the large surface area and good scalability compared to other carbonaceous materials, carbon fiber brushes are often used as anode electrodes in MFCs. However, in most of the studies, a defined medium was used rather than real wastewater, so that clogging of the fibers did not have to be considered. Clogging of the fibers reduces the anode surface area accessible to microorganisms, which in turn may result in lower power output from the MFCs. Therefore, the objective of this work was the investigation of the interplay between mechanical stability and active surface area of carbon fiber brushes in a 1-L single-chamber MFC in brewery wastewater treatment. The carbon fiber brushes were analyzed with respect to their mechanical and electrochemical properties as well as their dimensions. Subsequently, the performance of MFCs with one 10 cm diameter brush (B1) and MFCs with two 5 cm diameter brushes (B2) were compared in a long-term experiment using brewery wastewater as an example of highly contaminated organic wastewater over six months. In addition, the chemical stability, biocompatibility and active surface area of the brushes could be investigated by FTIR and SEM.

Carbon fiber brushes with a fiber thickness of 300  $\mu\text{m}$  in MFCs for brewery wastewater treatment remained stable and biocompatible for 180 days. However, the MFC with configuration B2 consistently achieved more than twice the power density compared to the MFC with configuration B1, despite providing only half the theoretical area. The comparably high degradation rates in both cases indicated similar biofilm activity, so overall brush dimension was determined to be the main factor influencing power density. In summary, carbon fiber clogging was significantly reduced by the use of two 5 cm diameter brushes instead of one 10 cm diameter brush. The continuously more than doubled power density could be attributed to the reduction of the internal resistance by 58 %.

### 3.4 Key factors of brewery wastewater influencing treatment efficiency and power output of microbial fuel cells

The research article “*Key factors of brewery wastewater influencing treatment efficiency and power output of microbial fuel cells*” was published in “BrewingScience” in 2021 (doi: 0.23763/BrSc21-06brunschweiger). Sarah Brunschweiger developed the concept, conducted the laboratory work, collected and interpreted the data, wrote the manuscript and revised the publication according to the comments of the reviewers.

Although MFCs have great potential for power generation in wastewater treatment, the energy efficiency and especially the treatment performance of MFCs need to be improved to become commercially attractive. The concentration of wastewater and its composition are among the most important factors affecting COD removal and power densities in MFCs. However, the appropriate selection is at the same time a major challenge due to the molecular complexity and conductivity-dependent resistance in MFCs. Consequently, it is necessary to identify the main influencing factors in industrial wastewater. Thus, within a long-term operation of MFCs with brewery wastewater and diluted beer wort, in parallel the objective in this work was to clarify (1) whether pretreatment of brewery wastewater is necessary to be competitive, (2) which are the major influential components in the wastewater, (3) do methanogens need to be explicitly suppressed, and (4) whether a pre-grown biofilm remains stable after feeding with brewery wastewater. For stability and adaptability examination of biofilms, brewery wastewater was added at the end of the MFC fed with beer wort. By analyzing the efficiency of organic acid degradation and the amount and composition of gas produced in the MFCs, conclusions can be drawn about microbial composition and resulting organic degradation. In addition, considering nitrate and nitrite, phosphorus and sulfate levels, as well as the impact on power output, the MFC results could be classified by the benchmarks of conventional brewery wastewater treatment.

The high proportion of easily degradable organic acids in brewery wastewater, which affected the composition of the biofilm, and the high conductivity resulted in a mean COD degradation rate of  $82 \pm 8 \%$  and a mean power density of  $58 \pm 24 \text{ mW/m}^3$ , which were almost twice the performances of the MFC fed with beer wort. The fermentable sugars in the beer wort were first degraded to acetate, and no effect of the absence of cleaning and disinfecting agents could be observed. Furthermore, the change from beer wort to brewery wastewater indicated that the process conditions, not the inoculum, determine mainly the microbial composition of the biofilm. In addition to organic degradation, the removal of nitrogen and sulfur components was also successful. In summary, untreated brewery wastewater could be successfully degraded over 180 days with MFCs, but energy efficiency was low due to losses in Coulombic efficiencies. In summary, untreated brewery wastewater can be degraded in long-term operation with MFCs, but energy efficiency must be increased by inhibiting methanogens and adjusting process conditions.

## 4 Discussion

As the results demonstrated, energy efficiency and COD treatment performance are critical in evaluating MFC technology for industrial wastewater treatment. These key figures were considered in the development of scenario-based benchmarks (publication 2). The identification of major influencing factors in MFC design (publication 3) and wastewater composition (publication 4) enabled the improvement of these factors and the development of further enhancement strategies. The application of these strategies was investigated with the design and operation of additional MFCs. In the new MFC design (MFC 2.0), electrode packing density was significantly increased and clogging of fibers was prevented to achieve higher power densities. In addition, the operation mode was changed from batch to continuous and the HRT was significantly reduced to become commercially attractive with improved COD treatment performance.

For an overall assessment, the MFC performances of publication 3 and 4 and the achieved performances of MFC 2.0 were set in context with MFC performances treating industrial wastewater (publication 1) along with the developed scenario-based benchmarks (publication 2). The compilation of the calculated energy efficiencies and the calculated ORR together with the benchmark limits enabled a classification of the MFC performances. A final SWOT analysis allowed a summary evaluation of the application potential of MFCs for industrial wastewater treatment.

### 4.1 Application of enhancement strategies

The application of strategies to enhance MFC performance focused on MFC design and process operation, which both directly affect performance and can also indirectly affect electroactive biofilm efficiency.(Borole et al. 2011). In the long term, larger wastewater streams will require processing, so the scalability of the MFC design is also of great importance (Logan et al. 2015). Consequently, in addition to scalability, the results from publication 3 on avoiding clogging of fibers and long-term losses on performance due to membrane fouling were considered in the development of MFC 2.0 to improve the power density. In addition to the design, the transfer of the process control system to a larger scale has to be ensured, so an automatic process control system with integration of a measuring system was developed. In terms of potential commercialization, this system was used to study the effects on COD treatment performance when switching from batch to continuous operation and when HRT was reduced. Finally, in the interest of commercialization, the effects of replacing the MCA in one MFC 2.0 after one year of operation could provide results on system stability. The experimental conditions for the application of the enhancement strategies are described in **Appendix B**.

### 4.1.1 Enhancement of the MFC design

For the improvement of MFC performance, the design of the MFC was modified by engineering a new design (MFC 2.0) based on the outcomes of MFC 1.0, which was used to generate the results from publications 3 and 4. Similar electrode material was selected for MFC 1.0 and MFC 2.0, only the dimensions and electrode configuration were changed for MFC 2.0 (**Table 4.1**). Even if there are a lot of different electrode materials used, the combination of brush anodes with active carbon cathodes in MFCs can be considered as an effective and low-cost electrode material (Kim et al. 2015). In particular, tubular cathodes combined with brush anodes are a promising scalable design to create larger systems (Zuo et al. 2007).

The results of publication 3 using MFC 1.0 were considered in the development of MFC 2.0, especially the prevention of fiber clogging. Furthermore, an updated literature review identified additional influencing factors which were considered in the design. In comparison with MFC 1.0 (**Figure 4.1**):

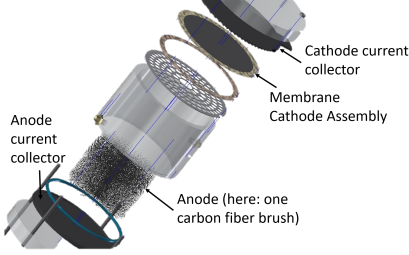
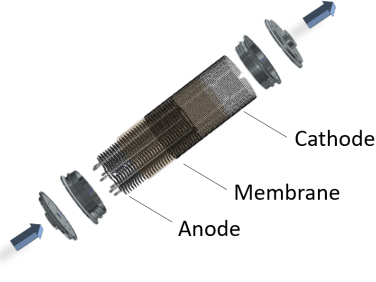
- (A) The electrode packing density was increased.
- (B) The clogging of carbon fibers and the distance between anode and cathode were reduced.
- (C) The scalability of single MFCs was provided.
- (D) Optical sensors were implemented.

A large surface area per volume **electrode packing density (Figure 4.1A)** on cathode and anode side will increase the power densities per unit volume of reactor (Liu et al. 2005), but the material costs especially on cathode side has to be considered (Lanas and Logan 2013). The anode surface area in MFC 2.0 was increased from  $0.027\text{ m}^2$  to  $0.096\text{ m}^2$  by using seven ( $d = 3\text{ cm}$ ) carbon fiber brushes instead of two ( $d = 5\text{ cm}$ ) in MFC 1.0. In addition, the cathode surface area was increased from  $0.011\text{ m}^2$  to  $0.046\text{ m}^2$  by the use of tubular cathodes. Overall, the anode and cathode surface areas per total volume were thus enlarged by 79 % and 104 %, respectively.

Furthermore, the anode to cathode surface area ratio should be large to maximize power production by the exoelectrogenic biofilm, but the size of the brush anode relative to the cathode has not been optimized yet (Lanas and Logan 2013). Increasing anode surface area do not have a clear effect on the power density, more the anode coverage of the cathode is critical (Lanas and Logan 2013), which was increased by 70 %. However, with higher anode surface area, the removal rates of organic compounds can be improved (Dong et al. 2015), because more microorganisms can accumulate on the anode.

The critical design factor for improving the volumetric power density is the cathode surface area (Logan et al. 2015). MFCs with larger cathode surface area per volume achieve higher power densities, although the cost factor should not be neglected (Kim et al. 2015). However, greater specific cathode surface area will lead to higher power densities compared to MFCs focusing on closer electrode spacing (Kim et al. 2015). The specific cathode areas of MFC 1.0 and MFC 2.0 were  $10\text{ m}^2/\text{m}^3$  and  $21\text{ m}^2/\text{m}^3$ , respectively. For classification of specific cathode surface area, SC-MFC studies in industrial wastewater

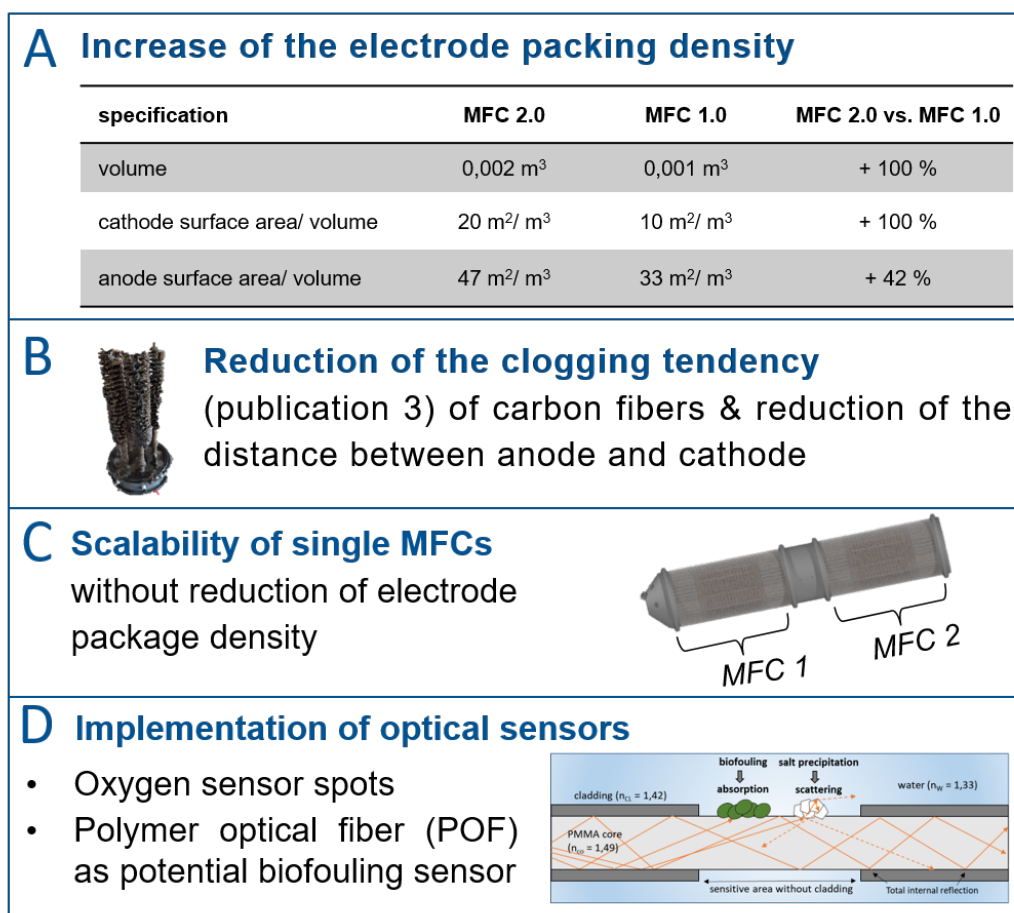
**Table 4.1:** Design and choice of electrode material of MFC 1.0 and MFC 2.0, respectively.

Configuration	MFC 1.0	MFC 2.0
Sketch		
Dimension	1 liter	2 liter
Anode	2 x carbon fiber brushes (d = 5 cm, l = 6 cm) screwed into gas-tight graphite plates	7 x carbon fiber brushes (d = 3 cm, l = 13 cm)
Membrane cathode assembly (MCA)	PFSA membrane coated on one side (adjacent to the cath- ode current collector) with 0.5 mg/cm <sup>2</sup> activated carbon	PFSA membrane and a gas dif- fusion electrode (VITOCORE <sup>®</sup> ) consisting of a stainless steel mesh coated with activated carbon and a gas diffusion layer
Anode to cathode configuration	vertical	horizontal

treatment were analyzed using carbon fiber brush anodes and air breathing cathodes. Here, the specific cathode surface area ranged from 1 to 33  $m^2/m^3$  (Huang and Logan 2008; Huang et al. 2009; Cusick et al. 2010; Haavisto et al. 2020; Dong et al. 2015).

**Clogging tendency of fibers and the distance between anode and cathode (Figure 4.1B)** also affects the power density of MFCs (Logan et al. 2015; Brunschweiler et al. 2020b). Reducing the brush diameter to 3 cm stabilizes the brush configuration and prevents clogging in MFC 2.0, as clogging could already be reduced by using 5 cm diameter brushes (Brunschweiler et al. 2020b). In addition, a reduced brush diameter allows the design of more compact reactors and may have the benefit of a larger number of current collectors (Lanas et al. 2014). Furthermore, the anode was placed horizontally instead of vertically to the cathode in MFC 1.0. Thus, the center of carbon fibers of the brush anode, which can be described as reference for anode-cathode distance (Kang et al. 2017), could be reduced from 40 to 15 mm. The reduction in electrode spacing can be expected to result in an increase in power density due to the lower internal resistance (Cheng et al. 2006; Logan et al. 2015). However, possible clogging due to biofilm growth or particle attachment (Cheng et al. 2006), as well as possible changes in oxygen transport and substrate diffusion (Ahn et al. 2014), need to be considered. In summary, electrode packing density, electrode spacing, and clogging tendency are critical factors affecting power densities in MFCs.





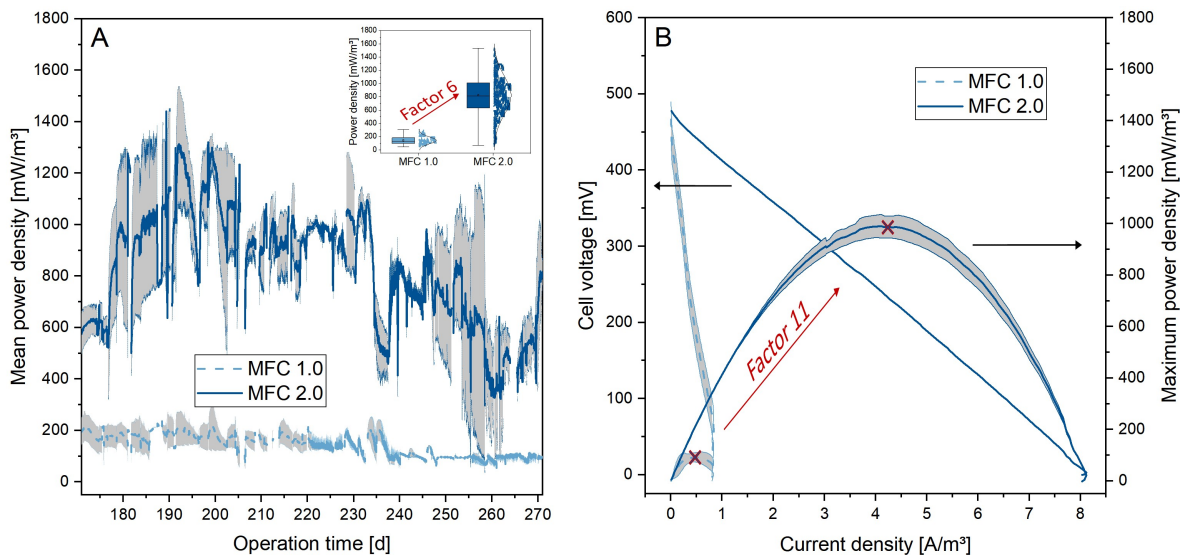
**Figure 4.1:** Changes in MFC 2.0 and differences in contrast to MFC 1.0.

In **scalability of MFCs (Figure 4.1C)**, maintenance of electrode packing density and electrode spacing are essential (Logan et al. 2015), which can be achieved by MFC stack systems. The more surface area available for substrate conversion, the faster COD degradation can occur. Consequently, maintaining electrode packing density during scale-up is important not only for achieving high power densities, but also for enhancing COD treatment performance in terms of minimizing HRT (Rossi et al. 2018; Logan et al. 2015; He et al. 2016a). The design of MFC 2.0 can be easily scaled up without changing these key factors by stacking them horizontally.

With the implementation of **optical sensors for oxygen and biofouling detection (Figure 4.1D)** the necessary anaerobic conditions in MFCs and the fouling of the membrane could be monitored. The sensor spot on the lid of the MFC 2.0 allowed an invasive measurement to monitor the oxygen concentration in the wastewater, which could be entered via the membrane or via process control. Fouling of the membranes can lead to a loss of power density after prolonged operation, as diffusion through the membrane is impeded and internal resistance increases (Brunschweiler et al. 2020b; Hiegemann et al. 2019). Thus, the decrease in power density over time described in publication 3 could be extended by initiating cleaning in time by the implementation of the fouling sensor.

For the [validation of MFC 2.0](#), MFC 1.0 and MFC 2.0 (in duplicate) were operated in parallel with the same inoculation process and operating conditions (details see [Appendix B1](#)). After the initial period in batch mode, one of each duplicate was switched to continuous mode in order to compare the process management described in the next subsection 4.1.2. After 171 days of operation, all four MFCs (two of MFC 1.0 and two of MFC 2.0) were investigated in continuous mode.

A comparison of the mean power densities of MFC 1.0 and MFC 2.0 over a 100-day period showed consistently higher power densities of MFC 2.0 (**Figure 4.2A**). Although there are fluctuations in the power densities, an increase in the mean power density by a factor of 6 was achieved with MFC 2.0 (MFC 1.0:  $144 \pm 53 \text{ mW/m}^3$ ; MFC 2.0:  $824 \pm 290 \text{ mW/m}^3$ ). For a complete evaluation, the COD treatment efficiency was also observed in this period, which was in a similar range in both MFC configurations with about  $83 \pm 13 \%$  (MFC 1.0) and  $87 \pm 6 \%$  (MFC 2.0) within an HRT of 24 h. In addition, by reducing the HRT to 12 h within these 100 days, the ORR could be increased in both MFC designs from  $1.9 \pm 0.6 \text{ kg}/(\text{m}^3 \cdot \text{d})$  to  $3.7 \pm 1.4 \text{ kg}/(\text{m}^3 \cdot \text{d})$  (MFC 1.0) and  $1.8 \pm 0.7 \text{ kg}/(\text{m}^3 \cdot \text{d})$  to  $3.6 \pm 1.2 \text{ kg}/(\text{m}^3 \cdot \text{d})$  (MFC 2.0).



**Figure 4.2:** **A** mean power density of MFC 1.0 and MFC 2.0 over 100 days; **B** Polarization and power densities curves of MFC 1.0 and MFC 2.0 after 227 and 233 days.

Moreover, even the difference between the maximum power densities of  $92 \pm 19 \text{ mW/m}^3$  (MFC 1.0) and  $990 \pm 45 \text{ mW/m}^3$  (MFC 2.0) determined by LSV (**Figure 4.2B**) during this period resulted in an increasing factor of 11, which could be attributed to the lower internal resistance of MFC 2.0 with  $26 \pm 2 \Omega$  compared to MFC 1.0 with  $334 \pm 62 \Omega$ . High increases in volumetric power density could already be attributed to the enlargement of the specific cathode area per volume (Zhang et al. 2011). This indicated that the increase by factor 6 (mean) and 11 (maximum) was mainly caused by the 104 % larger specific cathode surface area of MFC 2.0. Thus, it was confirmed that the specific cathode surface area needs to be considered as a critical factor for the volumetric power density during scale up. Furthermore, normalization of the power output to the cathode area showed that not only the cathode surface area affects the power output, because an

**Table 4.2:** Power densities of MFC 1.0 and MFC 2.0 and the calculated increasing factor

Design		MFC 1.0	MFC 2.0	Increasing factor
$P_{vol}$ [mW/m <sup>3</sup> ]	max <sup>a</sup>	92 ± 19	990 ± 45	<b>11</b>
	mean <sup>b</sup>	144 ± 53	824 ± 290	<b>6</b>
$P_{cat}$ [mW/m <sup>2</sup> ]	max <sup>a</sup>	9 ± 2	47 ± 2	<b>5</b>
	mean <sup>b</sup>	14 ± 5	39 ± 14	<b>3</b>

<sup>a</sup>calculated via slope of LSV

<sup>b</sup>calculated from the power densities between 171-271 days

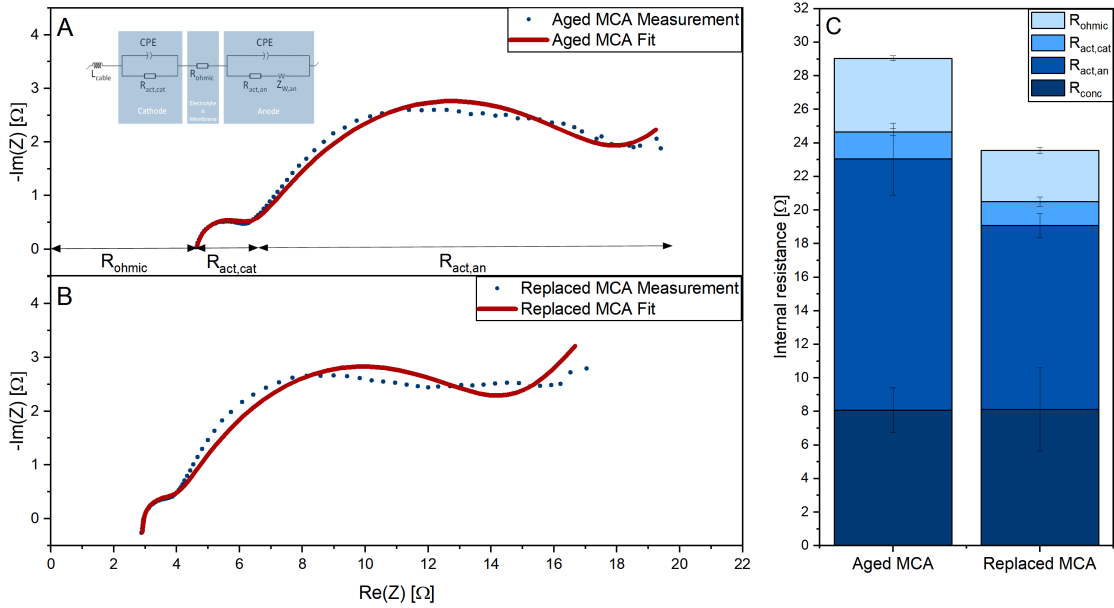
increase of the power density normalized to the cathode surface area by factor of 3 (mean) and 5 (maximum) was also observed (**Table 4.2**). In terms of power generation per electrode surface area, electrode spacing was described as a critical factor (Logan et al. 2015), so reducing the electrode spacing from 40 to 15 mm probably also impacted the increase in power density. In addition, the avoidance of clogging and the horizontal rather than vertical orientation of the anode likely also contributed to the improved performance of the MFC.

In conclusion, the sum of the described changes was the reason for the high power density increase. With the strategy of considering important design factors, it was possible to significantly enhance the MFC performance without reducing the COD treatment efficiency.

In addition to the validation of the new design, the [system stability of MFC 2.0](#), which is required to be commercially interesting, was also investigated. In publications 3 and 4, a general decrease in power density was observed over a period of about 180 days of operation, which was attributed to fouling of the membrane. The implemented fouling sensor proved to be too sensitive for the subsequent development of a cleaning concept on this basis. Therefore, after one year of operation, the membrane cathode assembly (MCA) of the MFC 2.0 was replaced to investigate the effects of membrane fouling on internal resistance and thus power density compared to an MFC with aged MCA. Already the analysis of the mean power densities and the ORR over one month revealed that the replacement of the membrane had mainly a positive effect on the power densities, as the MFC with replaced MCA had a mean power density of  $682 \pm 253 \text{ mW/m}^3$  compared to  $325 \pm 119 \text{ mW/m}^3$  for the MFC with aged membrane. However, the ORR was still very high in both cases (HRT = 6 h) at  $5.4 \pm 1.8 \text{ kg}/(\text{m}^3 \cdot \text{d})$  (aged MCA) and  $5.9 \pm 2.2 \text{ kg}/(\text{m}^3 \cdot \text{d})$  (replaced MCA), respectively.

The cause of the performance differences from the MFCs with aged and replaced MCA can be quantitatively assigned by determining the internal resistances and differentiating them into ohmic, activation and diffusion-limited concentration resistances (Littfinski et al. 2021). For this purpose, both LSV and EIS were periodically recorded over one month (details of settings in **Appendix B2**). The LSV provided the total internal resistance by calculating it from the slope of the linear intercept of the polarization curve, and the

MPP, which depend on the internal cell resistance, could be determined (Logan et al. 2006). In order to differentiate the internal resistance, the EIS data were fitted to an equivalent circuit model (ECM) to describe the electrochemical processes in the MFCs (He and Mansfeld 2009; Dominguez-Benetton et al. 2012; Kretzschmar and Harnisch 2021; Yoho et al. 2015). In several MFC studies, the EIS data measured with two electrode setups for the entire MFC have already been evaluated using ECMs (He et al. 2006; Aaron et al. 2010; Zhang et al. 2015; Kang et al. 2017; Wu et al. 2019; Littfinski et al. 2021). Subsequently, the EIS data of the MFCs with aged and replaced MCAs were fitted with an ECM (**Inlet Figure 4.3A**) using the EC-Lab<sup>®</sup> software.



**Figure 4.3:** Exemplary measured and fitted Nyquist plot of **A** MFC with aged MCA and **B** MFC with replaced MCA; **C** Internal resistance composition resulting from EIS and LSV analysis.

Inductance  $L_{cable}$  was attributed to leakage inductance caused by the cable connection between the potentiostat and MFC and did not occur within the MFC (Aaron et al. 2010). According to Littfinski et al. (2021), a resistor ( $R_{act}$ ) in parallel with a constant phase element (CPE) was chosen to describe the faradaic processes at the anode and cathode. The diffusion-limiting processes were represented by a Warburg element ( $Z_{W,an}$ ) (Zhang et al. 2015; Littfinski et al. 2021), which was assigned to the anode based on further individual measurements.  $R_{ohmic}$  represents the ohmic resistance of the electrolyte, material and membrane (Chen et al. 2019). **Figures 4.3A and 4.3B** show the measured and fitted EIS data of the MFC with aged and replaced MCA, respectively. For the complete differentiation of  $R_{int}$ ,  $R_{conc}$  was calculated according to **Equation 1.10** according to Wei et al. (2013a); Wu et al. (2019).

Thus, the evaluation of the LSV and the EIS provided the comparison of the internal resistance of the MFC 2.0 with aged and replaced MCAs (**Figure 4.3C**). As a result,

replacing the MCA caused a drop of  $6 \Omega$  or 19 %, contributing to differences in maximum power densities of  $832 \pm 62 \text{ mW/m}^3$  (aged MCA) and  $1101 \pm 80 \text{ mW/m}^3$  (replaced MCA). However, in contrast to the measured internal resistance of MFC 1.0 in publication 3 with  $377 \pm 184 \Omega$  (B2), the internal resistance of both MFCs was quite low after one year of operation with  $29 \pm 1 \Omega$  (aged MCA) and with  $23 \pm 2 \Omega$  (replaced MCA). In addition, the small difference between the internal resistance of  $26 \pm 2 \Omega$  (after 233 days) and  $29 \pm 1 \Omega$  (aged MCA) indicated that MFC 2.0 was a stable system.

However, differentiating the internal resistance, the main influence on the reduced power density could be identified. The internal resistance composition of both MFCs was similar, with  $R_{conc}$  being nearly the same at  $8.1 \pm 1.3 \Omega$  (aged MCA) and  $8.1 \pm 2.5 \Omega$  (replaced MCA), resulting in a percentage of  $28 \pm 5 \%$  and  $34 \pm 5 \%$ , respectively.  $R_{act,an}$  represented the major fraction,  $51 \pm 6 \%$  and  $47 \pm 5 \%$ , respectively, which could be attributed to electron transfer from the microbiology deposited on the anode. Thus, the comparably high percentage would be the result of biofilm thickness and microbial composition, which has already been discussed in publication 4. By replacing the MCA, differences in membrane resistance (included in  $R_{ohmic}$ ) and charge transfer at the cathode ( $R_{act,cat}$ ) were expected. In fact,  $R_{ohmic}$  could be reduced by  $1.3 \Omega$  and  $R_{act,cat}$  by  $0.2 \Omega$ , resulting in a 31 % and 12 % reduction, respectively.

In summary, the replacement of the MCA affected positively the internal resistance, which is even more evident in the differences in power densities. However, as already described in publication 4, the analysis of the anodic biofilm will play a crucial role, since the large contribution to the internal resistance is also likely to have been caused here by the biofilm thickness and the proportion of methanogens. Overall, the decreasing trend of power density after half a year of operation with the MFC 1.0 (publication 3 and 4) could be reduced and prolonged by the design of the MFC 2.0, but not avoided. The reason is probably the high cathode surface area.

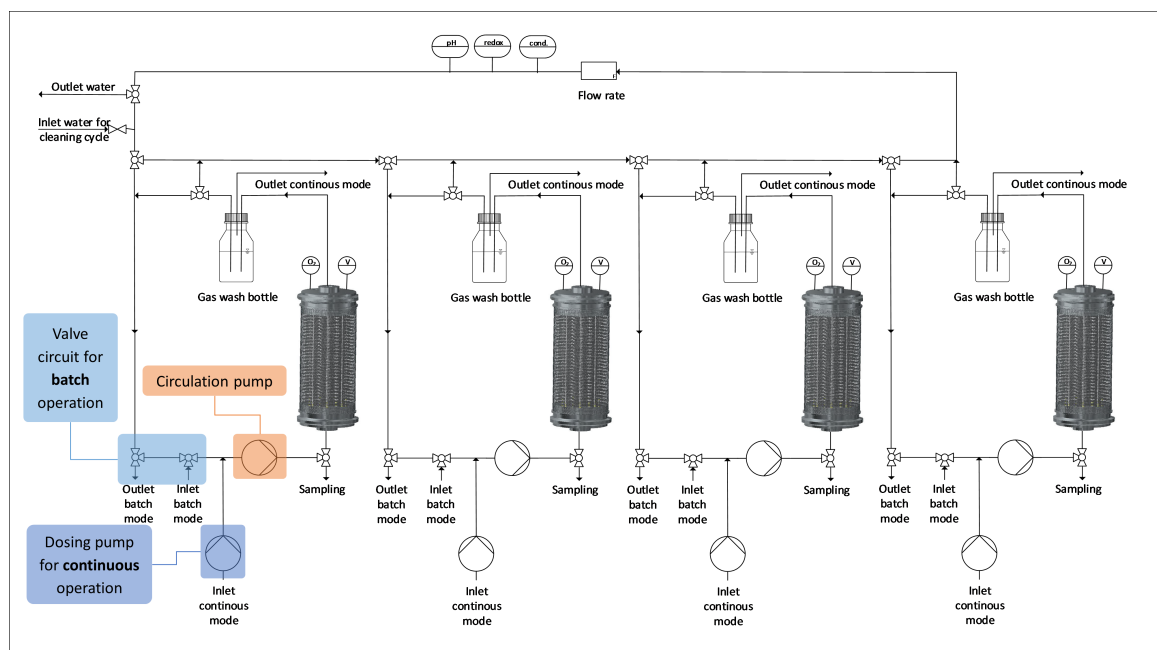
Finally, regarding the effects of the design enhancements (high specific cathode surface area, short electrode spacing, avoidance of fiber clogging), power densities were significantly increased at similar treatment efficiency. In addition, the stability of MFC 2.0 has been demonstrated for more than one year of operation by reducing the negative effects of membrane fouling, which is an important step for the commercialization of MFCs.

### 4.1.2 Adjustment of the process management

The evaluation of MFCs often focuses on the generated power density, but it requires effective treatment efficiency to make MFC treatment comparable to other technologies (Kim et al. 2016). For effective treatment, MFCs must be operated in continuous flow with short HRT (He et al. 2016b). In publication 3 and 4, the MFCs were operated in batch mode with an HRT of at least 48 hours to establish the knowledge base. Therefore, the operating conditions were changed from batch to continuous mode and the HRT was reduced up to 6 hours. Two MFCs 1.0 and two MFCs 2.0 were each investigated in parallel in batch and continuous mode.

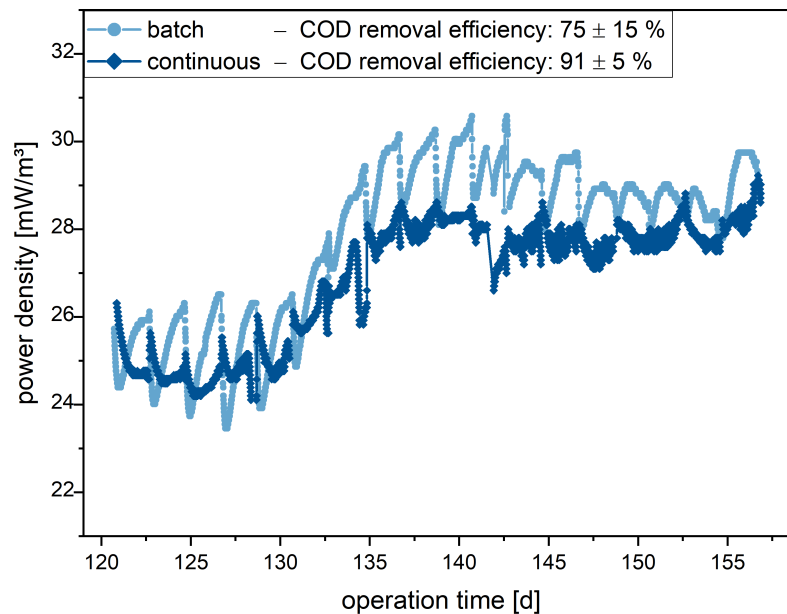
In order to compare the results of the different modes, flow rate and HRT have to be in a similar range. In the study of Wu et al. (2016), the four times higher flow rate in fed-batch mode resulted in a thinner liquid boundary layer, which caused higher kinetic power. Thus, superior COD removal rate and power density could be achieved compared to the continuous mode (Wu et al. 2016). The HRT controls the contact time between microorganisms and organic matter, thus a short HRT may result in low removal efficiency if there is insufficient time for substrate degradation (Abdallah et al. 2019). The mixed effects of HRT on power generation depend on operating conditions, such as influent concentrations and pH (Behera and Ghangrekar 2017).

For this purpose, a stackable MFC system (**Figure 4.4**) was developed. Thus, the MFCs can be operated comparably in batch and continuous mode because the flow rate was decoupled from the HRT and the mode of operation by using an additional diaphragm pump for circulating the wastewater. In addition, the valve system and peristaltic pump can be used to regulate HRT in batch or continuous mode, respectively.



**Figure 4.4:** P&ID of the MFC system for batch and continuous operation in parallel.

The [influence of the operating mode](#) on the power density and COD removal efficiency has already been investigated in a few studies (Wu et al. 2016; Lanas et al. 2014; Ahn and Logan 2010), but the MFCs were operated with different HRT and flow rates. Different power densities of MFC 2.0 in batch and continuous mode (**Figure 4.5**) could be observed over 36 days (18 batch cycles) with a constant HRT of 48 hours and a constant flow rate of about 220 ml/min. The mean power density of the MFC in batch operation with  $28 \pm 2 \text{ mW/m}^3$  was about 3 % higher than the mean power density of the MFC operated in continuous mode with  $27 \pm 1 \text{ mW/m}^3$ . However, the power density of the MFC was more stable in continuous operation because a constant COD concentration had probably adjusted inside the MFC. The observed fluctuation in power density in batch mode is likely caused by the decrease in COD concentration over time (Kletke et al. 2019), which can be described by first-order kinetics (Zhang et al. 2015; Wu et al. 2016). In addition, the higher COD concentration in the beginning of a batch cycle would be the reason for the slightly higher power density.



**Figure 4.5:** Power densities of batch and continuous operation of MFC 2.0 over 36 days.

Comparing the COD removal efficiency, the removal efficiency of MFC 2.0 in continuous mode with  $91 \pm 5 \%$  was higher and more stable than in batch mode with  $75 \pm 15 \%$ . Moreover, the same differences were observed for MFC 1.0 operated in parallel (**Table 4.3**), confirming this statement. Furthermore, these removal rates of batch operation ( $83 \pm 11 \%$ ) were comparable to the results of publication 4 (A2 with  $82 \pm 8 \%$ ). In both cases, MFC 1.0 was operated with an HRT of 48 h, but wastewater from different breweries was used. The proportion of organic acids in the wastewater was in the same range with about 21 % (publication 4) and 18 % (MFC 1.0 in batch mode). This proportion was identified in publication 4 as one key factor affecting MFC performance.

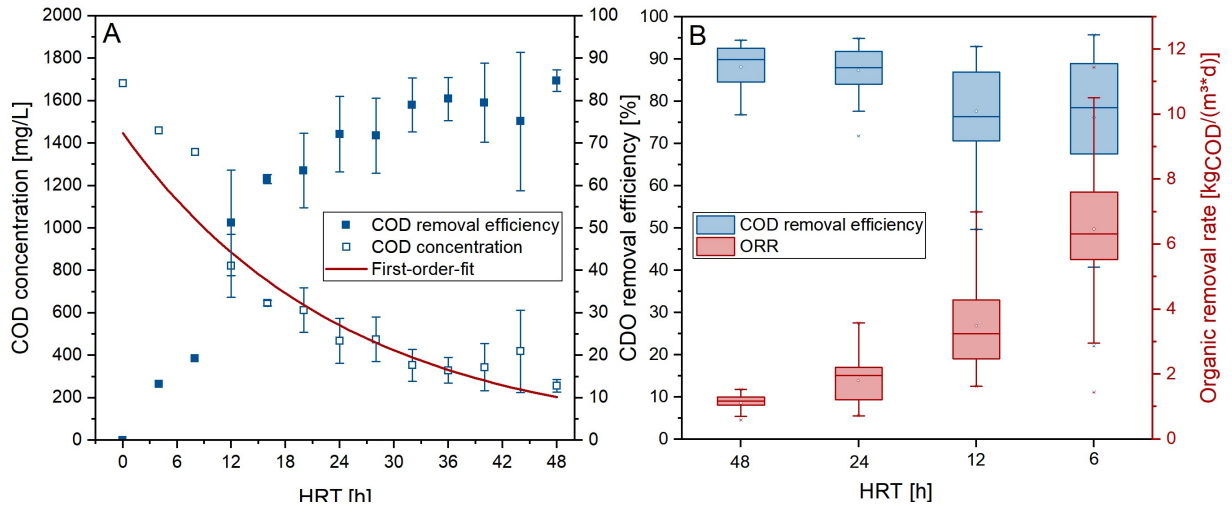
The results for higher removal rates in continuous mode are opposite to the results in literature. However, higher removal rates in fed-batch mode were attributed to the longer HRT (Lanas et al. 2014) and higher flow rate (Wu et al. 2016). Therefore, the next step

was to investigate the effect of HRT reduction on COD removal efficiency.

**Table 4.3:** COD removal rates of MFC 1.0 and MFC 2.0 in batch and continuous mode, respectively.

COD removal efficiency	batch	continuous
MFC 2.0 [%]	$75 \pm 15$	$91 \pm 5$
MFC 1.0 [%]	$83 \pm 11$	$91 \pm 4$
MFC 1.0 [%] (publication 4)	$82 \pm 8$	-

Furthermore, the [reduction of HRT](#) needs to be investigated in order to approach conventional wastewater treatment (Kim et al. 2015). The observed COD removal efficiency in batch operation of MFC 2.0 (**Figure 4.6A**) indicated that sufficient COD removal can be expected despite HRT shortening. According to the frequently applied first-order fit of the COD concentration (Wu et al. 2016; Kim et al. 2015; Zhang et al. 2015), an exponential decay of the COD concentration ( $R = 0.9$ ) is described. However, the reduction of HRT from 48 h to 6 h in continuous operation of the MFC 2.0 resulted in a decrease of the mean COD removal efficiency from  $88 \pm 6$  % to  $76 \pm 16$  % (**Figure 4.6B**). Nevertheless, by calculating the ORR, the successful reduction of HRT becomes visible. The ORR could be increased from  $1.1 \pm 0.3$   $kg/(m^3 \cdot d)$  (48 h) up to  $6.5 \pm 1.9$   $kg/(m^3 \cdot d)$  (6 h), achieving a significantly higher ORR than the median ORR of pilot-scale MFCs in industrial wastewater treatment of  $0.9$   $kg/(m^3 \cdot d)$  (Brunschweiler et al. 2020a).



**Figure 4.6:** **A** COD removal efficiency of MFC 2.0 in batch mode; **B** COD removal efficiency and ORR of MFC 2.0 in continuous mode

In conclusion, changing the operation mode into continuous mode lead to 3 % lower, but more stable power densities. In contrast, the COD removal rates in continuous mode are higher and more stable. In addition, the reduction of HRT to 6 h was successful with a removal efficiency of  $76 \pm 16$  % and ORR of  $6.6 \pm 2.1$   $kg/(m^3 \cdot d)$ .

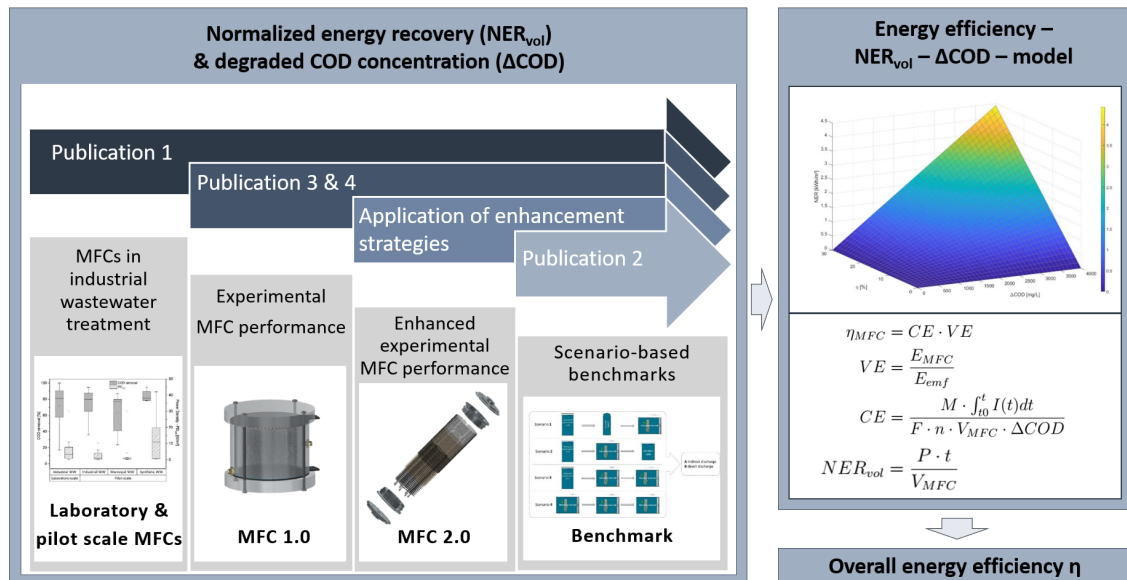


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Overall, the identification of key influencing factors in publications 3 and 4 enabled the development of targeted strategies to improve MFC performance in terms of energy efficiency and COD removal efficiency. However, for an evaluation of the examined MFC system, the key figures has to be set in relation to literature and the calculated benchmark to evaluate application possibilities.

## 4.2 Reflection of MFC performances using developed benchmarks

For an overall evaluation of MFCs, the energy efficiency and the treatment performance were reflected and classified. For this purpose, the MFC performances of publication 3 and 4 and the enhanced performances with MFC 2.0 were set in context with the MFC performances treating industrial wastewater (publication 1). Furthermore, these results were classified with the developed scenario-based benchmarks (publication 2). For an energy assessment of the MFC performances, the achieved and required energy efficiencies were calculated using the developed energy efficiency model, shown schematically in **Figure 4.7**. In addition, COD treatment efficiency was evaluated by calculating the ORR compared to the calculated benchmark. Finally, a SWOT analysis on MFCs in industrial wastewater treatment conclude the application possibilities of MFCs.



**Figure 4.7:** Schematic representation of the reflection process using the energy efficiency model

The basis for the reflection on the performance of MFC 1.0 and MFC 2.0 are the key figures summarized in **Table 4.4**. For MFC 1.0, the MFC configuration with higher performance from publication 3 and 4 was summarized in each case. Additional operating data for MFC 2.0 can be found in **Appendix B**. The key figures of the experiments with the highest energy efficiency or ORR (gray shaded columns) were consulted for comparison with MFC performances in industrial wastewater treatment and for classification in the context of the benchmark.

**Table 4.4:** Summarized key figures of MFC 1.0 and MFC 2.0 categorized into energetic MFC performance and treatment performance

	design	MFC 1.0		MFC 2.0									
	name	clogg.	comp.	MFC 1.0	MFC 2.0	batch	conti	48 h	24 h	12 h	6 h	aged	repl- aced
description	study	pub. 3	pub. 4	design		operation mode		HRT reduction				system stability	
	section	3.3	3.4	4.1.1		4.1.2		4.1.2				4.1.1	
	operation mode	batch		continuous		batch	conti	continuous				continuous	
	HRT	> 48	> 48	6 - 48 <sup>a</sup> / 12 <sup>b</sup>		48		48	24	12	6	6	
	$P_{cat}$ [mW/m <sup>2</sup> ]	11 ± 4	5 ± 2	14 ± 5	39 ± 14	0.6 ± 0.0	0.6 ± 0.0	17 ± 5	22 ± 3	14 ± 4	14 ± 5	7 ± 3	15 ± 5
energetic MFC performance	$P_{vol}$ [mW/m <sup>3</sup> ]	125 ± 51	58 ± 24	144 ± 53	824 ± 290	28 ± 2	27 ± 1	781 ± 235	1019 ± 152	649 ± 194	643 ± 231	325 ± 253	682 ± 119
	$NER_{vol}$ [kWh/m <sup>3</sup> ]	0.007 ± 0.002	0.003 ± 0.001	0.002 ± 0.001	0.008 ± 0.003	0.001 ± 0.000	0.001 ± 0.000	0.038 ± 0.015	0.024 ± 0.004	0.008 ± 0.003	0.004 ± 0.002	0.002 ± 0.001	0.004 ± 0.001
	$NER_{COD}$ [kWh/ kg <sub>COD</sub> ]	0.007 ± 0.004	0.004 ± 0.003	0.001 ± 0.001	0.005 ± 0.003	0.001 ± 0.001	0.001 ± 0.000	0.020 ± 0.012	0.016 ± 0.008	0.005 ± 0.002	0.003 ± 0.001	0.002 ± 0.001	0.003 ± 0.002
	CE [%]	1.3 ± 0.7	1.1 ± 0.5	0.16 ± 0.09	0.8 ± 0.3	0.1 ± 0.1	0.04 ± 0.02	2.1 ± 1.4	2.3 ± 1.2	0.8 ± 0.3	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.4
	$\eta$ [%]	<b>0.19</b> ± <b>0.10</b>	<b>0.11</b> ± <b>0.07</b>	<b>0.03</b> ± <b>0.02</b>	<b>0.14</b> ± <b>0.07</b>	<b>0.04</b> ± <b>0.03</b>	<b>0.02</b> ± <b>0.01</b>	<b>0.53</b> ± <b>0.33</b>	<b>0.43</b> ± <b>0.23</b>	<b>0.14</b> ± <b>0.07</b>	<b>0.08</b> ± <b>0.03</b>	<b>0.05</b> ± <b>0.02</b>	<b>0.09</b> ± <b>0.06</b>
treatment performance	$COD_{in}$ [mg/L] <sup>c</sup>	1292 ± 347	1202 ± 411	2214 ± 721	2012 ± 445	1572 ± 2409	2409 ± 744	2503 ± 561	2043 ± 721	2262 ± 762	2119 ± 430	2291 ± 283	2227 ± 275
	$\Delta COD$ [%]	83 ± 6	82 ± 8	83 ± 13	87 ± 6	75 ± 15	81 ± 5	88 ± 6	87 ± 6	78 ± 10	76 ± 16	59 ± 18	64 ± 20
	OLR [kg/(m <sup>3</sup> ·d)]	0.6 ± 0.2	0.6 ± 0.2	4.4 ± 1.4	4.0 ± 0.9	0.8 ± 0.3	1.2 ± 0.4	1.3 ± 0.3	2.0 ± 0.7	4.5 ± 1.5	8.5 ± 1.7	9.2 ± 1.1	8.9 ± 1.1
<b>ORR</b> [kg/ (m <sup>3</sup> ·d)]	<b>0.5</b> ± <b>0.2</b>	<b>0.5</b> ± <b>0.2</b>	<b>1.9</b> ± <b>0.6</b>	<b>1.8</b> ± <b>0.7</b>	<b>0.6</b> ± <b>0.3</b>	<b>1.1</b> ± <b>0.4</b>	<b>1.1</b> ± <b>0.3</b>	<b>1.8</b> ± <b>0.7</b>	<b>3.5</b> ± <b>1.2</b>	<b>6.5</b> ± <b>1.9</b>	<b>5.4</b> ± <b>1.8</b>	<b>5.9</b> ± <b>2.2</b>	

<sup>a</sup>evaluation of power density over 100 days (HRT = 6 - 48 h)<sup>b</sup>evaluation of treatment performance and calculation of  $NER_{vol}$ ,  $NER_{COD}$ , CE and  $\eta$  (HRT = 12 h)<sup>c</sup>calculated with determined corresponding DOC/COD factor

### 4.2.1 Application of the developed energy efficiency model

The energy efficiency of MFCs for industrial wastewater treatment was reflected with the application of the energy efficiency model developed in publication 2. The depiction of all calculated energy efficiencies (**Figure 4.8A**) enabled a classification of the energy efficiency of MFC 1.0 and MFC 2.0 in relation to the MFC results in literature and the calculated benchmarks. In this way, the application potential of MFCs could be estimated in terms of energy output.

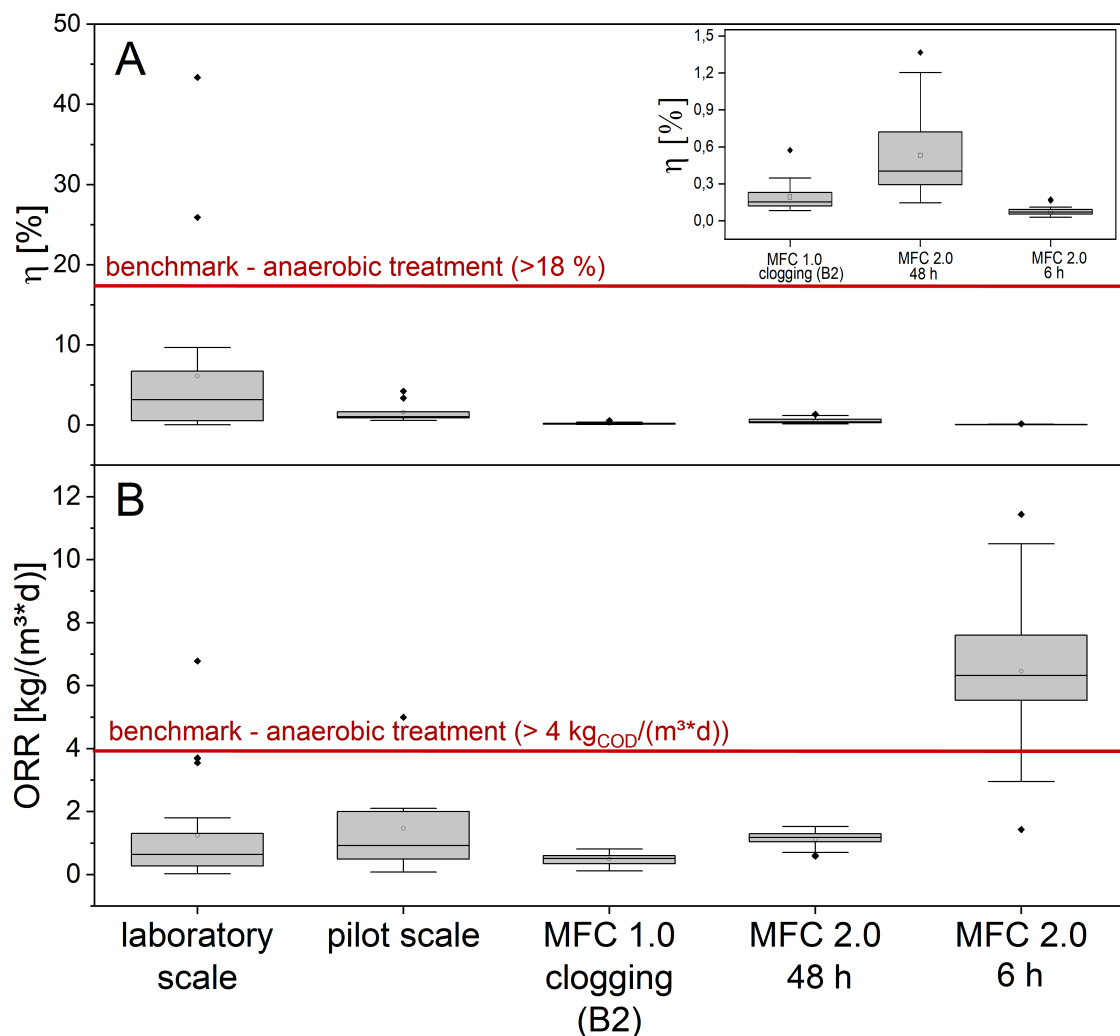
The median energy efficiencies of laboratory and pilot scale MFCs treating industrial wastewater were 3.2 % and 1.0 %, respectively. The energy efficiency is less than 10 % in almost all studies. However, in two laboratory-scale studies, energy efficiencies of 26 % and 43 % could be calculated. These extraordinary high efficiencies are likely the result of low electrochemical losses due to the small total volume (less than 0.3 ml) and very high CEs at 41 % and 27 %, respectively (Yu et al. 2014; Mardanpour et al. 2012).

In comparison with literature data, the mean energy efficiencies of MFC 1.0, MFC 2.0 (48 h) and MFC 2.0 (6 h) were relatively low with  $0.2 \pm 0.1$  %,  $0.5 \pm 0.3$  % and  $0.08 \pm 0.03$  %, respectively. Thus, although the power density has been highly increased the overall energy efficiency of MFC 2.0 remained below 1 %. The energy efficiency is the product of the CE and VE (Sleutels et al. 2016; Hamelers et al. 2010). Therefore, the low energy efficiency could be attributed to the fact that the VE could be increased by the MFC design, but the CE remained low. The competing microorganisms (e.g., methanogens) in MFC 1.0 and MFC 2.0 most likely negatively affected the CE value.

The classification of the currently achieved MFC energy efficiencies with the required energy efficiencies calculated by the benchmarks in publication 2 shows a strong discrepancy. An energy efficiency of at least 18 % (scenario 2B in publication 2) is required to theoretically achieve a comparable energy gain to conventional anaerobic treatment. The high energy efficiency of anaerobic treatment must serve as a benchmark, since both technologies aim to anaerobically degrade organic components in (high-strength) wastewater and simultaneously generate energy.

Nevertheless, other application possibilities of MFCs were also considered, since a combination of treatment technologies might be beneficial. In general, energy-intensive aerobic treatment enables compliance with required effluent limits and removal of additional nutrients. With regard to the negative energy balance ( $\eta < 0$ ), energy savings could be achieved by pre-treatment using MFCs prior to aerobic treatment. MFCs as post-treatment after anaerobic treatment (replacement of aerobic treatment) would certainly be an option from an energy point of view, but the MFC treatment performance would be reduced by the changed wastewater composition due to anaerobic treatment. In addition, the question of whether the required wastewater quality can be achieved at all must first be clarified due to their differences in treatment process (anaerobic vs. aerobic). The energetic benchmarks of a complete anaerobic-aerobic treatment are lower due to the aerobic fraction and range from 4 % to 21 %, depending on discharge option (direct/indirect) and pre-treatment step (with/without pre-treatment). However, especially with a stand-alone system, MFC scale-up needs to succeed first without performance suffering.

In conclusion, the overall energy efficiency of MFCs for industrial wastewater treatment is currently low due to a variety of influencing factors. However, the enhancement of the MFC design significantly improved the VE, but the CE remained low. Therefore, the next strategy needs to be the improvement of the CE by conditioning exoelectrogens and inhibiting methanogens. As already mentioned in publication 4, inoculum is not the key factor, but the composition of the wastewater (Brunschweiler et al. 2021a). The energetic benchmark of direct competing conventional anaerobic treatment is currently far away. Nevertheless, the energy requirement for the operation of MFCs is not as high as for aerobic treatment, as self-sufficient industrial wastewater treatment has already been reported (Dong et al. 2015; Zhang et al. 2013). Even more important than energy efficiency is the necessary COD treatment efficiency of MFCs, which is reflected in the next part.



**Figure 4.8:** **A** Overall energy efficiency  $\eta$  and **B** Organic removal rate (ORR) of laboratory and pilot scale MFCs in industrial wastewater treatment (data from publication 1), of MFC 1.0 (publication 3) and of MFC 2.0 (HRT = 48 h, HRT = 6 h).

## 4.2.2 Application of the benchmark for treatment efficiency

In the assessment of the MFC treatment efficiency, either the COD removal efficiency as a function of HRT or the ORR calculated from it can be taken into account. In addition to the removed COD concentration, the ORR directly considers the HRT, which is an important factor in becoming an alternative to conventional treatment technologies. Similar to the energy efficiency evaluation, the ORR was calculated for MFCs in industrial wastewater treatment (publication 1), the scenario-based benchmarks (publication 2), MFC 1.0 (publication 3 & 4), and MFC 2.0 (application of enhancement strategies) and depicted together in **Figure 4.8B**.

The ORR of laboratory- and pilot-scale MFCs varies widely, which could be attributed to differences in operating conditions, volumes, modes of operation, etc.. Nevertheless, the median ORR of MFCs in laboratory and pilot scale was in the similar range of about  $0.6 \text{ kg}/(\text{m}^3 \cdot \text{d})$  and  $0.9 \text{ kg}/(\text{m}^3 \cdot \text{d})$  with a median HRT of 96 h and 66 h, respectively.

Regarding the achieved mean ORR of MFC 1.0 with  $0.5 \pm 0.2 \text{ kg}/(\text{m}^3 \cdot \text{d})$  in 55 h (mean HRT) and the mean ORR of MFC 2.0 with  $1.1 \pm 0.3 \text{ kg}/(\text{m}^3 \cdot \text{d})$  in 48 h and  $6.5 \pm 1.9 \text{ kg}/(\text{m}^3 \cdot \text{d})$  in 6 h, a significantly higher ORR was obtained when HRT was shortened compared with literature results. Most MFC studies focus on achieving high power densities, in contrast to the experiment with MFC 1.0 and MFC 2.0, which concentrated on treatment efficiency, explaining the comparatively high ORR. In addition, the discrepancy between the treatment and energy performance becomes apparent, as energy efficiency decreases at high ORR at short HRT. This results from the direct correlation between HRT and  $\text{NER}_{\text{vol}}$ , which serves as the basis for calculating energy efficiency.

However, even the toughest ORR benchmark for anaerobic treatment of  $0.8 \text{ kg}/(\text{m}^3 \cdot \text{d})$  (Scenario 2B in publication 2) could be achieved if HRT is reduced. Moreover, the lower benchmarks for aerobic treatment and anaerobic-aerobic treatment ranged from 0.2 to  $0.8 \text{ kg}/(\text{m}^3 \cdot \text{d})$  (scenario 1) and 0.6 to  $5.7 \text{ kg}/(\text{m}^3 \cdot \text{d})$  (scenario 3 & 4), respectively. The ORRs of these scenarios might be the result of the long HRT needed to achieve the required discharge limits, especially for direct discharge.

In summary, the reduction of HRT positively affected the ORR of MFC 2.0, up to  $6.5 \pm 1.9 \text{ kg}/(\text{m}^3 \cdot \text{d})$ . Thus, even the anaerobic treatment benchmark would be realistically achievable. In terms of treatment efficiency, MFCs appear to become a potential alternative or complement to conventional technologies. Obviously, the high ORRs of MFC 2.0 were achieved with a volume of 2 L, so pilot-scale experiments must first confirm these results. Based on the promising results, the next step is to develop and evaluate a scale-up concept with stack systems. Therefore, a general SWOT analysis will provide an application overview of the MFC technology, in particular for industrial wastewater treatment. Thereby, the application aspects of energy efficiency and COD treatment efficiency were combined again.

### 4.3 SWOT Analysis on MFC technology in industrial wastewater treatment

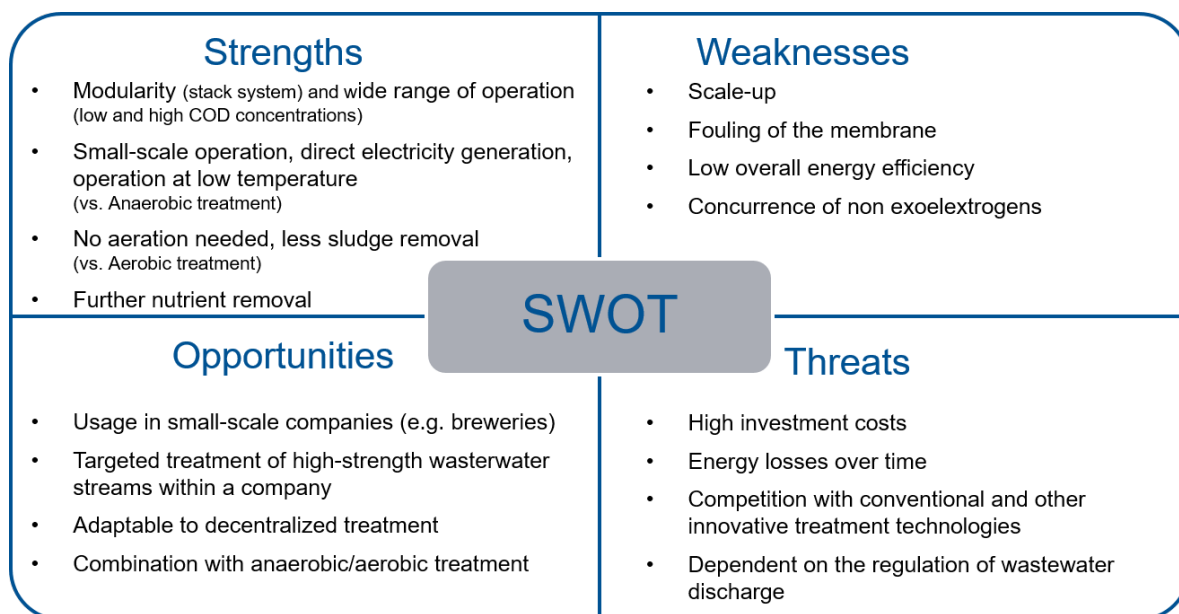
Overall, the feasibility of wastewater treatment with MFCs has already been demonstrated, and strategies are now being developed to exploit the strengths and overcome the current weaknesses to enable commercialization. An overview of the **strengths, weaknesses, opportunities and threats (SWOT)** of MFCs in the specific application of industrial wastewater treatment allows to highlight and identify the application possibilities and niches (**Figure 4.9**).

One of the main **strengths** is the modularity and compactness of MFCs, so that individually created MFC stacking systems can be adapted for each case according to the particular effluent conditions and wastewater compositions. Another important strength is the variability in operating conditions (e.g., temperature) that enable a wide range of applications and do not require harsh pretreatment (e.g., less use of chemicals). For example, MFCs can be operated with high- and low-organic loading wastewater. In addition, other nutrient removal such as nitrogen was also reported. With respect to MFCs versus anaerobic treatment, the strengths of MFCs are particularly the ability to operate on a small scale, generate electricity directly, and operate at low COD concentrations and low temperatures. The advantages of MFCs over aerobic treatment are additionally that no energy is required for aeration and less sludge is generated.

A number of application **opportunities** arise from the described unique combination of MFCs' strengths. The treatment of wastewater streams of smaller producing companies (e.g., breweries) relativizes the weakness of scalability and enables the companies to treat their wastewater, since conventional treatment is often only economically viable for large wastewater streams. In addition, MFCs can be used for targeted treatment of high-strength wastewater streams within a company and it can be determined whether MFCs are suitable for complex wastewater streams where conventional treatment technologies have difficulties. Due to the variable operating conditions, MFCs provide a complement to existing treatment systems or a combination option with anaerobic or aerobic systems. Nevertheless, there are still some **weaknesses**, so commercialization has not yet taken place. A major issue are electrochemical losses when individual MFCs are scaled up, but losses can be reduced by developing a smart stack system. In addition, fouling of the membrane during wastewater treatment reduces the MFC performance over time. However, with the MFC 2.0 the stability of the power output could already be prolonged, but cleaning concepts are necessary to further extend the lifetime of membranes. When considering MFC performances in the context of the benchmarks, overall energy efficiency remain still low. Significant performance improvements have already been demonstrated by applying the enhancement strategies. Nevertheless, the main losses are in CE due to the occurrence of non-electrogens. While these microorganisms are necessary to degrade the complex wastewater matrix, a balance must be achieved between them and the exoelectrogens for higher CE.

In addition, it will be important to identify **threats** of the MFC technology in order to overcome them through smart strategies and targeted improvements. The current high

investment costs are a major barrier for the commercialization of MFC technology, but already the materials become cheaper, the use of a membrane-less MFC was already shown and an economic assessment of an MFC implementation for wastewater treatment revealed MFCs with potential economic benefits (Trapero et al. 2017). The energy losses that occur over time are mainly due to fouling of the membranes, which needs to be overcome. The competition with conventional treatment technologies could be more seen as a combination of them with MFCs and specific niches for MFCs can be identified. The dependence on the discharge regulations concern all wastewater technologies, but stringent discharge limits could be expected.



**Figure 4.9:** Strengths, weaknesses, opportunities, and threats of MFCs in industrial wastewater treatment.

In addition, treating industrial rather than municipal wastewater could be more advantageous because (1) the energy potential is higher due to the higher organic load, (2) smaller treatment plants are needed, and (3) the discharge quality requirements are more variable, resulting in savings in discharge costs and, in the case of possible reuse of the water, freshwater costs. In summary, MFCs for industrial wastewater treatment will be an interesting technology for specific niche applications if scale-up is successful in terms of energy, treatment and economic aspects.



## 5 Conclusion & future perspectives

In order to evaluate the application of MFC technology in industrial wastewater treatment, both treatment and energy efficiencies must be considered and placed in context with efficiencies of conventional biological treatment technologies. In general, MFCs have been successfully used to remove organic loadings from industrial wastewater, revealing that the composition (e.g., conductivity) of industrial wastewater, rather than the type (e.g., brewery wastewater), is crucial. In addition to COD removal, nitrogen- and sulfate-containing constituents in wastewater could already be reduced with MFCs.

In terms of COD treatment efficiency, MFCs can compete technologically with conventional treatment technologies. However, no specific application fields have been identified yet, as no clear benchmarks have been established for the treatment of industrial wastewater. The immense theoretical energy potential of MFCs, based on the direct generation of electricity without combustion losses (as in anaerobic processes), is currently not being exploited. Therefore, an energetically self-sufficient treatment performance must be targeted first, as already reported for two MFC pilot-scale plants.

With respect to commercialization and enhancement of MFC performance, key design and operational factors were identified, applied, and evaluated through the developed benchmark in the context of industrial wastewater treatment.

In the long-term evaluation of the anode configuration using carbon fiber brushes for 180 days, by reducing the clogging of the fibers by using two 5 cm diameter brushes, doubling the power density was achieved compared to one 10 cm diameter brush. Based on these results, in combination with a high specific cathode surface area and short electrode spacing, the maximum power densities could be significantly increased by a factor of up to 11. In addition, long-term stability could be extended to one year with the new MFC design as the negative effects of membrane fouling could be reduced, representing an essential step towards the commercialization of MFCs.

The use of brewery wastewater with high content of easily degradable organic acids and high conductivity improved MFC performance in terms of treatment efficiency and power density, but also highlighted the need to inhibit competing methanogens. In addition, operating conditions were identified as mainly determining the microbial composition of the biofilm, rather than the inoculum. Considering these influencing factors, changing the operation mode to continuous mode resulted in slightly lower power densities (3 %) but higher COD treatment efficiencies and, in both cases, significantly more stable results than in batch mode. The successful reduction of HRT to 6 h with a COD removal rate of  $76 \pm 16$  % and ORR of  $6.5 \pm 1.9$   $kg/(m^3 \cdot d)$  demonstrates the great potential of MFCs with respect to commercialization.

This beneficial treatment performance is the basis for MFCs to become a potential alternative to conventional treatment technologies and also to address the weaknesses of anaerobic technology (narrow operating range, required gas treatment) and aerobic treatment (high energy consumption, high sludge yield). In this way, the advantages of the

wide range of operating conditions and the modularity of stack systems can be exploited, provided that the promising results can be confirmed on a large scale. However, the energy efficiency of MFCs is currently low, and their improvement is inevitable for possible commercialization.

Especially for the treatment of industrial wastewater, MFCs can become a promising option, since there is a high energy potential due to the higher organic load. In addition, a demand for smaller wastewater plants (e.g., in smaller breweries) also exists and the requirements for effluent quality vary according to local legal regulations. Therefore, savings in both discharge costs and fresh water costs are possible if the water is reused within the plant.

Although the potential exists, present limitations are still noticeable, providing both challenges and opportunities for future developments in MFC technology. Through the application of enhancement strategies, power densities and treatment efficiencies have been significantly improved, but the overall energy efficiency of MFC ( $\eta < 1\%$ ) remained low compared to required energy efficiency of anaerobic treatment ( $\eta > 18\%$ ).

The voltage efficiency has already been significantly increased by the enhancement strategies, but the coulombic efficiency remained low, indicating the need to increase the proportion of exoelectrogenic microorganisms. Therefore, in addition to the parameters already considered for the design and operating conditions, the biological influencing factors must also be investigated in a next step. Adjusting process conditions to an environment where appropriate exoelectrogens dominate the biofilm, using exoelectrogenic pure cultures along with a stable mixed culture, and inhibiting methanogens are some possible suggestions.

In addition, demonstrating high and stable performance in long-term pilot-scale trials is a prerequisite for potential commercialization. Therefore, efforts to scale up using appropriate compositions of industrial wastewater should be the focus of further developments. Parallel or serial stack plants are perfectly suited to verify the achievement of discharge conditions and to increase the treatment volume in the long-term, but the ideal setup for industrial wastewater has to be determined.

# A Publications

## A.1 Industrial wastewater treatment with simultaneous energy recovery using microbial fuel cells - a review

**Authors:** Sarah Brunschweiler; Thomas Hofmann; Karl Glas

**DOI:** 10.23763/BrSc20-15brunschweiler

**Keywords:** microbial fuel cell, industrial wastewater treatment, energy recovery, nutrient removal

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## A.2 Microbial fuel cells for brewery wastewater treatment - efficiency requirements and treatment performance

**Authors:** Sarah Brunschweiler; Laura Hörner; Thomas Hofmann; Karl Glas

**DOI:** 10.23763/BrSc21-02brunschweiler

**Keywords:** microbial fuel cell, brewery wastewater treatment, energy efficiency

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## A.3 The effect of clogging on the long-term stability of different carbon fiber brushes in microbial fuel cells for brewery wastewater treatment

**Authors:** Sarah Brunschweiler; Emile Tabu Ojong; Jana Weisser; Christian Schwaferts; Martin Elsner; Natalia P. Ivelva; Roland Haseneder; Thomas Hofmann; Karl Glas

**DOI:** 10.1016/j.biteb.2020.100420

**Keywords:** microbial fuel cell, carbon fiber brush, clogging, long-term performance, brewery wastewater

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## **A.4 Key factors of brewery wastewater influencing treatment efficiency and power output of microbial fuel cells**

**Authors:** Sarah Brunschweiler; Vera Kranich; Roland Haseneder; Thomas Hofmann; Karl Glas

**DOI:** 10.23763/BrSc21-06brunschweiler

**Keywords:** microbial fuel cell, brewery wastewater, different compositions, influence factors

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## B Operational conditions

The inoculation process and operation were initialized in parallel in the same way with MFC 1.0 and MFC 2.0. Both were inoculated with anaerobic sludge from a brewery (1:20 ratio), whose brewery wastewater from the mixing and equalization tank was subsequently treated with these MFCs. The pH of the wastewater was neutralized as needed in the range of 6 - 8 and atres c-plus™(0.1 mL/L), a complex trace element solution of cobalt, nickel, selenium and iron, was added.

The brewery wastewater used for the tests always came from the same brewery, but the respective batches may vary. The mean values of the concentrations including the standard deviation over the entire period are shown in **Table B.1**. Photometric tests (Machery Nagel, Germany) were used to analyze the unadjusted brewery wastewater for COD (Nanocolor® COD 1500), organic acids (Nanocolor® Organic Acids 3000),  $NH_4-N$  (Nanocolor® Ammonium 3) and  $P_{ges}$  (Nanocolor® ortho-and total Phosphate 50). DOC was determined using the TOC analyzer (liquiTOC II, Elementar, GER), with samples filtered through a 0.45  $\mu m$  syringe filter prior to measurement.

**Table B.1:** Characteristic of the unadjusted brewery wastewater over about one year of operation

Parameter	Unit	Value
COD	mg/L	2922 $\pm$ 600
DOC	mg/L	746 $\pm$ 225
Organic acids	mg/L	799 $\pm$ 214
$NH_4-N$	mg/L	3 $\pm$ 2
Ges-P	mg/L	47 $\pm$ 18
COD : DOC	-	4.1 $\pm$ 0.8
COD : Organic acids	%	29 $\pm$ 11

### B.1 Validation of MFC 2.0

In order to compare the MFC design, the MFC 1.0 and the MFC 2.0 were operated in duplicate for a period of 100 days. During this period, the MFCs were operated in parallel in continuous mode and the flow rate was gradually doubled. This included reducing the HRT from 24 to 6 hours for MFC 1.0 and from 48 to 12 hours in parallel for MFC 2.0. The voltage of the MFCs were continuously recorded via external resistance, transformed and recorded on a PC using a data logger (PicoLog ADC-20, Pico Technology, UK) and the associated software (PicoLog 6). The mean power output was calculated via Ohm's law using the measured voltage and external resistance. LSV was measured after 227 and

233 days of MFC 1.0 and MFC 2.0 in duplicate with a sampling rate of  $0.2\text{ mV/s}$  using a potentiostat (VSP, BioLogic, France) in a two-electrode setup at steady-state conditions.

## B.2 System stability of MFC 2.0

The operating period for comparison of the MFC with aged and replaced MCA was 28 days after one year of operation of both systems. The HRT was  $6\text{ h}$ . Periodic LSV and EIS measurements were performed using a potentiostat (VSP, BioLogic, France) in a two-electrode setup, each in duplicate. In order to determine internal resistance, LSV was measured at a sampling rate of  $0.2\text{ mV/s}$  after 30 minutes of equilibrium time. The EIS measurement was also started after an equilibrium time of 30 minutes with an applied potential of  $240\text{ mV}$  (close to midpoint potential). The sinus amplitude was set to  $10\text{ mV}$  and the frequency range was from  $100\text{ kHz}$  to  $5\text{ mHz}$  (forward scan) and vice versa from  $5\text{ mHz}$  to  $100\text{ kHz}$  (reverse scan).

## B.3 Adjustment of the process management

Parallel batch and continuous operation of MFC 1.0 and MFC 2.0, respectively, was conducted for 36 days. In the batch system, the wastewater in the MFC was completely replaced through the valve system every  $48\text{ h}$ , and the flow rate of the peristaltic pump for continuous operation was set to  $1.1\text{ L/d}$  to obtain a comparable HRT of  $48\text{ h}$ . The circulating pump was set to the same flow rate of about  $220\text{ mL/min}$  in both cases.

In order to estimate the effects of reducing HRT, the COD concentration profile was first measured during batch operation of MFC 1.0 and MFC 2.0. Reduction during continuous operation was performed over 195 days with a stepwise reduction of HRT from  $48\text{ h}$  to  $6\text{ h}$ .

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