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Voltage Recovery from Pesticides Doped Tomatoes, Cabbages and Loam Soil Inoculated with Rumen Waste: Microbial Fuel Cells

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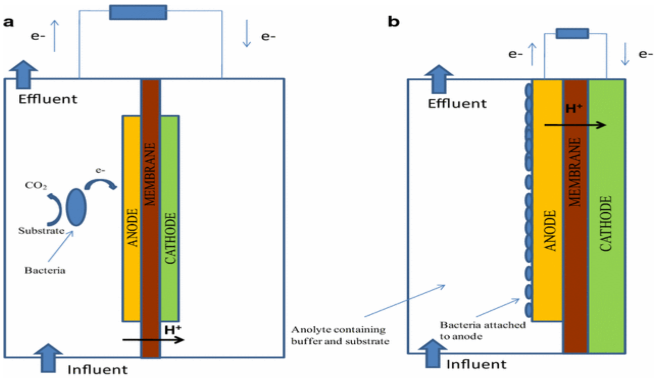
ABSTRACT

In the current study, voltage generation from loam soil, cabbages and tomatoes for a retention time of thirty days using double chamber microbial fuel cell is investigated. The anodic with 1.5 liters' substrate inoculated with rumen fluid from slaughter house and cathodic loaded with distilled water compartments were connected via a salt bridge made using 3% agarose in sodium chloride. The performance of microbial fuel cells was evaluated by measuring daily voltage using a multimeter. The observed results showed that the current produced increased for some time and levelled off on the tenth day. Current was highest in rumen fluid set up at 0.111±0.003 mA followed by tomato fruits, loam soil and least in cabbage at 0.101±0.008, 0.095±0.001 and 0.094±0.007 mA, respectively.
Keywords : Voltage, Current, Tomato, Cabbage, Loam Soil

I. INTRODUCTION

Fuel cells are voltaic or electrochemical cells which are able to convert chemical energy into electrical energy (Logan and Rabaey, 2012) through chemical reactions between hydrogen fuel and an oxidant, for example, oxygen. Fuel cells generally produce electricity smoothly till the fuel and oxidant are exhausted. Among many advantages of fuel cells include low or zero emissions, minimal noise and no air pollution. There are different types of fuel cells based on operational temperature, fuel, oxidant and electrolyte used (Marcella *et al,* 2007). Microbial fuel cells (MFCs) are a framework in which microorganisms oxidize natural/organic mixes into adenosine triphosphate (ATP) by consecutive responses where electrons exchange at the terminal electron acceptor to produce electricity (Torres *et al,* 2009). MFCs comprise of two compartments, namely the anode and cathode, separated by a cationic film. The microbes are usually placed in the anodic compartment where they feed on glucose (organic matter) that acts as a source of electrons. The metabolites produce both electrons and protons simultaneously, and exchange of electrons occurs on the surface of the anode (Logan, 2008). The electrons, then, move from the anode to the cathode via electrical circuit and protons are expended at the cathode chamber by oxidants. Examples of electron acceptors include oxygen and hexacyanoferrate (Rabaey *et al.* 2004). MFCs have two main partitions single and double chamber (Logan, 2008). The MFCs that have separate cathodic and anodic chambers are known as double chambered MFC while those having cathode and anode in a solitary chamber are called solitary chambered MFC. Figure 2.1(a) below indicates



double chamber MFC and Figure 2.1(b) shows single chambered MFC (Chaturvedi and Verma, 2016).

Figure 1: (a) a dual-chambered MFC and 2.1 (b) a single-chambered MFC (Chaturvedi and Verma, 2016)

Materials with a large population of microorganisms and high content of organic matter have been used to generate power in MFCs. Some of this material include marine sediment (Bond et al., 2002; Scott et al., 2008), sewage sludge (Zhang *et al.,* 2012), garden compost (Parot *et al.,* 2008), industrial/domestic waste- water (Rabaey and Verstraete, 2005) and animal waste (Yokoyama *et al.,* 2006). Soil generally has a bacterial population of approximately 109 cells g–1 (Whitman et al., 1998) and organic matter content of about 100 mg g^{-1} (Bot and Benites, 2005). It is, however, known that different soil types may have varying bacterial population, for example, in organic soil, the abundance of bacteria and organic matter can be much higher compared to other types of soil (Troeh and Thompson, 2005). Various types of substrates are available for biomass-electricity production (Jia *et al.,* 2013; Mbugua *et al.,* 2020). They range from simple to complex substrate matters in wastewater. Studies have been carried out to produce power using wastewater, different crop matter, cow manure, glucose and starch components of food, acetate, rice water, etc. (Jia *et al.,* 2013). Kamau *et al.* (2018) utilized market wastes inoculated with rumen waste as a carbon source in production of electricity. They observed that a peak voltage of 0.584V was obtained on day 19 from the avocado fruit waste while the maximum voltage of 0.701V was observed on day 20 for tomato waste. . Water melon and fruits mixture were observed to produce the least voltage. The maximum power and current densities were observed in the tomato waste, and were in the range of 1.825 to 60.041mW/m^2 and 6.762 and 99.174mA/m^2 respectively. Deng *et al.*, (2014) investigated factors that influenced the performance of MFS. They observed that the in soil MFC with 5 cm deep soil and 3 cm overlaying water exhibited the highest open circuit voltage of 562 mV and a power density of 0.72 mW m^{-2}. They also observed that ohmic resistance increased with the increase in the quantity of soil and water. They noted that the polarization resistance of the cathode increased with the quantity of the soil, while that of the anode increased with increase in the amount of water. During the 30-day operation, the cell voltage positively correlated with temperature (25 ^{0}C) and reached a maximum of 162 mV with a 500 Ω external load. Therefore, in this study, we investigate the potential of voltage generation from tomato, cabbage, loam soil inoculated with rumen fluid and doped with pesticide as a green approach to deal with persistent organic pollutants.

II. Methodology

The proximate composition was done on homogenized samples i.e the cabbage and tomato were subjected to size reduction by choppin and blending to obtain uniform substrate. The analysis included; energy, fat, nitrogen-free extract, ash, moisture content, protein, fiber, carbohydrates by the techniques of AOAC, (2003) as described by Mbugua *et al.* (2020). Loam soil from Kirinyaga County where tomato and cabbage farming is done was sampled and analyzed as described by Mbugua *et al.,* (2014).

1) Microbial Fuel Cells Construction

Two 1.5 liters containers were prepared as anodic and cathodic chambers. Two small holes were made on the caps of the containers, and were used as inlets for the wire that served as the external conductor. One end of the copper wire was attached to 5.7cm long and 0.7cm diameter graphite rod electrodes.

2) Salt bridge

A salt bridge was prepared using 2.5 litres of 1M NaCl, 3% agarose solution and lamp wicks. The wicks were boiled in the NaCl and agarose solution for 10 minutes after which it was kept in the freezer at -4°C for solidification. The solidified salt bridge was passed through PVC pipes and attached to the chambers using Araldite adhesive, which made them leak-proof.

Figure 2: Salt bridge prepared wicks, 3% agarose in NaCl

3) Electrode's preparation

The electrodes used in this study were made of carbon graphite rods stuck together using a zero-resistance copper wire as shown in figure 3 below. The carbon rods were obtained from zinc acid batteries. They were thoroughly cleaned using water and later scrubbed using a sand paper. They were then socked in concentrated sulphuric acid for 24 hours before stacking them together. There was 0.00399 m^2 operating electrodes surface area

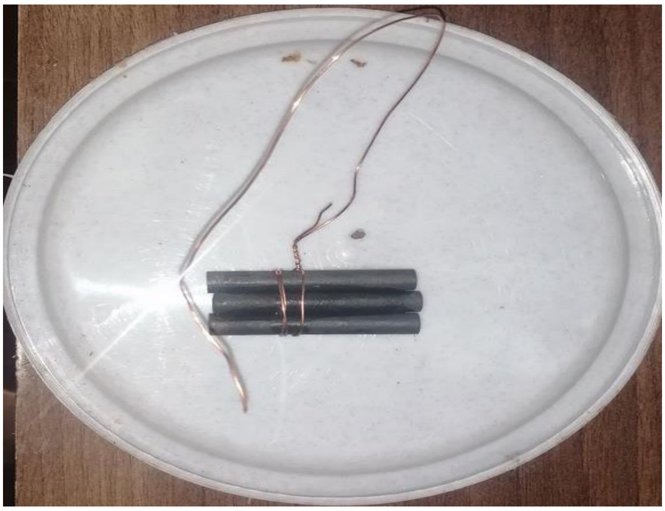


Figure 3 : The electrode made of graphite rods with copper wire

4) Circuit Assembly

The assembly of the H-shaped MFC was done, as shown in figure 4 as earlier described by Mbugua *et al.,* 2018. The anodic chamber was loaded with the substrate mixed with inoculum at 1:1 ratio doped at 10 ppm with chlorpyrifos, lambda cyhalothrin, malathion and pesticide mixture while the cathodic compartment was loaded with 1.5 liters distilled water A digital voltmeter was attached to the copper wires from the cathodic and anodic chambers, and the voltage and current were monitored daily.

Figure 4 : Set-up of H-shaped microbial fuel cells with a multi-meter

5) Control experiments

The control experiments were setup by blending about 750 g of decomposing cabbage and tomato wastes separately and 750g loam top soil, about 750 milliliters of rumen wastes and loading them into the different anodic chamber of the fabricated microbial fuel cell as shown in figure 4. The waste was then mixed with 750ml of distilled water after which the prepared electrodes were loaded. The anodic chamber was flushed with carbon dioxide before air tight sealing. The cathodic chamber was loaded with 1.5 liters of water. The voltage and current generated was recorded using a multimeter daily until the voltage generation plateaued (Kamau *et al.,* 2018, Kamau *et al.,* 2020).

6) Voltage from pesticide doped tomatoes, cabbages, loam soil and rumen wastes

About 750g of tomato and cabbage wastes were independently chopped, minced using a meat mincer and blended using a kitchen blender, thoroughly homogenized with 750 milliliters of rumen waste, then added to the anodic chamber of the H-shaped MFC. The substrate was doped with 10ml of 10ppm mixture of lambda cyhalothrin, malathion and chlorpyrifos pesticides from the stock solutions. The cathodic chamber was fed with 1500ml distilled water. The same was done with top loam soil where 750g of soil was mixed with water to make a homogeneous mixture before loading to the anodic chamber and adding 750ml rumen waste. Another set was made using 1500 mL of rumen waste water mixture loaded into the anodic chamber. The current and voltage emanating from the cells were recorded daily for 24 days.

III. Results and Discussions

Proximate analysis

The proximate study results on fresh basis are shown in table 1. High moisture levels were recorded in tomato at 95.16±4.00 compared to 94.87±2.56 in cabbages. The nitrogen-free extract (NFE) represents soluble carbohydrates, while crude fiber gives the insoluble carbohydrates (Dhont and Els, 2003). From table 1, the NFE reported in this study was in the range of 15.08±1.11- 3.22±0.89 %. The energy levels for the tomatoes were 2.93±0.05 Kcal/100g while in cabbages, 16.64±4.01Kcal/100g was observed.

Table 1 : Proximate analysis on wet weight fruit and vegetable waste

Sample	% Moisture	% Protein	% Fat	% Ash	% Fiber	% Carb.	% NFE	Energy (Kcal/100g)
Tomato	95.16±4.00	0.57±0.01	0.12±0.01	0.46±0.01	0.76±0.01	2.93±0.09	15.08±1.11	2.93±0.05
Cabbage	94.87±2.56	0.83±0.07	0.05±0.01	0.49±0.02	0.54±0.06	3.22±0.92	3.22±0.89	16.64±4.01

From table 1, the carbohydrates levels were higher compared to proteins and fats. This is because of sugars from the fundamental blocks in most tissues. This further translates to higher energy/100g of each waste. This had earlier been reported on similar samples by *Mbugua et al.,* 2020.

Loam soil properties

The properties of the loam soil used in this study is shown in table 2. The soil obtained from cabbage and tomato farm in Kirinyaga County was sampled at 2 cm depth after removing the plant matter from the surface. The soil pH was observed to be 6.5 ±0.51 while total nitrogen and phosphorous were reported at 0.25±0.08% and 44.00±5.00 ppm, respectively.

Profile	Properties	Profile	Properties
Soil depth cm	Top	Calcium milli-equivalent%	44.4±2.11
Soil pH-H_2O (1:2.5)	6.5±0.51	Magnesium me%	3.1±0.09
Elect. Cond. ms/cm	0.3±0.01	Potassium me%	1.5±0.66
Carbon %	2.7±0.32	Sodium me%	3.6±1.11
Sand %	40±3.56	Sum me%	52.6±3.44
Silt %	40±4.55	Base %	100+
Clay %	20±2.88	ESP	14.4±6.74
Texture Class	Loam	Total nitrogen %	0.25±0.08
Cat. Exch. Capacity. me%	24.8±2.67	Phosphorus ppm	44±5.00
Zinc ppm	62.9±10.22	Iron ppm	96.2±12.90
Copper ppm	1.22±0.11	me is milli-equivalent	

The carbon content of the soil influences the voltage generated by microbes in microbial fuel cells. Carbon serves as the microbe's food and therefore is a vital factor in soil microbial fuel cells. The carbon levels were observed at 2.7±0.32 % in the loam soil. Similar results had been observed from Limuru loam soil as reported by Mbugua *et al.,* 2014.

The microbial fuel cell voltage and current

The voltage and current generated in the respective experiments are shown and discussed in ths section. The mean voltage and current were used to plot the curves.

7) Control Experiment

The control experiments were done using the substrates without inoculum. The maximum voltage levels were observed in rumen fluid at 0.568 V compared to 0.508 V, 0.265V and 0.478 V in tomatoes, loam soil and cabbages, respectively (figure 5). The voltage generated from rumen fluid showed a steady increase from day zero to day 30th while the voltage in the showed a decrease in day 26. The high rate of voltage production is attributed to high population of microbes in rumen compared to the other substrates.

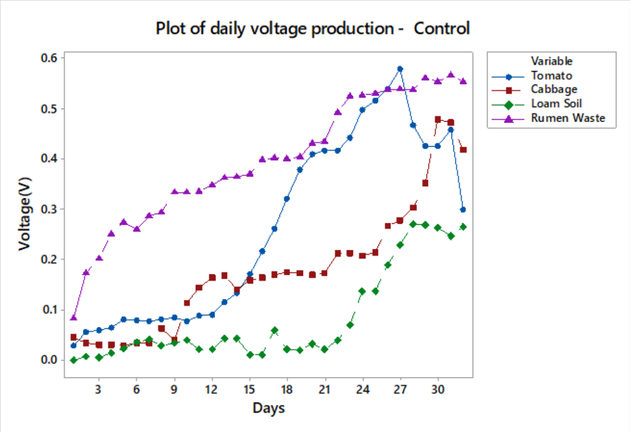


Figure 5 : Daily voltage generated from tomato, cabbage, loam soil and rumen waste

Figure 6 below shows the results obtained when an inoculum is added to the tomato, cabbage and loam soil substrates. The results indicated that when microbes were introduced, there was a spike in voltage generation for the first ten days of the experiments in tomato and cabbages substrates, after which voltage generation dropped. This could be explained by a change in pH which greatly affect the system stability and microbial activities. An aerobic process generally involves two sets of bacteria. The first one involves acid fermentation whereby complex carbohydrates are broken down into simpler, short chain molecules using facultative bacteria. The second process is the methane fermentation by anaerobic bacteria, whereby the simpler molecules formed in the first process are converted into gases (mainly methane and carbon dioxide. This second process is usually very pH sensitive and is considered to be the rate limiting step. When acid accumulates from the first process and the system is not properly buffered then the pH of the system drops and this affects the methane forming bacteria and generally affects the entire fermentation process. Similar observation, of changes in pH influencing microbial processes like anaerobic digestion and microbial fuel cells have been reported by Mbugua *et al.* (2018).

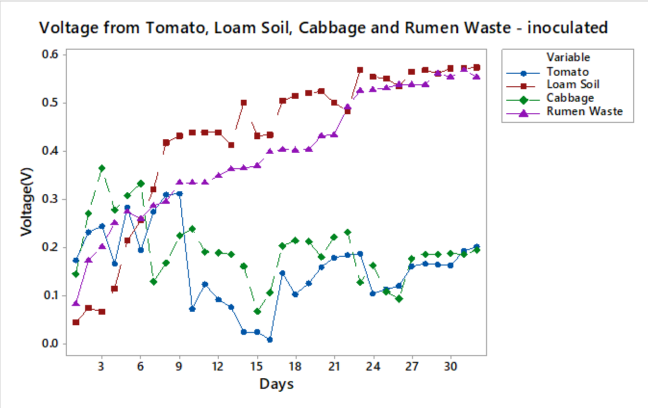


Figure 6 : Voltage generated from inoculate loam soil, tomato and cabbage set ups

The substrate pH is a vital factor which influences the microbial activity. the high calcium ions in soil and rumen fluid samples means that an acid- base balance is achieved as calcium ions buffers the mfc systems against pH changes The pH profile of the bio-anode chamber showed gradual increases (from 7 ± 0.02 to 8.3 ± 0.01) as the coulombic efficiency increases (Puig *et al.,* 2010).. Generally, the bio-electrochemical reaction in the MFCs shows an increase in pH after the bacterial growth (1 or 2 days), and then it exhibited a declined pH, which is due to the proton consumption by the cathodic reaction. It was also postulated that the bacteria would produce weak acid compounds as a metabolic product to maintain the intercellular pH (Sánchez-Clemente *et al.,* 2018; Puig *et al.,* 2010).

The changes in pH are due to the different components in the substrate in terms of the microbiota and structural components, such as glucose, which can be the producer of energy (Zhang *et al.,* 2009). However, due to the different microbial groups that can grow in the substrate, it is possible that the pH affects the ability of these microorganisms to produce bioelectricity, because there is an optimal pH in which the production performance is improved, which a variation above or below the optimum pH stopping the production. Neutral pH (6 to 8) is the optimal pH for an excellent performance of MFC and metabolic activity of microbes in both anode or cathode chambers (Shukla *et al.,* 2018; Puig *et al*., 2010). However, proton production during MFC process in MFCs leads to acidification of the anode compartment. Accumulation of protons creates a pH gradient resistance, which causes a loss of pH gradient potential over time (Strik *et al.,* 2011), which can reduce current density and electrode potential (Zhang *et al.,* 2013; Timmers *et al.,* 2010).

The movement of electrons from the anode results in increased proton concentration in the anodic chamber. High electric current also results in low pH at anode as reported by Zhang *et al.,* (2013). Effective approach should be employed to buffer the anodic pH, so that it remains close to neutral, when optimizing the power output of MFCs, especially at high current discharge (Zhang *et al.,* 2013).

Figure 7 shows the voltage generated when the rumen fluid was doped with chlorpyrifos, lambda cyhalothrin, malathion and the pesticides mixture and exposed to voltage generation using microbial fuel cells technology. The residues were added during the set up stage to allow initial system stability. From the figure 7, voltage generated showed an upward trend in the investigated retention time plateauing on day 21. The rate of voltage generation was higher in lambda cyhalothrin and malathion with lower voltage productions in pesticide mixtures.

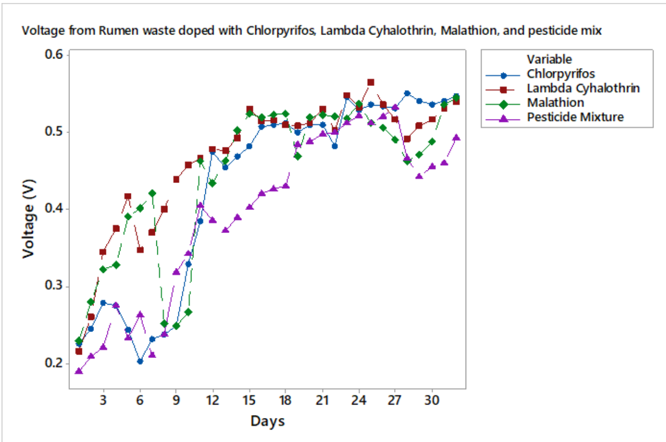


Figure 7 : Voltage from rumen waste doped with Chlorpyrifos, Lambda Cyhalothrin, Malathion and pesticide mix

The voltage produced by pesticide doped cabbages is shown in figure 8. An increasing trend was observed from day 0 to day 7 where 0.304 V, 0.219V, 0.410 and 0.335V were recorded in cabbages doped with chlorpyrifos, lambda cyhalothrin, malathion and the pesticides mixture, respectively.

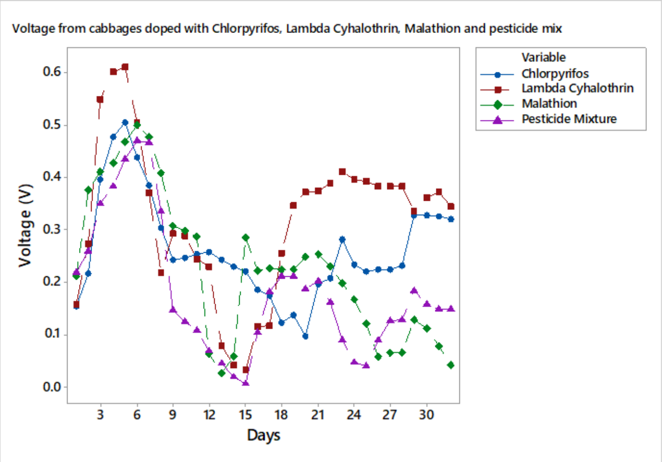


Figure 8 : Voltage from cabbages doped with Chlorpyrifos, Lambda Cyhalothrin, Malathion and pesticide mix

The downward trend is observed until day 14 where the voltage starts and upward trend up to day 21 for lambda cyhalothrin setup and then platoos. While the other setups show upward trend and downward trend interchangeably. This trend is observed due to the influence of pH on microbial activities. In the tomato doped with pesticides setup (figure 9), the trend is similar to the one observed in cabbages samples. An upward trend is observed for the first seven days and then a downward trend in voltage generated. The voltage starts increasing from day eight until day 23 where it starts dropping again. The highest recorded voltage was in lambda cyhalothrin set up at 0.582V on day 3. Influence of the acid formed during microbial breakdown is witness by the fluctuating trends of the voltage generated as shown in figure 9.

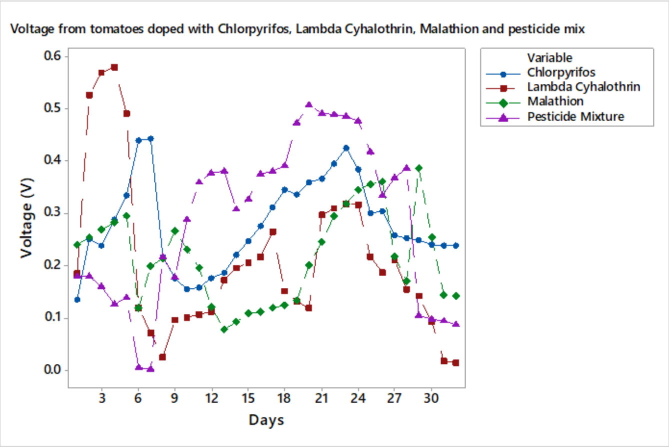


Figure 9 : Voltage from tomatoes doped with Chlorpyrifos, Lambda Cyhalothrin, Malathion and pesticide mix

The volatile matter in loam soil was the least compare to the other matrices. This means that the influence of pH on voltage generation in pesticide doped loam soil is minimal. This explains the voltage smooth curves in figure 10. The voltage produced increased from initial set up to day 12 and then stabilize up to day 25 where a downward trend is observed. The voltage generation showed a Gaussian/ normal distribution trend.

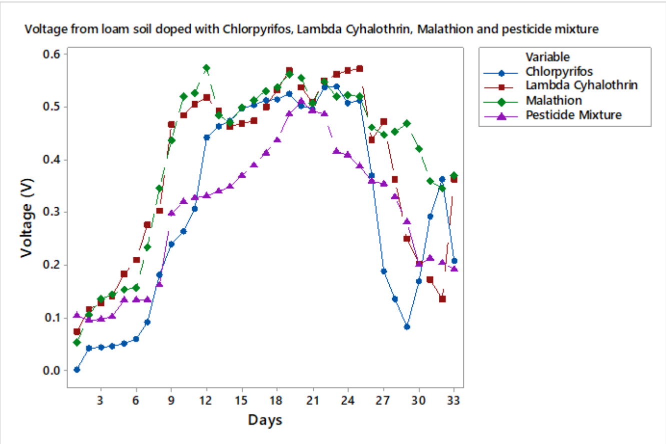


Figure 10 : Voltage from loam soil doped with Chlorpyrifos, Lambda Cyhalothrin, Malathion and pesticide mix

The voltage shown in figure 10 is almost twice compared to the control set for the same matrix. This clearly shows that microbe feeds on the pesticide's molecule and thereby bio-remediation of the molecules in the loam soil is possible.

IV. CONCLUSION

The results obtained shows that current (voltage) increased as the days went on but decreased from the 10th day on wards. Current was highest in the rumen followed by the cabbage and lowest in the loam soil. The number of carbon atom in the various substrate influenced the voltage output in the microbial fuel cell. In directly carbon seems to be food for the microbes. When the inoculum was introduced to the substrates this affected the pH hence voltage generation shot up notably. This is due to microbe activities verses voltage increase. When the substrate was doped with pesticide there was a general increase in voltage generated, this can be attributed to pesticides increasing carbon number and this continued till the pH, changed and this killed the microbes and voltage generation went down.

The higher the concentration of the microbes the greater the voltage and the vice versa. Also the higher the carbon content the higher the bioelectric energy production.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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