Effects of wastewater constituents and operational conditions on the composition and dynamics of anodic microbial communities in bioelectrochemical systems

Marika Kokko[a,c], Stefanie Epple[b], Johannes Gescher[b], Sven Kerzenmacher[a,*]

[a] Laboratory for MEMS Applications, IMTEK – Department of Microsystems Engineering, University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany

[b] Institute for Applied Biosciences, Department of Applied Biology, Karlsruhe Institute of Technology, Fritz-Haber-Weg 2, 76131 Karlsruhe, Germany

[c] Current address: Laboratory of Chemistry and Bioengineering, Tampere University of Technology, Tampere, Finland

* Corresponding author. E-mail: sven.kerzenmacher@imtek.de; Tel.: +49 761 203 73218; fax: +49 761 203 73299

Abstract

Over the last decade, there has been an ever-growing interest in bioelectrochemical systems (BES) as a sustainable technology enabling simultaneous wastewater treatment and biological production of, e.g. electricity, hydrogen, and further commodities. A key component of any BES degrading organic matter is the anode where electric current is biologically generated from the oxidation of organic compounds. The performance of BES depends on the interactions of the anodic microbial communities. To optimize the operational parameters and process design of

BES a better comprehension of the microbial community dynamics and interactions at the anode is required. This paper reviews the abundance of different microorganisms in anodic biofilms and discusses their roles and possible side reactions with respect to their implications on the performance of BES utilizing wastewaters. The most important operational parameters affecting anodic microbial communities grown with wastewaters are highlighted and guidelines for controlling the composition of microbial communities are given.

Keywords: bioelectrochemical system, microbial community, exoelectrogen, wastewater

1. Introduction

Bioelectrochemical systems (BES) are devices that enable a transfer of electrons to or from a biocatalyst via a working electrode. In case this electrode is operated as anode (i.e. accepting electrons) it can sustain the biological oxidation of organic or inorganic substrates under anoxic condition. Such a biobased current flow is for instance used in microbial fuel cells (MFC) for the generation of electrical energy from the chemical energy if biomass. For electricity generation in a MFC usually oxygen is reduced at the corresponding cathode. In microbial electrolysis cells (MEC) or microbial electrosynthesis cells (MES), the reductive current is used to produce hydrogen or other compounds, such as acetate at the cathode, with the help of an additional voltage. For further information, see for instance the reviews by Hamelers et al. (2010) and Logan and Rabaey (2012).

At the anodes of BES the organic carbon content of various wastewaters, including domestic, distillery and dairy wastewaters, can be oxidized biologically (Ha et al., 2012; Montpart et al., 2015; Yu et al., 2012). If the wastewaters contain complex organics, such as cellulose, hemicellulose, fats, proteins, sugars, alcohols, or volatile fatty acids (VFAs), the biological degradation often requires syntrophic interaction of mixed species microbial communities or in other words, the capabilities of an interacting microbiome. In nature, the first degradation step is often the hydrolysis of a biopolymer. Thereafter, fermentative bacteria metabolize the monomeric sugar compounds, fatty acids, and amino acids into alcohols, VFAs, H_2, and CO_2 (Fig. 1). Current producing bacteria, called exoelectrogens, can oxidize different sugars, alcohols, and VFAs through anaerobic respiration using the anode of the BES as terminal electron acceptor. The wastewaters can, however, also contain inorganic compounds, such as sulfate or nitrate, which

55 serve as electron acceptors instead of the anode and thus, result in competing metabolic reactions
56 that direct electrons away from current production (Fig. 1).
57 By optimizing process design and environmental parameters (such as anode potential, pH and
58 presence of O_2, see section 3), it is possible to direct the growth of microorganisms and improve
59 the performance of BES. However, to be able to select for the right microbial species and to control
60 the oxidation of organic compounds, it is important to understand the microbial community
61 dynamics and interactions at the anode. Different microbial communities at the anode (Logan and
62 Regan, 2006) as well as syntrophic interactions of bacteria utilizing different substrates for
63 electricity generation (Kiely et al., 2011b) have been reviewed earlier. However, the number of
64 publications on microbial community compositions in BES anodes has increased significantly in
65 the past years. Furthermore, the wastewaters used as substrate at the anode may contain inorganic
66 compounds, which may result in competing metabolic reactions (such as nitrate- or sulfate-
67 reduction, see section 2.1). These metabolic reactions that compete with electricity generation need
68 to be taken into account when designing a bioelectrochemical system, but have not been considered
69 in previous review articles.
70 The purpose of this review is to describe the roles of different microbial groups at the anodes of
71 bioelectrochemical systems. Furthermore, the abundance of different bacterial phyla and species
72 in the anodic microbial communities are compared and their capabilities to improve or hamper
73 current production are discussed. Based on the literature, the effects of operational parameters on
74 microbial communities are illustrated. In addition, some guidelines are derived regarding the
75 selection of desired microbial communities and inhibition of microbial species hindering current
76 production by changing environmental and technological parameters. At the end, further necessary
77 research topics are highlighted.

2. Anodic microbial communities

The microbial communities enriched at the anode (as biofilms and/or planktonic cells) are diverse and depend on i) the composition of waste streams, i.e. presence of organic and inorganic compounds or microorganisms, ii) the type of BES being used, i.e. one- or two-chamber BES and anode electrode material (Sun et al., 2011) , and iii) the operational conditions.

Currently, only a few microorganisms that are capable of directly producing electrical current from sugars or other complex organics are known. The ecological role of exoelectrogens, which directly transfer respiratory electrons to anode surfaces, seems to be the oxidation of typical fermentation end products and not a full oxidation of a biopolymer to CO_2. Thus, very similar to natural ecosystems the hydrolysis of biopolymers in BES is conducted by fermentative species (Fig. 1). The hydrolysis is supported by exoelectrogens via the removal of fermentation end products, which increases the available Gibbs free energy in the synthetic ecosystem formed by the microbial community.

In addition to the bacterial species required for current production from organic compounds, the microbial communities may also contain other microbial species that direct electron flow away from current production. These microbial species include methanogens, aerobic or facultatively aerobic bacteria, sulfate- and nitrate-reducers, and homoacetogens (Chung and Okabe, 2009; Kiely et al., 2010; Lee et al., 2009; Zhang et al., 2013b). These microorganisms use other electron acceptors than the anode, which likely results in decreased current production or at least in a decreased coulombic efficiency. While sulfate- or nitrate-reduction may direct electrons away from current production (Kim et al., 2004), some sulfate- and nitrate-reducers have also been shown to be capable of donating electrons to an electrode (Bond and Lovley, 2004; Xing et al., 2010). Thus, their role in the anodic microbial communities likely depends on the wastewater

composition as well as on the potential of the anode. The diversity of oxidation or reduction reactions that can occur in an anodic biofilm community can be sorted according to the corresponding redox potentials under standard state conditions (298 K, 1 bar, 1 M) or typical experimental (pH 7, 30ºC, 10 mM, 0.2 bar) conditions (Table 1) (Logan et al., 2006).

2.1 Roles of different microbial groups

2.1.1 Exoelectrogens

Exoelectrogenic species are most often found within the physiological groups of iron reducers (e.g., *Geobacter sulfurreducens*, *Shewanella oneidensis*) (Kim et al., 2002; Reguera et al., 2005), sulfate-reducers (e.g., *Desulfuromonas acetoxidans* and *Geothrix fermentans*) (Bond et al., 2002; Bond and Lovley, 2004), nitrate-reducers (e.g., *Comamonas denitrificans* and *Paracoccus pantotrophus*) (Kiely et al., 2010; Xing et al., 2010), and phototrophic purple nonsulfur bacteria (e.g., *Rhodopseudomonas palustris*) (Xing et al., 2008). Most currently known exoelectrogens belong to the phylum *Proteobacteria*, including α-, β-, γ-, δ-, and ε-*proteobacteria*. Exoelectrogens have also been characterized from the phyla *Acidobacteria*, *Firmicutes*, and *Actinobacteria* (Bond and Lovley, 2004; Marshall and May, 2009; Wang et al., 2008).

Electrons can be transferred from bacteria to the anode either by direct electron transfer via outer-membrane cytochromes or nanowires, bound-flavin semiquinone mechanism (Okamoto et al., 2014), or by mediated electron transfer (for reviews, see Lovley (2008) and Rabaey et al. (2007)). For more information on pure exoelectrogenic species and on their electron transfer mechanisms see, e.g., the reviews by Koch et al. (2016), Kumar et al. (2016), and Semenec and Franks (2015). Most exoelectrogens can oxidize simple organic acids directly to current, while sugars are often fermented into soluble metabolites before they can be converted into current. Thus, syntrophic interaction between hydrolytic, fermentative and exoelectrogenic microorganisms is required for

current generation from more complex substrates. In summary, exoelectrogenic species are required for current generation.

2.1.2 Hydrolytic and fermentative bacteria

Hydrolytic bacteria are required, if waste materials contain polymeric carbohydrates, such as cellulose, sucrose, starch and molasses, fats, or proteins. Hydrolytic bacteria excrete enzymes that degrade either cellulose and hemicellulose into sugars (for a review, see (Schwarz, 2001)), fats into long chain fatty acids, or proteins into amino acids (Ramsay and Pullammanappallil, 2001). Sugars, long chain fatty acids and amino acids are degraded into VFAs by fermentative bacteria and further to acetate, H_2 and CO_2 through syntrophic interactions by different microorganisms (in Fig. 2 marked as syntrophic organisms). VFAs and H_2 can be further utilized by, e.g., secondary fermenters, exoelectrogens, methanogens, or nitrate- and sulfate-reducing bacteria (Fig. 2). Although exoelectrogenic bacteria are often required to oxidize the soluble metabolites into current, a pure culture of *Thermoanaerobacter pseudethanoliaus* has been shown to ferment sugars into soluble metabolites and to convert these metabolites to current simultaneously (Lusk et al., 2015). In addition to the microbial consortium, hydrolysis efficiency also depends on the substrate composition. Lu et al. (2012a) studied hydrogen production in MECs from waste activated sludge and reported that most of the organic carbon content removed at the anode resulted from protein degradation. Alkaline pretreatment of waste activated sludge, on the other hand, increased hydrolysis of carbohydrates compared to protein degradation (Lu et al., 2012a). Hydrolysis is often the slowest step in the degradation of complex organic compounds due to, e.g., the crystallinity and particle size of cellulose and low rates of hydrolysis (Schwarz, 2001; Zhang et al., 2012). For example, Zang et al. reported that 54% of cellulose, 39% of hemicellulose and 78% of lignin from plant material (*Canna indica*) could not be degraded in ca. 15 days and this way not be used for

current generation (Zang et al., 2010). Thus, in order to increase the current densities with more complex waste materials as substrate, the hydrolytic steps need to be optimized and their rates have to be increased. This can be done, for example, i) by optimizing the growth conditions of hydrolytic bacteria that prefer, e.g., near neutral pH and longer hydraulic retention times (Chyi, Y. T. and Levine, A. D., 1992; Hu et al., 2004), ii) by adding hydrolytic enzymes (Rezaei et al., 2008), iii) by using a two-stage process (e.g. Lalaurette et al., 2009), (Mohanakrishna et al., 2010), or iv) by pretreating the biomass with, e.g. hydrothermal methods (Liu et al., 2015). The two-stage processes enable the separation of hydrolysis and electricity production into different reactors that can be optimized separately.

Bacteria belonging to the phyla *Bacteroidetes* and *Firmicutes* are often able to hydrolyze complex organic compounds and have been found to dominate, e.g., in anode biofilms utilizing cellulose (Rismani-Yazdi et al., 2013) or waste activated sludge (Lu et al., 2012a) as electron donor. *Bacteroidetes* can degrade complex organics, such as proteins and carbohydrates (Montpart et al., 2015). In addition to fermentation and hydrolysis, some *Firmicutes* can utilize oxygen leaking to the reactor (resulting in aerobic respiration and CO_2 production) (Jung and Regan, 2007; Rismani-Yazdi et al., 2007) or take part in current production. In addition, bacteria from the phylum *Actinobacteria* able to hydrolyze, e.g. cellulose and chitin, have often been detected in anodes fed with more complex substrates, such as sewage sludge (Zhang et al., 2012). Hydrolytic and fermentative bacteria have been detected both in the anode suspension and biofilm (Beecroft et al., 2012; Rismani-Yazdi et al., 2007) suggesting that substrate is degraded in both phases.

As was discussed before, fermentation of complex substrates into compounds that can be used by exoelectrogens is often prerequisite for current generation. Zhao et al. (2012) reported that cattle dung was hydrolysed and fermented to acetate, butyrate and propionate before current generation.

Food waste leachate was first fermented into acetate, butyrate and hexanoic acid, from which only hexanoic acid was not completely utilized for current production (Li et al., 2013). Accumulation of acetate and lactate (Rismani-Yazdi et al., 2013) or acetate and propionate (Ishii et al., 2008) preceded current production from cellulose. Although fermentation is required for current generation, it often decreases the coulombic efficiency and current production due to the accumulation of side products not utilized for current production; especially when compared to the results obtained with VFAs as substrate (Chae et al., 2009; Zhang et al., 2011b).

In summary, hydrolytic and fermentative bacteria are required to degrade complex substrates, if they are used as feedstocks at the anodes. However, fermentation may result in side products not used for current production.

2.1.3 Methanogens

Methanogens belong to the domain Archaea and their activity at the anode can result in significant electron losses, since methanogenesis competes with current production. For example, Chung and Okabe (2009) deduced electron losses of 16% due to methanogenesis in bioelectrochemical systems fed with glucose. Methanogens can be divided into hydrogenotrophic and acetoclastic methanogens that produce methane from hydrogen and carbon dioxide ($4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$) or acetate ($CH_3COOH \rightarrow CH_4 + CO_2$), respectively. Hydrogenotrophic methanogens are more often detected at anodes than acetoclastic methanogens, especially when fed with acetate (Jung and Regan, 2010; Lee et al., 2009; Lu et al., 2011; Lu et al., 2012b) or ethanol (Parameswaran et al., 2010). The higher abundance of hydrogenotrophic over acetoclastic methanogens is likely due to outcompetition of acetoclastic methanogens by facultative anaerobes and exoelectrogens (Kim et al., 2011; Shehab et al., 2013), while hydrogenotrophic methanogens

have been reported to outcompete non-exoelectrogenic homoacetogens (see section 2.1.6) (Kim et al., 2011; Lee et al., 2009; Parameswaran et al., 2010).

In natural environments, hydrogenotrophic methanogens enable secondary fermentative pathways via a syntrophic interaction based on the consumption of H_2. Secondary fermentative bacteria convert longer chain alcohols and carboxylic acids into hydrogen, acetate and carbon dioxide. The depletion of hydrogen to very low partial pressures by hydrogenotrophic methanogens renders some fermentative pathways exergonic that are endergonic under standard state conditions. While exoelectrogens seem to be able to compete with methanogens for acetate, methanogens might have a competitive advantage regarding hydrogen oxidation (Parameswaran et al., 2009; Parameswaran et al., 2010), since they are – as autotrophic organisms – specialized in the formation of biomass from hydrogen and carbon dioxide. The use of hydrogen together with favoring the fermentative consumption of primary fermentation end products might be the reason why current production is negatively influenced by methanogenesis.

To increase current yields, the growth of methanogens has to be inhibited. This can be achieved by i) adjusting the anode temperature to ≤ 15 °C (Lu et al., 2011; Lu et al., 2012b), ii) decreasing the pH (Chung and Okabe, 2009), iii) using short HRTs, e.g. below 32 h in batch MEC fed with acetate (Lee et al., 2009), or iv) by constantly sparging the anode with nitrogen to strip out H_2 (Montpart et al., 2015). However, stripping the anode with N_2 has not always inhibited methanogenesis completely due to the presence of acetoclastic methanogens (Montpart et al., 2015). Rismani-Yazdi et al. (2013) reported that after 90 days of electricity production from cellulose, methanogenesis was completely suppressed indicating that the activity of methanogens can decline during operation. The suppression of methanogenesis was related to changes in VFA concentrations and decrease in microbial diversity (Rismani-Yazdi et al., 2013). Fast acetate

consumption by exoelectrogens has also resulted in suppression of methanogens, since no acetate was left for their growth (Ishii et al., 2008). Methanogens are often found mainly in suspension (Parameswaran et al., 2010), which indicates that continuous feeding can wash out methanogens. In summary, the presence of methanogens always hinders current production and thus, there presence at the anodic microbial communities should be prevented.

2.1.4 Sulfate-reducing and sulfur-oxidizing bacteria

Sulfate and sulfide (as well as other sulfur species) are present in various wastewaters and thus, the importance of sulfate-reducing and sulfur-oxidizing bacteria in BES cannot be ignored. Sulfur metabolism in BES anodes can be complex and include both oxidative and reductive reactions. Sulfate-reducing bacteria use sulfate (SO_4^{2-}) as electron acceptor reducing sulfate to sulfide (H_2S, Eq. 1). Sulfides may inhibit the growth of exoelectrogens (Lee et al., 2015), but can be electrochemically oxidized to solid sulfur (Eq. 2) on the electrode surface (Ha et al., 2012; Reimers et al., 2007), which will decrease the inhibitory effects of sulfides. In addition to electrochemical sulfide oxidation, sulfide can be removed from the anode chamber by volatilization, adsorption to the anode, chemical oxidation, or biological sulfide oxidation, from which electrochemical and biological sulfide oxidation were reported to dominate (Zhang et al., 2013a). Dutta et al. (2009) on the other hand, reported that with and without active biofilms, 95% of the sulfide was electrochemically oxidized to elemental sulfur and 5% to thiosulfate, suggesting that biological sulfide oxidation did not play an important role. Sulfur-oxidizing bacteria can oxidize sulfide (Eq. 2), sulfur (S^0), sulfite (SO_3^{2-}, Eq. 3), and thiosulfate ($S_2O_3^{2-}$, Eqs. 4 and 5). In addition, polythionates can be hydrolyzed at the anodes (Eq. 6) (Sun et al., 2010).

$$SO_4^{2-} + 8\ e^- + 10\ H^+ \rightarrow H_2S + 4\ H_2O \quad (1)$$

238 $H_2S \rightarrow S^0 + 2\ e^- + 2\ H^+$ (2)

239 $SO_3^{2-} + H_2O \rightarrow SO_4^{2-} + 2\ e^- + 2\ H^+$ (3)

240 $S_2O_3^{2-} + 5\ H_2O \rightarrow 2\ SO_4^{2-} + 8\ e^- + 10\ H^+$ (4)

241 $2\ S_2O_3^{2-} \rightarrow S_4O_6^- + 2\ e^-$ (5)

242 $S_4O_6^{2-} + H_2O \rightarrow S^0 + S_2O_3^{2-} + SO_4^{2-} + 2\ H^+$ (6)

243

244 Although sulfate-reducers often divert electrons from current production by using sulfate as
245 electron acceptor while oxidizing organic compounds (Zhang et al., 2013b), they also have other
246 roles at the anode. When marine plankton was used as substrate in MFCs to mimic seafloor
247 conditions, sulfate reduction enhanced the substrate degradation rates (although only 11-16% of
248 the electrons were used for current production). It was unclear whether current was produced
249 directly from substrate oxidation or from the oxidation of sulfide produced by sulfate reducers
250 (Reimers et al., 2007). In addition, the sulfate-reducer *Desulfobulbus propionicus* has been
251 reported to oxidize S^0 to SO_4^{2-} with an anode as electron acceptor (Holmes et al., 2004), thus acting
252 as an exoelectrogen. Sulfate-reducers may also play an important role in biofilm production since
253 they can secrete exopolysaccharides and produce protein filaments (Beecroft et al., 2012).
254 Sulfide can produce current (Eq. 2) when electrochemically deposited on the anode (see Table 1).
255 Sulfur deposition on the anode can increase the ohmic losses in long term (Sangcharoen et al.) due
256 to slow inactivation of the electrode. Solid sulfur can be used as a mediator for acetate oxidation
257 (Dutta et al., 2009). Examples of sulfate-reducing bacteria detected at the anodes include bacteria
258 from the phylum *Firmicutes*, while sulfur-oxidizers from the phylum *Proteobacteria* have been
259 detected (Sangcharoen et al; Sun et al., 2009; Sun et al., 2010; Zhang et al., 2013a). In a
260 thermophilic MFC treating distillery wastewater, 60% of sulfate was reduced by

Thermodesulfovibrio aggregans (Ha et al., 2012). At anodes, where current was produced at acidic conditions (pH 1.2-2.5), *Acidithiobacillus* spp. capable of tetrathionate disproportionation (Eq. 6) were detected (Sulonen et al., 2015).

In summary, some sulfate reducing bacteria can act as exoelectrogens and may enhance the biofilm growth. However, the presence of sulfate in wastewaters results in electron losses when sulfate is used as electron acceptor instead of the electrode. In addition, the H_2S produced by sulfate reducers may inhibit the growth of exoelectrogens.

2.1.5 Denitrifiers

Although denitrifying bacteria can consume electrons at the anode chamber by using nitrate as electron acceptor (Eq. 7), some denitrifiers might also be capable of transferring electrons to the anode electrode. For example, *Paracoccus denitrificans* accounted for 30% of an anodic biofilm fed with formic acid (Kiely et al., 2010). When nitrate was added to the anode, the cell voltage decreased by 45% until the nitrate was consumed over time. Kiely et al. (2010) suggested that *P. denitrificans* oxidizes formate either with nitrate or an anode as electron acceptor. Similar observations were made with *Comamonas denitrificans* and *Geobacter metallireducens*, denitrifying bacteria that have been reported to produce current from acetate (Kashima and Regan, 2015; Xing et al., 2010). A carbon/nitrogen ratio of lower than 7.4 (mg C/mg N) in the feed solution was reported to increase the anode potential and decrease the coulombic efficiencies due to nitrate reduction (Srinivasan and Butler, 2017). Kashima and Regan (2015) reported that the change in the metabolism of *G. metallireducens* from anode to nitrate reduction did not depend on the anode potential, but was affected by the biofilm thickness that likely hindered the availability of nitrate in thicker biofilms.

$$2\ NO_3^- + 12\ H^+ + 10\ e^- \rightarrow N_2 + 6\ H_2O \quad (7)$$

Beecroft et al. (2012) reported that bacteria capable of denitrification displaced other bacteria in an anodic biofilm growing on sucrose suggesting denitrifiers took part in current production. Denitrifiers were considered as putative exoelectrogens due to their high abundance in the biofilm and their ability to secrete exopolysaccharides (EPS), which likely helped in the attachment on surfaces and in biofilm formation.

In summary, some denitrifiers have been shown to have exoelectrogenic capabilities. However, if nitrate is used as electron acceptor, it reduces current production.

2.1.6 Homoacetogens

Homoacetogens produce acetate from hydrogen and carbon dioxide (Eq. 8 (Montpart et al., 2015)). Although H_2 consumption directs H_2 away from current production by hydrogen-oxidizing exoelectrogens, the presence of homoacetogens does not necessarily decrease current production. For example, Parameswaran et al. (Parameswaran et al., 2011) reported that acetate produced by homoacetogens in an anode fed with H_2 could be used for current generation by exoelectrogens, mainly *G. sulfurreducens*. The presence of homoacetogens was also reported in MFCs, where the fermentation of ethanol resulted in the production of H_2 (Parameswaran et al., 2009; Parameswaran et al., 2010). When methanogenesis was inhibited, H_2-utilizing homoacetogens channeled the electron flow to current through acetate as interspecies electron shuttle (Fig. 2). However, without inhibition of methanogens hydrogenotrophic methanogens outcompeted homoacetogens (Parameswaran et al., 2010) due to thermodynamic and kinetic advantage of hydrogenotrophic methanogens. Similar results were reported by Montpart et al. (2015), who concluded that homoacetogenesis took place only before methanogenesis became active.

$$4\ H_2 + H^+ + 2\ HCO_3^- \rightarrow CH_3COO^- + 2\ H_2O \quad (8)$$

306 Potential homoacetogenic bacteria have been reported to belong to the genera *Acetobacterium* and
307 *Eubacterium* (obligate homoacetogens) from the phylum *Firmicutes*, and *Spirochaeta* from the
308 phylum Spirochaetes (Parameswaran et al., 2010; Parameswaran et al., 2011). The presence of
309 homoacetogens at the anode can be affected by operational parameters. For example,
310 Parameswaran et al. (2011) showed that homoacetogens were mainly present in suspension and
311 were washed out at hydraulic retention times (HRTs) lower than 4.5 h.
312 In summary, homoacetogens direct electrons away from the electrode. However, their syntrophic
313 interactions with exoelectrogens may result in unaffected current production.
314 ***2.1.7 Co-cultures and their interactions***
315 The interactions of different microbial groups can have a syntrophic, commensalistic or
316 competitive character (Table 2). Competitive interaction, i.e. interaction where the other
317 organism's performance is decreased, has been shown with exoelectrogens and methanogens
318 (Chung and Okabe, 2009). Syntrophic interaction, where the performance of both microorganisms'
319 increases, has been shown between exoelectrogens and fermenting bacteria (Ren et al., 2007), and
320 between exoelectrogens and homoacetogens (Parameswaran et al., 2011). The syntrophic
321 interaction of hydrolytic and/or fermenting bacteria and exoelectrogens benefits both; Hydrolytic
322 and fermentative bacteria produce soluble metabolites ensuring substrates for exoelectrogens,
323 while exoelectrogens eliminate feedback inhibition for fermenting bacteria by consuming the
324 fermentation products (Table 2). For example, the consumption of the soluble metabolites of *C.*
325 *cellolyticum* by *G. sulfurreducens* improved the removal of organics from cellulosic substrates by
326 27-38% (Ren et al., 2007). Facultative aerobes present in the community can have negative and/or
327 positive effects. They can utilize oxygen as electron acceptor consuming organic and decreasing

328 current yields and coulombic efficiency, but their presence in the anode may also be beneficial due
329 to oxygen scavenging (Qu et al., 2012).

330 **2.2 Microbial communities enriched with different substrates**
331

332 Microbial communities producing electricity from organic materials can be enriched from various
333 sources, including aerobic and/or anaerobic sludge from wastewater treatment plants (Chae et al.,
334 2008; Kim et al., 2006; Parameswaran et al., 2009), rumen (Rismani-Yazdi et al., 2007), palm oil
335 mill effluent (Jong et al., 2011), and rice paddy-field soil (Miyahara et al., 2013). Methods for
336 enriching current producing communities have been previously reviewed by Rimboud et al (2014).
337 Various research groups have examined the anodic microbial communities in the biofilms and in
338 the planktonic phase. Based on literature research, the dominance of certain phyla depends to a
339 certain extent on the substrate (Fig. 3). For Fig. 3, results were combined from research articles
340 where the percentages of different phyla have been calculated. Although the articles used for this
341 figure represent only a fraction of articles discussing microbial communities (only in these articles
342 the percentages of different bacterial phyla have been reported; due the expertise required to
343 acquire the data, it is not included in many articles), they indicate a general trend that is further
344 discussed in this chapter. In addition to substrate, the microbial community structure depends, e.g.
345 on various compounds present in the anolyte, i.e. wastewaters (Borole et al., 2009c), cell design
346 that may result in oxygen diffusion to the anode (Quan et al., 2012) , temperature (Lu et al., 2011),
347 and anode potential (Torres et al., 2009). The differences in experimental setups make the
348 comparison between studies difficult. However, some trends can be observed based on the
349 combined results illustrated in Fig. 3.

350 The microbial community structure seems to be dependent on whether the substrate requires
351 hydrolysis and/or fermentation before exoelectrogenesis or if the substrate is readily oxidisable for

current generation (Fig. 3). For example, Velasquez-Orta et al. (2011) studied current generation and the resulting microbial communities from three different substrates: i) starch requiring hydrolysis and fermentation before exoelectrogenesis, ii) glucose requiring fermentation, and iii) acetate that is readily available for exoelectrogenesis. More diverse communities were obtained with glucose and starch than with acetate due to the increased number of metabolic reactions required (Velasquez-Orta et al., 2011). In addition, Heidrich et al. (2016) examined the most probable number of exoelectrogens with different substrates and reported that with more complex substrates, such as starch, less exoelectrogens were present than with acetate due to requirements to degrade starch before it can be utilized by exoelectrogens.

There are, however, differences in bacterial species between experimental runs with similar substrates (Fig. 3), which can partly be explained by differing reactor configurations, for example different oxygen concentrations and electrode surface areas. For example, Lee et al. (2016) reported that microbial communities enriched in MFC anodes with molasses wastewater were different depending on whether a one- or a two-chamber setup was used; more *Proteobacteria* were enriched in the anodes of the two-chamber MFC and also the methanogenic communities differed. However, they did not report on an effect by the use of membrane on the anodic microbial communities (Lee et al., 2016). In addition, Koch et al. (2014) reported that using identical wastewater from primary clarifier as inoculum and substrate in parallel reactors resulted in different reactor performances and microbial communities (planktonic and biofilm) suggesting that various parallel reactors for each researched parameter are required, especially when using wastewaters as substrate. The change in substrate during a run has also been shown to alter microbial communities (Yu et al., 2012; Zhang et al., 2011b), which implies that changes in wastewater composition will alter microbial communities.

2.2.1 Acetate

With acetate as substrate, most of the bacterial species of the anodic biofilm have been reported to belong to the phylum *Proteobacteria* (Fig. 3). Most exoelectrogens currently identified belong to the *Proteobacteria* and known to oxidize simple organic molecules. Thus, when simple organic acids, such as acetate, are used as substrate, exoelectrogens can oxidize them directly to current. However, the microbial communities have also contained other species that can be unknown exoelectrogens or have other metabolic functions. These other metabolic reactions can support current production by, e.g. oxygen scavenging (Butler and Nerenberg, 2010; Chae et al., 2009) or production of mediator (Aelterman et al., 2006), be independent of current production or decrease current production by, e.g., methane production (Lee et al., 2009; Rago et al., 2015). In addition, the inoculum used, e.g. activated sludge, may contain complex organics that lead to the enrichment of fermenting bacteria (Santoro et al., 2015).

2.2.2 Fermentable substrates

Bacterial communities in biofilms enriched with fermentable sugars as substrate are more diverse than the ones enriched with acetate (Fig. 3). Also in biofilms fed with fermentable sugars, the proportion of bacteria from the phylum *Proteobacteria*, that contain bacteria oxidizing simple organics to current, is large (~ 50%, Fig. 3) suggesting the presence of exoelectrogenic species. In addition, bacteria from the phyla *Firmicutes* and/or *Bacteroidetes* have been detected in anodes fed with monomeric sugars. The phylum *Bacteroidetes* consists of mesophilic fermentative bacteria that can, for example, ferment glucose into propionate, acetate, lactate, formate, succinate and fumarate (Lu et al., 2012b). The phylum *Firmicutes* consists of bacteria that are ubiquitously distributed and have been detected in a multitude of studies as part of the fermentative bacterial community. In addition, it has been suggested that certain *Firmicutes* species could take part in current production (Chung and Okabe, 2009).

399 The higher diversity of bacterial species in BES fed with fermentable monomeric sugars compared
400 to acetate (Sun et al., 2015; Velasquez-Orta et al., 2011) is due to the necessity of glucose
401 fermentation prior to current production since most known exoelectrogens can only utilize simple
402 organic acids. For example, Lu et al.(Lu et al., 2012b) reported that glucose fermentation both at
403 4 and 25°C proceeded through syntrophic interactions of fermenting bacteria and exoelectrogens.
404 Beecroft et al. (2012) studied sucrose fermentation and observed both fermenting and
405 exoelectrogenic species in the biofilms of three parallel reactors.
406 The requirement of fermenting bacteria in biofilms degrading monomeric sugars has been reported
407 to decrease the relative abundance of exoelectrogens, which has led to lower coulombic
408 efficiencies with xylose than acetate (Sun et al., 2015). In addition to fermenting bacteria and
409 exoelectrogens, bacteria with other metabolic characteristics have been detected in sugar-fed
410 exoelectrogenic biofilms and may compete with current production and hence lead to decreasing
411 coulombic efficiencies (Chae et al., 2009). These other bacterial species include facultative
412 anaerobes, microaerophiles as well as denitrifiers and sulphate reducers (see Chapter 2.1).
413 Nevertheless, even these organisms might also have exoelectrogenic capabilities (Beecroft et al.,
414 2012; Chae et al., 2009).

415 ***2.2.3 Waste streams***

416 It is important to study microbial communities enriched on real wastewaters, since the composition
417 of these waste streams is complex and can vary significantly with time. For example, Yu et al.
418 (2012) reported that biofilms enriched with synthetic wastewaters differed phylogenetically from
419 biofilms enriched with real domestic wastewaters. As with monomeric sugars, anodes fed with
420 wastewaters also contain diverse bacterial communities, which contain *Proteobacteria* often as a
421 major phylogenetic group (Fig. 3). The lowest proportions of bacteria from the phylum
422 *Proteobacteria* are often associated with more complex waste materials, such as cellulose (Cheng

et al., 2011), starch (Montpart et al., 2015), and distillery wastewater (Ha et al., 2012) that require hydrolysis and/or fermentation of the biomass before current generation. For example, in biofilms fed with cornstalk hydrolysate, the dominant bacterial species were hydrolytic and fermenting bacteria and not bacteria from phylum *Proteobacteria* where many of the exoelectrogens belong to (Liu et al., 2015). Although the wastewater microbial communities are diverse, several known exoelectrogens, including *Geobacter* sp.(Cusick et al., 2010; Ishii et al., 2012; Jia et al., 2013; Jong et al., 2011; Li et al., 2013; Lu et al., 2012a; Sciarria et al., 2013), *Desulfobulbaceae* sp. (Yu et al., 2012), *Pseudomonas* sp.(Koch et al., 2014; Yu et al., 2012), *Shewanella* sp.(Koch et al., 2014) as well as suggested exoelectrogens, such as *Geovibrio ferrireducens* (Katuri et al., 2012), *Magnetospirillum* sp. (Li et al., 2013), *Dysgomonas* sp. (Zhang et al., 2009), and *Clostridium* sp. (Wang et al., 2013; Zhang et al., 2012) have been detected in anodic biofilms.

Petrimonas sp. has been associated with protein degradation in biofilms fed with waste activated sludge (Lu et al., 2012a), while cellulose and chitin degrading strains *Oscillibacter*, *Chitinophagaceae* and *Acidobacter* (Wang et al., 2013) as well as bacteria from the phyla *Chloroflexi* and *Actinobacteria* (Zhang et al., 2012) were enriched in biofilms treating sewage sludge. Other fermentative and hydrolytic bacteria enriched in biofilms include *Pelobacter*, *Bacteroides*, *Veillonella*, *Enterococcus*, *Eubacterium*, *Spirochaeta*, *Fusobacterium*, and *Clostridium* sp. (Cusick et al., 2010; Jia et al., 2013; Jong et al., 2011; Li et al., 2013). Luo et al. (Luo et al., 2017) reported that increasing concentration of yogurt wastewater (from 1 to 13 g COD/L) resulted in higher microbial diversity and increased relative abundance of bacteria from the phylum *Bacteroidetes*, while the relative abundance of *Proteobacteria* decreased.

Since waste stream compositions are often complex and can also contain inorganic compounds, metabolic reactions that may lead to decreasing current production are often detected. For example,

sulfate- and/or sulfur-reducing bacteria have been enriched with distillery wastewater (Ha et al., 2012) or marine plankton (Reimers et al., 2007) as substrate. In addition, the fermentation products, acetate and H_2, of waste activated sludge or cattle dung have been shown to enhance the growth of acetoclastic (Lu et al., 2012a) and hydrogenotrophic (Lu et al., 2012a; Zhao et al., 2012) methanogens. Intrusion of oxygen from cathode to anode has enabled the growth of aerobic and facultative aerobic bacteria in biofilms fed with a mixture of domestic and olive mill wastewater (Sciarria et al., 2013).

3. Parameters affecting the microbial community compositions

3.1 Anode potential

In order for the microorganisms to grow, thermodynamics need to be favorable for the production of ATP, i.e. the potential difference of the electron acceptor and electron donor has to remain positive. The thermodynamics also influence the microbial communities. While with the same electron acceptor (e.g. electrode) the organic substrate affects the microbial community composition (section 2), with the same substrate the electron acceptor affects the microbial communities. Thus, reductive microorganisms compete with each other when many e-acceptors are available and anode potential determines whether electrode reducing microorganisms outcompete other microorganisms. When the electrode is the only available electron acceptor, the anode potential is of great importance and affects the microbial community composition. In addition, the anode potential can dictate the kinetics of extracellular electron transfer (Prokhorova et al., 2017).

Moreover, the physiology of microorganisms capable of transferring electrons to an electrode surface might be adapted to certain potential windows, which would lead to selective advantages

for organisms that are specifically adapted to the anode potential that is prevailing in the applied bioelectrochemical reactor. An example for this adaptation can be seen in organisms that use endogenously produced shuttles for current production. These shuttles can for instance have a midpoint redox potential of -34 mVvs. SHE as is the case for the *Pseudomonas aeruginosa* that shuttles pycocyanine, or -220 mV vs. SHE as is the case for flavin mononucleotide that can be found in culture supernatants of *S. oneidensis* (Glasser et al., 2017). Hence, under low redox potential conditions. *P. aeruginosa* would not be capable of interacting with electrode, while *S. oneidensis* still could.

Moreover, the anode potential can also lead to specific adaptations of an organism by the use of regulatory routines as was observed for *Geobacter sulfurreducens*. This organism uses different enzymes for the transfer of respiratory electrons from the cytoplasmic membrane into the periplasm depending on the applied electrode potential (Levar et al., 2014; Zacharoff et al., 2016). Interestingly, the way the electrons take is connected to variations in the yield per mol of electrons transferred. Hence, one organism might have a growth advantage over the other organism at one anode potential but this advantage does not necessarily have to exist over the whole range of applied anode potentials. All these factors can lead to variations in the community composition of anode biofilms thriving under different potentials but with similar electron donors and carbon sources. In principle, the anode potential can be controlled with a potentiostat or with an external resistance. However, relating the findings only to the values of external resistance is likely misleading due to the possibility of changing anode potentials during the experimental runs. The anode potential depends on the current density of the BES, which in turn depends on the size of the electrodes and the potential difference between the anode and cathode electrodes. In any case,

491 the anode potential can be controlled externally (e.g. by choosing a suitable load resistor, or load
492 current in case of MECs), which also enables the control of the microbial community composition.
493 Zhu et al. (2014) reported that changing the anode potential from -0.25 V to 0.81 V (vs. SHE) in
494 single-chamber MECs fed with acetate did not affect microbial community compositions, which
495 were mainly dominated by *G. sulfurreducens*. Cercado et al. (2013) also reported that using garden
496 compost leachate for current production from acetate at three different anode potentials resulted in
497 similar dominant species, although higher currents were produced at the highest anode potential
498 of +0.1 V (vs. SCE). Torres et al. (2009) tested the effects of anode potentials on microbial
499 communities growing on acetate in MECs containing four anodes in the same chamber. At the
500 lowest anode potential of -0.15 V (vs. SHE), *Geobacter* sp. dominated (99%) but was also highly
501 present at anode potentials of +0.02 V (90%) and -0.09 V (92%). At higher anode potential (+0.37
502 V vs. SHE) the biofilm community was more diverse. Sun et al. (Sun et al., 2012) compared the
503 microbial communities enriched on formate at different anode potentials and reported similar
504 microbial community composition (ca. 52% *G. sulfurreducens* and 22% *Acetobacterium*) in the
505 anodes (-0.30 and -0.15 V vs. SHE) excluding anode potential of +0.15 V vs. SHE, where
506 *Acetobacterium* was not present. All of these studies were conducted in one-chamber cells with
507 simple organics as substrate, which may not give a representative picture on which species would
508 be enriched on real waste streams or in two-chamber cells.
509 Dhar et al. (Dhar et al., 2016) studied the effect of ohmic drop on the anodic microbial
510 communities at the anodes placed at different distances from the reference electrode, which
511 resulted in more positive anode potentials with increasing distance. At the lowest anode potential
512 of -0.01 V vs. SHE the dominant bacteria were *Geobacter* species, while at +0.14 V vs. SHE
513 *Rhodocyclaceae* sp. dominated and at +0.30 V vs. SHE the bacterial community was highly diverse.

514 These results highlight the effect of reactor configuration and the resulting ohmic drop on the
515 bacterial communities and shows why the comparison between different studies is difficult, if the
516 ohmic drop effects are not reported. Thus, when reporting results, it is important to also consider
517 and quantify the effects of ohmic drop and uncompensated resistance (IR drop) which can
518 noticeably falsify the actual electrode potential (Madjarov et al., 2017).

519 **3.2 Temperature and pH**

520 The current production, metabolic pathways as well as microbial community compositions are
521 affected by pH and temperature. Furthermore, according to the Nernst Equation a change in pH
522 directly affects the redox potential of any reaction involving protons. For instance, by increasing
523 the pH by one unit the redox potential of hydrogen oxidation is shifted by – 59 mV. For hydrogen
524 oxidation at a given anode potential this shift translates into a by 59 mV increased electrode
525 polarization, which in turn leads to an increased oxidation current.

526 Ishii et al.(2008) reported that at neutral pH in the beginning of the experiment methane production
527 was observed. Chung and Okabe (2009) tried to inhibit methanogenesis by lowering the pH to 5-
528 6, but this led also to decreased current production. Current production from unbuffered paper mill
529 effluents resulted in pH increase from 7.5 to highly alkaline (up to 9.5), which did not affect the
530 current production but resulted in the disappearance of *Geobacter* sp. in the biofilm and appearance
531 of *Desulfuromonas acetexigens* suggesting that it played an important role in current production
532 at alkaline pH (Ketep et al., 2013). An optimum pH as high as 11 for current generation was
533 reported by Zhang et al. (2016) who enriched an anodic biofilm from aerobic activated sludge on
534 glucose and reported for the first time that the *Eremococcus* genus dominated. In addition, Luo et
535 al. (2017) reported current generation from yogurt wastewater at an alkaline pH of 10.5 with
536 *Geoalkalibacter* as the dominant species. Zhang et al. (2011a) studied the effect of low pH values

on current production. Low pH values of ≤ 5 resulted in cracking of biofilms and detaching of bacteria and pH values ≤ 4 may have resulted in long term and irreversible decrease in current production. However, current production at acidic conditions (pH < 4) is also possible (for a review, see (Dopson et al., 2016)).

Bacteria can grow in a wide range of temperature conditions from psycrophilic (<15°C) and mesophilic (25-40°C) to thermophilic (50-60°C). Temperature has been found to have a large effect on methane production in a one-chamber MEC. Change of operating temperature from 25-30°C to 4 or 9°C decreased microbial diversity and inhibited methane production completely at the anode, but also decreased H_2 yields at the cathode due to lower current densities (Lu et al., 2011; Lu et al., 2012b). At 15°C, methane production was low (5%) but the cathodic H_2 yields remained lower than at 30°C where hydrogen production was accompanied with methane production. *Geobacter* dominated the bacterial communities at each temperature, from 4 to 30°C (Lu et al., 2012b). It has been reported that the dominating bacterial species from the phylum *Proteobacteria* change due to changes in temperature (Liu et al., 2013). For example, decrease in temperature from 25°C to 4 or 9°C resulted in a change from *Geobacter chapelleii* to *Geobacteri psychrophilus* (Lu et al., 2011), from 25 to 15°C changed the dominant bacterium from *Simplicispira psychrophila* to *Geobacter psychrophilus* (Liu et al., 2013), and a gradual temperature decrease shifted the dominant species from *Geobacter* and *Azonexus* (30°C) to *Pelobacter* (20°C) and *Acidovorax*, *Zoogloea* and *Simplicispira* (10°C) (Mei et al., 2017).

In a thermophilic MFC treating distillery wastewater, thermophilic bacteria from the phylum *Bacteroidetes* dominated (Ha et al., 2012). At 60°C, in an acetate-fed MFC bacteria from the phyla *Firmicutes* and *Deferribacteres* were present, from which *Thermincola carboxydophila* from the phylum *Firmicutes* dominated (Mathis et al., 2008). Also at an anode fed with cellulose at 60°C,

560 *Thermincola* and *Thermoanaerobacter* from the phylum *Firmicutes* dominated, while fermenting
561 bacteria *Tepidmicrobium* and *Moorella* dominated in the anodic solution (Lusk et al., 2017).
562 In summary, pH values between 5 and 9.5 have resulted in stable current production with different
563 microbial communities. However, current production at acidophilic and alkaline conditions is also
564 possible. The temperature range between 25 and 60°C is promising for current production.

565 **3.3 Oxygen**

566 Oxygen can leak towards the anode from an oxic cathode or be produced at the anode by, e.g.,
567 photosynthetic bacteria. Bacteria grow faster by using O_2 as electron acceptor and thus, under oxic
568 conditions organic removal rates are often improved (Cusick et al., 2010; Quan et al., 2012), which
569 however results in electron losses and decreased current generation (Jung and Regan, 2007). Kiely
570 et al. (2011a) suggested that the presence of oxygen was essential for the functioning of the anode
571 community, i.e. substrate degradation and current production, when dairy manure was used as
572 substrate likely due to aerobic oxidation of complex organics. Shebab et al. (2013) also reported
573 that 72% of the organics removal from acetate was due to aerobic degradation or anoxic reactions
574 other than exoelectrogenesis. In some MFCs, the membrane separating the anode and cathode has
575 been shown to face fouling with, e.g. microaerophilic bacteria (Cárcer et al., 2011), which has
576 prevented oxygen diffusion from cathode to anode. Although oxygen scavengers on the membrane
577 enable the growth of obligate anaerobes at the anode, it might also hinder ion transfer between
578 cathode and anode compartments and thus increase internal resistance and contribute to a pH
579 imbalance between the chambers.
580 Liu et al. (2010) reported that starting the fuel cell as MEC instead of MFC on acetate resulted in
581 increased richness and diversity of the microbial communities due to strictly anaerobic conditions.
582 According to Chae et al. (2008) and Butler and Nerenberg (2010), O_2 leakage from the MFC

583 cathode likely supported the growth of aerobic or facultatively aerobic β-*Proteobacteria* in anode
584 biofilms, while in MEC anodes with anaerobic cathodes δ-*Proteobacteria* dominated.
585 Current production has also been reported with continuously aerated anodes, which however has
586 reduced coulombic efficiency (Quan et al., 2012). The power generation fully recovered after
587 aeration was stopped, although the microbial diversity decreased after air-exposure and contained
588 highly oxygen-tolerant microorganisms. After aeration, α-*Proteobacteria* disappeared and the
589 share of *Firmicutes* decreased, while the presence of *Bacteroidetes* and *Actinobacteria* increased
590 significantly (Quan et al., 2012). *Shewanella* sp. have been shown to produce current both under
591 oxic and anoxic conditions (Biffinger et al., 2008; Kipf et al., 2013; Ringeisen et al., 2007). Quan
592 et al. (2013) compared the enrichment of exoelectrogenic communities under oxic and anoxic
593 conditions. The bacterial communities in suspension were highly similar despite of the enrichment
594 methods, while the biofilms were only 77% similar.
595 In summary, the presence of oxygen has both beneficial and harmful effects. Presence of oxygen
596 may result in aerobic degradation of organic matter and hinder oxygen transfer to anodic biofilms,
597 while it also decreases coulombic efficiencies and current generation.

598 **3.5 Hydraulic retention time and hydrodynamic conditions**

599 Bioelectrochemical systems are mostly based on the activity of productive biofilms. The
600 disadvantage of the surface limitation of electrochemical processes is compensated by the
601 advantages of using anaerobic biofilm biocatalysts. Biofilms are natural retentostats (Halan et al.,
602 2012). This is especially true for biofilms of exoelectrogenic organisms, since these organisms can
603 only thrive with the organic substrates if they use the anode surface as terminal electron acceptor
604 of their respiratory chain. In other words, exoelectrogenic organisms that cannot contact the
605 electrode surface directly and which generation time is longer than the HRT will be washed out in

606 a continuously operated system. Moreover, growth and substrate consumption are at least to a large
607 extent uncoupled in biofilms meaning that the production of biomass is suppressed at high
608 substrate consumption rates. This is further accentuated by the – compared to oxic processes – low
609 amount of energy that is available for the organisms.

610 The activity of biofilms is affected by the hydrodynamic conditions within the reactor. Pham and
611 colleagues tested the effect of shear force on the activity of exoelectrogenic biofilms (Pham et al.,
612 2008, 2008). The authors observed that microbial fuel cell biofilms were more effective in terms
613 of chemical to electrical energy conversion if higher shear forces were applied. Certainly, there
614 was an optimum of shear force and higher shear rates than optimum lead to lower current
615 production, which might be a result of biofilm abrasion. Still, moving with the shear rate from 0.3
616 to 120 s^{-1} resulted in a two to three times higher current production and a biomass density increase
617 with a factor of 5.
618 Biofilms offer a further advantage; it might be possible to control the biology of the biofilm
619 biocatalyst, since there is a selection not only for organisms that can gain the most energy out of
620 the process prerequisites but also for those that are good biofilm formers. Dolch et al. (2016)
621 showed that a preformed biofilm composed of the exoelectrogenic model organisms *S. oneidensis*,
622 *G. sulfurreducens* and *G. metallireducens* had a rather high resilience in terms of cell number
623 retention even under non axenic conditions. Still, the experiments were conducted under fed batch
624 conditions and it is so far not clear how these model biofilms would react to higher shear forces in
625 flow through systems. Nevertheless, the results make way for the consideration of the
626 biotechnological use of non-axenic substrates by exoelectrogenic designer organisms that are
627 developed to efficiently form robust biofilms and to produce valuable compounds from mixed
628 biomass streams.

Typical substrates for microbial fuels cells are a mixture of volatile fatty acids. Generally, acetate is the preferred substrate and is hence consumed in the shortest amount of time. Freguia et al. (2010) measured the individual COD removal rates in a reactor inoculated with a mixture of sludge, soil and sediment and could show the consumption of typical VFAs in the order acetate, n-valerate, n-butyrate, i-valerate, hexanoate, propionate, i-butyrate. Hence, by varying the hydraulic retention time in a reactor it might be possible to steer the composition of its effluent, for example to be further used in biorefineries. Still, it is also necessary to account for competing reactions like secondary fermentations. High coulombic efficiencies that are partly reaching over 90% are usually reached for acetate as a substrate while the use of other VFAs usually leads to efficiencies around 50% (Freguia et al., 2010; Teng et al., 2010; Torres et al., 2007). Hence, the elimination of these carbon sources is only partly sustained by a bioelectrochemical process.

Primary and secondary fermentations usually lead to hydrogen as a by-product. Hence, high efficiencies will depend on the oxidation of hydrogen by the anode community. Different researchers provided evidence for the possibility of an anode coupled hydrogen oxidation, albeit its use seems to be connected only to rather limited current production at least in some studies (Lee et al., 2003; Teng et al., 2010). Interestingly, the addition of hydrogen as electron donor to anode compartments seems to select for co-cultures of homoacetogens and exoelectrogens. Hence, hydrogen is first converted with carbon dioxide to acetate, which is then consumed by the anode community (Parameswaran et al., 2012). Nevertheless, the average coulombic efficiencies presented in a variety of studies in which mixed VFAs were added as a substrate suggest that part of the electrons are lost in the form of hydrogen. Therefore, strategies to raise coulombic efficiencies in microbial fuel cells might include the recirculation of substrate at high shear rates

651 to raise the hydraulic retention time and/or the use of reactors that can be operated under pressure
652 to keep volatile compounds in the reactor.
653 In summary, continuous operation will wash out microorganisms that are not attached to the anode
654 biofilm, formation of which is more beneficial if shear forces are increased.

655 4. Challenges and possibilities for controlling the microbial
656 communities

657 To be able to control and direct the metabolic pathways of the microbes as well as to optimize
658 the current yields it is important to understand the behavior of the exoelectrogenic communities
659 at the anode. The exoelectrogenic communities can be controlled by changing process
660 parameters at the anode (see Table 3). The main challenge related to anodic microbial
661 communities is the inhibition of methanogenesis that decreases current yields and coulombic
662 efficiencies. Methane production has been decreased by decreasing the temperature or pH, which
663 has also led to low current densities (Table 3). Thus, the effects of lower temperature and/or pH
664 on microbial communities should be further studied before using them as the main controlling
665 parameters for inhibiting methanogenesis. Nevertheless, continuous pH adjustment can help to
666 avoid fluctuations in current as was reported by Ishii et al (Ishii et al., 2008).
667 The presence of oxygen has also an inhibitory effect on the growth of methanogens (Chae et al.,
668 2010) and has been shown to increase COD degradation (Table 3). However, the presence of
669 oxygen has decreased current production and coulombic efficiencies due to acting as electron
670 scavenger and has, in some cases, resulted in biofouling of the membrane which increased ohmic
671 losses of the cell (Table 3). Intermittent air sparging has been used to control the growth of
672 methanogens (Chae et al., 2010) and could still enable high coulombic efficiencies. Thus, it needs
673 to be taken into consideration whether the aim of the bioanode is to produce maximum current or
674 achieve high COD removal efficiencies.
675 Real waste streams contain various inorganic and organic compounds, all of which may affect to
676 current production with anodic biofilms. While most of the organic substrates can be used for
677 current production, inorganic compounds (such as sulfate or nitrogen) often divert electrons to

678 other metabolic processes (Table 3). Sulfate reduction at the anode can also result in precipitation
679 of solid sulfur on the anode electrode surface, which increases the losses of the cell. However,
680 sulfate reduction or sulfur oxidation reactions may also result in current production through, e.g.,
681 abiotic oxidation of hydrogen sulfide. In addition, part of the electrons present in organic
682 compounds may end up in fermentation products and oxygen leaking to the cells may result in
683 aerobic instead of anaerobic metabolism, both of which decrease the current yields (Table 3). Thus,
684 it is of major importance to recognize the different waste stream constituents and follow the
685 possible side metabolic reactions, when wastewaters are used as feed for anodic microbial
686 communities. From an engineering point of view, the avoidance of dead zones in the reactor
687 construction will also be of importance. The microbial reactions in these dead zones remain largely
688 unclear and should be further delineated.
689 Since the wastewater composition cannot be changed and pretreatment of the wastewaters is likely
690 not feasible, the effects of wastewater constituents on current production should be determined
691 separately for each wastewater. The inhibition of the competing metabolic pathways is likely
692 difficult due to chemical and microbial wastewater composition that cannot be altered. To a certain
693 extent, process control can help to increase the current yields and diminish competing pathways.
694 Suggested process parameters to be adjusted include pH (continuous control), temperature, HRT,
695 and prevention of oxygen leakages. The feasibility of current production in terms of wastewater
696 treatment and current production efficiencies should be determined. One possibility to generate
697 electricity from wastewaters containing potential alternative electron acceptors could be the
698 enrichment of an anodic community that cannot use these compounds as electron acceptors, which
699 however may be difficult to achieve.

700 The microbial community results of different studies with similar substrates vary a lot, likely due
701 to varying electrode materials and reactor configurations that affect, e.g. oxygen diffusion to the
702 cathode. Further, microbial communities of anodes fed with identical wastewater as inoculum and
703 substrate have shown large differences (Koch et al., 2014). These conclusions indicate the
704 importance of parallel experiments, especially when using wastewaters as substrate, as well as the
705 use of similar reactor configurations and materials for the microbial community, co-culture, and
706 current production potential investigations. Such a standardized reactor configuration is yet to be
707 developed. In addition, standard methods for designing experiments and choosing operational
708 parameters need to be determined within the research community. While tutorials and techniques
709 have already been reported for, e.g. electrochemical analysis of biofilms (Harnisch and Freguia,
710 2012; Harnisch and Rabaey, 2012) and taking into account uncompensated resistance (Madjarov
711 et al., 2017), detailed instructions for experimental design are still lacking. Examples of such
712 detailed instructions can be found in the field of anaerobic treatment, where specific instructions
713 are given for designing biomethane potential experiments (Angelidaki et al., 2009; Holliger et al.,
714 2016).

715 ## 5. Conclusions

716 Although the main possible metabolic reactions at the anodes of BES are known, the syntrophic
717 and competing interactions among different microbial species should be further studied. This can
718 be done, e.g., by studying different co-cultures and their responses at various operational
719 conditions. In addition, finding novel ways to inhibit the competing metabolic pathways is required
720 to be able to produce current from real wastewaters effectively and the operational parameters for
721 each wastewater should always be separately optimized. Finally, there is a requirement for

standard methods for designing experiments to enable better comparison between different laboratories.

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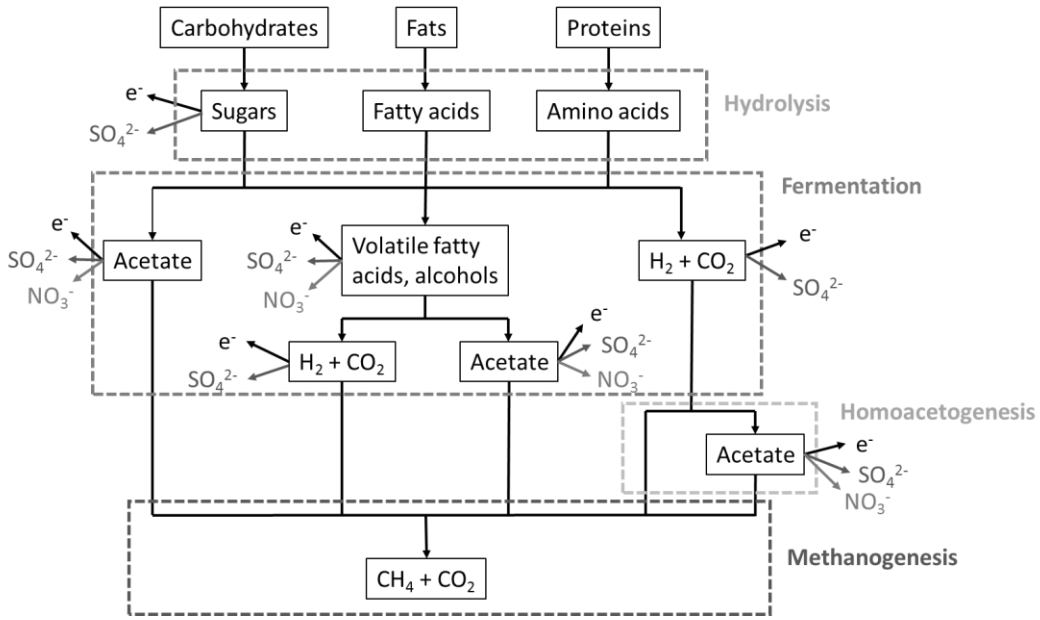
Figure captions

Figure 1. Anaerobic biological degradation of complex organics proceeds through to the following metabolic reactions: hydrolysis, fermentation, homoacetogenesis and methanogenesis. Organic intermediates and H_2 of these reactions can be used for electrogenesis (Chae et al., 2009; Kim et al., 2006; Parameswaran et al., 2009), which has been marked with e^- in the figure. Methanogenesis as well as sulfate and nitrate reduction (marked with SO_4^{2-} and NO_3^-, respectively, in the figure) are competing metabolic processes that direct electrons away from current production.

Figure 2. Syntrophic interactions of different microorganisms in wastewater-fed anodes. The light grey reactions describe metabolic reactions that precede current production, dark grey reactions direct electrons away from current production, and black reactions produce current. H_2 can also be produced by fermentative bacteria.

Figure 3. The distribution of bacteria in an anodic biofilm into different phyla with acetate (Borole et al., 2009a; Chae et al., 2008; Chae et al., 2009; Eyiuche et al., 2017; Ha et al., 2012; Jong et al., 2011; Lu et al., 2011; Quan et al., 2012; Xing et al., 2009; Zhang et al., 2011b), sugars (Borole et al., 2009b; Chae et al., 2009; Chung and Okabe, 2009, Eyiuche et al., 2017, 2017; Jong et al., 2011; Kim et al., 2006; Kim et al., 2011; Xing et al., 2009; Zhang et al., 2011b), or waste materials (Borole et al., 2009c, Cerrillo et al., 2017a, 2017b; Eyiuche et al., 2017; Ha et al., 2012; Hassan et al., 2017; Ishii et al., 2012; Jong et al., 2011; Kim et al., 2004; Kumar et al., 2017; Lu et al., 2012a; Luo et al., 2017; Pannell et al., 2016; Patil et al., 2009; Shen et al; Zhang et al., 2009; Zhang et al., 2012; Zhao et al., 2012) as substrate based on literature research. N shows the number of experiments used for calculations. Standard deviations are shown in the figure.



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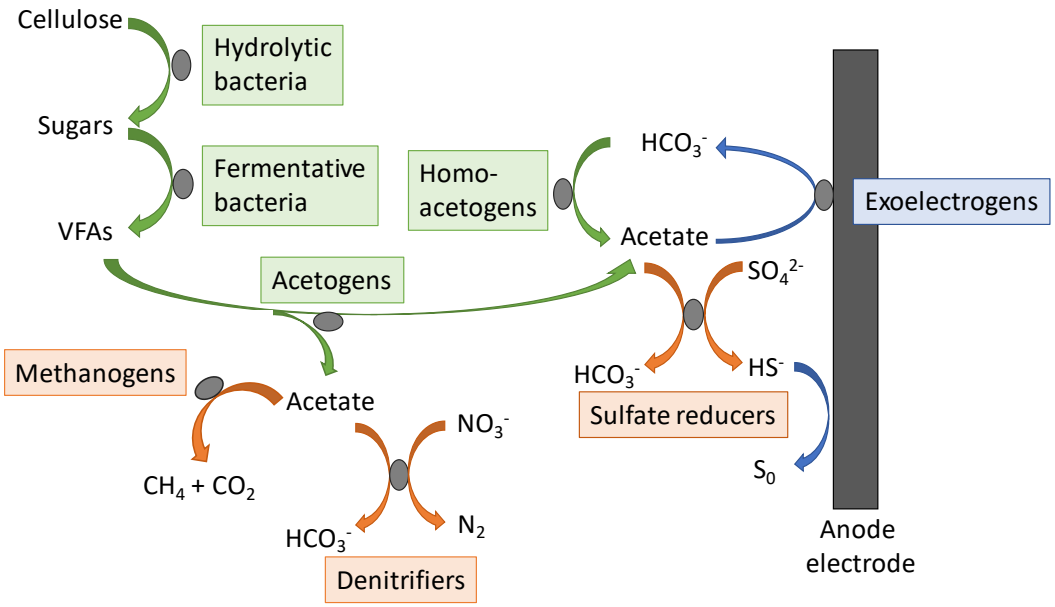

Figure 2. Syntrophic interactions of different microorganisms in wastewater-fed anodes. The green reactions describe metabolic reactions that precede current production, red reactions direct electrons away from current production, and blue reactions produce current. H_2 can also be produced by fermentative bacteria.

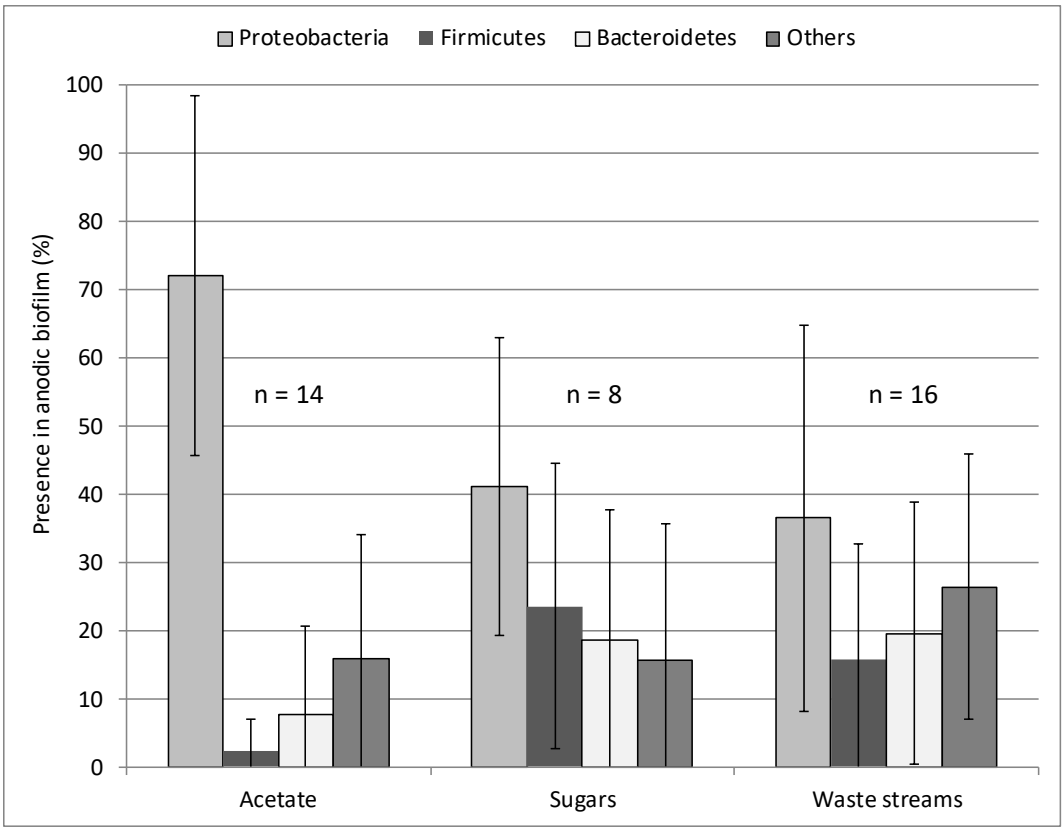

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Table 1. Possible oxidation and reduction reactions at the anode, their standard redox potential (E^0), and theoretical potentials at typical anodic conditions of pH 7 and 30°C (E). All potentials are given against normal hydrogen electrode (NHE).

Compound	Reaction	E^0 (V)	E (V)
Reduction reactions			
Oxygen	$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$	1.229	0.798
Nitrate	$2\ NO_3^- + 12\ H^+ + 10\ e^- \rightarrow N_2 + 6\ H_2O$	1.244	0.719
Sulfate	$SO_4^{2-} + 9\ H^+ + 8\ e^- \rightarrow HS^- + 4\ H_2O$	0.249	-0.225
	$SO_4^{2-} + 10\ H^+ + 8\ e^- \rightarrow H_2S + 4\ H_2O$	0.308	-0.228
Carbon dioxide	$HCO_3^- + 9\ H^+ + 8\ e^- \rightarrow CH_4 + 3\ H_2O$	0.227	-0.256
Hydrogen	$2\ H^+ + 2\ e^- \rightarrow H_2$	0	-0.400
Carbon dioxide	$2\ HCO_3^- + 9\ H^+ + 8\ e^- \rightarrow CH_3COO^- + 2\ H_2O$	-0.427	-0.916
Oxidation reactions			
Sulfide	$H_2S \rightarrow S^0 + 2\ e^- + 2\ H^+$	0.174	-0.187
	$HS^- \rightarrow S^0 + 2\ e^- + H^+$	-0.062	-0.213
Thiosulfate	$S_2O_3^{2-} + 5\ H_2O \rightarrow 2\ SO_4^{2-} + 10\ H^+ + 8\ e^-$	0.272	-0.269
Acetate	$CH_3COO^- + 4\ H_2O \rightarrow 2\ HCO_3^- + 9\ H^+ + 8\ e^-$	0.187	-0.301
Ethanol	$C_2H_5OH + 5\ H_2O \rightarrow 2\ HCO_3^- + 16\ H^+ + 14\ e^-$	0.144	-0.346
Propionate	$C_2H_5COO^- + 7\ H_2O \rightarrow 3\ HCO_3^- + 19\ H^+ + 17\ e^-$	0.159	-0.350
Glucose	$C_6H_{12}O_6 + 12\ H_2O \rightarrow 6\ HCO_3^- + 36\ H^+ + 30\ e^-$	0.084	-0.441
Butyrate	$C_3H_7COO^-\ 10\ H_2O \rightarrow 4\ HCO_3^- + 23\ H^+ + 20\ e^-$	0.196	-0.495
Sulfite	$SO_3^{2-} + H_2O \rightarrow SO_4^{2-} + 2\ H^+ + 2\ e^-$	-0.015	-0.496

Conditions used for calculations of E: concentrations of solutions = 10 mM, partial pressure of gases = 0.2 bar, pH 7, 30°C

1222 **Table 2.** Co-cultures of different bacterial species and their interactions.

Substrate	1st bacteria	2nd bacteria	Reference	Interaction
	Hydrolytic/fermentative bacterium	***Exoelectrogen***		Syntrophic*;* exoelectrogen consumed metabolites of hydrolytic/fermentative bacterium for current production, which decreased feedback inhibition of metabolites for hydrolytic/fermentative bacterium.
Cellulose	*Clostridium cellulolyticum*	*G. sulfurreducens*	(Ren et al., 2007)	
Cellulose	*Clostridium thermocellum*	*G. sulfurreducens*	(Cheng et al., 2011)	
Food waste	*Bacteroides*	*Geobacter* sp.	(Jia et al., 2013)	
Glucose	*Lactococcus lactis*	*Shewanella oneidensis*	(Rosenbaum et al., 2011)	
Glucose	*Enterobacter aerogenes*	*Pseudomonas aeruginosa*	(Venkataraman et al., 2011)	
Ethanol	*Pelobacter carbinolicus*	*G. sulfurreduces*	(Richter et al., 2007)	
Ethanol	*Pelobacter* sp.	*Geobacter* sp.	(Parameswaran et al., 2009)	
	Hydrolytic/fermentative bacterium	***Exoelectrogen***		Syntrophic : the metabolite of fermentative bacterium was used for growth of exoelectrogen, exoelectrogen produced mediators that were also used by fermentative bacterium
Glucose	*Escherichia coli*	*S. oneidensis*	(Wang et al., 2015)	
	1st exoelectrogen	***2nd exoelectrogen***		Syntrophic; The growth of 1st exoelectrogen was increased due to consumption of its metabolite by the 2nd exoelectrogen, which used the metabolites for growth and current production.
Lactate and acetate	*S. oneidensis*	*G. sulfurreducens*	(Dolch et al., 2014)	
Acetate	*G. sulfurreducens*	*Hydrogenophaga sp.*	(Kimura and Okabe, 2013)	
	Faculative anaerobe	***Exoelectrogen***		Syntrophic*;* Facultative anaerobe consumed oxygen, which improved current production by exoelectrogen
Acetate	*Escherichia coli*	*G. sulfurreducens*	(Qu et al., 2012)	
	Green sulfur bacteria	***Exoelectrogen***		Commensalism: green sulfur bacteria produced a metabolite that was used for current production by exoelectrogen.
Photosynthetic CO_2 fixation	*Chlorobium* sp.	*Geobacter* sp.	(Badalamenti et al., 2014)	

1223

1224 **Table 3**. Effects of different process parameters on anodic performance.

Process parameter	Condition/Change	Effect	Reference(s)
Anode potential	More negative	Fermentation overtook anaerobic respiration	(Rismani-Yazdi et al., 2011)
	More positive	More diverse microbial community	(Torres et al., 2009)
Temperature	Decrease from 25-30°C to ⩽15° C	Decrease in methane and current production	(Lu et al., 2011; Lu et al., 2012b)
pH	Neutral pH	Methane production	(Ishii et al., 2008)
	Decrease to ≤5	Cracking biofilms, detaching bacteria	(Zhang et al., 2011b)
	Decrease from neutral to 5-6	Decreased methane and current production	(Chung and Okabe, 2009)
	Increase from 7.5 to 9.5	Exoelectrogenic species changed, current production remained high	(Ketep et al., 2013)
	No pH adjustment	Fluctuations in current production	(Ishii et al., 2008)
Oxygen	Presence	Decreased current generation and CE	(Jung and Regan, 2007; Quan et al., 2012)
		Essential for COD degradation and current production with dairy manure	(Kiely et al., 2011a)
		Increased COD removal	(Shehab et al., 2013)
		Fouling of the membrane	(Cárcer et al., 2011)
		Inhibition of methanogenes	(Chae et al., 2010)
	Absence	Increased community richness and diversity	(Liu et al., 2010)
Sulfate	Presence	Current can be produced directly or indirectly by sulfate-reducing or sulfur-oxidizing bacteria	(Holmes et al., 2004; Reimers et al., 2007)
		Sulfur deposition on the anode can increase ohmic losses	(Sangcharoen et al.)
		Presence often decreases current generation and CEs	(Zhang et al., 2013b)

1225 COD = chemical oxygen demand, CE = coulombic efficiency

1226