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Electricity Generation by *Clostridiumspp* and *Proteus* V*ulgari*s from Rotten Tomatoes in a Double Chamber Microbial Fuel Cell

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Abstract: *Low electron transfer efficiency from bacteria to electrodes remains one of the major bottlenecks that limit industrial applications of microbial fuel cells (MFCs). Elucidating biological mechanism of the electron transfer processes is of great help in improving the efficiency of MFCs. The electricigens and its activity have an important influence on the power generation capacity and organic matter degradation ability of MFC system.*

In the current study, rotten tomatoes were used as a substrate in a double chamber microbial fuel cells using Clostridiumspp. and Proteus vulgarisbacteria cultures. Proximate analysis of tomatoes wastes shows 4.38% and 85.63 % volatile matter and15.08 and 292.37 Kcal/100g energy for fresh and dry weight respectively. Maximum generated voltage was 0.622V, 0.465V and 0.759V from Clostridium Spp., Proteus and rumen fluid respectively. Daily current recorded was in the range of 0.04 to 0.059mA for rumen fluid inoculum.

The power density and current density of 8.12mW/m2 and 13.02mA/m2 were generated using Clostridium spp. which is five times compared to what was generated using Proteus vulgaris culture.

Keywords: *Clostridium, Proteus, Voltage, Microbial fuel cell, Tomatoes*

1. INTRODUCTION

Fruits and vegetables wastes are generated in large quantities and subsequently dumped on landfills to decay in the process emitting an odour and attracts birds, rats and vectors responsible for many diseases (Du Haixia, (2017). Effective and efficient management of both fruits and vegetable wastes require its characterization to understand its composition which influences its net microbial yield and kinetics activities during digestion (Afifi *et al.*, (2011). A research by Manley (1983) shows that the United States tomato packing house produces about $6.13*10^5$ tons of defective tomatoes annually. The culls are produced during processing, washing, pulping, juice finishing, packaging and storage. A kilogram of tomato produces 20g of culls and 20g of peels and skin. This requires a budget to dispose of the culls (Riggi and Avola (2008) and Toor *et al.*, (2012).

In Florida, nearly 400,000 tons equivalent to 40 percent of tomatoes produced do not end up in the produce section which lead to massive environmental waste (Alex Fogg, (2016). Pomance contains tomatine (i.e. solanine like alkaloid) which would make it inappropriate to be fed to livestock (Downard and Roddick (1995) and Lyons and Keeley (2008)). Tomatoes are rich source of indigenous redox mediators, carbon and electrons. Natural lycopene found in tomatoes is a strong mediator that encourages the generation of electricity (Fogg A *et al.*, (2015). A gram of tomato cull (i.e. defective tomato) contains half a micro-gram of riboflavin and thiamin both of which can serve as electron transfer mediators to promote extracellular respiratory capabilities of exoelectrogens in MFCs (Curtis *et al., (*2010). The pomace can also serve as a source of electron-donor in the anode of a microbial fuel cell (MFC).

Electric gens are a kind of microorganism which can completely oxidize organic matter by using the electrode as the sole electron acceptor. They are essential for the bioelectrochemical processes in MFCs (Vilajeliu - Pons A. *et al.*, (2016). Electric gens in the anode chamber oxidize organic matter to release electrons to the anode electrode. Electrons donated to the anode flow to the cathode through electrical wires, where they are reunited with the protons generated in the anode chamber and combine with oxygen or other electron acceptors to form reduced product.

Various types of microbes have been used in MFCs. A major challenge to the development of MFC as a novel electricity generating technology is harnessing the linkage of electricigens to the electrode substrate *via* electron transport. To prove up the species distribution, growth characteristics, metabolic characteristics and electricity mechanism of electricigens will contribute to the development of MFC *(*Fan, L., & Xue, S. (2016).

In this study, electricity production was done using defective tomatoes from Kangemi and Wakulima market in a double chamber microbial fuel cell using *Clostridium* and *Proteus* cultures isolated from a running MFC using rumen fluid as inoculum. Voltage, current, power and current density were characterized for 30 days.

2. MATERIALS AND METHODS

Rotten tomatoes used in this study were obtained from Kangemi/Wakulima market in Nairobi County while rumen fluid was obtained from Dagoretti slaughterhouse in Kiambu County. The defective tomatoes were homogenized using a kitchen blender before loading to the anodic chamber of a double chamber MFC. Bacteria containing sample was obtained from anodic chamber of a voltage generating microbial fuel cell with cull as the substrate previously inoculated with rumen fluid.

2.1. Analytical Methods

The physico-chemical parameters in defective tomatoes were performed according to the standard methods by APHA (1998). Results are expressed as average of replicate ± standard deviation

2.2. Bacteria Studies

2.2.1. Growth Media

The isolation and routine sub-culturing of MFC bacterial isolates were carried out in Blood and MacConkey nutrient media.

2.2.2. Isolation of Bacteria from MFC

For the isolation and screening of bacteria, about 1ml of MFC sample was measured and poured into a test tube containing 9ml of autoclaved nutrient media and incubated under anaerobic condition for 24h at 37ºC to enhance the growth of the anaerobic bacteria. Following incubation, the test tube was then manually agitated to form a uniform solution and allowed to stand for some seconds for the larger particles to settle down. The obtained dilution of 10^{-1}(w/v) was further serially diluted to 10^{-5} (w/v) whereby 100µl of the 10^{-5} dilution was then spread plated on the nutrient agar plates and further incubated at 37°C for 24h. Following incubation, distinct pure colonies obtained on the plates were isolated and purified using streak plate method and transferred to nutrient agar slants as stock. The bacterial isolates were then used as inoculum in the anaerobic anodic chamber of MFC containing defective tomatoes as the substrate to check their individual potential for electricity generation.

2.3. Fabrication of MFC

Double chamber microbial fuel cells were fabricated as described by Mbugua *et al.*, (2017). The electrodes used were graphite rods from spent batteries with a maximum surface of 0.00399m^2. The salt bridge was made using lamp wicks and 3% agarose in sodium chloride. The assembly of the H-shaped MFC as shown in figure 2.1 (Kamau *et al.*, (2017).

Figure2.1. *Double chamber MFC with a voltmeter and batch MFC.*

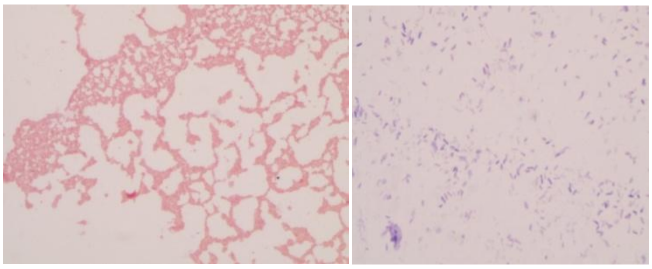
2.4. Voltage Generation

Voltage generation was investigated for every isolate as well as a combination of the cultures. Blank set up was made using defective tomatoes. Voltage and current were recorded daily using a voltmeter while power, current and power density were as per Mbugua *et al.*, (2017).

3. RESULTS AND DISCUSSIONS

3.1. Bacteria Cultures

Microscopic and bio-chemical studies of the cultures confirm that *Proteu*s and *Clostridiumspp*. are present in the anodic chamber of a microbial fuel cell. The images obtained from an scanning electron microscope is shown in figure 3.1.



(a) Proteus *(b) Clostridium*

Figures 3.1. *Electronic microscope images of Proteus and clostridium*

Proteus vulgaris is a rod-shaped, nitrogen reducing gram-negative bacterium which can be found in soil and fecal matter. On the other hand, *Clostridium* is obligate anaerobes gram positive bacteria (Harley *et al*., 1996; Brooks *et al.*, 2007). The two cultures were isolated from the anodic chamber of microbial fuel cell.

3.2. Tomato Analysis

According to Rossini *et al.,* (2013) tomato is one of the widely produced fruits in the world for direct use or for processing to other products like sauce. Table 1 shows the proximate properties of tomatoes in wet and a dry weight basis. The moisture content on fresh tomato wastes was 95.16 compared to 4.84% on dry weight basis. Previous studies by Mohammed S. *et al.*, 2017 showed moisture content of 90.75%. Moisture content reported in this study is slightly higher but in range with previous studies by Oko-Ibom and Asiegbu, (2007), Adubofuor *et al.*, (2010) and Hossain *et al.*, (2010) who have reported the moisture content in the range of 88.19 - 90.67%.

Table1. *Proximate properties of tomatoes in percentages*

Property	Wet Weight	Dry Weight
Moisture	95.16±1.23	4.84±0.06
Volatile Matter	4.38±0.03	85.63±1.09
Carbohydrates	2.93±0.02	55.42±0.56
Protein	0.57±0.01	11.89±0.69
Fat	0.12±0.01	2.57±0.02
Ash	0.46±0.02	9.53±0.32
Mineral Matter	0.51±0.03	10.48±0.25
Energy (Kcal/100g)	15.08±0.09	292.37±1.56

The average ash content in fresh tomatoes was in range with previous proximate studies by Mohammed *et al.*, (2017), Adubofuor *et al.,* (2010) and Suleiman *et al.*, (2011) who reported values ranging from 0.2 – 0.4%. The reported average protein levels were lower than those found by Mohammed *et al.,* (2017) of 2.26%.

3.3. Voltage

Voltage produced was recorded daily using a multimeter and used to plot figure 3.2. High voltage was recorded in the set up inoculated with rumen fluid. Rumen fluid contains a wide range of microbes which in the process breakdown substrate and in the process generate electricity (Mbugua *et al.*, (2018). Previous studies carried out to compare power generation from different fruits wastes reported tomato inoculated with rumen fluid generated open circuit voltage of up to 0.702V (Kamau *et al.*, (2018).

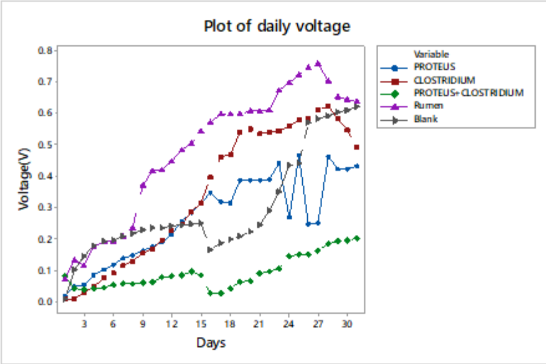


Figure3.2. *Plot of daily voltage using different culture*

Lower voltage was recorded in mix culture of *Clostridium* and *Proteus* compared to pure cultures. This contradicts what was observed by S. Fatemi *et al*., (2012) who observed that mix culture produce more voltage than pure ones. Rismani-Yazdi *et al*.,(2007) used rumen microorganisms as inoculum to produce electricity from cellulose, in an H-type MFC; the voltage reached a steady state level of 470±2mV after 14 days and an external load of 1000Ω. In another study voltage was generated by Ren *et al.,* (2007) using a binary culture of *Clostridium cellulolyticum* for the fermentation of cellulose into fermented compounds, such as acetate and ethanol, and *Geobacter sulfurreducens* to transfer electrons from these fermented products to generate electricity, without enzymatic pretreatment or an exogenous catalyst.

The daily current generated is shown in figure 3.3. Rumen fluid inoculated set up registered the highest current explained by higher microbe's population resulting in higher substrate breakdown rate (Mbugua *et al.,* (2017).

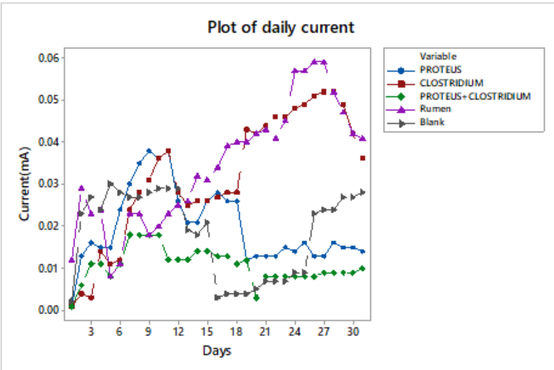


Figure3.3. *plot of daily current production*

The current generated using Proteus was highest on the 10th day at 0.038mA with voltage of 0.191V. In another study using the same culture a voltage of 0.5V was recorded at 37^{0}C (Namjoon K. *et al.*, (2002). The figure (3.4) shows daily power calculated by multiplying voltage by current. Power was highest in the set inoculated with rumen fluid followed by the set with *Clostridium.*

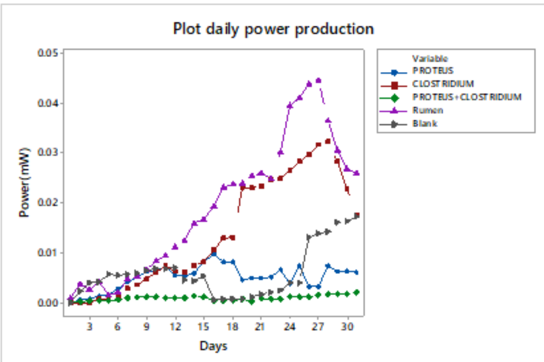


Figure3.4. *Plot of daily power production*

The current density shown in figure 3.5 was obtained by dividing current with the anodic electrode surface area.

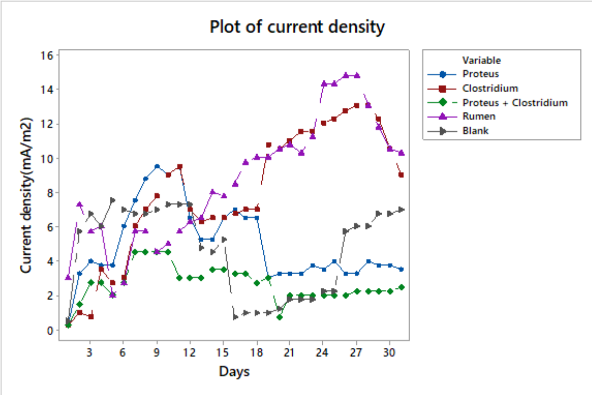


Figure3.5. *Plot of daily current density*

Figure 3.6 shows surface plots of daily power and current densities for the different cultures. Power density was obtained by dividing power by electrode surface area.

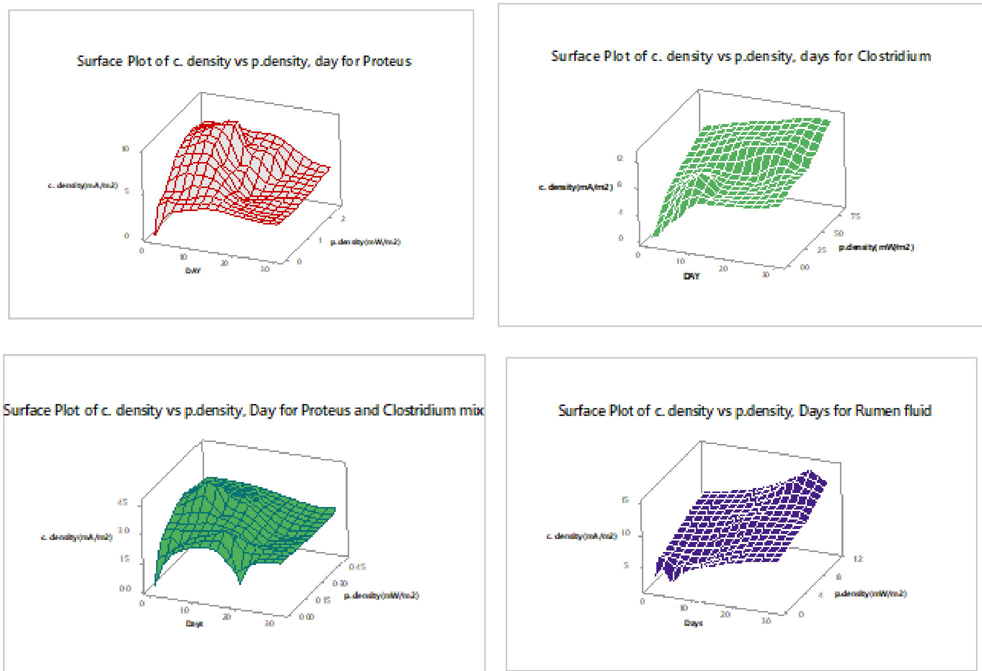


Figure3.6. *Surface plots of daily power and current densities*

4. Conclusion

In the current study, we tested the viability of power production from rotten tomatoes using rumen fluid, pure and mix cultures of *Proteus* and *Clostridium*. High power production from rumen fluid and *Clostridium* was recorded. Deep understanding on electricigens and their electron transfer mechanism will help to provide more effective methods to improve the performances of MFCs and subsequent application in electric devices.

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