JREE: Vol. 4, No. 2&3, (Spring & Summer 2017) 33-38

Journal of Renewable Energy and Environment

Research Article

Journal Homepage: www.jree.ir

Improved Performance of Soil Microbial Fuel Cell by Adding Earthworms

Yukimoto Hiroshi*, Ebe Shohei, Ohike Tatsuya, Okanami Masahiro, Ano Takashi

Division of Biotechnological Science, Graduate School of Biology-Oriented Science and Technology, Kindai University, Wakayama, Japan.

P A P E R I N F O

Paper history:
Received 23 March 2018
Accepted in revised form 8 July 2018

Keywords:
Microbial Fuel Cell
Earthworm
Power Generation
Sustainable Energy

A B S T R A C T

Soil microbial fuel cells (SMFCs) could generate electricity from the organic matters in the soil. We focused on the soil ecosystems, specifically the earthworms which were known to improve the soil-fertility. The aim of this study was to investigate the effect of earthworm on power generation of the SMFC. The maximum power density and the internal resistance were compared to the SMFC with and without earthworms. The power density increased 9 times and the internal resistance decreased by 8.5 times. The soil structure of the SMFC with and without earthworms was different and the clear soil aggregate structure was found in the SMFC with earthworms, which had been made with the passage of soil through the earthworm gut. The results indicated that adding earthworms had a significant effect on the SMFC performance, especially the power, internal resistance and soil structure. It was considered that the soil environments were changed biologically and physicochemically by adding earthworms into SMFC and these changes had a positive influence on the SMFC.

1. INTRODUCTION

Currently, energy demand is increasing in the world, and it has been reported that 80 percent of the energy supply source was produced from fossil fuels such as petroleum, coal and natural gas in 2016 [1]. However, these energy supply sources have problems such as depletion of petroleum and emission of greenhouse gases. Under the circumstances, demand for renewable and sustainable energy are increasing and the alternative energy sources other than fossil fuels are expected [2].

Microbial fuel cells (MFCs) are devices that use microorganisms as biocatalysts to directly convert chemical energy to electricity through the oxidization of organic and inorganic matters [3, 4]. MFCs can use wastewater and wastes as the fuel source of electricity generation and are expected in the recent years as an application that can produce sustainable energy [5, 6]. There are several kinds of MFCs, and a kind of MFC that uses soil as the fuel source is called soil MFCs (SMFCs) [7]. There are various kinds of bacteria in the soil and the number of bacterial species per gram of soil are estimated to be between 2000 to 8.3 million [8]. There exist various bacterial populations, and some bacteria in the soil such as metal or sulfate-reducing bacteria produce electricity in the MFCs [9-11]. The anode is embedded at a certain depth within the soil, while the cathode rests on the surface of the soil [12]. Basic principles of MFC and SMFC are similar, but the SMFC doesn't require proton exchange membrane and it can be inexpensive and easy to be constructed [13-15]. However, it is still a long way to go before the SMFC is practically applied [16]. One problem is the low power generation by SMFC operation. Several studies are being conducted to increase the power generation.

It has been reported that increased power generation was observed by reducing the internal resistance through the addition of graphite powder, silica colloid or sodium chloride in the soil [17-19]. In other studies, optimization of electrode distance or addition of substrate such as rice straw was effective for SMFC to increase the power generation [20, 21]. Most studies for SMFC have been focused on soil composition, bacteria in the soil or on the shape and materials of device. Another approach for improving the power generation was a hybrid system with other organisms, such as plants [22, 23]. Combining SMFCs with the rice plant in the rice paddy field allows the electricity generation effectively in the daytime, but it is not at nighttime because of the photosynthesis [24, 25].

We tried another hybrid system that uses earthworms, which degrade fallen leaves or plant litter [26]. Furthermore, there have been reports that plant growth is promoted by addition of earthworms to soil and plant pathogens are inhibited by microorganisms derived from earthworms, which means they are useful for

*Corresponding Author's Email: 1733710007j@waka.kindai.ac.jp (Y. Hiroshi)

agriculture [27, 28]. Charles Darwin, famous for his theory of evolution, had researched earthworms for over 30 years, and published "The formation of vegetable mould, through the action of worms, with observation on their habits". He described the importance of earthworms in production of fertile soil [29]. As the existence of earthworm improves the soil-fertility, we expected the earthworms to increase the performance of SMFC. As far as we know, there have been only reports on SMFC using vermicompost which is casts of earthworms [30, 31]. There have been no reports on SMFC which saw the effect of adding of earthworm, and this experiment is the first report that combines SMFC with earthworms. This is a novel approach to use earthworms as enhanced power generation through the SMFC. This hybrid SMFC combining the earthworm with SMFC is named as eMFC (earthworm microbial fuel cell).

2. MATERIALS METHOD

2.1. Soil and earthworm sampling

The soil and earthworms used in this study were collected at the campus of Kindai University in Wakayama, Japan. The top soil was collected at a depth of about 0-10 cm, and was sieved with 2 mm mesh to remove the big particles such as pebbles, rocks and twigs, and the fine earth fraction of the sample (less than 2 mm) was used. The external form of earthworm species were examined, and the collected earthworms were identified from external shape and position of the annulus. The earthworms with annulus were determined as Megascolecidae family and others without annulus were immature earthworms, because the immature earthworms don't have the annulus.

2.2. Preparation of mud from soil and MFC setup

The sieved soil described above was mixed with water thoroughly to make a homogeneous mud. The plastic case (9.0×9.0×7.5 cm) (clear craft case MD, Yamada chemical Co., Ltd., Japan) was used as MFC device. Carbon felt (GF-20-5FH, GF-20-10FH, Nippon Carbon Co., Ltd., Japan) with the inserted wire was used as anode and cathode electrodes and their sizes were 7.0×7.0×0.5 cm and 7.0×7.0×1.0 cm, respectively (Fig. 1). A layer of mud was packed over the bottom of the fuel vessel up to the 1 cm mark of the vessel. The anode electrode was placed on it, and the vessel was filled with mud up to the 5 cm mark. Then, the cathode electrode was gently placed on the surface of the mud, and the MFC was covered with a lid, and the wires were passed through the hole made upside of the bottom vessel (Fig. 2). The circuit was completed using an external resistor (10 kΩ)(variable carbon composition resistors RV24YN20S B104, TOCOS, Japan) [32]. Five earthworms of about 2.5 g were added per unit reactor. The device was operated at the room temperature about 25 °C.

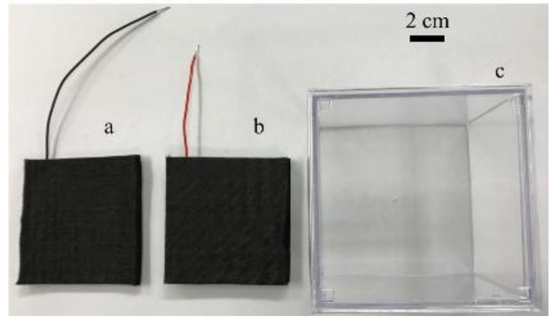

Figure 1. Components of the used SMFC in this study (a: anode electrode, b: cathode electrode and c: plastic case)

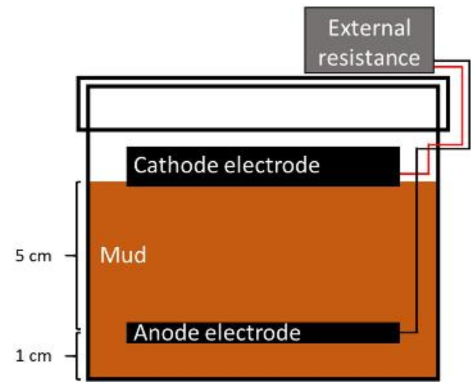

Figure 2. Schematic diagram of SMFC setup

2.3. Data acquisition and calculations

The voltage was recorded every day, by using a 3804-50 digital multimeter (Hioki Co., Japan). To evaluate the performance of the device, the polarization curve and the power curve were investigated. The voltage drops of the MFC were measured by employing a variable resistance that ranged from 10 k to 50 Ω [33]. The current values were calculated by the measured values of the voltage from Equation (1), according to Ohm's law.

$$I = V/R \tag{1}$$

(Where I, V and R are current (A), voltage across each resistor (V) and resistance of each external load (Ω), respectively.)

Current densities were obtained by normalizing the calculated currents to the anode surface area (0.0112 m^2). The internal resistance was calculated by polarization curve. The power was calculated from Equation 2 using the measured values of the voltage.

$$P = V^2/R \tag{2}$$

(Where P, V and R are power (W), voltage across each resistor (V) and resistance of each external load (Ω), respectively.)

Power densities were obtained by normalizing the calculated powers to the anode surface area (0.0112 m^2).

The maximum power density was obtained from the power curve [34].

2.4. Measurement of microbes in soil

Sub-samples were randomly collected at 5 sites from the superficial layer (1 cm) of the soil in the MFC reactor and were homogenized to make a composite sample. The soil composite sample of 0.5 gram was added in 1 mL of sterilized distilled water. After homogenization for 5 minutes on the mixer, the mixed sample was decimally diluted (10^{-1} to 10^{-7}) and aliquots of the resulting solutions were plated on Trypto Soy Agar (TSA) (20.0 g/L peptone, 5.0 g/L sodium chloride, 2.5 g/L glucose, 2.5 g/L K_2HPO_4), 1/10-strength TSA, 1/100-strength TSA, 1/1000-strength TSA, 1/10000-strength TSA, 1/100000-strength TSA and water agar, respectively. After incubation at the room temperature for up to 3 days, the colony forming units (CFU) were counted and the number of bacteria per gram of dry soil was obtained [35].

2.5. Analysis of soil component

The pH and electrical conductivity (EC) of soil in the MFC were measured by the standard procedure. Soil and water solution in the ratio of 1:2 were mixed together for 10 minutes, followed by centrifugation at 25,000×g. The supernatant of the soil was used to measure the pH and EC by the pH meter (Pocket-sized pH Meter S2K712. ISFETCOM Co., Ltd.) and EC meter (AQUAtwin EC meter., B-771 Horiba, Ltd.), respectively.

3. RESULTS AND DISCUSSION

3.1. Effect of earthworm on microbial fuel cell

The soil MFCs used in this study were operated without any outside source of substrate and inoculation. SMFC with earthworms showed the higher voltage than SMFC without them (Fig. 3).

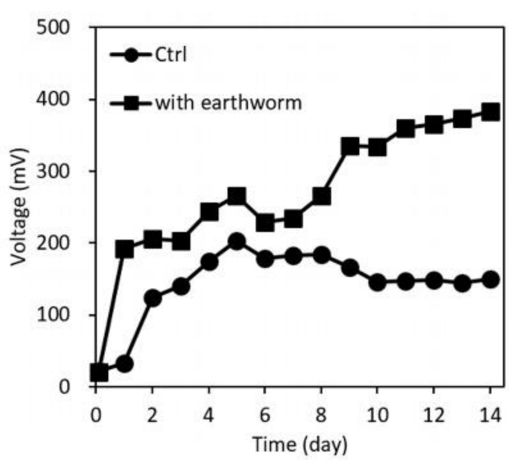

Figure 3. Comparison of the voltage in SMFC with and without earthworms (The square and circle symbols show the voltage of SMFC with and without earthworms, respectively.)

To examine whether this effect on the voltage is caused by earthworms, two SMFCs were set up and operated for 2 weeks and after confirming the power-similarity of the SMFCs, earthworms were added to one of them on the day shown with a vertical arrow in Fig. 4. On the third day after the addition of earthworms, a rise in voltage was observed as shown in Fig. 4. The maximum voltage was 441 mV on the 10 th day after earthworms were added. Polarization and power curve after 17 days of operation are shown in Fig. 5.

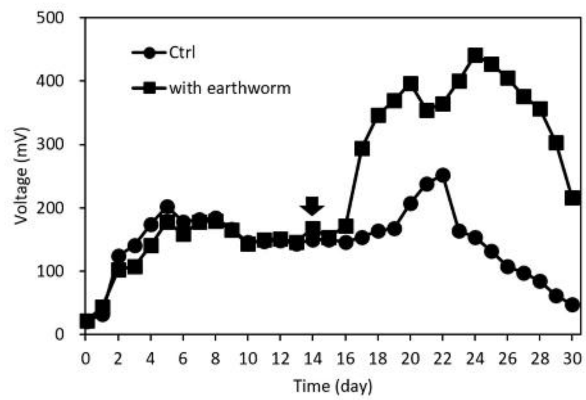

Figure 4. Effect of the addition of earthworm on SMFC (The triangles and circles symbol show the voltage of the SMFC with and without earthworms, respectively.)

⬇; Vertical arrow indicates the day of the addition of earthworms to one of the MFC.

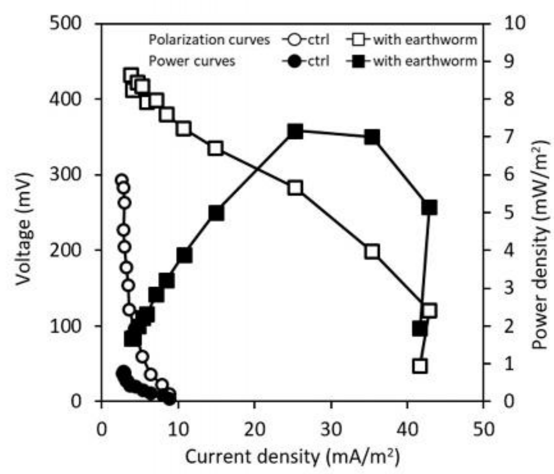

Figure 5. Polarization and power curves of the SMFC with and without earthworms (Polarization curves of the SMFC are shown with (open circle) and without (open square) earthworms and power curves of the SMFC are shown with (closed circle) and without (closed square) earthworms.)

The maximum power density and the internal resistance of the SMFC with earthworms were obtained from power and polarization curve. The maximum power densities were 7.2 mW/m^2 and 0.8 mW/m^2 at the SMFC with and without earthworms, respectively. The SMFC with earthworms showed a maximum power density of 9 times higher than that of control. This value was higher than the value of Hong et al. (2.4 mW/m^2) was investigated, but it was lower than the value of Simeon et al. (65.4 mW/m^2) [7, 13]. However, the important thing in this study was that improved the performance of SMFC by adding earthworms. The internal resistances of the SMFC with and without earthworms were 658 Ω and 7745 Ω, respectively (Table 1). Internal resistance of the SMFC with earthworms showed more than 91.5% decrease compared to the control. The maximum power density was increased by SMFC with earthworms.

In the previous study, it was reported that the SMFC was increased the power by the decreasing the internal resistance, and the decreasing internal resistance was observed by SMFC with earthworms in Fig. 5 [36]. The following can be considered as the cause of the increasing the power or the decreasing the internal resistance by adding earthworms: (1) Enhancing microbial activity in the soil [37], (2) Changing soil structure by eating soil and making casts [38], (3) Influencing the decomposition of the organic matter by microorganisms [39].

3.2. Effect of earthworm to microbes in soil

The effect of added earthworms on the number of microorganisms in the soil was investigated by counting the colony forming units (CFU) per gram of dry soil on the media with the different nutritional condition. It was suggested that increasing the power was not due to the increasing the number of microbes, because the CFU per gram of dry soil in the SMFCs with and without earthworms were similar (Fig. 6).

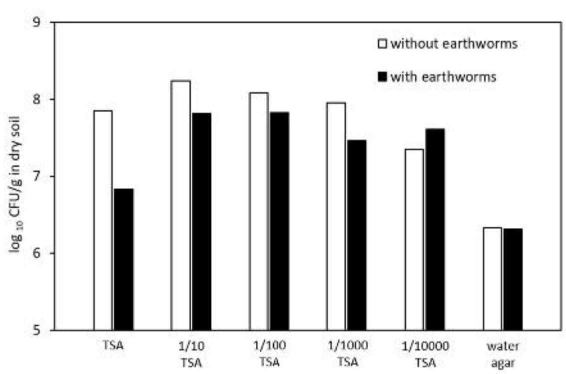

Figure 6. The colony forming units per gram in dry soil of the SMFC with and without earthworms on the different nutritional condition

Suor et al. were reported that rotating-cathode MFC by using aquatic worms, which are also annelids like earthworms, enhanced MFC performance because aquatic worm predation produced easily biodegradable organic matter for electrogenic bacteria [40]. It was suggested that the proportion of microorganisms involved in electricity generation was increased and the activity of electrogenic bacteria in the soil was enhanced, because the number of bacteria between the MFCs with and without earthworms was similar.

3.3. Effect of earthworm to soil component and structure

The soil structure with earthworms was very different from that without earthworms as shown in Figs. 7a, b. In the SMFC with earthworms, clear soil aggregate structure was found as shown in Figs. 7a and 7c. This structure was made with passaging soil through the earthworm gut. Both pH and EC of the soil samples with and without earthworms were measured. The values of pH and EC from the soil with earthworms were 7.9 and 0.26, respectively, and the counterparts from the soil without earthworms were 7.2 and 0.21, respectively (Table 1). The soil with earthworms showed slightly alkaline pH, and higher EC than those of control SMFC.

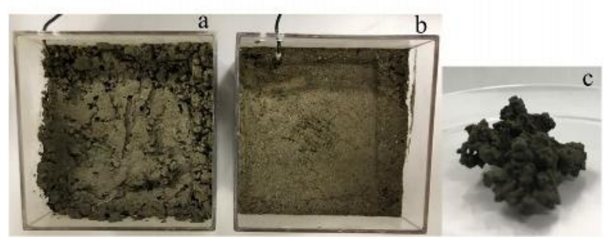

Figure 7. The soil structure of the SMFC (a: with earthworm, b: without earthworm and c: aggregate structure in SMFC with earthworm.)

TABLE 1. The soil components of SMFC with and without earthworms

	R_{in} (Ω)	P_{max} (mW/m^2)	pH	EC (mS/cm)
Ctrl	7745	0.8	7.2	0.21
Earthworm	658	7.2	7.9	0.26

(R_{in}: internal resistance, P_{max}: maximum power density, EC: electrical conductivity.)

The soil aggregate structure was clearly observed in the SMFC with earthworms and it had been reported that the soil aggregate structure of vermicompost, made by earthworms, increased the power density of SMFC [30]. It was reported that the soil with the aggregate structure had a high cation exchange capacity (CEC) [41]. It was suggested that the loss of voltage was decreased by increasing CEC and forming the aggregate structure, which had a high cation exchange capacity and enhanced proton transfer in the SMFC. The aggregate structure by earthworm was called vermicompost. Ammaraphitaka et al. were shown that vermicompost liquid had potential for production of renewable energy [31].

4. CONCLUSION

It was considered that the soil environments were changed biologically and physicochemically by adding earthworms into SMFC. These changes had a positive influence on SMFC. There was no report of hybrid type SMFC combined with the earthworms. It was a very novel approach to use earthworm as enhanced power generation through the SMFC and we would like to call this eMFC (earthworm MFC).

5. ACKNOWLEDGEMENT

We gratefully acknowledge Ms. Naomi Backes Kamimura, Department of Biology-Oriented Science and Technology, Kindai University, for English editing.

REFERENCES

1. Key World Energy Statistics, International Energy Agency, (2016).
2. Ieropoulos, I., Greenman, J. and Melhuish, C., "Urine utilization by microbial fuel cell; energy fuel for the future", ***Physical Chemistry Chemical Physics,*** Vol. 14, (2012), 94-98.
3. Logan, B.E. and Regan, J.M., "Electricity-producing bacterial communities in microbial fuel cell", ***Review, Trends in Microbiology***, Vol. 14, (2006), 512-518.
4. Mostafa, R., Arash, A., Soheil, D., Alieza, Z. and Sang-Eun, O., "Microbial fuel cell as new technology for bioelectricity generation: A review", ***Alexandria Engineering Journal***, Vol. 55, (2015), 745-756.
5. Rabaey, K. and Verstraete, W., "Microbial fuel cells: novel biotechnology for energy generation", ***Trends in Biotechnology***, Vol. 23, No. 6, (2005), 291-298.
6. Parot, S., Marie-Line, D. and Alain, B., "Forming electrochemically active biofilms from garden compost under chronoamperometry", ***Bioresource Technology***, Vol. 99, (2008), 4809-4816.
7. Hong, S.W., Chang, I.S., Yong, S.C. and Tai, H.C., "Experimental evaluation of influential factors for electricity harvesting from sediment using microbial fuel cell", ***Bioresource Technology***, Vol. 100, (2009), 3029-3035.
8. Roesch, F.W., Luiz, Fulthorpe, R.R., Alberto, R., George, C., Alison, K.M.H., Angela, D.K., Samira, H.D., Flavio, A.O.C., William, G.F. and Eric, W.T., "Pyrosequensing enumerates and contrasts soil microbial diversity", ***The ISME Journal***, Vol. 1, (2007), 283-290.
9. Kouzuma, A., Kasai, T., Nakagawa, G., Yamamuro, A., Abe, T. and Watanabe, K., "Comparative metagenomics of anode-associated microbiomes developed in rice paddy-field microbial fuel cells", ***PLOS One,*** Vol. 8, No. 11, (2013), 1-10.
10. Yamamuro, A., Kouzuma, A., Abe, T. and Watanabe, K., "Metagenomic analyses reveal the involvement of syntrophic consortia in methanol/electricity conversion in microbial fuel cells", ***PLOS One***, Vol. 9, No. 5, (2014), 1-8.
11. Quan, X., Quan, Y., Kun, T. and Xiao-man, J., "Comparative investigation on microbial community and electricity generation in aerobic and anaerobic enriched MFCs", ***Bioresource Technology***, Vol. 128, (2013), 259-265.
12. Reimers, E.C., Leonard, M.T., Stephanie, F. and Wei W., "Harvesting energy from the marine sediment-water interface", ***Environmental Science & Technology,*** Vol. 35, (2001), 192-195.
13. Simeon, M., Imologie, R.O.A., Agidi, G. and Okoro-Shekwaga, C.A., "Performance of a single chamber soil microbial fuel cell at varied external resistances for electric power generation", ***Journal of Research in Rural Education***, Vol. 3, No. 3, (2016), 53-58.
14. Mostafa, R., Gholamreza, B., Mostafa, G. and Alieza, Z., "A review on the role of proton exchange membrane on the performance of microbial fuel cell", ***Polymers for Advanced Technologies,*** Vol. 25, (2014), 1426-1432.
15. Mostafa, R., Gholamreza, B., Ghasem, N., Mostafa, G., Alieza, Z. and Sang-Eun, O., "A review on the effect of proton exchange membranes in microbial fuel cells", ***Biofuel Research Journal***, Vol. 1, (2014), 7-15.
16. Li, Z., Qian, C., Ding, Y., Shaoyuan, B. and Xiaojun, L., "A review of microbial fuel cells coupled with constructed wetland", ***Advances in Engineering Research***, Vol. 63, (2016), 1-4.
17. Fujinaga, A., Tei, K., Ozaki, H., Takanami, R. and Taniguchi, S., "Evaluation of the effect of graphite powder in decreasing the internal resistance for microbial fuel cell using soil", ***Journal of water and Environment Technology,*** Vol. 14, No. 3, (2016), 141-148.
18. Ainara, D.G., Antonio, B., Irene, O.B. and Abraham, E.N., "Silica colloid formation enhances performance of sediment microbial fuel cells in a low conductivity soil", ***Environmental Science and Technology***, Vol. 47, (2013), 2117-2122.
19. Zahra, N., Mostfa, R. and Ghasem, N., "Effect of electrolyte conductivity and aeration on performance of sediment microbial fuel cell", ***Journal of Research in Rural Education***, Vol. 2, No. 1, (2015), 43-48.
20. An, J., Bongkyu, K., Jonghyeon, N., How, Y.N. and In, S.C., "Comparison in performance of sediment microbial fuel cells according to depth of embedded anode", ***Bioresource Technology***, Vol. 127, (2013), 138-142.
21. Gurung, A. and Oh, S.E., "Rice straw as a potential biomass for generation of bioelectrical energy using microbial fuel cells (MFCs)", ***Energy Sources, Part A: Recovery, Utilization and Environmental Effects***, Vol. 37, (2015), 2625-2631.
22. Doherty, L., Yaqian, Z., Xiaohong, Z., Yuansheng, H., Xiaodi, H., Lei, X. and Ranbin, L., "A reviw of a recently emerged technology: Constructed wetland-Microbial fuel cells", ***Water Research***, Vol. 85, (2015), 38-45.
23. Zahra, N. and Mostafa, R., "Improvement of sediment microbial fuel cell performance by application of sun light and biocathode", ***Korean Journal of Chemical Engineering,*** Vol. 33, (2016), 154-158.
24. Kouzuma, A., Kaku, N. and Watanabe, K., "Microbial electricity generation in rice paddy fields: recent advances and perspectives in rhizosphere microbial fuel cells", ***Applied Microbiology and Biotechnology***, Vol. 98, (2014), 9521-9526.
25. Kaku, N., Yonezawa, N., Kodama, Y. and Watanabe, K., "Plant/microbe cooperation for electricity generation in a rice paddy field", ***Applied Microbiology and Biotechnology***, Vol. 79, (2008), 43-49.
26. Bunemann, E.K., Schwenke, G.D. and Zwieten, L.V., "Impact of agricultural inputs on soil organisms - a review", ***Australian Journal of Soil Research***, Vol. 44, (2006), 379-406.
27. Plavsin, I., Mirna, V., Sandra, E., Karolina, V. and Jasenka, C., "Inhibitory effect of earthworm coelomic fluid on growth of the plant parasitic fungus *Fusarium oxysporum*", ***European Journal of Soil Biology***, Vol. 78, (2017), 1-6.
28. Laossi, K.R., Amandine, G., Diana, C.N., Manuel, B. and Sebastien, B., "Erthworm effects on plant growth do not necessarily decrease with soil fertility", ***Plant Soil***, Vol. 328, (2010), 109-118.

29. Sinha, K.R., Dalsukh, V., Krunal, C. and Sunita, A., "Embarking on a second green revolution for sustainable agriculture by vermiculture biotechnology using earthworms: Reviving the dreams of Sir Charles Darwin", ***Journal of Agricultural Biotechnology and Sustainable Development,*** Vol. 2, No. 7, (2010), 113-128.
30. Nandy, A., Vikash, K., Moumita, K. and Patit, P.K., "MFC with vermicompost soil: power generation with additional importance of waste management", ***The Royal Society of Chemistry***, Vol. 5, (2015), 41300-41306.
31. Ammaraphitaka, P., Piyachon, K. and Rattapoom, P., "Electricity production from vermicompost liquid using microbial fuel cell", ***International Journal of Energy and Environmental Engineering***, Vol. 12, (2018).
32. Liu, j., Cumaraswamy, V. and Yang, M., "Biosurfactant production from used vegetable oil in the anode chamber of a microbial electrosynthesizing fuel cell", ***Waste and Biomass Valorization***, Vol. 9, (2018), 1-7.
33. Wang, A., Cheng, H., Nanqi, R., Dan, C., Na, L. and Weimin, W., "Sediment microbial fuel cell with floating biocathode for organic removal and energy recovery", ***Frontiers of Environmental Science & Engineering,*** Vol. 6, (2012), 569-574.
34. Agnieszka, W., Zofia, S., Arletta, B. and Jakub, C., "Bioelectricity production from soil using microbial fuel cells", ***Biotechnology and Applied Biochemistry,*** Vol. 173, (2014), 2287-2296.
35. "Basic Practical Microbiology", Society for General Microbiology, (2006).
36. Li, X., Wang, X., Zhao, Q., Lili, W., Yongtao, L. and Qixing, Z., "Carbon fiber enhanced bioelectricity generation in soil microbial fuel cells", ***Biosensors and Bioelectronics***, Vol. 85, (2016), 135-141.
37. Sinha, K.R., Sunil, H., Dalsukh, V. and Krunal, C., "Vermiculture and sustainable agriculture", ***American-Eurasian Journal Of Agricultural & Environmental Sciences,*** Vol. 5, (2009), 42-55.
38. Zirbes, L., Mark, M., Veronique, V., Jean, P.W., Francois, J.V., Philippe, T. and Eric, H., "Earthworms use order cues to locate and feed on microorganisms in soil", ***PLOS ONE***, Vol. 6, Issue 7, (2011), 1-7.
39. Chaoui, I.H., Larry, M.Z. and Tsutomu, O., "Effects of earthworm casts and compost on soil microbial activity and plant nutrient availability", ***Soil Biology and Biochemistry***, Vol. 35, (2003), 295-302.
40. Suor, D., Ma, J., Wang, Z., Li, Y., Tang, J. and Wu, Z., "Enhanced power production from waste activated sludge in rotating-cathode microbial fuel cells: The effects of aquatic worm predation", ***Chemical Engineering Journal***, Vol. 248, (2014), 415-421.
41. Oyedele, D.J., Schjonning, P. and Amusan, A.A., "Physicochemical properties of earthworm casts and uningested parent soil from selected sites in southwestern Nigeria", ***Ecological Engineering***, Vol. 28, (2006), 106-113.