Chapter 5

Rumen Microbial Fuel Cells

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Introduction

Converting renewable biomass into electricity by microbial fuel cells (MFCs) can produce clean and transportable energy, with minimal impact on the environment. The capacity and efficacy of MFC have been extensively evaluated on laboratory scales (Rabaey and Verstraete, 2005). Thus far, electron suppliers from biomass for MFCs have been primarily limited to those soluble and rapidly metabolized organic compounds such as simple carbohydrates (Rabaey *et al*., 2003; Chae *et al*., 2009), small organic acids (Liu *et al*., 2005a; Chae *et al*., 2009), starch (Niessen *et al*., 2004) and amino acids (Logan *et al*., 2005). Although good performance has been obtained, these materials are valuable and have other economical uses. Indeed, when used to produce other biomass energy such as ethanol, they give better energy unit yields. Another potential limitation for these substrates for MFCs is that they are hydrolysed at a rapid rate by microbes, and fermentation or metabolic end-products can build up quickly. The accumulation of these products has been shown to affect microbial ecology (Mohan *et al*., 2007) and the electrical property of the MFC system (Logan *et al*., 2006).

Transformation of Plant Fibre into Electricity by MFCs

Plant fibre carbohydrates, including wastes from agricultural and industrial activities, are the most abundant and renewable biomass on Earth (Niessen *et al*., 2005). There are three types of existing renewable energy technologies related to plant fibre biomass: bio-ethanol (Chen, W.H. *et al*., 2008), bio-hydrogen (Huang, 2008) and power generation. At relatively lower cost than other substrates described above, direct use of fibre to produce electricity would enable MFC to utilize a full spectrum of organic compounds and potentially become a more sustainable source of energy supply.

In a plant fibre-fed MFC system, the biocatalyst is fibre-hydrolysing microbes, such as rumen microbes (Rismani-Yazdi *et al*., 2007; Chen, 2010; Wang *et al*., 2011, 2012) and *Clostridium* spp. (Ren *et al*., 2007). The microbes produce enzymes to hydrolyse fibre with the release of hydrogen ions and electrons required for the MFC system.

Compared to other organic substrates, plant fibre is relatively insoluble and is a large polymer with diverse and complex structure, which varies greatly with species and plant living environment (Malherbe and Cloet, 2002). The biodegradation of fibre coupled with

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electrical output by MFCs requires cooperated actions of various microorganisms. Consequently, as summarized in Table 5.1, contemporary attempts for power generation utilize mostly purified plant fibre sources as cellulose (Niessen *et al*., 2005; Ren *et al*., 2007; Rismani-Yazdi *et al*., 2007; Rezaei *et al*., 2008). Although high efficiency of power output has been acknowledged, such fibre substrates require considerable pretreatment, which would add to the cost of power output.

Pretreatment of plant fibre removes hemicellulose and lignin, which hinder cellulose hydrolysis by microorganisms (His *et al*., 2002). Cellulose after treatment can become soluble, which would further increase susceptibility to microbial degradation. The rate of substrate hydrolysis has been demonstrated as a constraint for power output by MFCs (Rezaei *et al*., 2007; Huang and Logan, 2008; Mathis *et al*., 2008; Jadhav and Ghangrekar, 2009; Rezaei *et al*., 2009). Table 5.1 illustrates variations in electrical performance by MFCs under various experimental conditions, which could reflect solubility of cellulose employed. However, some studies argued that the loss of the activation polarization, Ohm polarization and concentration polarization of the cell system may have greater impact on power output (Zhang and Halme, 1995; Logan *et al*., 2005; Rismani-Yazdi *et al*., 2007; Huang *et al*., 2009; Wang, X. *et al*., 2009).

In contrast, little work has been conducted to evaluate native plant fibre for generating electricity by MFCs (Rezaei *et al*., 2009; Chen, 2010; Wang *et al*., 2011, 2012). This is likely due to the fact that native fibre is virtually insoluble and is much less degradable than purified fibre by microorganisms.

Power output via native plant fibre by rumen MFCs

The rumen of ruminant animals harbours a variety of symbiotic microorganisms, consisting of bacteria, protozoa and fungi. These microorganisms produce assorted enzymes and, working in concert, can effectively degrade structurally complex plant fibre and starch under anaerobic conditions (Hobson and Stewart, 1997; Krause *et al*., 2003). Reduced metabolites such as organic acids (acetate, propionate and butyrate) are produced and used in term by ruminants as an energy source. The organic acids can also be further catabolized to carbon dioxide (CO^2) by rumen microorganisms.

During these processes of anaerobic metabolism of organic matter, reducing equivalent is produced, which is accompanied by the release and translocation of protons and electrons (Offner and Sauvant, 2006). These products could theoretically be connected to power generation. Such potential is further manifested by the fact that in normal conditions, the ruminal milieu is anaerobic with an oxidation reduction potential (ORP) that is markedly negative (Marden *et al*., 2005), indicating a strong power of reduction.

Resent research work has demonstrated that purified cellulose could be converted into electricity by rumen microorganisms in MFCs, in the absence of exogenous electron transfer mediators (Rismani-Yazdi *et al*., 2007). These results indicate that rumen microorganisms could degrade cellulose in MFC conditions and process electrical-chemical properties to reduce the anode, with simultaneous generation of electricity.

In nature, cellulose is always inevitable associated with other components present in the fibre. To date, direct use of native fibre for power generation in rumen MFCs (RMFC) has not been adequately investigated. Our preliminary work shows that electricity could be produced from forage plant fibre (Bermuda grass straw) via transformation by rumen

Table 5.1. Plant fibre substrate applied in MFCs.

C/N [a]	Anode electode (m^2)	Culture	Substrate (g l^{-1})	P (mW m^{-2})	Reference
2*	Platinum sheet and net (—)	C. cellulolyticum	Cellulose powder (3)	—	Niessen *et al*. (2005)
		C. thermocellum			
	Graphite brush (0.22)	Anaerobic sludge	Cellulose (1)	35	Rezaei *et al*. (2007)
	Graphite plates (0.0084)	Rumen microbes	Microcrystalline cellulose (7.5)	55	Rismani-Yazdi *et al*. (2007)
	Graphite plates (0.00152)	C. cellulolyticum	Carboxymethyl cellulose [b] (CMC ; 1 / 2)	1.16 / —	Ren *et al*. (2007)
		Co-culture	CMC (1 / 2)	143 ± 7.2 / 151	
			MN301 (1) [c]	59.2 ± 3.5	
		Mixed culture (sludge)	CMC (2)	42.2 ± 6.1	
			MN301 (2)	33.7 ± 4.9	
	Graphite rods (0.00039)	C. cellulolyticum	Cellulose (—)	—	Sund *et al*. (2007)
	Bundled graphite fibres (—)	G. sulfurreducens PCA	Cellulose (6)	—	Ishii *et al*. (2008b)
	Carbon electrodes (0.00004)	Palm oil sludge (strain Bb)	CMC (5)	1840	Aslizah *et al*. (2007)
			Ethyl cellulose (Eth cel; 5)	1300	
			Native cellulose (Nat cel; 5)	3300	
			Empty fruit bunch (EFB; 5)	1365	
		Palm oil mill effluent (strain P9)	CMC (5)	787.5	
			Eth cel (5)	892.5	
			Nat cel (5)	1400	
			EFB (5)	1470	
	Graphite brush electrode (0.22)	Anaerobic sludge	Microcrystalline insoluble cellulose with cellulase (1.3) [e]	98 ± 0.05	Rezaei *et al*. (2008)
	Graphite plates (0.0016)	C. cellulolyticum G. sulfurreducens [d]	CMC (1)	153	Ren *et al*. (2008)
			MN301 (1)	83	
	Carbon paper (0.0042)	Waste water	Wheat straw hydrolysate (1) [e]	123	Zhang *et al*. (2009)

	Graphite plates (0.02024)	Rumen microbes	Bermuda grass (3.3)	66.2 / 273.5 [f]	Chen (2010)
**	Ammonia-treated carbon cloth (1.13)	Enterobacter cloacae ATCC 13047[T]	Pure cellulose of plant origin (4)	5.4 ± 0.3	Rezaei *et al*. (2009)
		Enterobacter cloacae ER mixed culture		4.9 ± 0.01	
1				18 ± 2.2	
***	Carbon cloth (0.0045)	Mixture of sediment and sand	Cellulose (1)	83 ± 3	Rezaei *et al*. (2007)
	Plain carbon paper (0.001125)	H-C culture with domestic waste water (20%, v/v)	Corn stover powder (CSP; 1)	333	Wang, X. *et al*. (2009)
			Corn stover residual solids (CSRS; 1)	390	
		H-C culture	CSP (1)	—	
****	Carbon paper (0.00071)	Domestic waste water [g]	Neutral hydrolysates (1) [e]	371 ± 13	Zuo *et al*. (2006)
			Acid hydrolysates (1) [e]	367 ± 13	
	Graphite-fibre brush (—) [h]	Paper recycling water unamended waste water	Cellulose (1.464) [i]	672 ± 27	Huang and Logan (2008)
*****	Stainless steel (0.004049/ 0.005101) [j]	Rumen microbes	Bermuda grass (3.3)	0.021102 / 0.014492	Chen (2010)

[a] chamber number; [b] soluble; [c] insoluble; [d] co-culture; [e] g-COD l^{-1}; [f] $K_3Fe(CN)_6/KMnO_4$ catholyte; [g] 5 ml, 0.3 g-COD l^{-1}; [h] 5418 m^2 m^{-3}; [i] g l^{-1} initial TCOD; [j] with/without obstacle at Re = 496.18
* H-type; ** U-type; *** bottle-type air cathode; **** tube-type air cathode; ***** plate-type air cathode

microorganisms in MFCs. Although comparatively high efficiency could be obtained, the capacity of power output was low (average 305 mV; Chen, 2010).

Biotic Factors Affecting Power Output by RMFCs

Degradation rate of plant fibre

Plant fibre varies greatly in the inherent rate of degradation by ruminal microorganisms (Yang, 2002; Chang, 2005). Fibre hydrolysis can be the rate-limiting step for power generation in fibre-fed MFCs. It is anticipated that fibre sources with a faster rate of breakdown in MFCs by rumen microorganisms would also lead to greater production of reducing equivalents available for electricity output. Research work using a defined binary

culture by Ren *et al*. (2007) illustrated that amorphous and microcrystalline cellulose, comparable to native cellulose, produced less electricity by MFCs in comparison to carboxyl methyl cellulose, which is soluble and presumably degraded faster.

Several studies with other microorganisms have also pointed out that the rate of purified cellulose breakdown could affect power output (His *et al*., 2002; Zuo *et al*., 2006; Ren *et al*., 2007; Huang and Logan, 2008; Rezaei *et al*., 2008; Wang, X. *et al*., 2009). However, the correlation between cellulose degradation and power generation remains inclusive.

End-products of substrate fermentation

The major end-products from substrate fermentation in the rumen are short chain fatty acids (SCFA), mainly acetate followed by propionate and butyrate. These reduced products are potential fuels for power output in MFCs. Rumen bacteria which could oxidize SCFA and reduce the electrode have been detected in cellulose-fed MFCs (Rismani-Yazdi *et al*., 2007).

Production of electricity from acetate or butyrate in MFCs with other microorganisms has also been demonstrated (Bond and Lovley, 2003; Min and Logan, 2004; Liu *et al*., 2005a). Acetate was found to produce more electricity than butyrate (Liu *et al*., 2005a) and converted to more oxidized product in the presence of the anode (Bond and Lovley, 2003).

Although SCFA can serve as a source of fuel for electrical generation, accumulation of them has been found to affect microorganisms in MFCs (Mohan *et al*., 2007; Jeong *et al*., 2008) and functional properties of MFCs (Logan *et al*., 2006; Jeong *et al*., 2008; Wang *et al*., 2012). Therefore, optimal SCFA and other fermentation end-products for power output by RMFC require further investigation. In particular, quantitative analyses on fermented organic acids in relation to reduction potential and cell electrical property are warranted.

Profiles of substrate fermentation

Rumen microbial communities and their fermentation pathways alter when switching ruminants from forage diets to increasing proportions of grain (Tajima *et al*., 2000). Therefore, changes in diet or substrate for ruminal microorganisms are the major impacts on rumen SCFA profiles and fermentation efficiency (Guan *et al*., 2008; Yu *et al*., 2010). When substrates contain less forage (mostly fibre) and more grain (mostly starch), the concentration of total SCFA and the proportion of propionate increase, but acetate proportion decreases (García-Martínez *et al*., 2005; Wang *et al*., 2012).

The ratio of forage to concentrate for ruminant diet also affects ORP in the rumen (Mishra *et al*., 1970; Wang *et al*., 2012). Chen (2010) observed that the ratio of acetate to propionate in the rumen is highly correlated to ORP in a negative fashion ($r = -0.712$). This is consistent with the fact that acetate-dominated fermentation pattern produces more reducing equivalents in comparison to propionate-type of fermentation (Russell and Wallace, 1997). A more negative ORP would be apt to reduce the anode. Regardless, how relative alterations in SCFA profile affect RMFC performance is currently inclusive.

Composition and function of rumen microorganisms

Within the rumen, microorganisms exist as attached either to rumen epithelium or feed particles, and as free floating cells in fluid fraction (Chen *et al*., 1998). In addition, distinct distribution of microbial communities forms among ruminal locations (Yang and Varga, 1989). The establishment of these microorganisms is an integral part of normal rumen function and the degradation of resistant plant fibre.

It is expected that to utilize plant fibre by rumen microorganisms for electricity production via biotransformation with efficacy would depend on: (i) the hydrolytic ability by fibre-associated microbial consortia; (ii) the catabolic rate on fibre fermentation products by liquid-associated microbial consortia; and (iii) electron-transmitting microbial consortia attached on the anode. All these individual metabolic sectors cannot be accomplished by any single microbe.

Work by Rismani-Yazdi *et al*. (2007) illustrates that the inoculum source and substrate type could affect composition of attached and suspended microbial communities enriched in rumen MFC. Similarly in other MFCs, substrate composition and electrochemical conditions have been shown to alter composition of MFC-adapted microbes (Jung and Regan, 2007; Reimers *et al*., 2007; Aelterman *et al*., 2008c; Chae *et al*., 2009). Therefore, it appears that microbial composition and function can vary due to differences in fibre substrate composition and changes in MFC environment.

How changes in microbial composition in MFCs affect power output is not yet clear (Back *et al*., 2004; Phung *et al*., 2004; Logan and Regan, 2006; Freguia *et al*., 2007). In the rumen, the existence of protozoa may affect the variations in ruminal pH, ORP and SCFA (Abe and Kumeno, 1973; Mathieu *et al*., 1996; Wang *et al*., 2012). Chen (2010) observed that, in the presence of protozoa, ruminal ORP was more negative, and higher maximal voltage output (595 versus 480 mV) from rumen MFCs fed Bermuda grass straw was recorded. Identification of changes in microbial consortia under various substrate and MFC conditions should help to understand microbial physiology and ecology in MFCs and increase efficiency of electrical output from fibre by MFCs.

Abiotic Factors Affecting Power Output of RMFCs

An MFC is a renewable energy device that converts energy available in organic compounds to electricity via the catalysation of microorganisms. Its power output has been greatly improved in recent years, but the maximum power is still several orders of magnitudes lower than that of chemical fuel cells (CFCs). At present, the development and application of MFCs are decided by factors such as bacteria, electrode material, transportation at proton exchange membrane, etc. These result in a necessity to elevate the power generation of the cell.

Electrode materials that can increase system efficiency and the electron transfer from bacteria to electrode should be helpful for elevating the power generation efficiency of MFCs. The anodes associated with microbial metabolism and the cathodes related to oxygen reduction reaction are often the limiting factors that affect power performance. A conductive film-modified anode material was demonstrated to have a beneficial impact on enhancing the power density of an MFC by increasing the surface area and the biocompatibility of the electrode substrate.

An RMFC is one type of microbial fuel cell. In principle, rumen bacteria are cultivated in an anode trough into which a cellulose source is introduced and there oxidization takes place. The electrons and protons generated during the oxidation process reach the cathode through wire and proton exchange membrane, respectively. Then, oxygen reduction on the cathode completes the power generation process and water synthesis.

In RMFC, the bacteria in the anode transfer electrons by respiration or fermentation. There are two types of electron transfer model between microorganism and anode. One is that an electron transfers from cell membrane to anode directly without any electron mediator as shown in Fig. 5.1. In this case, the *Rhodoferax ferrireducens* and *Geobacter sulfurreducens* are the typical two. They can reduce the dissimilatory metal under an anaerobic environment. The electron transfers from cell membrane to anode by direct attaching of microorganism on the metal oxide surface (Lovley *et al*., 2004).

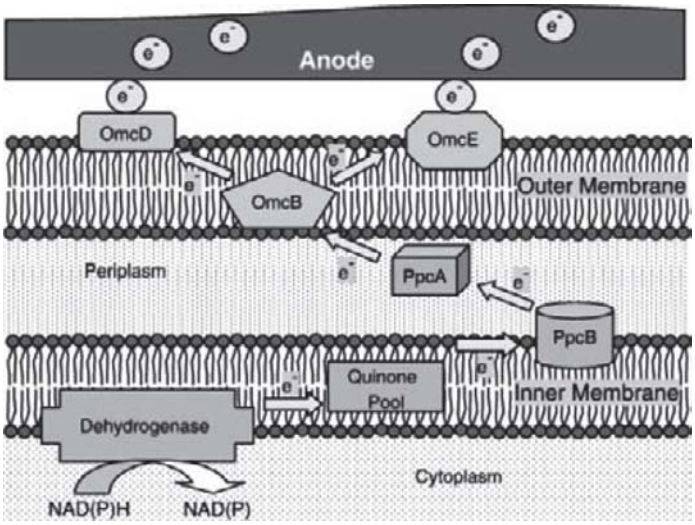

Fig. 5.1. Illustration of electron transfer from microorganism (*Geobacter* species) directly to anode without any electron mediator (Lovley *et al*., 2004).

Another type of electron transfer is shown as Fig. 5.2 (Lovley *et al*., 1996), with the electron transfer occurring at a biofilm formed by the bacteria on the anode surface. The attachment of bacteria on the anode is achieved by producing a matrix in the biofilm. The matrix is composed of extracellular proteinase, sugar and cells, and is enriched with matter that can potentially transport electrons.

It has been proved that the matrix contains conductive nanowires, which can accelerate electron transport. In this case, *Actinobacillus succinogenes*, *E. coli*, *Proteus mirabilis*, etc. are the typical species, which need the electron mediator for achieving electron transfer. Power production in MFCs depends mainly upon factors such as bacteria, water-power retention time, organic charge, feeding matrix, electron transfer, inner resistance, electrode material, proton exchange membrane, etc.

Requirements for the electrode in MFCs

Lower power generation ability limits practical application of MFCs. Therefore, to elevate power generation is one goal of MFC development. The anode is the attachment substrate for bacteria in MFCs. It affects not only the amount of bacterial adsorption but

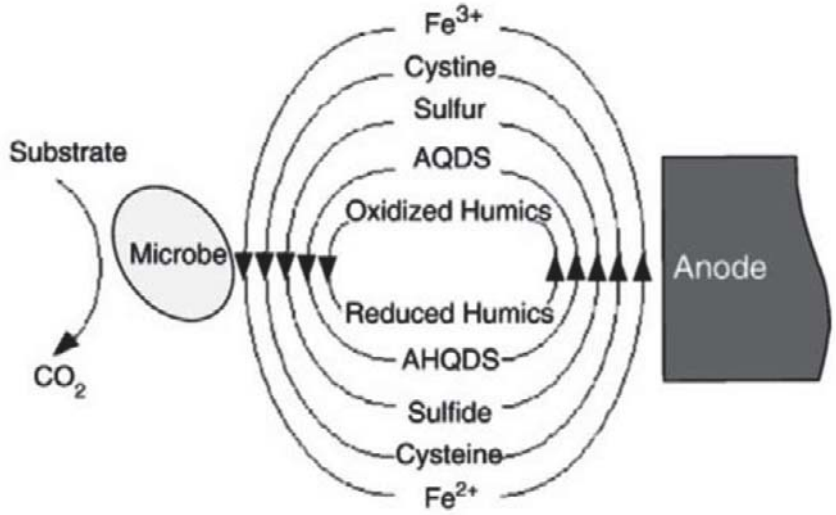

Fig. 5.2. Illustration of electron transfers from microorganism to anode through the electron mediator (biofilm) (Lovley *et al*., 1996).

also the direction of electron transfer from microorganism to anode (Rabaey and Verstraete, 2005; Du *et al*., 2007). Therefore, the anode plays an important role in the power generation of MFCs.

Previous studies and literature (Lee *et al*., 2003; Kim *et al*., 2004; Rabaey *et al*., 2004; Logan *et al*., 2006; Davis and Higson, 2007; Mohan *et al*., 2008; Chung and Okabe, 2009; Prathap *et al*., 2009) pointed out the three key factors of MFC performance: (i) total organic substrate (fuel); (ii) biomass accumulation on anode; and (iii) potential of biofilm-anode. For the third factor, if the biofilm on the anode plate is too thick, it does not favour transfer of electrons to the anode. If the biofilm is too thin, the bacteria of the film cannot quickly consume fuel to generate power (Lovley *et al*., 1996; Ieropoulos *et al*., 2005).

The reaction on the electrode is controlled by the electron transformation. Therefore, the material comprising the electrode is important to the reaction rate. For cathode material, the reduction rate of hydrogen ion to hydrogen varies with different materials (Kong *et al*., 2010). This is due to the different catalytic effects of the metals on hydrogen generation. For example, platinum and palladium are very effective catalysts for hydrogen generation. In addition, both anode and cathode with high electronic conductivity could enhance the reaction rate of MFCs and also the performance.

The anode of an MFC should be conductive, biocompatible and chemically stable. It may be metal or non-metal. A majority of non-metal anodes are made of close carbons or fibrous carbons. The former include graphite plate, graphite stick and graphite powder. The latter include carbon carpet, carbon cloth, carbon paper, carbon fibre and carbon cotton. Graphite has typically been the material of choice for the construction of anodes of MFCs. Other conductive materials may be preferable, either because they enhance electron transfer between the microorganisms and the anode material or because they are better adapted to specific applications. The high electron conductivity metal could be employed as the anode. Metal anodes include stainless steel, copper, alloy, etc. Although metal is a good conductor it is not bio-compatible and chemically stable; it should be under control when applied. How to prevent the oxidation of metal and the toxicity of metal is a key point.

Except for the nature of material, the electrode surface area is also another important factor of MFC performance. This is because the electrode reaction takes place at the interface between the electrode and liquid electrolyte, and the reaction rate is proportional to electrode surface area (Logan *et al*., 2007). Therefore, developing an electrode with high specific surface area is very important. Furthermore, producing anodes with nano and porous surface leads to the increasing of specific surface area and is useful for producing a thinner and large area biofilm on the anodes. Such a biofilm could accelerate the electron transfer. Meanwhile, higher power would be generated by the large amount of bacteria processing the fuel consumption.

Proton exchange membrane (PEM) has a great influence on the proton transfer efficiency of MFCs (Rabaey and Verstraete, 2005; Rozendal *et al*., 2006), and will affect the internal resistance and concentration polarization loss of cells (Logan *et al*., 2006). Nafion proton exchange membrane manufactured by Du Pont is more often used because of its high proton selective permeability (Du *et al*., 2007). In the research by Rozendal *et al*. (2006), the balance of positive ions by using Nafion proton exchange membrane was tested. The ratio of proton exchange membrane surface area to battery size has a great influence on cell performance. The larger contact area between proton exchange membrane and the electrode, the higher performance of the MFCs, so as to reduce the internal resistance of the MFC (Oh and Logan, 2007). In addition to the Nafion, Park *et al*. (2000) employed kaolin to produce porcelain septum to replace the Nafion proton exchange membrane. The porcelain septum installed in the MFCs with wastewater sediments can yield power density with 788 mW m^{-2}. The porcelain septum is cheaper than commercial film (Nafion), but it is not easy to make.

Electrode materials in MFCs

There are two sorts of common MFC anode materials. One is the original plate type, such as carbon paper, graphite, soft graphite, metal plate, etc. The other is the compound plate type, material powder mixed with binder and then compressed to form a plate, such as a carbon anode made from carbon powder by press-mould process with polytetrafluoroethene (PTFE) as the binder (Zhang *et al*., 2007). A high performance MFC anode should be easily attached by bacteria, transfer electrons easily from bacteria to anode, have a low inner resistance, have a high electronic conductivity and be voltage stable.

Among the commonly used materials at present, graphite plate and graphite stick are cheap and easily available, but their effects are not better than carbon carpet and any other fibrous materials. This can be attributed to the lower surface area of graphite plate and graphite stick than carbon carpet, which has a surface area of 0.47 m^2 g^{-1}. The order in surface area is carbon carpet > carbon cotton > graphite powder. The generated electric power is increased with the surface area of the anode. In general, an electrode coated with platinum or platinum black powder is better than one made of graphite granule, graphite carpet, carbon black, carbon cloth, etc.

In an MFC system with *E. coli* as the microorganism, a carbon cloth electrode coated with platinum black powder is better in electricity density than the one without coating. The former has an electricity density of 0.84 mA cm^{-2} and the latter has 0.02 mA cm^{-2}. However, platinum or platinum black powder are very expensive, and platinum is possibly toxic to bacteria (Trinh *et al*., 2009). It is necessary to seek methods that avoid toxic

substances produced by platinum. In recent years, it has been discovered that a layer of polyaniline (conduction layer) coated on platinum is able to avoid the production of toxic substances on platinum and retain the activity of platinum, and cause no interference in the growth of bacteria. Compare with the platinum-coated carbon cloth, coating of polyaniline on a platinum carbon cloth electrode can elevate the electricity density to 1.45 mA cm^{-2}. Due to the better catalysis ability of platinum for oxygen ionization, it can accelerate the reduction of oxygen on the cathode (Jang *et al*., 2004; Oh *et al*., 2004; Moon *et al*., 2006).

Gold is a potentially attractive anode material for some MFC applications because it is highly conductive and because gold provides a high degree of versatility for electrode manufacture. However, a previous study (Richter *et al*., 2008) suggested that bare gold is a poor electrode material for the anode of MFCs. Current production with gold electrodes was low and increased 100-fold when the gold surface was coated with a surface-associated monolayer (SAM) of 11-mercapto-undecanoic acid (Crittenden *et al*., 2006), even though the SAM would be expected to have insulating properties. These results indicate that the gold surface was either toxic to the cells or otherwise poorly suited to interact with electron-transfer cell components. Redox-active proteins, such as cytochromes, may adsorb strongly to gold, resulting in denaturation and loss of their electron-transfer capabilities.

There are also other research findings related to MFC anodes. Rosenbaum *et al*. (2007) studied WC-Nafion/graphite anode. They showed that the tungsten carbide displayed electric catalysis. WC can efficiently oxidize the products from fermentation, such as chlorine, formates, lactates, etc. This can enhance the electric generation ability of MFC. Electrode resistance is also one factor for power generation. To increase the power generation, experimental catalytic anodes were made of low inner resistance metals or metal oxides, which were dispersed in carbon or conductive polymer substrate (Maksimov *et al*., 1998; Gloguen *et al*., 1999). Lowy *et al*. (2006) made graphite anode via Fe_3O_4, Fe_3O_4 and Ni^{2+}, and graphite-ceramic anode via Mn^{2+} and Ni^{2+} in a similar way. The power density of MFCs were 1.7~2.2 times that without modification.

Carbon nanotube (CNT) has specific pore structure, high mechanic strength, toughness, large specific surface area, high thermostability, chemical inertness and high electric conductivity. The electrons in the surface are highly reactive. Electrons can migrate easily between the CNT and the surrounding matter. CNT is becoming an ideal material for electrodes. Qiao *et al*. (2007) prepared an anode by the mixture of CNT and polyaniline, and the maximal output of MFC was 42 mW m^{-2}. This indicates that addition of CNT can elevate power generation of MFCs. Morozan *et al*. (2007) pointed out that an anode with CNT displayed good biocompatibility.

The charge in the surface of the electrode is increased by pretreating the electrode with high-temperature ammonia. This speeds attachment of bacteria and increases the attached bacteria number. Electron transfer between bacteria and electrode is therefore elevated. Cheng and Logan (2007) prepared an anode by using amine-treated carbon cloth. The anode surface charge density increased and power generation elevated to near 2000 mW m^{-2}. The power generation was 300 mW m^{-2}, more than MFC using normal carbon cloth as the anode. Logan *et al*. (2007) achieved 2400 mW m^{-2} of power density by treating a graphite anode with ammonia. With regard to the effects of electrode area on power generation rate, Oh *et al*. (2004) proved that power density would be elevated 22% by increasing cathode surface area three times more. In addition, an increase of one-third of

cathode surface area increased the voltage by 11%. This indicates that increasing the cathode surface area will increase the power density of the MFC.

In summary, for the electric conductivity of electrode materials, an anode or cathode that has a high electric conductivity can elevate reaction rate in MFCs. Therefore, a material with a high electric conductivity could be selected as anode and the cathode material should have good catalytic capacity in hydrogen generation. It is certainly important to develop metals with high current collecting property and that are anticorrosive for the long-term durability of MFCs. For the surface area of the electrode, because electrode reaction takes place at the interface between electrode and solution, reaction rate is proportional to the electrode surface area. Therefore, developing electrodes that have high specific surface areas are very important. On the other hand, too thick biofilms are not good for electron transfer, and they are short of bacteria for consuming power to generate power when the biofilms are too thin. This problem may be solved by employing anodes with high specific surface area that are achieved through the nano and porous surface modification.

Cell configuration and operational condition

The design and operation have been shown to affect redox potential (Bretschger *et al*., 2010), metabolic activities required for microbial growth (Cheng *et al*., 2008) and the performance of MFCs (Liu *et al*., 2008). Several considerations are discussed pertaining to rumen MFCs.

Mixing mechanism: flow-field plate design

The contents in the rumen are churned continuously to be pushed forward and against by ruminal contractions. Such action results in three-dimensional mixing to facilitate contact between microorganisms and feed substrate and rumen wall, and minimize fermentation products from building up elsewhere in the rumen. For example, as stated above, the accumulation of SCFA can be toxic to rumen microorganisms (Mohan *et al*., 2007; Jeong *et al*., 2008). Adopting designs to mimic ruminal mixing is expected to play a vital role for maintaining a functional MFC. This has particularly been the case for dual-chamber MFCs, in which precipitation of microbes and floating of insoluble substrates could affect the performance of the MFC.

Because mixing *in situ* may not be applicable, a flow-field plate has been shown to distribute reaction fluid more evenly (Wang, C.T. *et al*., 2009, 2011). Chen (2010) coupled flow-field plates to single-chamber MFCs with mixed ruminal microorganisms. The flow-field plates were designed bionically to create various liquid flow patterns at various Reynolds numbers before entering the MFC. Such operation resulted in different cell performance, with improvement by 16% over previous flow-field plates (Dicks, 2006). Improving the efficiency of fluid mixing presumably could reduce concentration polarization in MFCs. Application of flow-field on electrical property was conducted on other occasions (Min and Logan, 2004; Ter Heijne *et al*., 2006), but the efficiency was not clear.

Use of oxidizers in cathode solution

Oxidizers, such as $K_3(Fe(CN)_6)$, applied to the cathode chamber can increase the cell power output (Logan *et al*., 2005; Rezaei *et al*., 2008) and decrease the cell internal resistance (Rabaey *et al*., 2003, 2007; Logan *et al*., 2005, 2007). If oxidizer concentration is sufficiently high, the replacement interval can be extended (Logan *et al*., 2005; Jia *et al*., 2008), though it can be toxic (Jia *et al*., 2008).

As mentioned earlier, the ORP of ruminal fluid is already very negative. Selection of proper oxidizers for the cathode to run the cell system smoothly can influence power output. Chen (2010) compared $KMnO_4$ with $K_3(Fe(CN)_6)$ as oxidizers in cathode solution for MFCs with rumen microbes. The performance was better with $KMnO_4$ (746.8 mW m^{-2}) than with $K_3(Fe(CN)_6)$ (352.3mW m^{-2}). In this work, when $KMnO_4$ was used as the catholyte, two cells of RMFCs were stacked and able to light an LED (Chen, 2010). Such difference could be explained by differentials in redox potential gradient present in MFCs (Rabaey and Verstraete, 2005; Fig. 5.3).

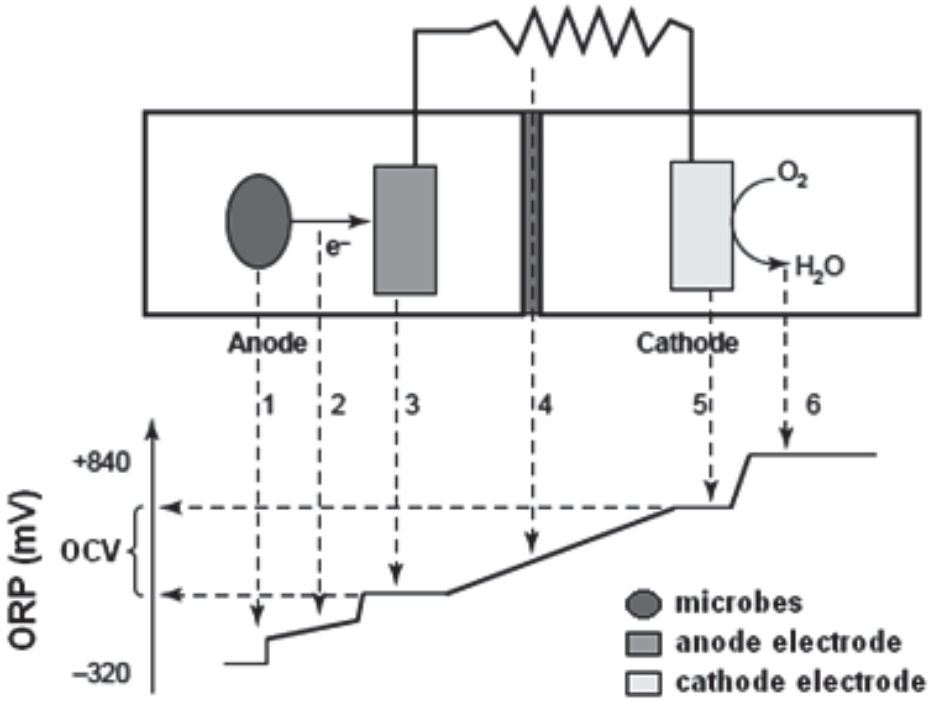

Fig. 5.3. Potential losses during electron transfer in a MFC. 1: Loss owing to bacterial electron transfer. 2: Losses owing to electrolyte resistance. 3: Losses at the anode. 4: Losses at the MFC resistance (useful potential difference) and membrane resistance losses. 5: Losses at the cathode. 6: Losses owing to electron acceptor reduction. (Rabaey and Verstraete, 2005).

Circuit and current

Ishii *et al*. (2008a) indicated that, compared with an open circuit MFC, the high current density operation could reduce methane generation, while increasing the concentration of short-chain fatty acids, such as acetic acid or propionic acid. Similarly, methanogenesis occurs during rumen microbial fermentation, which consumes hydrogen at the expense of

SVFA formation (Van Soest, 1982). Decreasing methane, a highly reduced compound, would conserve electron and hydrogen ion availability for power generation in MFCs.

Research work has revealed that electron flow may correlate to microbial diversity in MFCs (Holmes *et al*., 2004; Reimers *et al*., 2007; Aelterman *et al*., 2008a, b, c; Cheng *et al*., 2008; Ishii *et al*., 2008a; Erable *et al*., 2009; Virdis *et al*., 2009; White *et al*., 2009). However, it remains unclear how current in the cell affects rumen microbes (Bretschger *et al*., 2010).

Current status with RMFCs

As shown in Table 5.1, both Rismani-Yazdi *et al*. (2007) and Chen (2010) utilized dual-chamber microbial fuel cells to study power output with rumen microorganisms. Insoluble but purified plant fibre was used as substrate by them, as opposed to native plant fibre substrate (Chen, 2010). Carbon plates were used as electrodes for both studies, but differed in reaction surface areas (0.0084 versus 0.02024 m^2). The cathode solution of $K_3(Fe(CN)_6)$ adopted was at different concentrations (0.05 versus 0.5 M). Two kinds of proton exchange membrane with different surface area were mounted, the Ultrex proton-exchange membrane (CMI-7000; 28.3 cm^2) and Nafion 117 (136.3 cm^2), respectively.

A better cell performance (66.2 versus 55 mW m^{-2}) was observed by Chen (2010) than by Rismani-Yazdi *et al*. (2007). Such difference could be due to the fact that Chen (2010) used an electrode with a larger reaction area, which would have allowed microbes to generate biofilm more easily. Biofilm plays an important role in assisting electron transfer from substrate on to the electrode plate (Chae *et al*., 2009). Also, a sufficiently large reaction surface area decreases the activation polarization in the cell (Logan *et al*., 2005).

Second, the proton exchange membrane used by Chen (2010) was also larger in reaction area. Although such an increase in area is not immune to microbial-derived biological blocking (Li *et al*., 2009), which may suppress cell performance, it can reduce the resistance of ions passing through the proton exchange membrane (Rismani-Yazdi *et al*., 2008) or lessen the Ohm polarization in the cell (Logan *et al*., 2005).

Third, a higher concentration of oxidizer in cathode solution as $K_3(Fe(CN)_6)$ was applied by Chen (2010). An adequately high concentration favours cell power output (Logan *et al*., 2005; Rezaei *et al*., 2008) and decreases the cell internal resistance (Rabaey *et al*., 2003, 2004; Logan *et al*., 2005).

Conclusions

Comparing general MFCs with RMFCs

Ruminal microorganisms have evolved to adapt in the rumen environment and actively break down plant fibre therein for growth and survival. Plants have also evolved, but toward resistance by microbial degradation. Ruminal degradation of plant fibre is a rather complex process. Therefore, the magnitude of degradation relies heavily on the amounts and the make-up of rumen microorganisms. Technologies have been developed to culture rumen microorganisms in simulated conditions outside the rumen. Methods to enhance fibre degradation by ruminal microorganisms *in vitro* with acetate-dominating type of fermentation pattern are well documented. Recent technologies have been applied to

manipulate rumen microbial fermentation to decrease methane (a highly reduced compound). Such changes in fermentation profiles should spare hydrogen with concurrent generation of reducing equivalents. If such conditions could be realized in MFCs, the generated reducing power should translate into an elevated electricity output.

Therefore the prerequisite for feasible transformation of plant fibre into power output by rumen microorganisms in MFCs requires sustained survival and growth of rumen microorganisms. Only after biotic and abiotic considerations relevant to rumen conditions have been taken into account for designing rumen MFCs should the potential merit of converting plant fibre into electricity be practical.

Future perspectives

At present, power output from fibre of plant origin by RMFCs is relatively low. Linking RMFCs to treatment of fibrous wastes would seem applicable at the initial stage of RMFC development. By simultaneously treating wastes and generating electricity, the cost of waste treatment could be spread out and the added value of RMFCs should be appreciated. When the capacity of RMFCs is fully amplified, an independent power production system from plant fibre can be established.

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