Chapter 9
Harvesting Energy Using Compost as a Source of Carbon and Electrogenic Bacteria

Fabio Flagiello, Edvige Gambino, Rosa Anna Nastro, and Chandrasekhar Kuppam

Abstract Compost is widely used to improve soil fertility for its chemical–physical properties, with particular regard to the abundance of humic substances. Compared to the untreated organic solid waste, the use of compost in microbial fuel cells (MFCs) could offer different advantages like the strong reduction of fermentative processes. The use of compost in MFCs in combination with soil or mixed with other substrates had been reported by some researchers to improve the performance of MFCs fed with agro-industrial residues and plant MFCs. In this chapter, we report the results of an experiment carried out using a compost of vegetable residues as feedstock in a single chamber, air cathode MFCs. We investigated the behavior of two MFCs serially connected, the possibility to use compost as a long-term source of energy in MFCs, the influence of cathode surface/cell volume ratio on MFCs performance in terms of power and current density. Our results showed for MFCs serially connected a maximum PD and CD of 234 mW/m^2 and 1.6 A/m^2, respectively, with a maximum OCV of 557 mV. Unexpectedly, the compost-based MFCs kept significant electric outputs (854 mV, 467 mW/m^2 kg, and 114 mA/m^2 kg) after being reactivated 2 years later its set-up, thus demonstrating its potential as long-term operation energy system.

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9.1 Introduction

Since the Kyoto protocol agreement, the production of green energy to sustain the cities growth was the focus of worldwide public opinion and, because of this, an interesting topic of political debates. Despite the big evolution of green energy technology and the amount of money invested in research and development, we are still so far from reaching a worldwide stable economy based on green energy plants. We still need the “old good oil” to perform most of our daily tasks and support the big amount of overall energy request. Great efforts are being performed to replace fossil fuel, with some recent encouraging results: Costa Rica, using a very interesting mix of green technologies was recently able to fulfill the energy demand covering 300 days/year. This achievement did not include the local transport system, still based on fossil fuel. The secret of oil success can be summarized in two simple sentences: flexibility of use (Ferreira Coelho and Szklo 2015), enough amount to sustain the modern civilization growth for years and affordable costs, even for developing countries. Among renewables, biomass-based systems are very good candidate technologies to obtain both energy and energy vectors (H_2 and CH_4) for the availability of substrates produced in the agroindustry, agriculture practices, and everyday life (Florio et al. 2019).

9.1.1 Organic Waste: A Modern Gold Mine

Billions of tons of food are produced in every corner of the Earth (Food and Agriculture Organization of United Nations 2018), with the consequent increasing amount of waste. For this reason, waste management is becoming of outstanding importance to reduce environmental damages due to leachate leaking, greenhouse gas emission, microplastics diffusion in the environment, etc. (UNEP 2015). In recent years, organic waste has been considered more and more like a resource for both energy and commodity chemical production. The new approach towards waste management have to be essentially focused on the 3R concept (reduce, reuse, and recycle), cleaner productions, circular economy establishment, waste prevention, and, finally, the transformation of waste into a source of energy and commodity chemicals (Nastro et al. 2016; Florio et al. 2019; Venkata Mohan et al. 2016). With the advances in the green chemistry and consequent advances in biorefinery, the range of molecules obtainable from biomass is increasing more and more, thus changing the “waste” into “raw material for new biosynthesis processes.” Different biosynthetic routes are already available like acidogenic bacteria-based processes: biohydrogen and biohythane production and carbohydrates fermentation are two examples. Like oil, organic waste is mainly composed of carbon. Both of them can be used to produce fuel for the automotive sector (Basso et al. 2016) and, both of them, can be used to produced plastic compounds with the advantage, in the case of the organic waste, of the biodegradability (Sharma et al. 2018) and a very low carbon footprint. Furthermore, the organic waste can be converted in compost, a very well-known fertilizer useful for damaged soils and to recover bacteria communities able to act as biochemical refinery under different environmental condition (Aresta et al. 2012). These examples of use open to a wide range of growing economic opportunities as highlighted by Levidow (2018).

9.1.2 Compost: From Where All Start

Whenever we want to recover a portion of our garden's soil or we want to improve the performance of our cultivar, we buy a fertilizer. Not all the fertilizers are the same, and they gave rise to a lot of scientific debates on their long-term effects on the environment (Xin et al. 2016). In the last decade, even thanks to the circular economy philosophy, the focus of the scientific community has been mainly on the organic fertilizers that can be recovered from organic waste. Compost is one of the best examples of this recovering and is widely used as a soil amendment (Adugna 2016). The composting is a three-phase process: intensive decomposition, stabilization, and maturation, with the stabilization as the shortest phase (Dimambro et al. 2016). In Fig. 9.1 we report a diagram of the German Rottegrad classification of the different phases succeeding each other during the composting process.

In the first step, organic matter is degraded by thermophile bacteria at a temperature near 60 °C. The most common bacteria strains at this stage are *Bacillus* spp., *Thermoactinomyces* sp., *Stearothermophilus* sp. (Daas et al. 2016). Soon after the oxidation phase (intensive decomposition), the temperature cools down (stabilization stage): in this phase, compost becomes more rich in aromatic structures while aliphatic and alcoholic structures are degraded. During the maturation phase the temperature goes down at 30 °C, the organic elements are already completely converted and lots of mesophilic biochemical reactions complete the chemical enrichment of the matter. The big complexity of these reactions is still under investigation by scientists, who see the opportunity to use biowaste as a "biorefinery" (Fava et al. 2015), able to produce biodegradable chemical compounds in a change of the ones coming from oil. Anyway, there is another aspect not well known about the compost: the ability to participate, directly or indirectly to electrical energy production.

9.1.3 The Blood of Our Society: Electrical Energy

According to the definition of the famous "Encyclopaedia Britannica (2019)," energy is: "*the capacity of doing work...*" and all we know there are lots of different

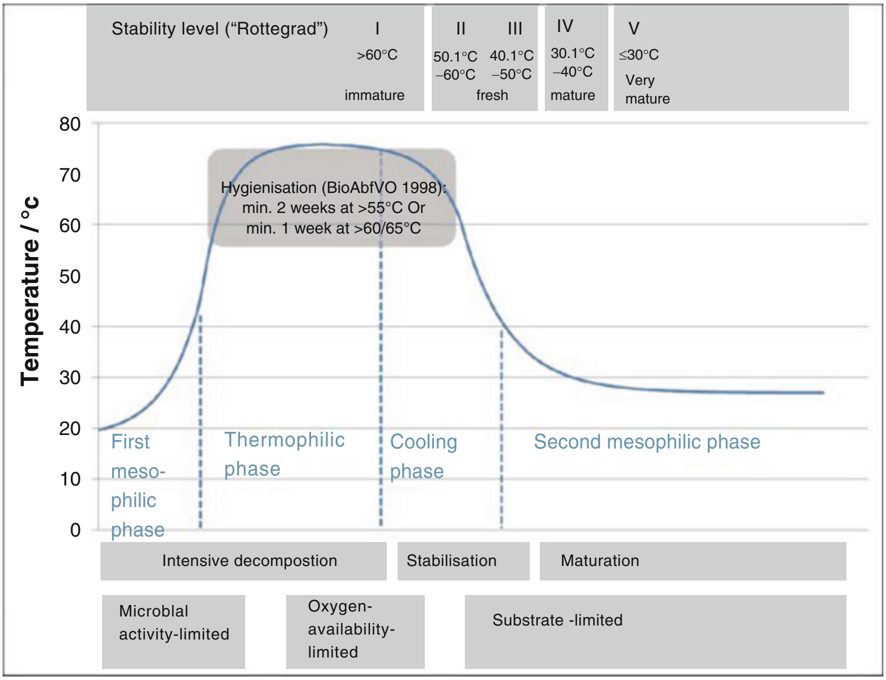

Fig. 9.1 A diagram of the German Rottegrad classification for compost maturity. The graph shows the temperature plotted over time (from Dimambro et al. 2016).

types of energy, as potential, chemical, thermal, and so on but, probably, the best-known energy is the electrical energy. Electricity is the "blood" of modern society; its flow powers our lamps, notebooks, smartphones, medical devices, cars, our houses. Without electricity we could never had the big development of the last centuries, so taking into account this, it is worth noting that the production of electricity was, and actually is, always based on the use of oil and its derivate products that are, unfortunately, very pollutant. Limiting pollution is a modern mission of governments and scientists have discovered different alternative sources to produce electricity with a nearly zero environmental impact like solar panels, wind farms, biomasses. One of the problems with these technologies is related to their low energy production efficiency and their discontinuous working operation. If we add also the big costs and the maintenance of the production plants, it becomes understandable why so difficult a definitive worldwide shift is. So, scientific research needs to face the above problems by finding new ways to produce electrical energy, limiting greenhouse gas emissions and costs while granting the satisfaction of world energy needs. In this framework, a combination of biology and engineering could give an important contribution to shift from a fossil-fuel-based to a green energy society.

9.1.4 Microbial Fuel Cells: A New Paradigm in Green Energy Production

The production of electrical current from bacteria was known since 1911, when Potter experienced for the first time *Escherichia Coli* as "bio-extractor" of electrons from organic matter. The results obtained were discouraging compared with the energetic alternative performances of the historic period. We had to wait until 1993 to obtain a serious interest in the argument, when Allen and Bennetto (1993) realized the first prototype of an electrochemical bioreactor able to reach interesting results in terms of current density using *Proteus vulgaris* as microorganism and, as substrate, glucose. That reactor was one of the first modern double chambers "microbial fuel cell" (MFC). A basic MFC is a bio-electrochemical device composed of two physical elements, an anode and a cathode, which can be placed in two chambers where different environmental conditions are realized (Logan et al. 2006). Each chamber contains an electrode, generally made by carbon-based material. An ion exchange membrane divides the two compartments, thus preventing the flow of unwanted substances among the two chambers while allowing anions or cations (according to the chosen membrane) to pass from the anode to the cathode compartment. In this last configuration, very useful for research tests, the catholyte and the anolyte are independent, and parameters as pH, bioecological dynamics, kind of substrate, the effect of different electrolytes can be easily taken under control. Also, the internal resistance is very low due to the lack of physical or chemical obstacles as unexpected biofilm formation on the electrodes or chemical electron competitors. The side effects are related to the high costs of production and maintenance in the long run, the efficiency of the membrane, and its durability (Stoll et al. 2016). In most part of cases, proton exchanging membrane, like Nafion, is used for lab-scale experiments (Khan et al. 2017; Koók et al. 2017). Cations exchanging membrane made up by ceramic, eggshell, and other cheap materials are becoming more and more popular and recommended for an in-field application of MFCs to liquid/solid waste treatment (Ortiz-Martínez et al. 2016; Chouler et al. 2017; Khan et al. 2017; Nastro 2014). Unlike two-chamber MFCs, in a single-chamber MFC both anode and cathode share the same compartment (Fig. 9.2). In a single-chamber MFC, both electrodes are soaked in the same feedstock. A wide range of substrates could be used to power MFCs: pure solutions containing an organic molecule acting as a source of chemical energy for bacteria or more complex substrates like municipal/industrial wastewater, biomass, and even compost (Nastro et al. 2016; Khan et al. 2017; Santoro et al. 2017; Florio et al. 2019; Zhao et al. 2017; Gambino et al. 2017; Moqsud et al. 2015) are examples of energy sources for bacteria in MFCs.

Regardless of the carbon source available, all MFCs are based on the metabolism of electroactive or exoelectrogenic bacteria and on their ability to exchange electrons with the anode placed into an anoxic/anaerobic environment (Logan 2009). One of the factors affecting MFCs performance is the nature of the molecule acting as electrons acceptor at the cathode, i.e. the step of potential established between the anode and the cathode. Such a step is the force driving the electrons to flow towards

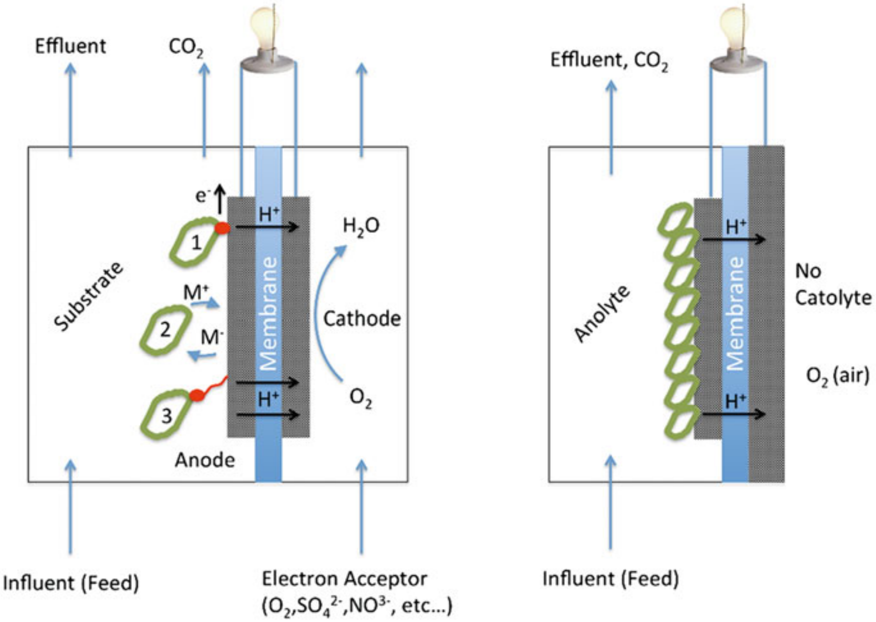

Fig. 9.2 Schematic of the double-chamber and single-chamber MFC (from Nastro et al. 2016)

the cathode: the higher is the step of electric potential between the electrodes, the higher is the current density potentially produced (Ucar et al. 2017). Other factors that can limit MFCs performance are: activation, concentration, and ohmic losses (which can be revealed during polarization experiments (Chandrasekhar et al. 2017)), pH of the substrate used as source of chemical energy, environmental temperature (Xu et al. 2018) as well as biofilm age (Paitier et al. 2017). Both biological and electrochemical processes in MFCs result in electrons able to power electrical devices like biosensors (Chouler et al. 2018), robots, small medical instruments and so on (Santoro et al. 2017; Dong et al. 2013).

9.1.5 Compost as a Source of Electrogenic Bacteria

Compost can be considered an important source of exoelectrogenic bacteria, thanks to its unique biochemical genesis. During the thermophilic and mesophilic phases, it is possible to isolate bacteria belonging to *Geobacillus* and *Bacillus* genera, using simple microbiological culture techniques and amplification/sequencing of rDNA 16S. It is worth noting that all these genera are also involved in other green chemical processes, like the production of biofuels or water depuration (Novik et al. 2018). *Geobacillus* spp. are widely used among different industrial fields, producing various metabolites of commercial use like enzymes, ethanol, and antibiotic substances like Geobacillin I and II (Garg et al. 2012). *Bacillus licheniformis* and *Geobacillus thermoglucosidasius* can easily survive and carry out their metabolism at high temperatures and, for this reason, can be used in reactors operating at $T > 50°$ (Choi et al. 2004). Many microorganisms among the *Bacillus* genus and other gram-positive bacteria have proved their electrogenicity in MFCs. For example, *Bacillus subtilis and Bacillus licheniformis* were used in amperometric biosensor systems for water BOD values (Su et al. 2011), increasing the speed of the analysis. *Bacillus firmus* was, instead, tested in a membraneless single-chambered MFC, working in batch mode using glucose, hydrolyzed potato peel, and hydrolyzed cyanobacterial biomass substrates. A maximum power density (PD) of 16.46 mW/m^2 at 62.48 mA/m^2 was achieved using cyanobacterial biomass as the substrate (Singh et al. 2016). Pure culture of *Brevibacillus borstelensis* STRI1 was tested with sugarcane molasses (1.15 g/L) removing up to 82% of COD and reaching 188 mW/m^2 of PD (Hassan et al. 2019). *Bacillus subtilis* was also tested in a double-chamber CEM membrane provided, as electrogenic bacteria to harvest energy from wastewater using graphite electrodes. The results in terms of COD removal and PD were, respectively, of 90% and 270 mW/m^2 (Ismail and Jaeel 2013a).

9.1.6 Compost as a Source of Energy in MFCs: A Short Overview

Recently, some papers confirmed the efficiency of bacteria from compost as inoculum in MFCs fed with different substrates, dairy and food waste included (Cercado et al. 2013; Reiche and Kirkwood 2012; Cercado-Quezada et al. 2010a, b). In some other papers, they report the utilization of compost as substrate alone or in combination (Wang et al. 2013, 2015; Khudzari et al. 2016; Moqsud et al. 2015; Nastro et al. 2016). In all cases, the authors report a significant increase in power production when compost is used as inoculum or substrate in MFCs. Moqsud et al. (2015), for example, report an increase of, respectively, two and three times in voltage and in power when compost is mixed in soil in plant MFCs (Carmalin and Sreeja 2017). Wang et al. (2015) explored the possibility to join composting of vegetable residues with power generation in MFCs, giving evidence that electrogenesis can occur during composting conditions. Even though the authors explored the influence of C/N ratio and moisture content on power generation, there is no data about the influence of temperature. Moreover, the whole process occurred in the anode compartment of MFCs, i.e. in the absence of oxygen, so even though they address the whole process as “anaerobic composting” that is not comparable to the well-known compost process. Nevertheless, Cercado-Quezada et al. (2010a) reported for MFCs fed with yogurt waste optimal working temperatures of 40 °C and 60 °C (maximum current density of 1450 mA/m^2 at 40 °C). In this chapter, we explored the utilization of homemade compost as a substrate for energy recovery in a single chamber, air cathode MFCs. We carried out two distinct yet connected experiments:

the first one was to investigate power production in two homemade compost MFCs serially connected in a stack and the possibility to use compost as a long-term source of power. The second experiment investigated the performance of MFCs fed with compost from a solid waste treatment plant, with particular regards to cell volume/cathode surface.

9.2 Materials and Methods

9.2.1 Homemade Compost-MFCs Stack

As a first step, we set up 500 mL MFCs by using a common PET bottle for sampling activity as cell and graphite rods as electrodes, as reported by Gambino et al. (2017). The feedstock was prepared by mixing 100 g of soil, 300 g of homemade compost, 200 mL of sodium acetate solution (10% w/v), 100 mL of PBS. The anode was buried in the mix of soil and compost at about 2 cm below the surface of the solid layer. The cathode was placed at about 5 cm from the anode, soaked in the liquid phase of the feedstock (Fig. 9.3). pH was set at 7.2 ± 0.2. Voltage was measured by a Keithley Multimeter and the current produced was calculated according to Ohm's law, normalized to the cathode surface (mA/m^2). Polarization experiments were carried out on a two-weeks base, using resistors in a range of 256 kΩ–100 Ω. These first MFCs (named MFCs$_0$) were prepared in double replica, connected to a 200 Ω external resistor for 24 h and, then, serially connected. The stack was kept at maximum power and incubated at 30° C for 4 weeks. When a voltage reversal occurred, 10 mL of the liquid substrate containing a source of energy/carbon (10% acetate solution, Trypticase Soy Broth purchased from Oxoid®, 10% glucose solution) was added to the MFCs$_0$ and the stack was left in OCV for 24 h. Once the data collection ended, each MFC was left in OCV for 72 h, then connected to a 1000 Ω resistor and stored at 20 °C. During the following months, the leachate level was

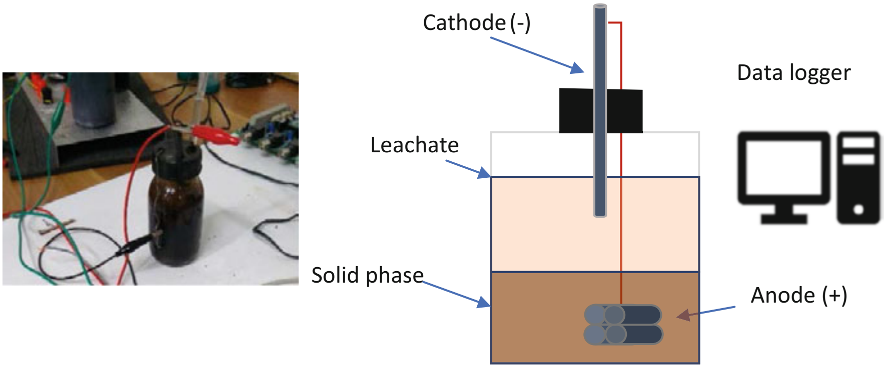

Fig. 9.3 A compost-MFC (on the right). On the left, a schematic of the MFC-data logger system

kept constant by adding sodium acetate solution (10% w/v) and PBS in a mix of 2:1 ratio. From time to time, the voltage was measured to verify whether both $MFCs_0$ were able to keep long-term performance.

9.2.2 Industrial Compost-MFCs

In order to collect more data about single compost-MFCs behavior and performance, after 2 years from the first experiment, we set up MFCs fed with fresh compost sampled at a waste treatment plant in Naples District (Italy). More in detail, we provided glass and plastic bottles of 100 mL (MFC_1 and MFC_2) and 250 mL (MFC_3 and MFC_4) in volume with an anode made up of four graphite sticks (5 cm in length, 0.5 cm in diameter). Like in previous MFCs, the anode was buried in compost and placed at an approximate distance of 5 cm from the cathode. This last one was made of a 5 cm graphite stick (0.5 cm in diameter, 8.0 cm^2 total surface) and put at the interface between the feedstock and the air (Fig. 9.3). An insulated copper wire was used to connect the electrodes. MFCs feedstock was a suspension of compost/saline solution (1:2 w/v ratio). The solution was prepared using NaCl in distilled water (0.9% w/v) and phosphate buffer solution (PBS, Oxoid) in a 1:2 ratio. The final pH was set at 7.5 ± 0.2. For this second experiment, we added no acetate but the organic compounds in compost were the only source of energy since the very beginning. Nevertheless, in order to reduce the MFCs start-up period, an inoculum of 25 mL and 10 mL of leachate from $MFCs_0$ was added to MFC_1/MFC_3 and MFC_2/MFC_4, respectively. To investigate the electrochemical performances, a data acquisition system made up of an ARDUINO based MEGA 2560 was set up to record the values of voltage. The current was calculated according to Ohm's law. MFCs were monitored for 4 weeks, with no nutrients refill. Polarization experiments were performed every week using a range of 256 kΩ to 100 Ω resistors as well. After every polarization experiment, MFCs were set at the maximum power for 5 days before being set in OCV for 6 h. Then, the same cycle was repeated and data was collected.

9.2.3 Anode Biofilm Screening

Anode biofilms were sampled with a cotton swab and treated according to Florio et al. (2019). A basic screening on viable microorganisms was carried out with media for bacteria culture (Oxoid©). A PureLink™ Microbiome DNA Purification Kit (INVITROGEN©) was used to extract genomic DNA from microbial isolates and 16S rDNA sequences were amplified by Real Time-PCR (UNO96 HPL Thermocycler, VWR). A sequencing similarity search was performed using the BLAST algorithm referring to the GenBank database.

9.3 Results and Discussion

9.3.1 Homemade Compost-MFCs Stack

The stack of two $MFCs_0$ serially connected, during the first month, achieved a maximum PD and CD of 234 mW/m^2 and 1.6 A/m^2, respectively, with a maximum OCV of 557 mV. pH ranged between 6.0 and 7.8. We occasionally observed a voltage reversal in one of the two MFC_0. We, then, added 10 mL of 10% acetate solution, TSB, and 10% glucose solution to the feedstock, at different times, to verify if such reversal could have been ascribed to nutrients depletion. Our results seem to support our hypothesis (Fig. 9.4). We observed MFCs highest performance after glucose addition, thus signifying the prevalent utilization of glucose as a source of energy and electrons by the electroactive bacteria. As to pH, we measured a decrease in values soon after adding glucose. Voltage, power, and pH trends suggested the presence of electroactive bacteria able to carry out mixed acid and/or lactic acid fermentation pathways. These last ones allow many microorganisms like bifidobacteria, enterobacteria, clostridia, bacilli, and lactobacilli to recover energy from glucose and other carbohydrates in anaerobic conditions (Ciani et al. 2013). Microbiological analyses of anode biofilm confirmed even in this case our hypothesis, showing the prevalence of Enterobacteria, with *Escherichia coli* and *Acinetobacter* spp. as prevalent strains. As to gram-positive bacteria, *Bacillus subtilis* and *Bacillus* spp. were present, even though in a less concentration. The growth of strictly aerobic bacteria like bacilli at the anode is not unexpected. Some strains like *Bacillus subtilis* can grow anaerobically, either by using nitrate or nitrite as a terminal electron acceptor or by fermentation (Nakano and Zuber 1998) and the

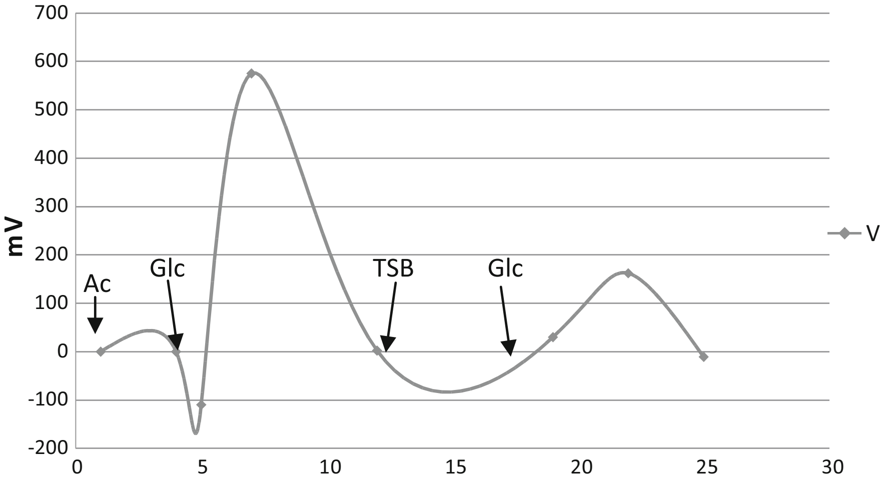

Fig. 9.4 MFC° stack trend over time according to added carbon sources. *Ac* acetate, *Glc* glucose, *TSB* tryptic soy broth

electroactivity of bacilli has been demonstrated by different researchers (Florio et al. 2019; Ismail and Jaeel 2013b). The quite unusual composition of anode microflora can find an explanation in the not perfect process occurring in the home compost-bin. Nevertheless, the performance achieved by the use of not mature compost is far higher than the outputs obtained by the same authors in MFCs fed with the Organic Fraction of Municipal Solid Waste (Nastro et al. 2017; Jannelli et al. 2017; Florio et al. 2019).

A certain voltage instability, with also negative values, was observed in one of the two MFCs° even after its disconnection from the stack, at the end of the experiment. It required several months to obtain stable positive voltages at 1000 Ω external load. After 2 years, we reactivated one of the two MFCs° by replacing 100 g of the spent substrate with fresh compost sampled at the solid waste treatment plant in Naples District. This MFC° after few hours in OCV achieved 413 mV, increasing to 854 mV 3 months later. Polarization curves were performed monthly for 4 months. The MFC° was kept at maximum power for 5 days before being put in OCV for 6 h and, then, perform a new polarization experiment. In Fig. 9.5 we report the polarization and power curves obtained from the 1st till the 4th month of operation, after the reactivation. PD increased with time, achieving 35 mW/m^2 the 4th month and a maximum CD of 140 mA/m^2 the 3rd month (Table 9.1).

9.3.2 Industrial compost-MFCs

Polarization experiments of MFC$_1$, MFC$_2$, MFC$_3$, and MFC$_4$ were performed on a weekly base. Power and polarization curves are reported in Figs. 9.6 and 9.7. MFC$_3$ and MFC$_4$ power curves revealed a certain irregularity, with more than one peak and the presence of overshoots. A constant increase in power production and stability was, instead, observed in 100 mL MFCs (MFC$_1$ and MFC$_2$), even though the highest PD was obtained in MFC$_4$ (4.2 mW/m^2) the third week of operation and the maximum CD was achieved in MFC$_3$ the 1st week (Table 9.1). In all MFCs, pH values ranged between 7.2 and 7.8. It is interesting to notice that, besides MFC$_0$, the highest performance was achieved in 100 mL MFCs. This result confirmed what was reported by Santoro et al. (2018): "*smaller the microbial fuel cell reactor is, the greater is the power output both density (express in function of the electrode geometric area) and volumetric (express in function of the reactor empty volume).*" In the case of compost-MFCs, the lower is the electrodes volume (or surface)/cell volume, the higher are the energy losses due to fermentative activities taking place in the feedstock to the detriment of electrogenesis.

Anodes biofilm qualitative analyses revealed the presence of *Bacillus licheniformis, Bacillus firmus, Bacillus subtilis, Geobacillus thermoglucosidasius, Brevibacillus borstelensis.* All these strains are among the most characteristic microorganisms in compost and, some of them are proved electroactive bacteria as reported in Sect. 1.5.

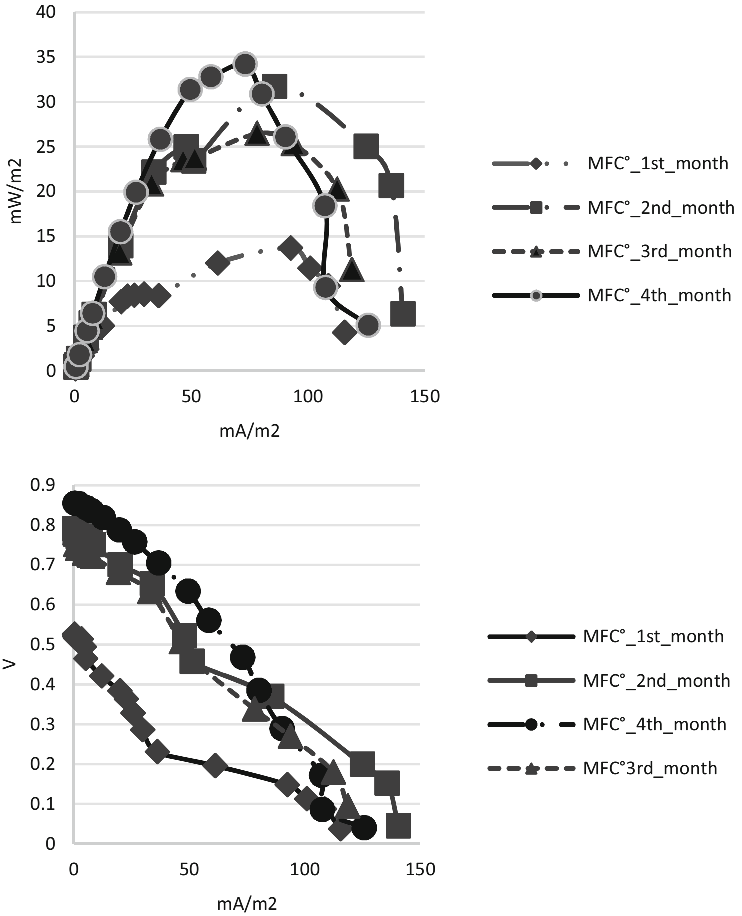

Fig. 9.5 Power and polarization curves after MFC reactivation

Table 9.1 MFCs electric outputs

	Cell volume (mL)	Compost (g)	CD$_{max}$ (mA/m^2)	CD$_{max}$ (mA/m^{2}kg)	PD$_{max}$ (mW/m^2)	PD$_{max}$ (mW/m^{2}kg)	V_{max} (mV)
MFC1	100	30	20.8	693	2.3	76.7	230
MFC2	100	30	11.5	383	1.9	63.3	532
MFC3	250	80	38.7	484	1.6	20	207
MFC4	250	80	37.4	467	4.2	52.5	520
MFC°	500	300	140	467	34.2	114	854

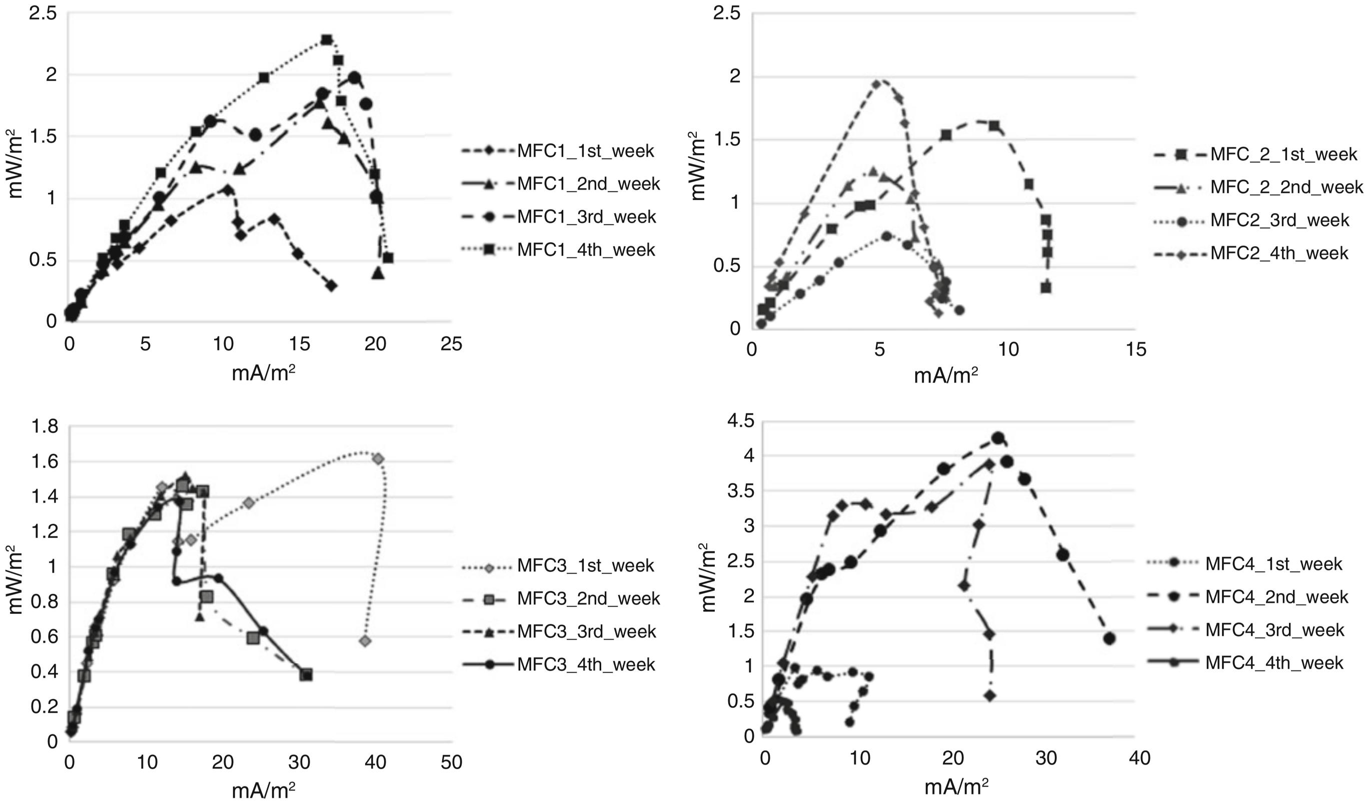

Fig. 9.6 Power curves of MFC1 and MFC2 (100 mL), MFC3 and MFC4 (250 mL)

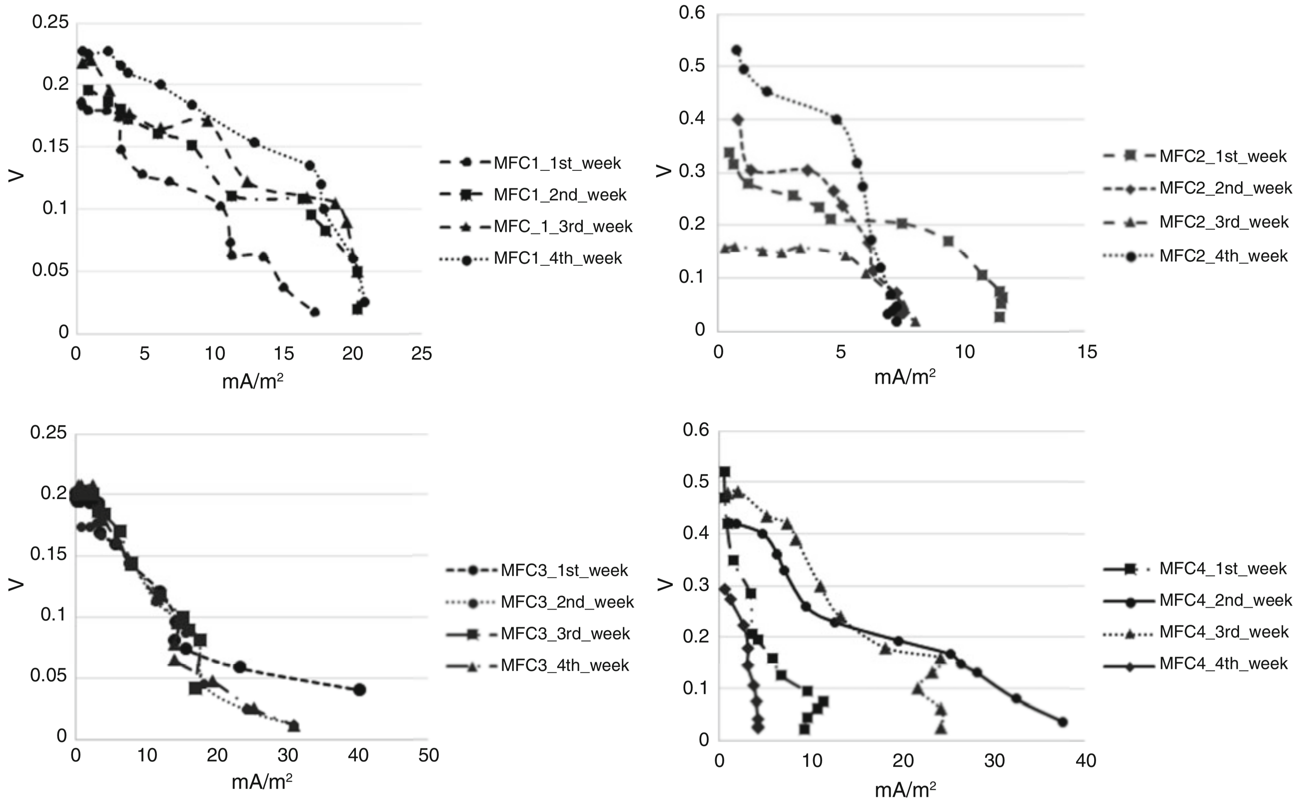

Fig. 9.7 Polarization curves of MFC_1 and MFC_2 (100 mL in volume); MFC_3 and MFC_4 (250 mL in volume)

9.4 Conclusions

Our research confirmed the potentialities of compost as feedstock in MFCs to be operated on both a short and a long-term basis. Interesting results in terms of PD and CD have been measured in MFCs with a lower electrode surface/cell volume ratio, confirming what is observed in MFCs fed with wastewater. As to MFC°, high power outputs were achieved after its reactivation, two years later its set-up. The high power and current outputs could be explained by the development of a robust electroactive biofilm ant both anode and cathode. The feedstock composition should have changed after such a long time. This last issue and how it could have contributed to increase in MFC_0 performance are still to be investigated as well as the dynamics of anode microflora over time. The prevalence of electroactive bacteria at the anodes supports the utilization of compost to produce inocula for MFCs potentially fed with other substrates.

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