

Electric Power Generation from Municipal, Food, and Animal Wastewaters Using Microbial Fuel Cells

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Abstract

Here, we explore if microbial fuel cell (MFC) technology can replace activated sludge processes for secondary wastewater treatment. We will discuss the *present* limitations and problems of electric current generation when a complex wastewater is treated with a diverse and undefined community of microbes in large-scale systems. These challenges include low coulombic efficiencies, slow kinetic rates, and nonlinear power density increases during scale-up efforts. Finally, we developed a prediction of the main economic gain from treating wastewater with microbial fuel cells. We conclude that electricity generation will not justify MFC operation, but that BOD removal with this more sustainable technology is attractive.

Keywords: Microbial fuel cells, Wastewater treatment, Economical evaluation, Coulombic efficiency, Fuel cells

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1. Introduction

Researchers in the fields of Biological and Environmental Engineering have shown a real potential to apply microbial fuel cell technology to wastewater treatment. Motivations of their work were based on the economic, environmental, and social needs for sustainable wastewater treatment systems and renewable energy. We will first give a short introduction into wastewater characteristics, followed by a review of the MFC technology, before we start our evaluation of this technology for large scale wastewater treatment applications.

1.1. Wastewater Characteristics

We project the treatment of municipal and industrial wastewater with MFC technology to reduce energy usage in wastewater treatment facilities. Municipal wastewater is included here as a source of organic compounds, despite a low concentration of biochemical oxygen demand (BOD) (ca. < 300 mg/L), and thus a low energy density. Initially, however, MFC technology will be explored with high-strength industrial wastewater with concentrations exceeding 2000 mg (BOD)/L because of the higher energy densities available in the wastewater. Many of these wastewaters are generated in the food industry and are rich in

easily degradable carbohydrates and organic acids with relatively low concentrations of organic nitrogen (e.g., proteins) [1–4]. Animal wastewaters from the livestock-related industry are often particularly high in organic material content (ca. 100 000 mg chemical oxygen demand [COD]/L for animal wastes) and may contain high levels of nitrogen-containing components, such as proteins, and harder to degrade organic materials, such as cellulose [5]. In addition, slaughterhouse wastewaters from the livestock-related industry may also include lipids besides carbohydrates, organic acids, and proteins. Despite considerable variability in the characteristics of wastewater depending on their sources, the following general characterization parameters were defined [6]:

- “Soluble” wastewater, which is characterized as non-settleable and noncoagulable, is composed of readily biodegradable COD, readily hydrolysable COD, and inert substrates.
- “Colloidal” wastewater, which is characterized as non-settleable, is composed of heterotrophic biomass, inert substrates, and slowly-biodegradable COD substrate.
- “Particulate” wastewater, which is characterized as settleable, is composed of biomass, slowly-biodegradable COD, and inert substrates.

The primary organic compound in wastewater is particulate organic matter. Particulate organic matter is 100 to 300 μm

in size and is mainly composed of proteins, polysaccharides, and lipids. To be utilized by bacteria, the polymeric compounds must first be converted to low molecular-weight organic substrates, such as sugars, amino acids, and volatile fatty acids. Particle conversion, which is accomplished through particle degradation and/or hydrolysis, is slow. Hydrolysis of entrapped organic particulates to lower molecular weight substrates can be regarded as the rate-limiting step in bacterial processing because the rate of breakdown of soluble and colloidal wastewater components is much faster [7]. To convert the particulate fraction of wastewater into electricity without the requirements of a very large MFC volume, engineers can design a two-step process in which particulate compounds are first hydrolyzed and preacidified in a mixed tank. Then, the preacidified solution with mainly carboxylic acids is fed to a MFC to treat mainly soluble wastewater. The addition of a preacidification tank before a high-rate anaerobic digester system is already standard for the treatment and bioconversion of high-strength wastewater, such as brewery wastewater, into methane gas.

1.2. MFC Technology

In 1911, Michael Potter, a botany professor at the University of Durham, United Kingdom, demonstrated that microbes created a voltage and conveyed a current [8]. This technology is now known as microbial fuel cell (MFC), which is an

example of bioelectrochemical systems (BES). During the last decade, increasing energy costs and the desire for environmentally sustainable energy sources have stimulated research on how to use MFCs for simultaneous wastewater treatment and energy generation. This surge in activity resulted in considerable improvements in power densities and power output, showing a real potential to scale up [9–11].

1.3. How It Works

The MFC for wastewater treatment is an engineered system designed to support a nondefined mixed culture of microbes in the anode chamber. These MFCs transform (treat) organic substrates (in wastewater) through oxidation-reduction reactions and transport electrons through an electric circuit for the generation of electric power. The oxidation reactions occur in the anode compartment where bacteria metabolize organic substrates to generate energy for cell maintenance and biomass synthesis. Bacteria, which are capable of extracellular electron transfer (called electricigens), can respire with the solid electrode (Figure 1), while conserving energy by oxidizing organic molecules, such as acetate, completely to carbon dioxide [12].

The electrons transfer from the anode electrode via an external circuit to the cathode electrode to participate in a reduction reaction. MFC cathodes have a number of different configuration and catholyte fluid options [11,

Lars Angenent's research interest includes mixed culture bioprocessing of wastes to produce energy. Currently, the Angenent laboratory studies anaerobic digestion systems to produce methane, bioelectrochemical systems, such as microbial fuel cells, to generate electric current, and anaerobic fermentation to produce butyrate or butanol. Molecular biology methods are used to ascertain the community composition and its function with the ultimate goal to engineer better bioprocesses or gene expression to understand the important mechanisms in bioprocesses.



Miriam Rosenbaum's research interest revolves around bioelectrochemical systems. Together with Uwe Schröder, she studied the exploitation of photosynthetic microbial processes with various electrocatalysts. With a team of graduate students, she is currently studying various bioelectrochemical systems in Lars Angenent's laboratory. For instance, they are investigating synergistic microbial interactions for a more efficient energy conversion in BES and for new types of biosensors.



Jeff Fornero's research interests include improving cathode operation conditions by pressurizing and carbon dioxide addition to enhance the practicality of bioelectrochemical system treatment of wastewater. Currently, Jeff is exploring opportunities to scale-up bioelectrochemical systems through novel reactor configurations and materials of construction selection.



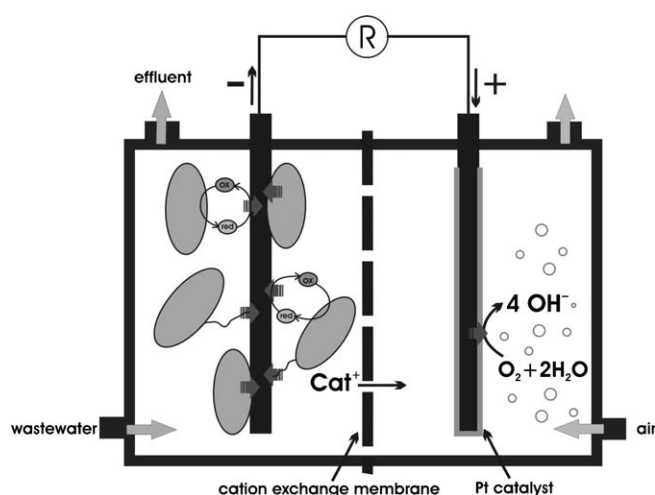


Fig. 1. The working principle of a microbial fuel cell: substrate (in wastewater) is metabolized by bacteria, which transfer the gained electrons to the anode electrode through three mechanisms: direct cell contact, shuttling via electron mediators (red/ox), or shuttling via nanowires. Electrons flow from the anode (negative pole) to the cathode (positive pole) via an electric circuit and power is generated because of an external resistor (R). Cation transfer from the anolyte to the catholyte ensures electroneutrality when a cation-exchange membrane is installed. On the cathode, oxygen is reduced to form hydroxide ions with protons available from water ($\text{O}_2 + 4\text{e}^- + 2\text{H}_2\text{O} \rightarrow 4\text{OH}^-$).

13–18]—in the conventional design, platinum (Pt) catalyzes the reduction of oxygen in an aerated catholyte solution; with oxygen being the terminal electron acceptor of the overall fuel cell reaction (Figure 1). With the electron transfer from the anode to the cathode, it is necessary to maintain electroneutrality in the fuel cell. Charge balance maintenance is mediated by an ion exchange membrane that allows the passage of cations/protons or anions/hydroxide ions, depending on the pH and membrane selected between the anode and cathode chambers. For cation exchange membranes at neutral pH levels, researchers have found a movement of cations, such as sodium and potassium, from synthetic wastewater to the catholyte [19, 20]. This is in contrast to hydrogen fuel cells in which protons are moving through an electrolyte [21]. The membranes also function to separate the environmental conditions with the goal to maximize the potential difference between anode and cathode electrodes to optimize the electric power generation.

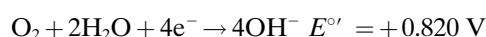
1.4. Bacteria and Energy Management

Microorganisms gain energy for their function by transferring electrons from an electron donor to a terminal electron acceptor. Aerobic heterotrophic bacteria gain energy by transferring electrons from a reduced organic substrate through metabolic and respiratory pathways to their terminal electron acceptor oxygen (with the help of the intermediate electron carrier nicotinamide adenine dinucleotide (NAD^+/NADH)). Under anaerobic conditions (no oxygen present) the reoxidation of NADH can still be

accomplished through respiration when alternative electron acceptors, such as nitrate [22], soluble or insoluble iron (Fe^{3+}), or solid electrodes [12], are present. An alternative pathway for heterotrophic bacteria under anaerobic conditions is fermentation, during which the reoxidation of NADH occurs with primary metabolites acting as terminal electron acceptors [23].

In the anaerobic environment of a MFC anode, the solid anode electrode acts as an electron acceptor facilitating anaerobic respiration and the reoxidation of NADH to NAD^+ (even though oxygen remains the terminal electron acceptor in the cathode of a conventional MFC; Figure 1). The electron transfer from the intracellular NADH can be accomplished through direct cell contact with the anode [24], shuttling via electron mediators [25–27], or shuttling via nanowires or other filamentous appendages [28, 29] (Figure 1).

The theoretical potential difference (maximum energy gain) between the biological standard potential (E° [V]) of the terminal metabolic electron donor NADH and the terminal electron acceptor oxygen is 1.14 V ($+0.820\text{ V} - (-0.320\text{ V})$) [30].



Other terminal electron acceptors in the cathode of the MFC, such as nitrate in biological cathodes, were also proposed or proven to facilitate electron transfer [31, 32]. This may be important for wastewater treatment because nitrate removal is critical in regards to nutrient removal. However, with nitrate ($E^\circ = +0.421\text{ V}$), the theoretical potential difference to NADH decreases (0.741 V for nitrate), and the amount of energy available for the transforming bacteria decreases accordingly [32].

The cathode oxygen reduction reaction, however, will not yield the theoretical +0.84 V potential, because the potential is reduced by activation polarization losses. Further, as resistances are lowered and the current increases, ohmic losses and concentration polarization losses become more prominent. A more achievable potential for the MFC cathode when considering losses is +0.51 V [30]. Bacteria that are able to exploit the relatively higher potential of the anode electrode compared to other electron acceptors, such as carbon dioxide for autotrophic methanogens, will transfer electrons to the electrode to gain more energy, and therefore, deriving a competitive advantage. Besides generating a high power output, the measured potential difference between anode and cathode must also be maximized to select for a microbial community with enhanced electrochemical activity [33].

2. Wastewater Treatment with MFCs

Lab-scale MFCs have been operated on synthetic (e.g., sucrose, glucose, acetate) and real wastewater (e.g., munic-

Table 1. Coulombic efficiencies and maximum power densities or output achieved in MFCs with undefined mixed cultures in the anode chamber using various anode volumes, substrates, and external resistances. The data are ordered based on coulombic efficiency with the highest percentages on the top. Real wastewater substrate solutions (without preacidification) are given in bold.

§ [a]	Anode volume (mL)	Synthetic or real wastewater substrate type [b]	Concentration (mg/L) [c]	Maximum power [d]	External resistor (Ω) [e]	Coulombic efficiency (%) [f]	Ref.
F	560	Acetate	458	48 W/m ³	20	98	[17]
D	40	Glucose	2000	3600 mW/m ²	< 100	89	[18]
D	240	Glucose	NP [g]	4310 mW/m ²	< 100	81	[27]
F	390	Glucose	467	35 W/m ³	20	74	[17]
B	22	Acetate	1000	286 mW/m ²	33	65	[16]
B	22	Butyrate	1000	220 mW/m ²	33	50	[16]
F	390	Hospital wastewater	332	25 W/m³	NP [g]	36	[17]
C	28	Acetate	800	506 mW/m ²	218	29	[36]
B	22	Starch	1000	242 mW/m ²	33	21	[16]
H	28	BSA	1100	354 mW/m ²	> 50	20.6	[35]
F	390	Municipal wastewater	429	10 W/m³	75	20	[17]
B	22	Dextran	1000	150 mW/m ²	33	17	[16]
E	440	Sucrose	800	29 W/m ³	20	14.2	[34]
B	22	Glucose	1000	212 mW/m ²	33	14	[16]
E	520	Sucrose	1000	170 mW/m ²	66	8.1	[13]
I	28	Swine waste (soluble fraction)	8320	261 mW/m²	200	8	[37]
C	28	Butyrate	1000	305 mW/m ²	1000	7.8	[36]
A	22	Municipal wastewater	379	72 mW/m²	470	6	[16]
H	28	Peptone	500	269 mW/m ²	> 50	6.0	[35]
H	28	Slaughterhouse wastewater	1420	80 mW/m²	> 50	5.2	[35]
G	5400	Brewery wastewater	1168	5 W/m³	10	3.6	This paper

[a] Described in the paragraphs A–I.

[b] Real wastewater substrate in bold, these wastewaters were not preacidified.

[c] COD concentration was used for the real wastewater substrates

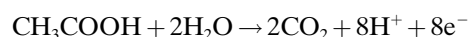
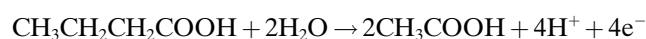
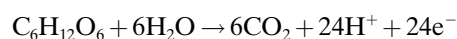
[d] Maximum power density in mW per m² anodic electrode surface area and maximum power output in W per M³ of total anodic volume.

[e] Used to obtain the maximum power except for the Min et al. [16] for which we reported the average values.

[f] Data from maximum power density of output was used to calculate the coulombic efficiency except for the Min et al. [16]

[g] NP: information not provided by authors

ipal, hospital, brewery, animal wastewater) [13, 17, 18, 34–37]. Hexose, butyrate, and acetate were chosen here as model components for a complex wastewater with a diverse composition of organic compounds. The MFC half reactions with these substrates are:



The removal of reducing equivalents (electrons) from the anode chamber is basically similar to decreasing the chemical oxygen demand (COD) concentration from the wastewater. Therefore, the calculation of the coulombic efficiency for the organic substrate oxidation in the anode chamber is performed based on the amount of BOD or COD removed by the mixed culture in the anode chamber and the electric current generated (Equation 1).

In the following paragraphs A–I, we give an overview of different MFC research studies that used either synthetic or real wastewater as the electron donor. We discuss the operation parameters and the specific performance data. Table 1 summarizes all performance data and lists the compared studies ordered from highest to lowest coulombic efficiency.

A. Min and Logan [16] reported that a lab-scale MFC with an anode volume of 22 mL was able to continually generate electricity from wastewater while at the same time reducing the COD concentration of the waste stream. The batch MFC was able to produce a maximum power density of 72 mW/m² (per anode electrode surface area) with a hydraulic retention time (HRT) of 1.1 h and a COD removal efficiency of 42%. The coulombic efficiency for domestic wastewater was ca. 6% at an average power density of 56 mW/m² and a 1.4-h HRT.

B. In the same study, the power densities and coulombic efficiencies for various chemical compounds (i.e., acetate, starch, glucose, dextran, and butyrate), which were introduced into the same acclimated MFC, were higher than for the wastewater substrate [16]. For example, adding acetate increased the power density to 286 mW/m² and the coulombic efficiency to 65% at a 0.68-h HRT and an external resistor of 33 Ω . However, the COD removal efficiencies for those compounds were lower than the COD removal efficiencies for the municipal wastewater due to a shorter HRT; with higher COD removal efficiencies for the fermentable substrates (i.e., starch and glucose) than the nonfermentable substrates (i.e., acetate and butyrate) while the coulombic efficiencies showed an opposite trend (Table 1).

C. In another MFC study from the Logan laboratory with a 28-mL anode volume, Liu et al. [36] generated electricity

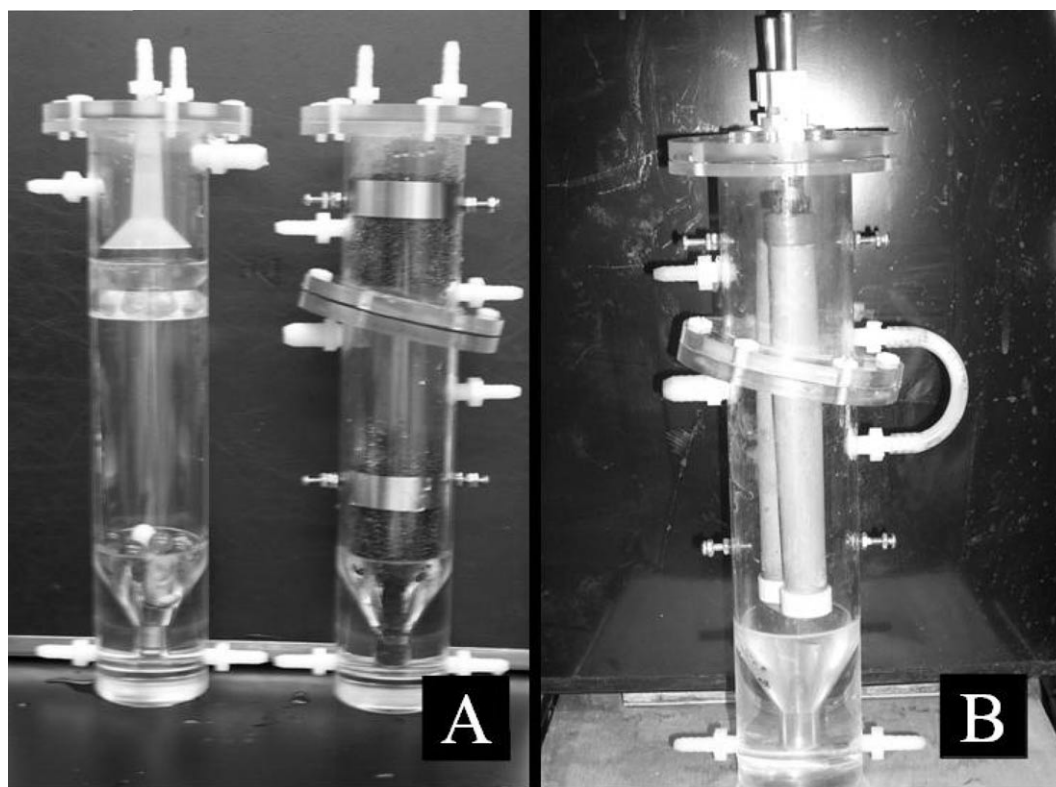


Fig. 2. Pictures of lab-scale bioreactors: A. UASB reactor (left) and first-generation UMFC reactor (right); and B. second-generation UMFC reactor with an interior cathode configuration. The anodic electrode that consisted of granular activated carbon was omitted in picture B to show the cathode configuration.

from butyrate and acetate. The individual batch periods lasted ca. 20 h with substrate removal efficiencies of 98 and 99% for butyrate and acetate, respectively. For acetate the power density was 506 mW/m^2 with a coulombic efficiency of 29%, while butyrate showed a lower power density of 305 mW/m^2 and coulombic efficiency of 7.8%.

D. Rabaey et al. [18] obtained a maximum power density of 3600 mW/m^2 and coulombic efficiency of 89% in a batch-operated MFC fed with glucose at a concentration of 2000 mg/L (40 mL anode volume). In a follow-up study in the same lab with a similar MFC with an anode volume of 240 mL, a maximum power density of 4310 mW/m^2 and an 81% coulombic efficiency was reported when fed glucose [27]. The very low volatile fatty acid levels in the anolyte and hydrogen concentrations in the headspace (below detection) explain the relatively high coulombic efficiency for glucose, while methane generation in the well functioning MFC was suppressed [27]. This is important because it shows that MFCs can be efficient energy conversion devices in regards to yields if the internal losses (e.g., ohmic and polarization losses) are minimized. However, the maximum volumetric loading rates for the two studies were only 2.5 g COD/L/d and 1 g COD/L/d , respectively [18, 27]. Such loading rates are much lower compared to high-rate anaerobic digestion systems, such as the upflow anaerobic sludge blanket (UASB) or the anaerobic migrating blanket reactor

(AMBR) for which volumetric loading rates $> 20 \text{ g COD/L/d}$ have been reported [38–40].

E. Rather than using a batch-fed MFC, He et al. [13] in our laboratory fed a sucrose solution at a concentration of 1 g/L continuously to an upflow microbial fuel cell (UMFC) with an anode volume of 520 mL (Figure 2). We obtained a maximum power density of 170 mW/m^2 and a coulombic efficiency of 8.1% at an external resistor of 66Ω . In a follow-up study with a second-generation UMFC (440 mL anode volume – Figure 2), we lowered the internal resistance compared to the first-generation UMFC by decreasing the distance between the electrodes and increasing the membrane surface area, resulting in a higher power output (a maximum volumetric power of 29.2 W/m^3 and a coulombic efficiency of 14.2% was achieved at a lower external resistance of 20Ω with 800 mg/L sucrose) [34]. The volumetric loading rate with the highest power output in this study was 3.4 g COD/L/d .

F. A continuously-fed tubular MFC (total anode compartment volume of 560 mL for acetate and 390 mL for the other substrates) with an outside cathode was tested for four different wastewater solutions: synthetic acetate, synthetic glucose, prefiltered municipal wastewater, and prefiltered hospital wastewater [17]. The maximum power outputs for these wastewaters were 48 ($20\text{-}\Omega$ external resistor), 35 (20Ω), 10 (75Ω), and 25 (Ω not reported) W/m^3 at COD concentrations of 458, 467, 429, and 332 mg/L and coulombic efficiencies of 98, 74, 20,

and 36%, respectively. The MFC power output increased when acetate, peptone, or digester effluent (with a high acetate concentration) was augmented to municipal wastewater influent to show that power was limited by the absence of readily degradable organics because of slow degradation kinetics of complex organics in real wastewater.

G. A low coulombic efficiency during treatment of a complex wastewater was also shown in our lab with a high-strength waste stream from the brewery industry (no preacidification was performed for this clarified spent grain liquor). We choose this wastewater with a COD concentration of 533–2800 mg/L (after dilution with make-up water) because of its high energy density. The COD and BOD removal efficiencies with a 5.4-L anode volume were relatively low and varied from 28.8–32.9% and 47–85%, respectively, because of the predominance of slowly degradable organic material. Treatment with this MFC resulted in a maximum power output of 5.0 W/m³ (external resistance of 10 Ω and a pH of 7.3) and a sustainable coulombic efficiency of 1.5–3.6% with an external resistor of 100 Ω (total current of 6.6–6.8 mA). After an operating period of five months, a thick biofilm developed on the anodic electrode, and entrapment of organic particles from the wastewater may explain the low coulombic efficiency during removal of BOD. In addition, we observed an increase in methane generation over time due to the development of pockets in the thick anodic biofilm where electricigens could not compete with methanogens due to insulating effects of the biofilm on the electrode material. We had already published that methane generation was an important factor that will reduce the coulombic efficiency in MFCs [13]. Unexpectedly, however, the internal resistance (measured by the steady discharging method) remained at 3.83 Ω before and after a thick biofilm developed during an operating period of five months while the coulombic efficiency decreased from 1.5–3.6% to 0.6%.

H. Protein mixtures make up a large fraction of the organic material in municipal wastewater and wastewater from livestock-related industries. Heilmann and Logan [35] performed a study on the electricity generation from protein-rich wastewaters using a MFC with a 28-mL anode. In their study, three different protein containing waste streams (bovine serum albumin [BSA] added to municipal wastewater [1100 mg/L], peptone added to municipal wastewater [500 mg/L], and a high protein content slaughterhouse wastewater [1420 mg BOD/L]) were evaluated. The maximum power densities were 354, 269, and 80 mW/m² for the BSA, peptone, and slaughterhouse wastewater, respectively (external resistance > 50 Ω). In addition, BOD removal efficiencies were 90, 86, and 93% with maximum coulombic efficiencies of 20.6, 6.0, and 5.2%, respectively. While this experiment involved batch rather than continuously operating MFCs, the high removal efficiencies indicate the ability of bacteria to convert protein into low molecular-weight substrates. The highest power was achieved with BSA,

which is a less complex protein than either peptone or proteins in slaughterhouse wastewater [35].

I. Swine waste with a complex substrate composition, consisting of proteins, celluloses, and lipids, was also tested as a substrate for a MFC with an anode volume of 28 mL [37]. The authors obtained a maximum power density of 261 mW/m² with an external resistor of 200 Ω . The soluble COD removal efficiency was 27% and the coulombic efficiency was 8%. One likely reason for the relatively low coulombic efficiency was the diffusion of oxygen into the anodic chamber (indicated by ammonia oxidation in the anode chamber), which would be the terminal electron acceptor during the removal of COD.

We have compared these MFC studies to verify that complex wastewater substrates result in lower coulombic efficiencies compared to easily degradable synthetic substrates, such as glucose and acetate (Table 1 is ordered based on coulombic efficiency). Comparisons between studies are difficult because anode sizes, electrode and membrane materials, inocula, reactor configurations, anolyte and catholyte conductivity, oxygen influx to the anode, cathode electron transfer mechanisms, temperatures, pH levels, etc. are different, resulting in variable internal resistances. Despite these problems, a trend emerged with hospital wastewater, municipal wastewater, slaughterhouse wastewater, swine waste, and brewery wastewater (in bold) showing lower coulombic efficiencies with a highest level of 36% compared to synthetic wastewaters with a highest level of 98%.

2.1. Anodic Foodweb

One primary reason for the trend of higher coulombic efficiencies for glucose and carboxylic acids compared to the lower values for higher-molecular weight substrates or more complex wastewaters, is the loss in efficiency of electron transfer in the anodic foodweb. For a complex substrate, fermenters must first convert polymers, such as (poly)saccharides and proteins, to acetate and hydrogen, which can then be converted to electrons (to the anodic electrode), protons, and CO₂ by electricigens. For Rabaey et al. [17] the highest coulombic efficiency was achieved with acetate (vs. glucose) because the electricigens, such as *Geobacter* sp., convert acetate to CO₂ (bicarbonate), protons, and electrons directly without having to sustain a fermentative population. Indeed, we have observed a nonvisible, microscopic biofilm when feeding solely acetate to the anode compartment while a thick biofilm was visible with the naked eye when feeding sucrose or carbohydrate rich brewery wastewater. The same effect is also reflected in the work of Liu et al. [36], who found a higher coulombic efficiency with acetate compared to butyrate, which first needs to be converted to acetate by acetogenic bacteria. For the metabolic processes and biosynthesis to sustain the anodic foodweb, energy (electrons) is required from the organic compounds in wastewater.

The anodic foodweb of nondefined mixed cultures has not been studied in detail and the question remains if complex compounds in wastewater are first converted to acetate and hydrogen by fermenters after which electricigens convert acetate and hydrogen into current, or if some of the fermentable compounds are directly converted into current by fermenters or other anaerobic respirers? Workers have shown that undefined mixed cultures acquired electrochemical activity over the operating period of the fuel cell [13, 27]. For instance, Rabaey et al. [27] showed increased electrochemical activity in an enriched community of diverse bacteria from the phyla Firmicutes (e.g., *Lactococcus* sp., *Enterococcus* sp., *Clostridium* sp.), Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria (*Pseudomonas* sp.). The DNA finger-printing technique that was used to characterize the community did not detect typical electricigens, such as *Geobacter* spp. However, they may have been important in the foodweb albeit at a relatively low level of 1–5% of the bacterial community, and therefore remained not detected due to the nonsensitive nature of this molecular biology technique. *Pseudomonas* sp., which can oxidize acetate in the presence of a working electrode, was detected, isolated, and able to excrete metabolites that can mediate electron transfer [27]. Indeed, under anaerobic conditions when no alternative electron acceptors were present, the activity of *Pseudomonas* sp. was enhanced when an anodic electrode was present in a MFC compared to a serum bottle without an electrode (this organism does not ferment and needs a terminal electron acceptor to respire with). In a follow-up study, these workers showed that production of electron mediators by *Pseudomonas* sp. benefited the electron transport for other microbes, such as *Enterococcus faecium*, possibly explaining why a mixed culture of microbes can generate a higher current density compared to a pure culture [26]. Kim et al. [41] isolated various bacteria from an anodic biofilm and besides the known electricigens of the genera *Geobacter* and *Shewanella* they also found additional phylogenetic bacterial groups, including fermenters (e.g. *Bacteroidetes* sp.) and anaerobic respirers, which proved to be electrochemically active. However, we do not know the relative importance of each of these electrochemically active bacteria in a dense and diverse anodic biofilm. Therefore, measuring electrochemical activity for a bacterium isolated from a community may not necessarily identify an electron transferring bacterium of importance to the overall electric current generation. But also finding groups of organisms that have shown to be electrochemically active before does not necessarily mean they are in any case: Kim et al. isolated two *Pseudomonas* sp. strains which were not electrochemically active [41].

3. Present Challenges

3.1. Low Coulombic Efficiency

Thus far, we have discussed that the: 1. entrapment and accumulation of organic particles from wastewater in the anodic biofilm [17]; 2. production of methane [13]; 3. losses in efficiency due to the energetic requirements to sustain fermentative and acetogenic communities in the anodic foodweb [16]; and 4. diffusion of the terminal electron acceptor oxygen into the anode [37] are all responsible for low coulombic efficiencies, especially during the treatment of real wastewater. Other factors that could also contribute to lowering the coulombic efficiency are the: 1. presence of other terminal acceptors, such as nitrate, soluble Fe(III), and sulfate, in the wastewater; 2. energetic requirements for electricigens to sustain bacterial metabolism and biosynthesis; and 3. escape of gaseous products, such as hydrogen, with the off gas. In this chapter we have highlighted that the coulombic efficiency for real wastewater treatment with MFC systems must be improved by, for example, placing preacidification tanks in front of MFC systems. Pre-acidifying wastewater would reduce the biofilm thickness of the anodic electrode by shifting the anodic foodweb from predominant activity by fast-growing fermenters towards predominant activity by slow-growing electricigens, similarly as found in anaerobic digesters [42]. A thin biofilm would reduce the likelihood of harboring methanogens, and thus methane generation.

3.2. Slow Kinetic Rates

A low coulombic efficiency limits power density or power output, however, the slow kinetic rate of electron transfer from anodic microbes to electrodes in MFCs may be more crucial. It was reported that beyond a volumetric loading rate of 2.5 and 3.4 g COD/L/d, an extinction effect of the power density was observed [13, 27]. Such volumetric loading rates are competitive with high-rate-aerated activated sludge systems but not with high-rate anaerobic digestion systems [40]. One way of increasing the rates is to increase the activity of the biomass by changing operating conditions. Indeed, an optimum anode electrode potential has increased the biofilm activity (per biomass concentration) of electron transfer to the anode electrode [43]. It also was shown that for certain microorganisms (e.g., *Shewanella oneidensis*) low levels of oxygen can increase the reaction rates (albeit at lower coulombic efficiencies) due to an overall more energetic state of the bacterial culture [44, 45].

Another way of increasing the rates may be by optimizing biofilm thickness. A modeling paper by Picioreanu et al. [46] showed that the resistance to substrate diffusion increases with biofilm thickness. This would slow microbial conversion rates, and thus reduce the current output and volumetric loading rate. Thus, maintaining thin anodic biofilms is imperative to improve the volumetric loading rates, which has a direct effect on the required MFC volume. However,

this creates a problem that was observed in a paper by Aelterman et al. [43]. The thin biofilm requirement dictated by substrate diffusion resistances, resulted in low levels of active biomass in their MFC (ca. 30 times lower than anaerobic digestion), which explained the lower kinetic rates of substrate removal for MFCs compared to anaerobic digesters despite similar biomass activities of ca. 3.4 g COD/g volatile suspended solids/d. They described the unique nature of anodic biofilms: "Biofilms growing on electrodes are subject to a duality that is rarely observed in natural conditions: the substrate concentrations are the highest at the outer layers of the biofilm while the electrode is only available at the inner layer of the biofilm. This feature requires the development of thin and open biofilm structure, which allows for a sufficient migration of substrate without hampering the transfer of electrons to the electrode" (cited from [43]). Development of an electrode material with a vast surface area may resolve this problem, but it should, at the same time, assure high shear forces to slough of biofilm layers and remove entrapped organic particles from wastewater. Having a high surface area electrode while at the same time providing a high shear may prove to be a real challenge.

3.3. Reactor Scale Up

Electrochemical techniques, such as impedance spectroscopy, have shown that high internal resistance in MFCs is influenced by the slow movement of ions between the electrodes due to a high electrolyte resistance of the anode and cathode solutions or the ion exchange membrane (i.e., ohmic limitations). Researchers have, therefore, often miniaturized MFCs, because this inherently increases the membrane surface area to volume ratio and reduces the distance between electrodes, resulting in decreased electrolyte resistances (and thus increased power output) [16, 47–49]. Such a miniaturization approach is helpful for research purposes to circumvent internal resistances due to reactor configuration limitations, however, this approach is not practical for wastewater treatment systems that require large reactor volumes.

Knowledge that was gained from hydrogen or methanol fuel cell fields cannot automatically be translated into MFC optimizations, especially when it comes to scale up. The energy density per unit volume of hydrogen gas or methanol is much higher than for wastewater (hydrogen gas [300 bar]: 119.9 MJ/kg, methanol: 19.9 MJ/kg [21], and wastewater: 0.58 J/kg [our calculation example below]). In accordance, the volumes of fuel streams are much higher for MFCs than for hydrogen and methanol fuel cells. With large treatment volumes, scale up is of the highest importance, but we cannot use a single MFC volume because of the requirements of high potential and current levels. To reach these high potential and current levels, we have to place individual MFC units in an electrical array (as explained in the outlook section below), and thus the scale up needs to be only from the mL scale to the L scale. This three orders of magnitude

scale up without performance losses, however, is still a great challenge. Here, we discuss three tasks/challenges that are important/must be overcome during scale up:

1. *To maintain low internal resistance while increasing the levels of electrochemically-active biomass.* For a low internal resistance, a close proximity of anode to exchange membrane to cathode, as well as a sufficiently large exchange membrane surface area is crucial. On the other hand, we need high surface, porous anode materials to offer enough surface area for the desired thin biofilm of electricigens. A high power density cell would, thus, call for a more or less two-dimensional thin layer assembly of anode/exchange membrane/cathode to minimize the internal resistance, while an optimized coulombic efficiency cell would call for a three-dimensional, porous anode that allows for optimized biotransformation. Achieving close proximity, and, still, an efficient three-dimensional anode design has been accomplished for a mL-scale MFC but will be a challenge for a L-scale MFC. In addition, clogging by accumulating biomass and particles from wastewater must be prevented.

2. *To optimize reactor designs.* One way, to realize this, could be with a tubular MFC design (shell and tube design). However, this raises another challenge - with our present knowledge, the electrical connection of one individual L-scale MFC to another MFC (in series) with current collectors would happen at one end of the long tubular cell, which means that electrons must flow to this specific connection site. This problem has been circumvented for flat panel hydrogen and methanol fuel cells by using bipolar plates as current collectors to directly connect the anode from one cell to the cathode of the next cell, without electrical wiring, which allows a direct transfer of electrons through the chain of individual cells in a fuel cell stack without major resistance losses [21]. For a L-scale MFC this would only work in a very large flat panel design, which, too, is under investigation for its scalability. Another challenge for a scaled-up MFC design is the distribution of the performance within one individual fuel cell and between fuel cells. If anywhere a low biotransformation activity zone exists this will consume energy from the system, reducing the overall power output. This phenomenon is well known to occur in hydrogen and methanol fuel cells [21], and has also been reported for stacked MFCs [50].

3. *To Develop new ways of separating the anode and cathode.* The selection of an ion exchange membrane will also become more and more crucial during scale up. Several research groups showed that the highly-selective (and expensive) proton exchange membrane for hydrogen fuel cells (Nafion) is not ideal for MFCs because at neutral pHs cations are moving from the anode to the cathode rather than protons [19, 20, 51]. Another study showed that the performance of an anion exchange membrane is better than a cation exchange membrane [52], and our own work proved that the selection of the ion exchange membrane is very much depending on the anolyte and catholyte composition [51]. One approach to reduce the internal resistance is to remove the ion exchange membrane and work without any membrane or with an ultrafiltration membrane [15, 53]. This

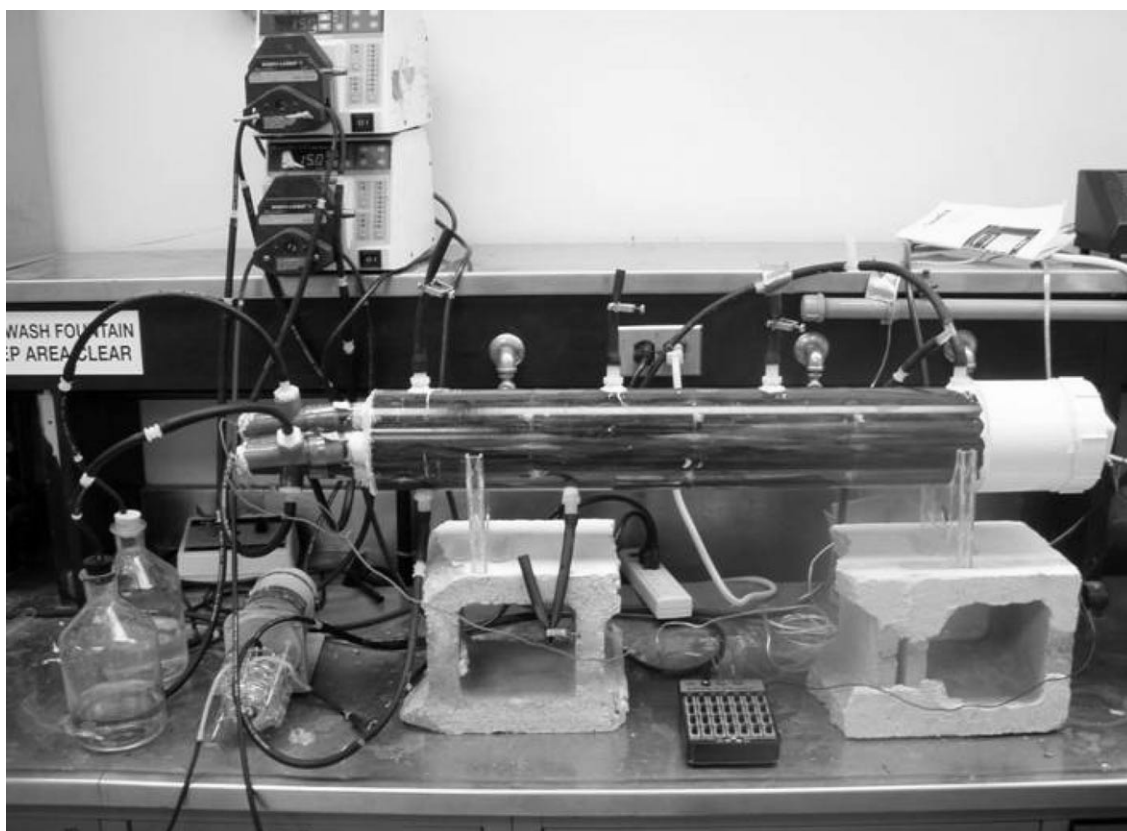


Fig. 3. A 6-liter MFC setup before the treatment of real brewery wastewater. The anodic and cathodic electrode material consisted of 2-m strings of carbon fiber (Panex 30 - unsized, Zoltek, St. Louis, MO) and the four internal cathode chambers (two cathode loops) were fabricated by rolled up cation exchange membrane material (CMI-7000, Membrane International, Glen Rock, NJ). Ferricyanide solution was recirculated through the cathode tubes. Real wastewater from a brewery was pumped into the anode chamber at a flow rate of 1.4 L/d with 7.2 L/d of make-up water for dilution (the overall hydraulic retention time was 15 h), which resulted in a volumetric loading rate of 0.9–1.9 g COD/L/d. Effluent from the 5.4-liter anode chamber was recirculated and mixed with the diluted brewery wastewater.

may be useful for mL-scale MFCs, however, in a L-scale MFC a considerable liquid pressure gradient would cause massive mixing of anolyte and catholyte. We anticipate a low coulombic efficiency as a result due to the intrusion of oxygen into the anode. On the other hand as mentioned above, the overall rates of electricity production could be enhanced with some oxygen. This effect has now been shown for pure cultures [44, 45], but it remains to be verified if the selection for an oxygen tolerant or even promoted mixed microbial community is possible, too.

4. Outlook: Economic Evaluation

The treatment of high organic content wastewater represents the most promising application of MFC technology at a large scale because of the higher energy density of the solution compared to, for example, municipal wastewater. This can be further illustrated with an overview of MFC wastewater treatment economics for a theoretical and relatively small 100 000 L/d wastewater stream with an organic concentration of 2000 mg BOD/L. We assume here that our MFC has an 8-h HRT (and thus an

anode volume of 33 333 L), an 85% BOD removal efficiency, and a 20% coulombic efficiency (Table 1 shows an average coulombic efficiency of 20% for real wastewater). With these assumptions the current generation is:

$$\text{Coulombic efficiency} = \frac{(\text{molecular weight O}_2)(\text{current})}{F(e^{-1}/\text{mol O}_2)(\text{flow rate})(\text{BOD removal})} 100\% \quad (1)$$

$$20\% = \frac{(32 \text{ g O}_2/\text{mol O}_2)(\text{current})}{(96485 \text{ C/mol e}^{-})(4 \text{ mol e}^{-}/\text{mol O}_2)(1.157 \text{ L/s})(1.7 \text{ g O}_2/\text{L})}$$

$$\Rightarrow \text{Current} = 4,744 \text{ A}$$

where F is the Faraday constant

Since the generated power will be the product of the current and the cell voltage (Joule's law), we have to make assumptions on achievable cell voltages. We achieved a sustainable potential of ca. 0.67 V at an external resistor of 100 Ω for our 6-liter MFC described in this chapter, and here we assume a potential of 0.6 V. In addition to estimating the MFC potential, some further assumptions for this theoret-

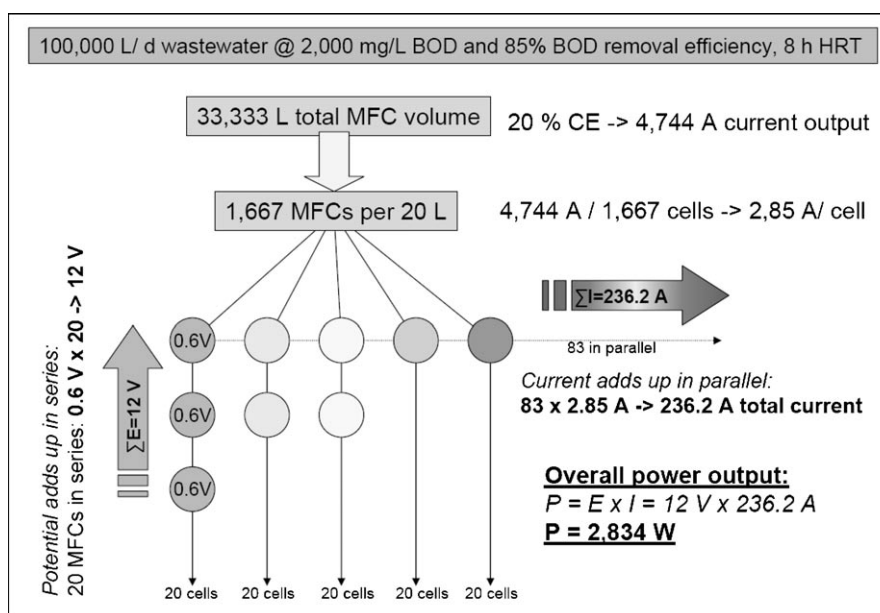


Fig. 4. Configuration of a potential full-scale MFC system. 100 000 m³/day wastewater with 2000 mg/L BOD will be treated in 1667 individual MFCs of 20 L anode volume at 85% BOD removal. To allow of optimum power output, the individual MFCs (at 0.6 V and 2.85 A) are arranged in an array of 20 MFCs in series (to build up voltage to 12 V) and 83 of these series in parallel (to build up current to 236.2 A). The overall power output of the wastewater MFC system will then be 2834 W (or 24 758 kWh annually).

ical wastewater treatment scenario are required (based on current understanding of MFC technology) (Figure 3):

A single 33 333 L anode chamber is not feasible. To achieve high MFC power densities, it is essential to maintain the anode and cathode reactions in close proximity while separated by an ion exchange membrane. The close spacing requirements result in the necessity of MFC designs with numerous smaller MFC cells. For this economic evaluation, we assume individual MFCs with anode volumes of 20 L. Then, the total MFC array will consist of 1 667 MFC cells (33 333 L of total anode volume/20 L of anode volume per one MFC cell).

The 1667 fuel cells need to be placed into an electrical array with both series and parallel connections to add potential and current, respectively (similarly to fuel cells and photovoltaic cells). The series connections will add potential – generating a 12-V potential will be desirable to charge batteries or to convert the current from a DC to AC. To achieve a 12-V potential, we assume that twenty 0.6-V MFCs must be placed in series. The parallel connections will add current, the 83 groups of 20 MFC cells in series will be connected in parallel (see Figure 4).

The current generation from the 83 MFC groups in parallel is determined with the assumption that the current generation from each MFC is proportional to the total current generation from the 33 333 L of total anode volume, or 2.85 A per 20-L of anode volume (equivalent to a power output of 85 W/m³ if we look at the individual MFC). This is according to a best-possible scenario because in series the cell with the lowest potential and in parallel the group of MFCs with the lowest current will dictate the total potential and current, respectively (similar to fuel cells) [50]. In

addition, the 85 W/m³ is much higher than the 5 W/m³ we achieved with the 6-L MFC, but we anticipate that the higher power output can be achieved by further improving the MFC configuration and by preacidifying the wastewater (the latter to improve the coulombic efficiency). The current generation for 83 MFCs in parallel will equal 236.2 A (83 multiplied with 2.85 A). While coulombic efficiency of each individual MFC was assumed to be 20% (4744 A/BOD_{removed}), the overall electricity recovery efficiency of the MFC system (array of parallel and serial cells) is only 1.7% (236.2 A/BOD_{removed}). Power generation will be 2834 W according to Joules law ($P = I \times E = 236.2 \text{ A} \times 12 \text{ V}$). On an annual basis, this represents an electrical power generation of 24 758 kWh. Thus, the total value of the power generated from this wastewater treatment is \$ 2971 per year when we assume an electricity value of \$ 0.12/kWh (a representative value in the USA).

Such a value of electricity generation will not recover the investment costs. Even if the anticipated 20% MFC coulombic efficiency could be tripled to 60% (which has not been achieved with real wastewater, but could be a reality when, for example, a preacidification tank is installed before the MFC), the resulting \$ 8937 value of annually generated electricity is also unlikely to recover the investment. With the more dilute municipal wastewater (ca. 300–500 mg BOD/L), the economics based on just electricity generation are even more unfavorable. The economic evaluation of our theoretical wastewater-to-electricity conversion, however, remains incomplete. A ca. 2000 mg BOD/L waste stream requires treatment, which presently is costly and energetically unfavorable due the requirement of aeration (i.e., adding a terminal electron acceptor) to the

mixed liquor in activated sludge treatment tanks. Currently, if our wastewater stream was discharged to a municipal wastewater treatment facility, the municipality would impose a waste treatment fee of ca. \$ 0.53/kg BOD to cover municipal costs (for a BOD concentration > 300 mg/L and based on data from municipalities in Chicago, IL and St. Louis, MO), which includes the electricity cost for aeration. Thus, the cost to treat our wastewater stream at a municipal wastewater treatment plant with activated sludge tanks (secondary treatment) is \$ 32 760 per year. Combined for our example, the total MFC cost justification will be \$35 731 per year. The present value of a MFC system can then be calculated with engineering economics and the following assumptions: inflation – 3%; electricity inflation – 6%; wastewater treatment inflation – 5%; and MFC service life – 10 years. For 100 000 L/d wastewater with a 2000 mg BOD/L and a coulombic efficiency of 20%, the net present value equals \$ 380 528 or \$228/20-L MFC for a 10 year time span. These MFC economic justifications are driven by electricity revenue generation and municipal waste treatment cost. However, the latter economic driver is more important regardless of energy costs. It is also clear from this very simplified economic evaluation that the capital costs of MFC construction must be relatively low to make this intricate system economically viable. Lastly, our example does not reflect any MFC operating costs, which will include maintenance, personnel and chemical costs. Even though the return of investment may never be high, social and environmental considerations (e.g., saving energy, recovering energy and reducing carbon dioxide emissions) may make MFC technology feasible.

To promote a successful scale up effort, we must now focus on the development of more efficient MFC configurations with low cost, sustainable materials. To further improve the coulombic efficiency of wastewater-to-electricity conversion, pretreatment of real wastewater (e.g., preacidification) is necessary while oxygen diffusion from the cathode into the anode must be circumvented. In addition, and maybe more importantly, the microbial kinetics must be improved to decrease the necessary volume (i.e., shortening the HRT by increasing the volumetric loading rates) compared to activated sludge systems and to make MFC technology competitive to commercial high-rate anaerobic digestion systems.

Economic value can also be added to the technology, if, besides BOD removal, the system is used for the production of valuable chemical products instead of the low-value electricity. Thus, bioelectrochemical systems (BES) are gaining great importance as innovative technological devices for the renewable generation of chemical products [54–56] and for the sequestration of CO₂ [57]. They also have shown promise as biosensors [58, 59], desalination devices [60] and for recalcitrant chemical conversion [38, 61]. Some of these applications, such as the production of hydrogen gas, caustic, or hydrogen peroxide that can directly be used in the treatment process, or the removal of persistent wastewater components might provide an additional cost benefit for the wastewater treatment plant.

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