Chapter 10
Rumen Fluid Microbes for Bioelectricity Production: A Novel Approach

Deepika Jothinathan, Prabhakaran Mylsamy, and L. Benedict Bruno

10.1 Introduction

Microbial fuel cell (MFC) is a promising technology for the production of sustainable energy. Almost all wastewater and waste have been tried out as the raw material for the energy production under mesophilic conditions. In this chapter, power production of rumen microbes is being discussed. The utilization of slaughterhouse waste is compared to the other wastewater collected from industries. Slaughterhouse waste comprises of blood, skin, digestive contents, etc. Ruminants such as cow, sheep, camel, etc. have a four compartmental stomach comprising of rumen, omasum, abomasum, and reticulum. While slaughtering the ruminants, the rumen fluid is thrown away as a waste. Million tonnes of rumen fluid gets wasted in slaughter houses. The wastewater from slaughterhouse is heavy in pollution, and, therefore, it should not be allowed to mix with the municipal drain system without pretreatment meeting sewage standards as per the Bureau of Indian Standards (BIS).

In a large slaughterhouse per day, more than 200 large animals are slaughtered, and annually 40,000 animals are slaughtered approximately, which create a waste of 6–7 tonnes/day. To efficiently convert this waste into energy, microbial fuel cell can be employed.

One milliliter of rumen contains roughly 10–50 billion bacteria and 1 million protozoa, and certain yeast and anaerobic fungi also comprise the group. *Fibrobacter (Bacteroides) succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*,

D. Jothinathan (✉)
Department of Life Sciences, Central University of Tamil Nadu, Thiruvarur, 610005, India

P. Mylsamy
Post Graduate and Research Department of Botany, Pachaiyappa's College, Chennai, 600030, India

L. Benedict Bruno
Department of Environmental Sciences, Bharathiar University, Coimbatore, 641046, India

V. Sivasankar et al. (eds.), *Microbial Fuel Cell Technology for Bioelectricity*,
https://doi.org/10.1007/978-3-319-92904-0_10

Butyrivibrio fibrisolvens, *Streptococcus bovis*, *Succinimonas amylolytica*, *Selenomonas ruminantium*, *Succinivibrio dextrinosolvens*, *Lactobacillus sp.*, *Anaerovibrio lipolytica*, *Eubacterium ruminantium*, *Oxalobacter formigenes*, *Methanosarcina barkeri*, *Wolinella succinogenes*, *Megasphaera elsdenii*, etc. are the common bacteria found in the rumen. These bacteria are anaerobic and carbohydrate fermenters.

Microbes in rumen exist either to the rumen epithelium or feed and free floating cells in rumen fluid portion (Chen et al. 2008). These microorganisms help in the degradation of ingested plant material. As per Cheng and Costerton (1980), rumen was considered as an ecosystem for studying the microbial behavior that is adhered to the biological surfaces. In 2007, Rismani-yazdi et al demonstrated that rumen microbes in MFC depend on the inoculum source and size and substrate composition. Cellulolytic bacterium is the most active species involved in the digestion of plant cell walls due to its high cellulase and hemicellulase activity. It produces hydrogen acetate and succinate as end products. Electrochemically active microorganisms are also present in the rumen. Similarly in other MFC reports, the physical and chemical parameters affected the performance of the microbes in the system (Reimers et al. 2007). Chen (2010) observed that, in the presence of protozoa, ruminal redox potential was more negative and produced a higher maximal voltage output of 595 mV (Chen 2010).

This chapter gives an insight of the power production by rumen microbes in MFC. The parameters that are favorable for the biofilm formation and electron transfer are tested. The bacterial strains isolated were checked for their efficiency in bioelectricity generation, and also their electrochemical activity was tested using cyclic voltammetry (CV) and electron impedance spectroscopy (EIS) techniques.

10.1.1 Optimization of Parameters for the Increased Electricity Production by the Microbial Fuel Cell Using Rumen Fluid

(a) The first parameter was electrodes where copper and zinc electrodes gave a maximum of 840 mV and 0.820 mA. However, the voltage dropped after the 4th day drastically and reached 100 mV. Carbon electrodes produced a stable voltage and current of 540 mV and 0.510 mA, respectively. Graphite and stainless steel produced 300 mV and 0.420 mA and 90 mV and 0.320 mA, respectively. Aluminum produced a negligible amount of voltage and current. Since carbon electrodes produced a considerable power, it was used for the further experiments. In an earlier report, carbon paper used as anode produced 14.92 mA with sugar industry wastewater as the anolyte (Mathuriya and Sharma 2009). MFC with carbon cloth utilizing beer brewery wastewater produced 63 mW/cm^2 as reported by Feng et al. (2008). In another report, plain graphite plates which were used as anode in a dual-chambered MFC produced 271.5 mV which has confirmed that carbon is the best electrode material in MFC (Venkatamohan et al. 2008).

(b) The second parameter being the pH which plays a vital role in many biological experiments was selected ranging from 5.0 to 9.0 in the anode chamber, and the results showed that pH 7.0 gave a maximum voltage of 590 mV and 0.420 mA. When the rumen fluid pH is changed to acidic, the voltage and current production is increased. When it is alkaline, the voltage production was stable. Various studies have focused mainly on the pH of the medium in anode chamber. For instance, the anodic sludge of pH 6.0–6.8 gave a power density of 10.4 W/cm^3, and when the pH was increased to 7.55, the power output increased to 11.8 W/m^3 (Jiang et al. 2009). Beer brewery wastewater produced 10.92 mA of current at pH – 6.4; municipal wastewater produced 9.01 mA of current at pH – 7.6 (Mathuriya and Sharma 2009).

(c) Substrates are the source of the bacteria during the process of bioelectricity generation and hence were selected as the third parameter. A variety of substrates in the final concentration of 2 g which contributed for the oxidation process were used in anode chamber. Among them, spinach gave the maximum voltage and current production of 600 mV and 0.300 mA, respectively. Cabbage peel produced 410 mV and 0.20 mA, respectively. All other substrates except paddy straw gave less amount of electricity. These results show that cheap substrates or agro-waste material can be used for the current production. In an earlier study, monosodium glutamate wastewater was used as a substrate in MFC inoculated with *Rhodoferax ferrireducens*, and it produced 0.18 V (Liu and Li (2007)). In another study, cheese whey was found to produce 29.1 W/m^2 (Kassongo and Togo 2011). Abattoir wastewater being an exceptional substrate used in a MFC produced 12.26 mW/cm^2 (Momoh and Neayor 2010). These reports support the finding implying that when an appropriate substrate is used based on the waste, a maximum power production can be achieved.

(d) Among the catholytes tested, acetic acid gave the maximum electricity production. Hydrogen peroxide produced the least current. Acetic acid gave the maximum voltage of 0.47 V and current of 0.05 mA. In the earlier reports, oxidizing agents like hexacyanoferrate (Rabaey et al. 2005) and acidic permanganate (You et al. 2006) have been used as catholytes in MFCs. The maximum power density of using a single-brush anode in a double-air cathode MFC was 154 ± 1 W/m^3, which is 108% more than the single-cathode MFC (Xiaoyuan Zhang et al. 2011). When calcium hypochlorite powder ($Ca(OCl)_2$) was used as a catholyte, it produced 12.26 and 20.71 mW/cm^2 for single- and dual-chambered MFC, respectively (Momoh and Neayor 2010). From our experimental results, the catholyte acetic acid produced 470 mV and 0.05 mA.

(e) Among the buffers ranging from pH 5 to 9 tested, acetate gave a maximum of 1.4 V and 0.140 mA. Phosphate buffer produced a maximum of 720 mV and 0.100 mA. Citrate phosphate buffer produced very less voltage among the buffers. From the previous literature, it is understood that phosphate-buffered saline of concentration 50/50 v/v was used for the efficiency (Cheng et al. 2009). A maximum power density of 1550 W/m^3 (2770 mW/m^2) was obtained and a current density of 0.99 mA/cm^2 using a pH 9 bicarbonate system. The power density was 38.6% higher when compared to the system using pH 7 phosphate buffer at the same concentration of 0.2 M (Fan et al. 2007).

Fig. 10.1 (**a**) Voltage production of MFC connected in series. (**b**) Current production of MFC connected in series

10.1.1.1 Scale-Up of MFC with Rumen Fluid

Upon analyzing the individual parameters in small MFC, scale-up of rumen fluid MFC has been demonstrated with 3 L plastic bottles. The working volume was 2.5 L in each MFC. When three individual MFCs, namely, MFC 1, MFC II, and MFC III, were connected in series, it produced 2.05 V and 20 mA. When connected in parallel, they produced 0.73 V and 62 mA to the maximum. Figure 10.1 (a) gives the voltage and (b) current production of MFC connected in series. This denoted that to achieve a long-term voltage and current, parallel connection is favorable,

and, for high voltage, series connection of MFCs is favorable. Similarly in a report, two individual MFCs were stacked together either in series or in parallel. The MFCs stacked in series produced a working voltage of 1.22 V (Gurung et al. 2012). Likewise, Aelterman et al. (2006), connected six individual MFCs in series and parallel which enabled an increase of the voltage by 2.02 V and current 255 mA while retaining high power output. The OCV of 0.67 and 4.16 V was obtained when they were connected in parallel and series, respectively (Aelterman et al. 2006).

The individual microbial fuel cell in the stacked series was observed for the potential and current readings separately, and the results were interpreted. Here among the three MFCs, MFC I gave a maximum production of 0.86 V. Though MFC III gave an initial peak in voltage of 0.85 V, it gradually decreased to 0.6 V in the course of time period. On the other side, MFC I also gave a stable current of 0.24 V. However, MFC II had the maximum production of 0.32 mA on the 10th day. The same observation was observed by Aelterman et al. (2006) where he has reported that during the connection of the individual MFCs together, the voltage diverged due to the microbial limitations at increasing currents. It is well known that a series connection could improve the voltage while maintaining the current (Aelterman et al. 2006). In a recent article, four membrane-electrode assembly MFCs were checked both individually and in series connection. Individually they showed 0.68 ± 0.05 V which sharply increased to 2.06 ± 0.03 V when connected in series (Kim et al. 2013). MFC stacked with bipolar plates made up of carbon blocks has been tested for their performance. Five single cells connected in series produced a maximum voltage of 2.5 V indicating that the individual cells generated 0.5 V (Shin et al. 2006). Figure 10.2 (a) gives the voltage and (b) current generation connected in series and parallel.

10.1.2 Comparative Analysis of Power Production of Pure, Co-culture, and Mixed Culture in Microbial Fuel Cell

10.1.2.1 Bacterial Strains

Bacterial strains which were isolated from the biofilm were streaked by quadrant plate method to obtain pure cultures. The isolated strains were named as Strain 1, Strain 2, Strain 3, Strain 4, and Strain 5. After the colony morphology observation, the strains were screened for various biochemical tests to infer the genus of the organism. Based on the gram staining, it was identified that Strains 1 and 3 are gram-positive rods, Strain 2 is a gram-negative coccobacillus, Strain 4 is a gram-negative rod, and Strain 5 is a gram-negative rod to ovoid. Based on the hanging drop technique, it was found that all the bacterial strains except the Strain 1 were motile confirming the presence of flagella or pili. This kind of projections is helpful for the electron transfer to the anode surface (Gorby et al. 2006). Based on the biochemical tests and 16s rRNA sequencing, the strains were identified as *Pseudomonas aeruginosa* DMR-3, *Bacillus tequilensis* DMR-5, *Bacillus thuringiensis* DRR-1, *Pseudomonas fragi* DRR-2, and *Paracoccus homiensis* DRR-3.

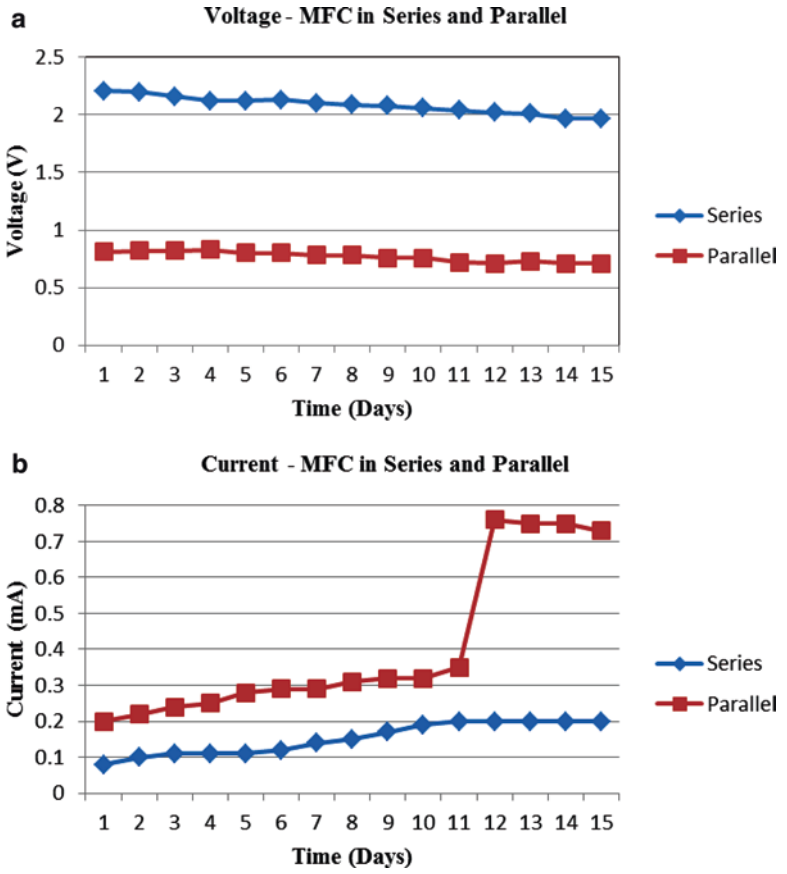

Fig. 10.2 (**a**) Voltage of MFC connected in series and parallel. (**b**) Current of MFC connected in series and parallel

10.1.2.2 Brief Pure Culture Study in Terms of Voltage Production and Cyclic Voltammogram

In this experiment, five cultures were inoculated as pure cultures in five separate MFCs. The readings were taken in multimeter for 12 days. Among the five cultures, *Paracoccus homiensis* and *Pseudomonas aeruginosa* produced the maximum voltage of 320 mV and 300 mV, respectively. *Bacillus thuringiensis* produced the least voltage of 150 mV. Likewise, *Paracoccus sp.* and *Pseudomonas sp.* gave the maximum current of 0.01 mA and 0.02 mA, respectively. Henceforth, *Paracoccus homiensis* was chosen for proton-exchange membrane study as a pure culture. Figure 10.3 (a) shows the potential and (b) current comparison between the five pure cultures.

Microbial fuel cell performance differs for each and every bacterium. *Saccharomyces cerevisae* and *Clostridium acetobutylicum* generated 10.89 mA and

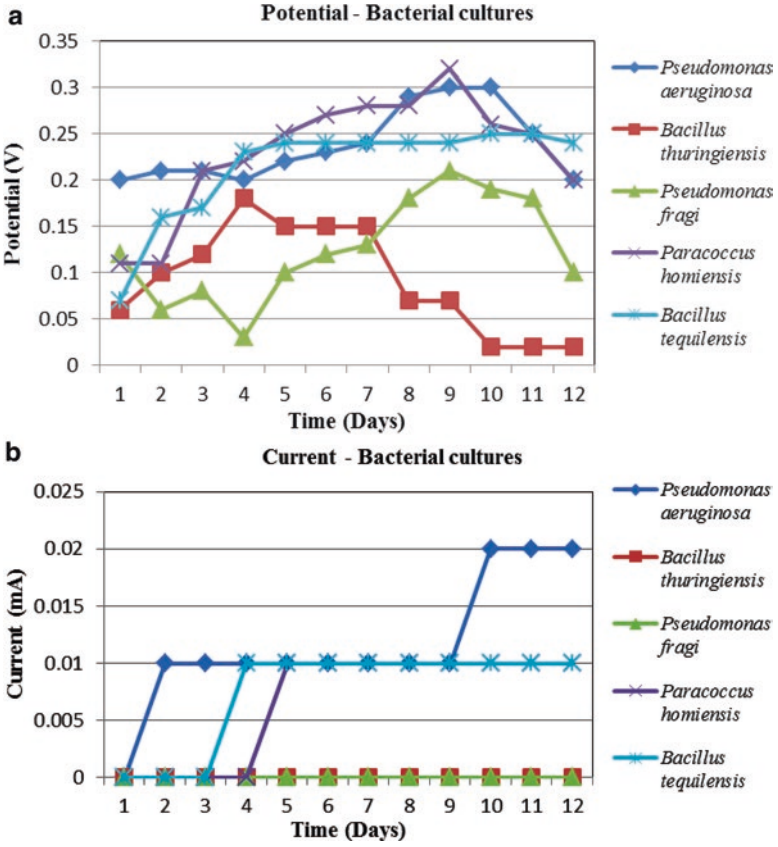

Fig. 10.3 (**a**) Potential of five pure bacterial strains. (**b**) Current of five pure bacterial strains

10.45 mA, respectively, after 10 days of operation (Mathuriya and Sharma 2009). On the other side, an aircathode MFC with *Enterobacter aerogenes* produced a maximum power density of 2.51 W/m^3 where no mediators were used (Zhuang et al. 2011). *Geobacter sulfurreducens* and *Geobacter metallireducens* exhibited lower current densities of 110 ± 7 A/m^3 (Call et al. 2009). *Shewanella oneidensis* DSP10 grown in medium with lactate exhibited 24 mW/m^2 for reticulated vitreous carbon, and once external mediators were used, the current and power increased by 30–100% (Ringeisen et al. 2006). *Hansenula anomala* yielded 2.34 W/m^3 with graphite felt as the anode material in a deaerated suspension of nutrient broth in anodic chamber (Prasad et al. 2007).

The cyclic voltammogram is a characteristic feature which confirms the electrochemical activity of the biofilm or individual bacteria. Hence, this technique has been widely used for the studies involving microbial fuel cell. The redox potential in the anode compartment and also information about the direct electron transfer

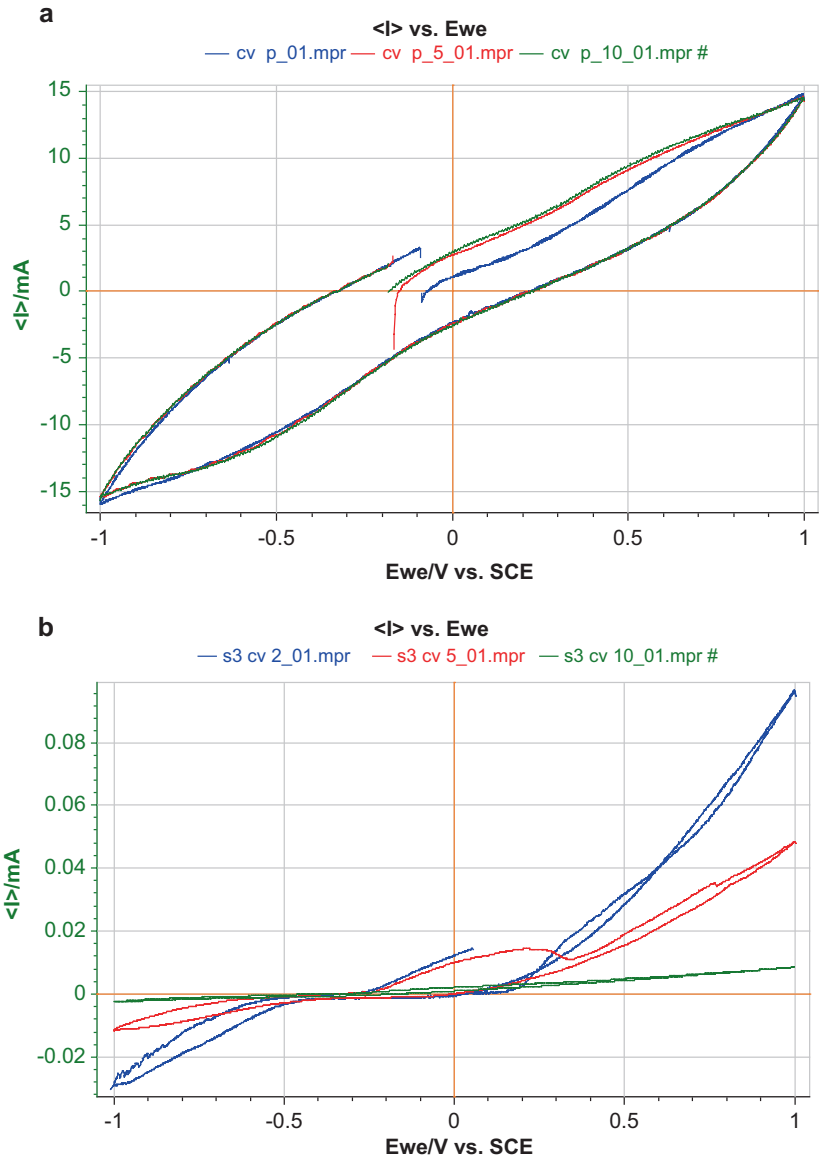

Fig. 10.4 (**a**) Cyclic voltammogram of *Pseudomonas aeruginosa* DMR-3. (**b**) Cyclic voltammogram of *Pseudomonas fragi* DRR-2. (**c**) Cyclic voltammogram of *Paracoccus homiensis* DRR-3

can be studied with the technique. For instance, the electrochemical activity of two enzymes has been demonstrated in a study where *Hansenula anomala* produced less peak currents when lactate has been added (Prasad et al. 2007). Figure 10.4a–c represents the cyclic voltammogram of *Pseudomonas aeruginosa*, *P. fragi*, and *Paracoccus homiensis*, respectively, showing prominent redox peaks which confirm

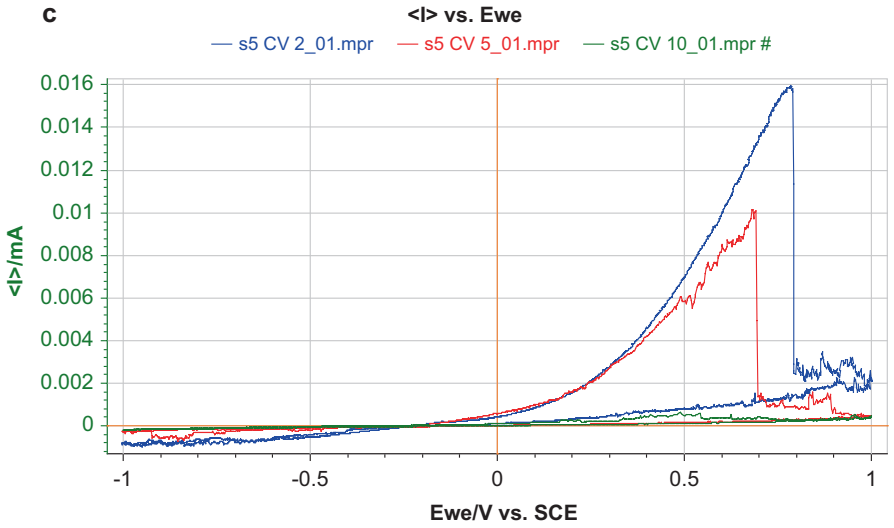

Fig. 4 (continued)

the electricity production in the voltage–current experiments ($V \times I$). *P. aeruginosa* showed an oxidation peak at −0.398 V and reduction peak at 0.587 V. *Pseudomonas fragi* showed a mild oxidation peak at −0.71 V and a reduction peak at 0.20 V. *Paracoccus homiensis* showed a reduction peak at high voltage of 0.77 V. *Bacillus thuringiensis* and *Bacillus tequilensis* did not show peaks in the voltammogram. These two bacteria produced less voltage in the previous experiment in MFC.

10.1.2.3 Co-culture and Mixed Culture Studies

The anodic chamber of MFC was inoculated with 110×10^5 CFU/mL of *Bacillus tequilensis*, 70×10^5 CFU/mL of *Pseudomonas aeruginosa*, and co-culture of *Bacillus tequilensis* and *Pseudomonas aeruginosa* (110×10^5 CFU/mL and 70×10^5 CFU/mL, respectively) in three separate MFCs on the same day. When inoculated as pure culture, *Pseudomonas aeruginosa* showed a maximum of 310 mV and 0.020 mA. *Bacillus tequilensis* produced a maximum of 250 mV and 0.010 mA. The co-culture of *Bacillus tequilensis* and *Pseudomonas aeruginosa* has shown a maximum of 450 mV and 0.040 mA. From the above results, it is evident that the co-culture produced high power density than the pure cultures.

In addition to microorganisms that can transfer electrons to the anode, the presence of other organisms appears to benefit MFC performance. It is reported that a mixed culture generates a current that was sixfold higher than a pure culture (Park and Zeikus (2002)). The anodic chamber was inoculated with 120×10^5 CFU/mL of *Paracoccus homiensis* and 100×10^5 CFU/mL of *Bacillus thuringiensis* and a co-

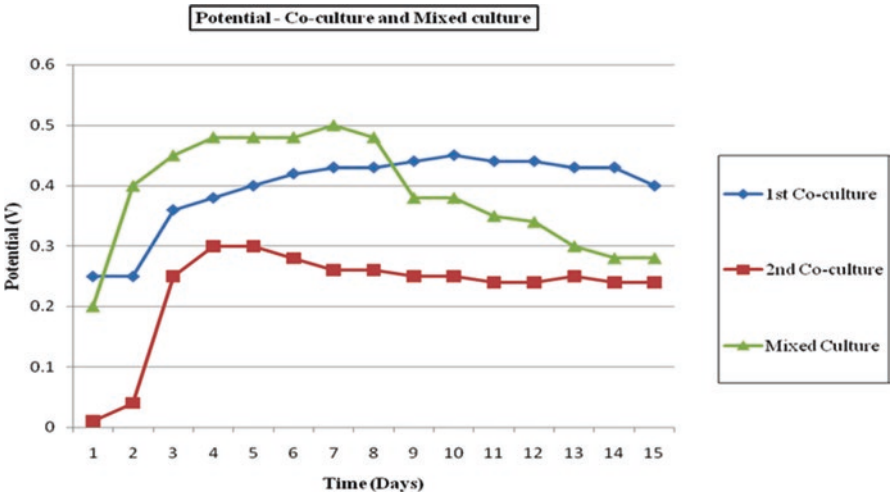

Fig. 10.5 Potential comparison of co-culture and mixed culture

culture of these bacteria in three separate MFCs. When *Bacillus thuringiensis* was tested as pure culture, it produced a maximum of 180 mV with no current, and *Paracoccus homiensis* produced a maximum of 300 mV and 0.010 mA. However, when the two bacteria were inoculated in the MFC, it produced 300 mV and 0.100 mA. Comparatively, the combination of the two cultures gave the maximum voltage and current. However, there is noticeable change in the current from 0.010 to 0.100 mA in co-culture. This shows that the pure cultures react on their own way, and when combined there might be some mechanism existing between the cultures which is the reason for the increase in current.

This experiment reveals the potential comparison between co-culture and mixed culture. The first co-culture is a combination of *Pseudomonas aeruginosa* and *Bacillus tequilensis*. The second co-culture is a combination of *Paracoccus homiensis* and *Bacillus thuringiensis*. Mixed culture is a combination of all the five bacterial strains used in this study. Figure 10.5 shows the comparison of potential between the cultures. Among the two different sets of co-culture, the first co-culture produced the maximum voltage of 450 mV. The second set of co-culture produced a maximum voltage of 300 mV. But compared to this, the mixed culture with five bacterial strains produced a maximum of 500 mV. Thus it is evident from the experiments that bacterial cultures in mixed form produce maximum power.

10.1.2.4 SEM Analysis

The anode subjected to scanning electron microscope analysis shows the biofilm formation attached on the surface of the electrode. Figure 10.6a shows the plain carbon sheet (control), and Fig. 10.6b shows bacteria (*Paracoccus homiensis*)

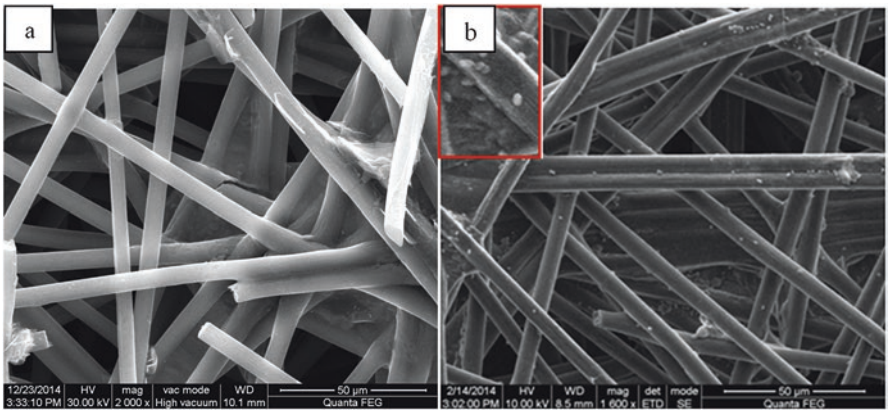

Fig. 10.6 (**a**) Scanning electron micrograph of the carbon sheet (control) (**b**) *Paracoccus homiensis* growth

adhering to the surface of the carbon sheet. From this image, it was found that biofilm was spreaded on the carbon sheet which facilitates bioelectricity production. A thick biofilm of *Aeromonas hydrophila* PA3 on the anode surface with uniform cells was observed through SEM. It has been reported that the biofilm has contributed to the maximum current (Pham et al. 2003).

10.1.2.5 Production of Bioelectricity in MFC by *Pseudomonas fragi* DRR-2 (Psychrophilic) Isolated from Goat Rumen Fluid

Over the period of time, MFC has been examined at ambient temperature with different microbes. There are many bacteria isolated from different places other than rumen such as *Rhodoferax ferrireducens* (Chaudhuri and Lovley 2003), *Shewanella putrefaciens* (Kim et al. 2002), *Geobacter sulfurreducens* (Bond and Lovley 2003), and *Desulfobulbus propionicus* (Holmes et al. 2004) which have been reported for power production in MFC. A recent study has focused on bioelectricity production from *Geopsychrobacter electrodiphilus* gen. nov., sp. nov., a psychrotolerant bacteria which can grow between 4 and 30 °C with an optimum temperature of 22 °C (Holmes et al. 2004). *Rhodoferax ferrireducens* is capable of transferring electrons to electrodes at 4 °C in a mediatorless microbial fuel cell (Chaudhuri and Lovley 2003). Previous studies show that mesophilic bacteria show higher growth rate, higher electron transfer, shorter lag phase, and lower respiration which are not found in low-temperature-adapted microbes (Hall et al. 2010). There are many cold-adapted microorganisms (psychrophilic) present in our environment which need to be explored for MFC research. The purpose of this study was to investigate bioelectricity generation by *Pseudomonas fragi*, psychrophilic bacterium growing in low temperature, so that they can be used in places of cold region. Based on the experimental results, it can be concluded that the bacteria showed higher growth rate,

higher electron transfer, and shorter lag phase when subjected to low temperature. This is the first report on *Pseudomonas fragi* for the production of bioelectricity at low temperature.

10.1.2.6 Growth Curve and Protein Content of *Pseudomonas fragi* DRR-2 at Different Temperatures

The bacterial growth was measured every 24 h for a period of 15 days at different temperatures. The maximum growth was observed on the 6th day at 20 °C. The protein content was maximum on the 6th day at 10 °C, whereas for other temperature bacteria showed less content. This confirms that the optimum temperature for growth is 20 °C and the bacteria has the ability to grow in low temperatures (>4 °C). At all the temperatures, the total protein was observed to be highest between the 6th day and 10th day.

Under high-nutrient conditions, bacteria tend to alter their membrane lipid composition to adapt to the changing temperatures (van de Vossenberg et al. 1999), by the method known as homeoviscous adaptation (Sinensky 1974). According to Hall et al. (2010) report, at low temperatures, membranes can be highly firm and prevent the efficient function of transmembrane proteins, important for resource utilization. This is due to the membrane fluidity which plays a main role in the proton-motive force. However, in bacteria the cellular membrane is also used to create an electrochemical gradient, which makes the synthesis of ATP as protons move down the proton gradient into the cell. As membrane lipids play a main role in maintaining the membrane fluidity, it has been observed that the organisms dominated in cold environments are rich in MUFA or branched-chain fatty acids, while organisms in warmer environments have saturated fatty acids (SAFA) (Kaneda 1991). Mesophilic bacteria show higher growth rate, higher electron transfer, shorter lag phase, and lower respiration which are not found in low-temperature-adapted microbes (Hall et al. 2010). Our experimental results confirm that *Pseudomonas fragi* (psychrophilic) shows higher activity at low temperature (10 °C) where the protein concentration was found to be maximum.

Power Production of the Bacterium Under Different Temperatures Using Salt Bridge and Nafion 117

The bacterium produced a maximum voltage of 540 mV on the 10th day at 20 °C indicating that the favorable temperature for the growth gave the maximum electricity production. The maximum current was only 0.020 mA since salt bridge was used as the proton exchanger due to higher internal resistance. Compared to the room temperature, the bacteria produced more voltage in low temperatures.

A maximum voltage of 380 mV on the 10th day at 20 °C and a maximum current of 0.070 mA on the 7th day at 4 °C confirm that the bacteria are active at low temperatures between 4 and 20 °C. When compared to the salt bridge, Nafion 117 mem-

brane gave a maximum current, indicating that the internal resistance of the fuel cell is decreased thereby improving the cell performance. *Geopsychrobacter electrodiphilus* produced a maximum current of 3.73 mA/cm^2 when acetate was provided as the electron donor (Holmes et al. 2004). Rumen microbes when they grow in low temperatures tend to produce less methane comparatively to the mesophilic conditions (Graham et al. 1959; Kennedy and Milligan 1978). Based on the above information, we prove that the isolated strain might have produced less methane and more hydrogen for the electron and proton transfer. This may be the reason for the increased bioelectricity production of *P. fragi* at low temperatures. Figure 10.7a and b represents the potential and current production of *P. fragi* at low temperatures with Nafion membrane.

Cyclic Voltammogram of the Strain in Low Temperatures

The cyclic voltammograms of the anodic biofilm clearly give an anodic potential and cathodic potential. This confirms that the bacteria grown in low temperatures exhibit a sigmoidal curve indicating that they are electrochemically active in nature. Figure 10.8 shows the voltammogram of the anodic biofilm at 20 °C. From the voltammograms, it has been observed that at 4 °C, a sharp oxidation peak at 0.04 V was found indicating the maximum substrate utilization of the microbe has taken place at low temperature. At the same time, a reduction peak at −0.2 V reveals that there the electron transfer has taken place. However, the electron transfer was found to be maximum at 20 °C, and the corresponding voltammogram confirms it with three reduction peaks in the reverse scan at −0.14, −0.8, and −0.6 V.

10.1.3 Performance of Paracoccus homiensis *DRR-3 in Microbial Fuel Cell with Membranes*

10.1.3.1 Power Production of *Paracoccus homiensis* DRR-3 with Nafion 117 in MFC

This research also focuses on to find an alternative membrane to the commercially available Nafion 117. Henceforth, Nafion 117 was tested for its efficiency in the 300 mL acrylic chamber which has a membrane holder. The other membranes which were tested are polyvinylidene difluoride (PVDF) and polycarbazole (PCZ) which are conductive in nature. This was the reason to choose them for the experiments.

Initially Nafion 117 was tested with three types of electrodes, namely, carbon cloth, carbon sheet, and graphite plate. The carbon paper produced the maximum potential and current with 0.8 V and 0.13 mA. The carbon cloth produced a maximum of 0.54 V and 0.7 mA, whereas graphite plate showed the least output of 0.24 V and 0.1 mA. This is due to the smooth surface of the graphite plate which did

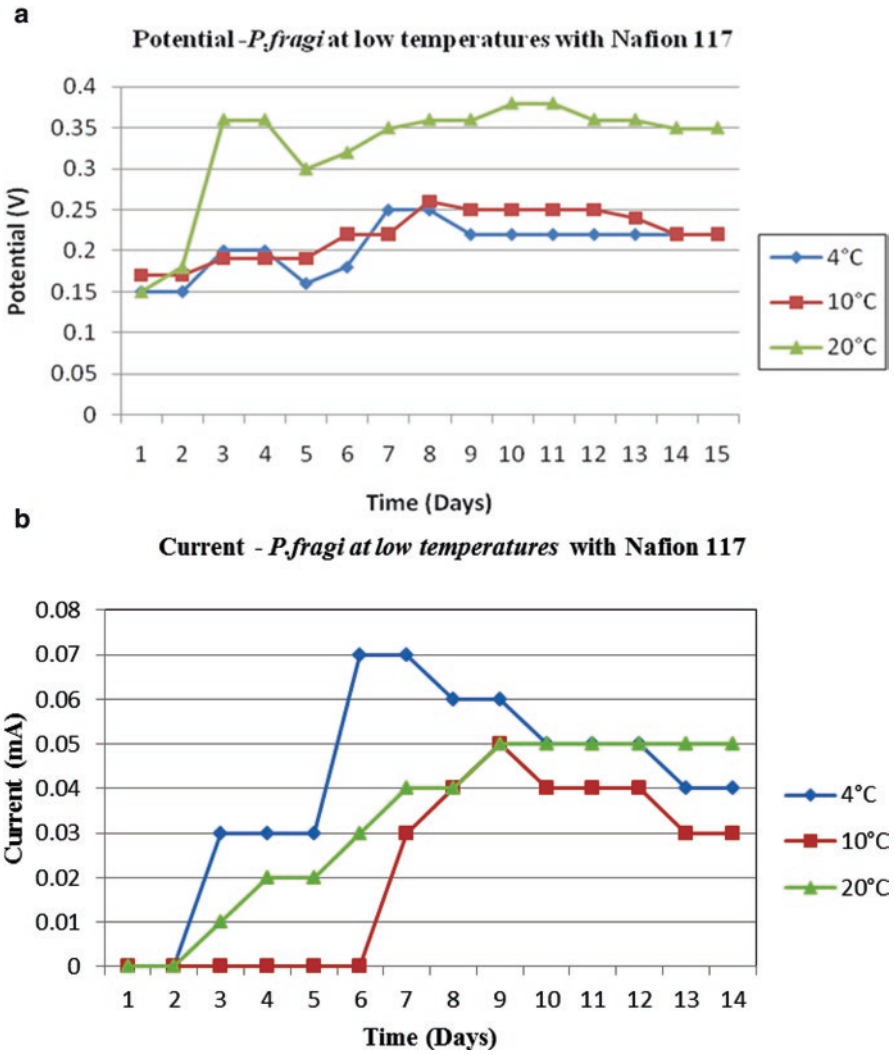

Fig. 10.7 (**a**) Potential curve of *P. fragi* at low temperatures using Nafion membrane. (**b**) Current curve of *P. fragi* at low temperatures using Nafion membrane

not help the bacterium to colonize the surface which is contradicting to the observation carried out by Junqiu Jiang where he observed the MFC yielding a maximum voltage of 0.687 V with a graphite fiber brush anode (Jiang et al. 2009). In a previous report, a modified CNT/PANI (carbon nanotube/polyaniline) increased the MFC performance with 1.18 V and current of 12.8 mA (Wang et al. 2013). In a MFC utilizing corn stover biomass, plain carbon paper was used as anode, and the cathode was made up of carbon cloth containing Pt catalyst. Reactors fed with the sample produced 437 mV (390 mW/m^2) (Wang et al. 2009). Carbon cloth of pro-

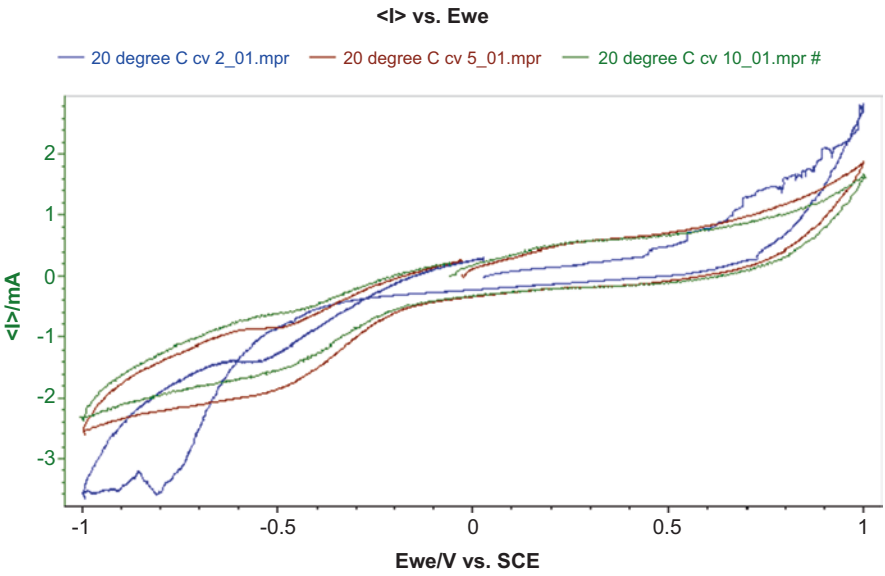

Fig. 10.8 Cyclic voltammogram Cyclic Voltammogram analyses of MFC inoculated with of *P. fragi* at 20°C with scan rates 1mV/s, 5mV/s & 10mV/s

jected surface area 7 cm^2 used in a MFC employing biodiesel waste as the organic matter produced a maximum of 450–500 mV (Yujie et al. 2011). These results confirm that carbon paper and modified carbon electrodes strongly play a main role in electron transfer when compared to other electrode materials.

10.1.3.2 Power Production of *Paracoccus homiensis* DRR-3 with PVDF and PCZ in MFC

Since carbon paper showed a maximum power production, it was used for the further experiments. *Paracoccus homiensis* produced a maximum of 0.64 V on the 8th day with the PVDF membrane as a proton exchanger. The voltage then gradually decreased to 0.37 V on the 17th day. Though PVDF could not achieve a high voltage as Nafion membrane (0.80 V), it produced a significant amount of power. However, PVDF produced a maximum of 0.16 A which is higher than that of Nafion membrane. Similarly, in a report polyether ether ketone was sulfonated and used as a proton-exchange membrane in a single-chamber MFC. *Escherichia coli* produced a maximum of 670 mW/cm^2 with SPEEK membrane, whereas Nafion 117 produced 300 ± 7 mW/cm^2 (Ayyaru and Dharmalingam 2011). This experiment has given us a hint that PVDF membrane might be a good alternative for Nafion in future in the field of microbial fuel cell.

Paracoccus homiensis gave a maximum voltage of 0.46 V on the 4th day which gradually decreased to 0.15 V on the 15th day. The maximum current production

was 0.10 mA on the 9th day which gradually declined to zero. This membrane seems to produce less voltage when compared to the commercial Nafion and PVDF. However, it was taken into account for the further experiments to check the efficacy in terms of power production.

10.1.4 Membranes, Their Performance, Electrochemical Analysis in MFC

10.1.4.1 Cyclic Voltammogram of *P. homiensis* Using Membranes

Paracoccus homiensis in the presence of Nafion membrane has given the cyclic voltammogram with two oxidations peaks at −0.57 V and 0.37 V, respectively, and one reduction peak at 0.07 V. Similar kind of results were observed in *Shewanella oneidensis* MR-1 in the presence of buffer and lactate as anolyte showing a reduction peak at −500 mV (−0.5 V) with Nafion 424, DuPont membrane. An oxidation peak was observed at the potential of 200 mV (0.2 V) which is comparable to the present study (Manohar et al. 2008).

Paracoccus homiensis in the presence of PVDF membrane showed two reduction peaks at −0.59 V and 0.49 V, respectively, and an oxidation peak at 0.42 V which indicates the transfer of electrons at the anode chamber has taken place. Likewise, *Shewanella putrefaciens* used as an EAB (electrochemically active bacteria) showed a characteristic reduction peak at – 250 mV (i.e., −0.25 V), and in the anodic scan, it showed an oxidation peak at 0.09 V (Khilari et al. 2015).

The reduction peaks observed in the voltammogram in the presence of PCZ membrane signify the reducing activity of *Paracoccus homiensis.* Two oxidation peaks at 0.127 and 0.36 V were found in the anodic scan which indicates the oxidation of substrate by the bacterium. A reduction peak was observed at −0.37 V which confirms that the bacterium is electrochemically active and the membrane which has been used in the MFC is transferring electrons at a good rate. To summarize the membrane study, all the three membranes were working quite efficient in terms of electron transfer. However, to further elucidate a better performance, the internal resistance and conductivity should be taken into account.

10.1.4.2 Impedance Spectra of *P. homiensis* Using Membranes

EIS was used to measure the internal resistance in the MFC before and during the course of reaction. The results were plotted as Nyquist curves and further fitted with an equivalent circuit. EIS curves usually consist of well-defined semicircle followed by a straight line. The intercept of semicircle with the real impedance axis presents the total ohmic resistance (R_{ohm}) of the electrochemical cell including the solution resistance (R_s) and charge transfer resistance (R_{ct}) at the electrode-electrode interface (Dominguez-Benetton et al. 2012). The internal resistance had two major

components, namely, the ohmic and non-ohmic resistance (Logan et al. 2006). The resistance produced by electrolyte and electrode material during electron transfer is known as ohmic resistance, and this is caused due to faradic reactions (He et al. 2006). The non-ohmic resistance due to the electrochemical reaction which happens on the surface of the electrode mainly because of the microbial metabolism is called as charge transfer resistance (Khan and Iqbal 2005). The present experimental study of the impedance spectra of *P. homiensis* with Nafion membrane MFC gave a solution resistance of 9.202 Ω. The polarization resistance is 70.34 Ω, and the charge transfer resistance is found to be 61.138 Ω.

The impedance spectrum obtained for PVDF membrane has given the possible circuit which depicts that a layer of biofilm has formed over the surface of the electrode. The solution resistance contributed by this MFC is 23.61 Ω, and the polarization resistance is 68.66 Ω. Figure 10.9 represents the Nyquist plot and the circuit of

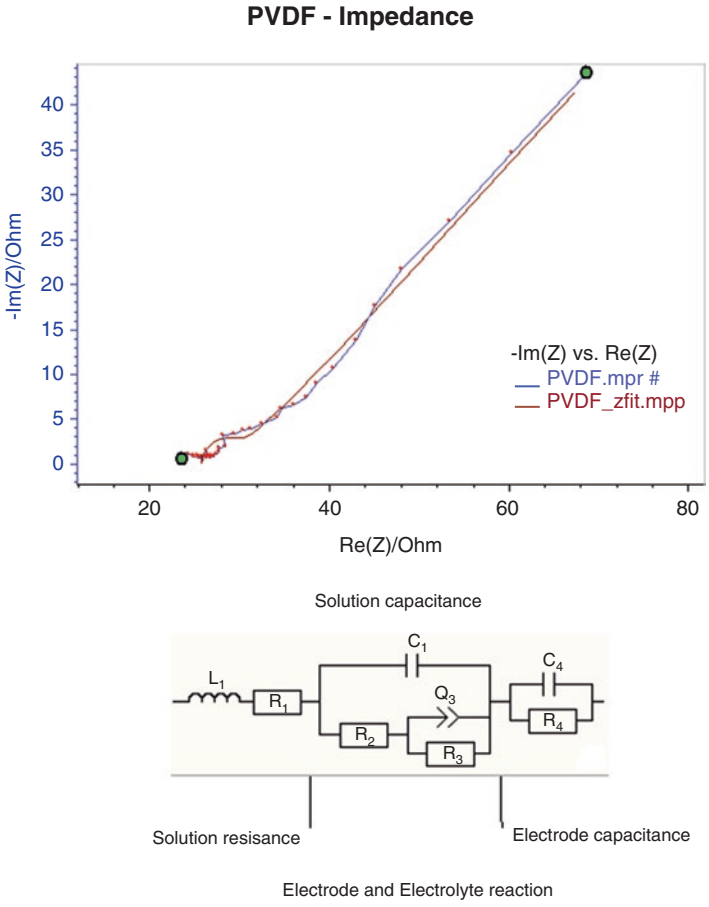

Fig. 10.9 Nyquist plot and equivalent circuit diagram of *Paracoccus homiensis* with PVDF membrane

MFC with *P. homiensis* employing PVDF membrane. Equivalent circuit modelling (ECM) was utilized to further explore the EIS results, specifically to determine the distribution of resistive and capacitive features in the operating MFC.

The solution resistance contributed in the MFC with the PCZ membrane is 23.45 Ω. The polarization resistance is 533.8 Ω. The charge transfer resistance is 510.35 Ω. The MFC performance using dairy waste with pure culture *E. coli* for 4 days operation was found to be maximum at low resistance (31.14 kΩ) with high conductivity as described by Patil et al. (2013). The measured ohmic resistance R_s for the SSFF-MFC, PANIche/SSFF-MFC, and PANIele/SSFF-MFC is 36.1 Ω, 36.5 Ω, and 32.7 Ω, respectively. The polarization resistance for the MFCs was 938.4 Ω, 279.1 Ω, and 215.6 Ω, respectively (Hou et al. 2015). From our EIS results, the PVDF membrane showed a better performance when compared to the others with a low resistance of 68.66 Ω.

10.1.5 Applications of Rumen Fluid MFC

Scale-up microbial fuel cell of four cells has been tested for various applications like glowing an 1.5 V LED, running a small fan, powering pocket calculator, powering digital wristwatch, and finally charging a mobile phone. The MFCs connected in series gave an output of 3.57 V and 60 mA. Figure 10.10 shows MFC powering

Fig. 10.10 Rumen fluid MFC glows a 1.5 V white LED

Fig. 10.11 MFC powering a pocket calculator. (**a**) Calculator soldered with the positive and negative ends of MFC. (**b**) Calculator getting powered by MFC

a 1.5 V white LED. Figure 10.11 shows MFC powering a calculator. In future, MFC can be used for various applications if they are worked in large scale.

10.2 Summary and Conclusion

MFC performance was primarly based on the reactor model, electrodes, organic matter, etc. Hence, various parameters such as electrodes, pH, substrates, catholytes, and buffers were tested to study the favorable conditions for the rumen MFC. The optimized parameters like carbon electrodes, pH 7.0, spinach, acetic

acid, and acetate buffer used in a single MFC gave better efficiency. The cyclic voltammogram of the anodic biofilm confirmed the electrochemical activity of the biofilm. Scale-up of rumen MFC was done both in series and parallel connection where series connection gave 2.05 V and 20 mA. In parallel it gave 0.73 V and 62 mA. Totally five bacterial strains isolated from the biofilm were identified by biochemical tests and 16srRNA sequencing. The phylogenetic tree was constructed to study the family structure. Among the bacterial strains, *Pseudomonas aeruginosa*, *Pseudomonas fragi*, and *Paracoccus homiensis* produced consistent power and showed electrochemical activity. From the co-culture study, it was understood that a bacterium with high electricity production and a bacterium with low production when combined together give a much higher amount of bioelectricity, thus enabling a weaker bacterium to work better. The cyclic voltammograms support the I–V graphs. A special bacterium *Pseudomonas fragi* was also tested under different temperatures. Only at 20 °C, the bacteria produced higher bioelectricity production. A mixed culture of all the five bacterial strains was also carried out to check the efficiency. Though mixed cultures give a large amount of power, study of the individual bacterium might help us in carrying out this research to the next step such as genetic modification, identifying the functional gene, etc. Among the membranes tested, PVDF produced a significant power and less internal resistance in par with the commercial Nafion membrane: R_{in} of PVDF −68.66 Ω and R_{in} of Nafion −70.34 Ω.

Acknowledgments Dr. J. Deepika is very much thankful to the Head of the Department of Chemistry SRM University for helping her with the instrumentation facility for the cyclic voltammogram and electron impedance spectroscopy experiments. She wishes to convey special thanks to Nanotechnology Research Centre, SRM University, for helping in the scanning electron microscope images. Dr. L. Benedict Bruno thankfully acknowledges the Science and Engineering Research Board, National Postdoctoral fellowship scheme (Grant No. PDF/2017/001074), for financial support.

References

Aelterman P, Rabaey K, Pham HT, Boon N, Verstraete W (2006) Continuous electricity generation at high voltages and currents using stacked microbial fuel cells. Environ Sci Technol 40: 3388–3394

Ayyaru S, Dharmalingam S (2011) Development of MFC using sulphonated polyether ether ketone (SPEEK) membrane for electricity generation from waste water. Bioresour Technol 102:11167–11171

Bond DR, Lovley DR (2003) Electricity production by *Geobacter sulfurreducens* attached to electrodes. Appl Environ Microbiol 69:1548–1555

Call DF, Wagner RC, Logan BE (2009) Hydrogen production by *Geobacter species* and a mixed consortium in a microbial electrolysis cell. Appl Environ Microbiol 75:7579–7587

Chaudhuri SK, Lovley DR (2003) Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. Nat Biotechnol 21:1229–1232

Chen ZS (2010) Evaluation and analysis of applying native fiber substrates I rumen microbial fuel cell. Masters thesis, Department of Mechanical and Electro-Mechanical Engineering, National Ilan University, Ilan

Chen WH, Guo JL, Huang WS, Wang GB (2008) The development of cellulosic ethanol technology. Agric Biotechnol 9:62–69

Cheng K-J, Costerton JW (1980) Adherent rumen bacteria-their role in the digestion of plant material, urea and epithelial cells. In: Ruckebusch Y, Thivend P (eds) Digestive physiology and metabolism in ruminants. MTP Press, Lancaster, pp 227–250

Cheng S, Xing D, Call DF, Logan BE (2009) Direct biological conversion of electrons into methane by electromethanogensis environ. Sci Technol 43:3953–3958

Dominguez-Benetton X, Sevda S, Vanbroekhoven K, Pant D (2012) The accurate use of impedance analysis for the study of microbial electrochemical systems. Chem Soc Rev 41:7228–7246

Fan Y, Hua H, Liu H (2007) Sustainable power generation of microbial fuel cells using bicarbonate buffer and proton transfer mechanisms. Environ Sci Technol 41(23):8154–8158

Feng Y, Wang X, Logan BE, Lee H (2008) Brewery wastewater treatment using air-cathode microbial fuel cells. Appl Microbiol Biotechnol 78:873–880

Feng Yujie, Qiao Yang, Xin Wang, Yankun Liu, He Lee, Nanqi Ren (2011) Treatment of Biodiesel Production Wastes with Simultaneous Electricity Generation using a Single-chamber Microbial Fuel Cell. Bioresour Technol 102: 411–415.

Gorby YA, Yanina S, McLean JS, Rosso KM, Moyles D, Dohnalkova A, Beveridge TJ, Chang IS, Kim BH, Kim KS, Culley DE, Reed SB, Romine MF, Saffarini DA, Hill EA, Shi L, Elias DA, Kennedy DW, Pinchuk G, Watanabe K, Ishii S, Logan B, Nealson KH, Fredrickson JK (2006) Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. Proc Natl Acad Sci U S A 103:11358–11363

Graham NM, Wainman FW, Blaxter KL, Armstrong DG (1959) Environmental temperature, energy metabolism and heat regulation in sheep. I. Energy metabolism in closely clipped sheep. J Agric Sci 52:13–17

Gurung et al (2012) The performance of serially and parallelly connected microbial fuel cells. Energy Sources A 34(17):1591–1598

Hall EK, Singer GA, Kainz MJ, Lennon JT (2010) Evidence for a temperature acclimation mechanism in bacteria: an empirical test of a membrane-mediated trade-off. Funct Ecol 24(4):898–908

He Z, Wagner N, Minteer SD (2006) An upflow microbial fuel cell with an interior cathode: assessment of the internal resistance by impedance spectroscopy. Environ Sci Technol 40(17): 5212–5217

Holmes DE, Nicoll JS, Bond DR, Lovley DR (2004) Potential role of a novel psychrotolerant member of the family *Geobacteraceae*, *Geopsychrobacter electrodiphilus* gen. nov., sp. nov., in electricity production by a marine sediment fuel cell. Appl Environ Microbiol 70(10):6023–6030

Hou J, Liu Z, Li Y (2015) Polyaniline modified stainless steel fiber felt for high-performance microbial fuel cell anodes. J Clean Energy Technol 3(3):165–169

Jiang J, Zhao Q, Zhang J, Zhang G, Lee D-J (2009) Electricity generation from bio-treatment of sewage sludge with microbial fuel cell. Bioresour Technol 100:5808–5812

Kaneda T (1991) Iso- and anteiso-fatty acids in bacteria – biosynthesis, function, and taxonomic significance. Microbiol Rev 55:288–302

Kassongo J, Togo CA (2011) Performance improvement of whey-driven microbial fuel cells by acclimation of indigenous anodophilic microbes. Afr J Biotechnol 10(40):7846–7852

Kennedy PM, Milligan LP (1978) Effects of cold exposure on digestion, microbial synthesis and nitrogen transformations in sheep. Br J Nutr 39:105–117

Khan MJ, Iqbal MT (2005) Modelling and analysis of electro-chemical, thermal, and reactant flow dynamics for a PEM fuel cell system. Fuel Cells 5(4):463–475

Khilari S, Pandit S, Varanasi JL, Das D, Pradhan D (2015) Bifunctional manganese ferrite/polyaniline hybrid as electrode material for enhanced energy recovery in microbial fuel cell. ACS Appl Mater Interfaces 7:20657–20666

Kim HJ, Park HS, Hyun MS, Chang IS, Kim M, Kim BH (2002) A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. Enzym Microb Technol 30:145–152

Kim B, An J, Kim D, Kim T, Jang JK, Lee B-G, Chang IS (2013) Voltage increase of microbial fuel cells with multiple membrane electrode assemblies by in series connection. Electrochem Commun 28:131–134

Liu Z-D, Li H-R (2007) Effects of bio- and abio-factors on electricity production in a mediatorless microbial fuel cell. Biochem Eng J 36:209–214

Logan BE, Hamelers B, Rozendal R, Schrorder U, Keller J, Freguia S, Aelterman P, Verstraete W, Rabaey K (2006) Microbial fuel cells: methodology and technology. Environ Sci Technol 40(17):5181–5192

Manohar AK, Bretschger O, Nealson KH, Mansfeld F (2008) The polarization behavior of the anode in a microbial fuel cell. Electrochim Acta 53:3508–3513

Mathuriya AS, Sharma VN (2009) Bioelectricity production from various wastewaters through microbial fuel cell technology. J Biochem Technol 2(1):133–137

Momoh OLY, Neayor B (2010) Generation of electricity from Abattoir waste water with the aid of a relatively cheap source of catholytes. J Appl Sci Environ Manag 14(2):21–27

Park DH, Zeikus JG (2002) Impact of electrode composition on electricity generation in a single-compartment fuel cell using Shewanella putrefaciens. Appl Microbiol Biotechnol 59:58–61

Patil VD, Patil DB, Pawar SS, Otari SV, Deshmukh MB, Pawar SH (2013) Studies on electrochemical performance of microbial fuel cell based on dairy waste for energy conversion. Int J Chem Sci Appl 4(2):111–115

Pham CA, Jung SJ, Phung NT, Lee J, Chang S (2003) A novel electrochemically active and Fe (III)-reducing bacterium phylogenetically related to *Aeromonas hydrophila*, isolated from a microbial fuel cell. FEMS Microbiol Lett 223:129–134

Prasad D, Murugesan AS, Padmanaban M, Satyanarayanan S, Sheela RS (2007) Direct electron transfer with yeast cells and construction of a mediatorless microbial fuel cell. Biosens Bioelectron 22:2604–2610

Rabaey K, Clauwaert P, Aelterman P (2005) Tubular microbial fuel cells for efficient electricity generation. Environ Sci Technol 39:8077–8082

Reimers CE, Stecher HA III, Westall JC, Alleau Y, Howell KA, Soule L, White HK, Girguis PR (2007) Substrate degradation kinetics, microbial diversity, and current efficiency of microbial fuel cells supplied with marine plankton. Appl Environ Microbiol 73:7029–7040

Ringeisen BR, Henderson E, Wu PK, Pietron J (2006) High power density from a miniature microbial fuel cell using *Shewanella oneidensis* DSP10. Environ Sci Technol 40:2629–2634

Rismani-Yazdi H, Christy AD, Dehority BA, Morrison M, Yu Z, Tuovinen OH (2007) Electricity generation from cellulose by rumen microorganisms in microbial fuel cells. Biotechnol Bioeng 97:1398–1407

Shin S-H, Choi Y, Na S-H, Jung S, Kim S (2006) Development of bipolar plate stack type microbial fuel cells. Bull Kor Chem Soc 27(2):281–285

Sinensky M (1974) Homeoviscous adaptation – a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. Proc Natl Acad Sci U S A 71:522–525

van de Vossenberg JLCM, Driessen AJM, da Costa MS, Konings WN (1999) Homeostasis of the membrane proton permeability in *Bacillus subtilis* grown at different temperatures. Biochim Biophys Acta 1419:97–104

Venkatamohan S, Mohanakrishna G, Purushotham Reddy B, Saravanan R, Sarma PN (2008) Bioelectricity generation from chemical wastewater treatment in mediatorless (anode) microbial fuel cell (MFC) using selectively enriched hydrogen producing mixed culture under acidophilic microenvironment. Biochem Eng J 39:121–130

Wang X, Feng Y, Wang H, Qu Y, Yu Y, Nanli N, Wang E, Lee H, Logan BE (2009) Bioaugmentation for electricity generation from corn stover biomass using microbial fuel cells. Environ Sci Technol 43:6088–6093

Wang C-T, Huang R-Y, Lee Y-C, Zhang C-D (2013) Electrode material of carbon nanotube/polyaniline carbon paper applied in microbial fuel cells. J Clean Energy Technol 1(3):206–210. https://doi.org/10.7763/JOCET.2013.V1.47

You S, Zhao Q, Zhang J (2006) A microbial fuel cell using permanganate as a cathodic electron acceptor. J Power Sources 162:1409–1415

Yujie F, Yang Q, Wang X, Liu Y, He L, Ren N (2011) Treatment of biodiesel production wastes with simultaneous electricity generation using a single-chamber microbial fuel cell. Bioresour Technol 102:411–415

Zhang X, Cheng S, Liang P, Huang X, Logan BE (2011) Scalable air cathode microbial fuel cells using glass fiber separators, plastic mesh supporters, and graphite fiber brush anodes. Bioresour Technol 102:372–375

Zhuang L, Zhou S, Yuan Y, Liu T, Wu Z (2011) Development of *Enterobacter aerogenes* fuel cells: from in situ biohydrogen oxidization to direct electroactive biofilm. Bioresour Technol 102:284–289