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Research Article

Kinetic Studies and Simulation of Microbial Fuel Cells Voltage from *Clostridium Spp.* and *Proteus*

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ABSTRACT

Microbial Fuel Cells (MFC) can be employed in bio-remediation of organic pollutants. In In this study, MFC voltage generated from various fruit market wastes using *Clostridium spp*., *Proteus spp*. and rumen fluid microbes was fitted into linear, logistic and Gompertz growth models. The dual chamber MFC was constructed using 1.0 liters plastic containers. NaCl in 3% agarose based salt bridge was used to connect the two chambers while graphite rods and copper wires were used as electrodes. The study was done by inoculating microbes present in about 250 mL cow dung mixed with 250 mL water and 500 g of homogenized fruit wastes and market wastes respectively, then current/voltage generated measured for 24 days. The control experiment had 250 mL cow dung spiked with water to 1000 mL. From this study, microscopic and biochemical studies of the cultures confirmed that *Proteus* and *Clostridium spp*. were found in the anodic compartment of MFC. The rumen fluid inoculate registered the highest current (0.074 mA) explained by a higher microbe's population resulting in a higher substrate breakdown rate. Low voltage was recorded in a mixed culture of *Clostridium spp*. and *Proteus* ssp. compared to pure cultures. The Gompertz equation growth model was applicable, with regression values of 0.967 compared to 0.922 obtained in linear data fitting. The same was well reflected by the simulating growth model of *Clostridium spp*. In both cases, the voltage generated from the pure cultures could not be explained linearly due to low R^2 of 0.911 and 0.962 for *Clostridium spp* and *Proteus* respectively compared to 0.96 and 0.98 for the Gompertz equation fitting.

Keywords: Bacterial inoculation; Deficit irrigation; Fatty acid; Flax; Oilseed; Protein

INTRODUCTION

Microbial bioremediation strategies can be either *ex-situ* or *in-situ*. *Ex-situ* techniques consist of transporting pollutants from polluted sites to another site for treatment, while *in situ* techniques treat polluted substances at the site itself. *Ex-situ* remediation technique owing to its limitations is not considered as a choice of cleanup by many researchers. It may or may not be lucrative at particular sites and may be possible that the microorganisms which assisted in clean-up of pollutants under *in-vitro* conditions fail to remove them effectively under *in-vivo* conditions [1-3]. The mode of action and growth of microorganisms in polluted sites needs to be more studied for a better understanding [4]. Microbial fuel cells (MFC) can be employed in bio-remediation of organic pollutants like market wastes, pesticides etc. [5]. Microbial Fuel Cell is a device that uses bacteria as a biocatalyst to breakdown organic matter thereby generating current. These bacteria, called exoelectrogenic bacteria are oxidizing organic substrates to release electrons, which then harvested in an external circuit to produce bioelectricity [6]. Despite all the potential, the bioelectricity production from MFC is still low and its relation with MFC conditions is uncertain as it is highly affected by any parameter that influences microbial activities [5]. Therefore, this study models the voltage generated from various market wastes in attempt to simulate the amount of voltage that can be obtained from various market wastes.

METHODOLOGY

Microbial fuel cells construction

As anode and cathode chambers, two 1.2 liter containers were packed as shown in Figure 1. The wire was inserted through two small holes drilled into the caps of the containers. A 5.7 cm long and 0.7 cm diameter graphite rod electrode was connected to one end of the copper wire. 2.5 litres of 1 M NaCl, 3 percent agarose solution, and lamp wicks were used to make a salt bridge. The wicks were boiled in a NaCl and 3% agarose solution for 10 minutes before being placed in the freezer at -4°C to solidify. The solidified salt bridge was passed through PVC pipes and secured

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to the chambers with Araldite adhesive, ensuring that they were leak-proof.

Circuit assembly

The double chamber MFC was put together as depicted in Figure 1. The voltage and current was taken regularly *via* a multi-meter connected to copper wires joined to the carbon rods [7,8].

Figure 1: Set-up of H-shaped microbial fuel cells with a multi-meter.

Isolation and culturing and identification of microbes form anodic chamber

A sample containing microbes was taken from the anaerobic anodic chamber of a running MFC and microbial community cultured, isolated and identified, the following plates were obtained in Mac-Conkey and blood agar. In Figure 2, the anodic chamber sample was stained in a dish and two distinct cultures isolated. The isolates were then removed from the initial plate and cultured in blood and Mac-Conkey agar, as shown in Figure 2.

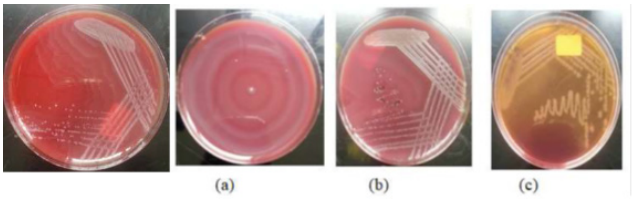

Figure 2: Anodic chamber sample stained plate, plates of microbes in the anodic chamber of MFC (a) and (b) in Blood agar and (c) in Mac-conkey agar.

Rumen fluid and cow dung were used as a microbe source in market waste microbial fuel cell. Further, the isolated microbes were also employed as bio-catalyst in voltage generation from the fruit wastes.

Investigation of the potential of fruit wastes and cow dung

Around 500 g each of watermelon, avocado, banana, tomato, and mango were cut into smaller sizes, minced with a meat mincer, and homogenized then put into the anodic chamber. About 500 ml distilled water was loaded in the cathodic chamber. A fruit mixture was also produced. To introduce the microbes, 250 ml cow dung in 250 ml water was added to each cell. The control experiment was 1000 ml cow dung in water. The current and voltage coming from the cells were measured every day for a period of 24 days.

RESULTS AND DISCUSSION

Microscopic and biochemical studies of the cultures confirmed that *Proteus* and *Clostridium spp*. were found in the anodic compartment of MFC. The images obtained from an electron microscope are shown in Figure 3. These results compare with a previous study by Gagandeep et al., (2017) who identified Bacillus subtilis, *Clostridium spp*, *Peptostreptococcus Species*, *Bacillus Cereus* and *Bacteroides Species* in the anodic chamber of a running MFC which aided in electricity generation in the MFC [9]. The isolated microbes found in this study are also comparable to others [10-15].

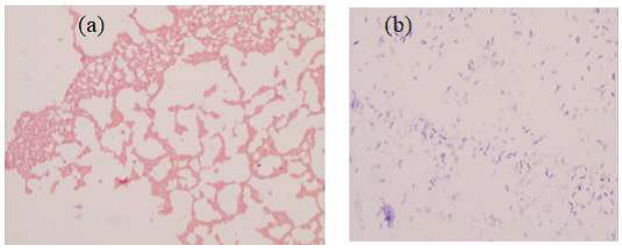

Figure 3: Electron microscope images of (a) *Proteus* and (b) *Clostridium sps* . bacteria.

Proteus spp is a gram-negative proteo-bacterium found in decomposing animal matter, sewage and manure soil. It is also widely seen in the mammalian intestine. Proteus Vulgaris commonly grow in the Mac-Conkey agar culture plate. *Clostridium* is a rod shaped genus of gram-positive bacteria that are obligate anaerobes. This means that they are killed by exposure to atmospheric oxygen (20.9 5%) [16]. The voltage produced from decaying tomato wastes is shown by plots (Figure 4). In a study using five cultures, *Paracoccus homiensis* and *Pseudomonas aeruginosa* produced the maximum voltage of 320 mV and 300 mV, respectively. *Bacillus thuringiensis* had the least voltage of 150 mV. Likewise, Paracoccus sp and *Pseudomonas ssp* gave the maximum current of 10 mA and 20 mA, respectively [17]. MFC performance differs for every bacterium. For example, 10.89 mA and 10.45 mA current were generated by *Saccharomyces cerevisae* and *Clostridium acetobutylicum* after 10 days of operation [17].

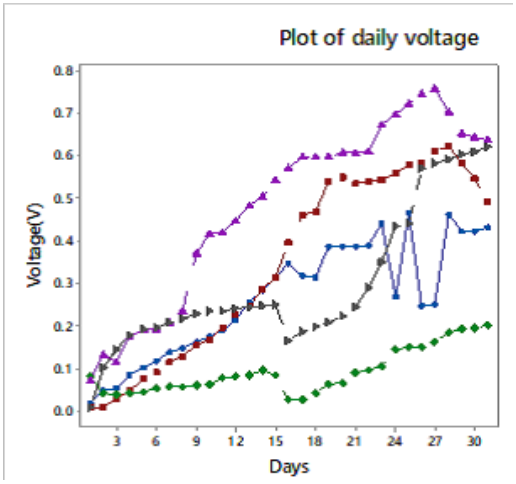

Figure 4 : Electron microscope images of (a) *Proteus* and (b) *Clostridium sps*. bacteria. **Note:** Variable (—●—) *PROTEUS*; (—■—) *Clostridium*; (—◆—) *PROTEUS+Clostridium* ; (—▲—) Rumen; (—►—) Blank.

Low voltage was recorded in a mixed culture of *Clostridium sps.* and *Proteus* sps. compared to pure cultures. This is explained by the fact that the two cultures require individual time to adapt to the anodic chamber environment in addition to collective time to adapt as a mixed culture [18]. This contradicts what was observed by Fatemi et al., 2012, who claimed that diverse culture produced more voltage than pure ones [19]. 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 ± 2 mV after 14 days and an external load of 1000 Ω [20,21]. In another study, the voltage was generated using *Clostridium cellulolyticum* utilizing cellulose as a substrate while electron transfer *Geobacter sulfurreducens* was used [22,23]. The daily current generated is shown in Figure 4. Rumen fluid inoculated set up registered the highest current (0.074 mA) explained by a higher microbe's population resulting in a higher substrate breakdown rate as per the total viable count data [8].

Voltage simulation and modeling

The main product of MFC is voltage generated from microbial breakdown of organic matter. The voltage generated was fitted into various kinetic models used to explain microbial activities. The models are linear, Gaussian and Gompertz models. In the linear model, it is assumed that voltage production rate will increase linearly with increase in time and after reaching a maximum point after sometime it would decrease linearly to zero with increase in time (Equation 1).

$$V = a + bt \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. (1)$$

Where V is voltage generated, a and b are constant while t is the retention time. The fitted linear plots are shown in Figure 5. The linear fitness is indicated by R^2 where in this case, mango fruit waste inoculated with rumen fluid was 0.9987 while for the other fruit wastes, the fitness ranged from 0.234 to 0.675 showing non –linear fitness.

In addition, voltage production was simulated using Gompertz (Equation 2).

$$V = ae^{-be^{-ct}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. (2)$$

Where V is voltage, a, b and c are constants while t is the retention time in days. The experimental voltage generated by *Proteus spp.*, *clostridium spp*, *Proteus spp.+clostridium spp* and rumen fluid microbes was fitted in linear and Gompertz growth models. The results obtained for melon and tomato are shown in Figure 6 where the fitness was observed to be 0.987 in melon compared to 0.786 in tomato wastes. The other fruits showed low regression values to Gompertz model in the range of 0.123 to 0.367 and therefore were unfit.

On modeling voltage from pure cultures, the daily voltage from *Proteus spp.* was fitted onto linear and Gompertz model as per equations 1 and 2 and the resultant plots are shown in Figure 7. The linear model showed a fitness value of 0.922 while Gompertz showed a fitness value of 0.976. This means that the voltage generated from the mixed market wastes inoculated with *Proteus spp.* can be explained by Gompertz model.

Similarly, the plots for the linear and Gompertz model for *Clostridium spp* is shown in Figure 8 where the fitness was 0.911 and 0.985 for linear and Gompertz model respectively.

The results shown in Figure 7 shows that the growth of *Proteus* culture, which translates to voltage production is well explained by the Gompertz equation growth model with regression values of 0.967 compared to 0.922 obtained in linear data fitting. The same is well reflected by the simulating growth model of *Clostridium spp.* as shown in Figure 8. In both cases, the voltage generated from the pure cultures cannot be explained linearly due to low R^2 of 0.911 and 0.962 for *Clostridium spp* and *Proteus* respectively compared to 0.96 and 0.98 for the Gompertz equation fitting.

Figure 9 shows the best fits for the rumen fluid voltage and the *Clostridium* ssp.+ *Proteus* culture mix simulated models. The voltage produced from rotten tomato wastes by rumen fluid microbes is better explained by the Gompertz growth model while the mixed culture voltage fitted the linear model best. Only the best-fit curves are shown.

The regression coefficient of the *Clostridium spp.+Proteus spp.* culture mix was 0.911 for linear plot compared to 0.87 for the Gompertz plot. This means that the Gompertz model should be employed in explaining electricity generation from MFC with a high concentration of microbes.

Finally, the voltage generated from mixed fruit market wastes was fitted onto modified Gompertz model depicted by equation 3 and the resulting plot is shown in Figure 10.

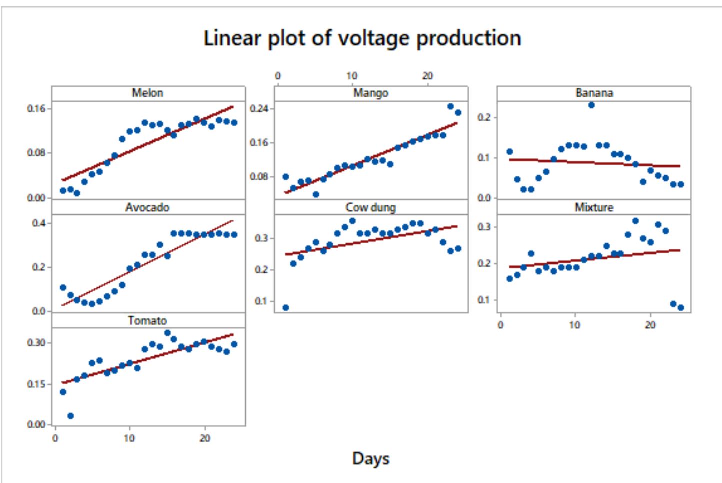

Figure 5: Linear plots of voltage generated from various market wastes.

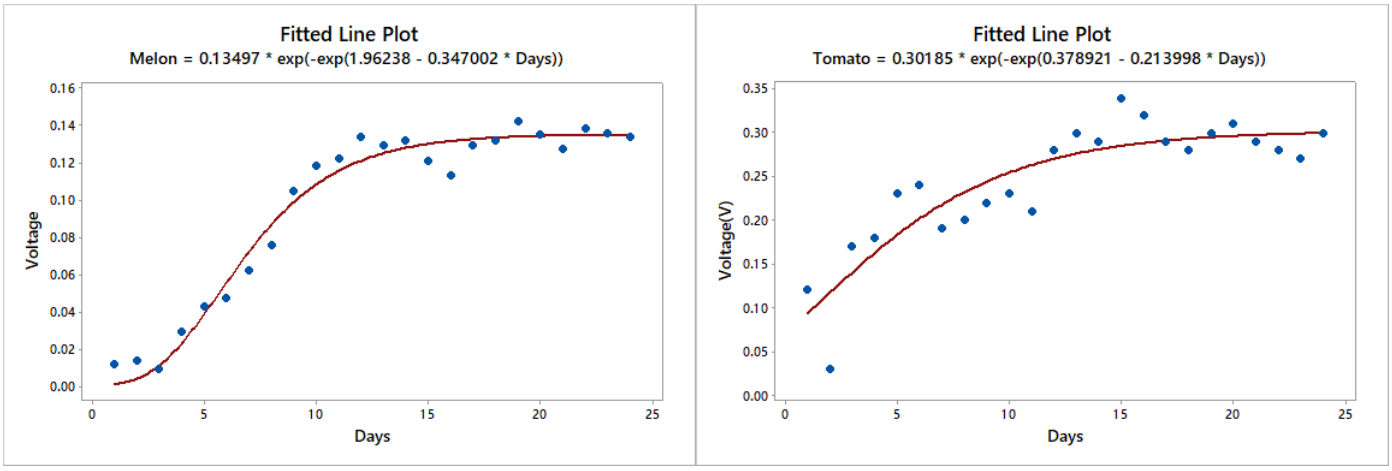

Figure 6: Voltage fits onto Gompertz model for melon and tomato wastes.

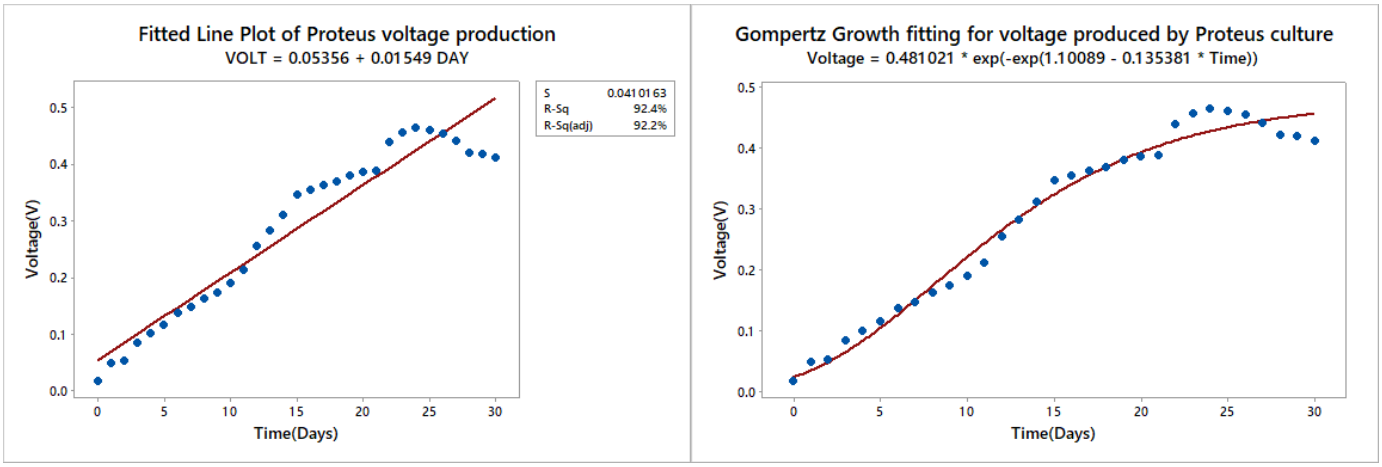

Figure 7: Fitted plots for voltage generation by *Proteus* a) linear b) Gompe rtz.

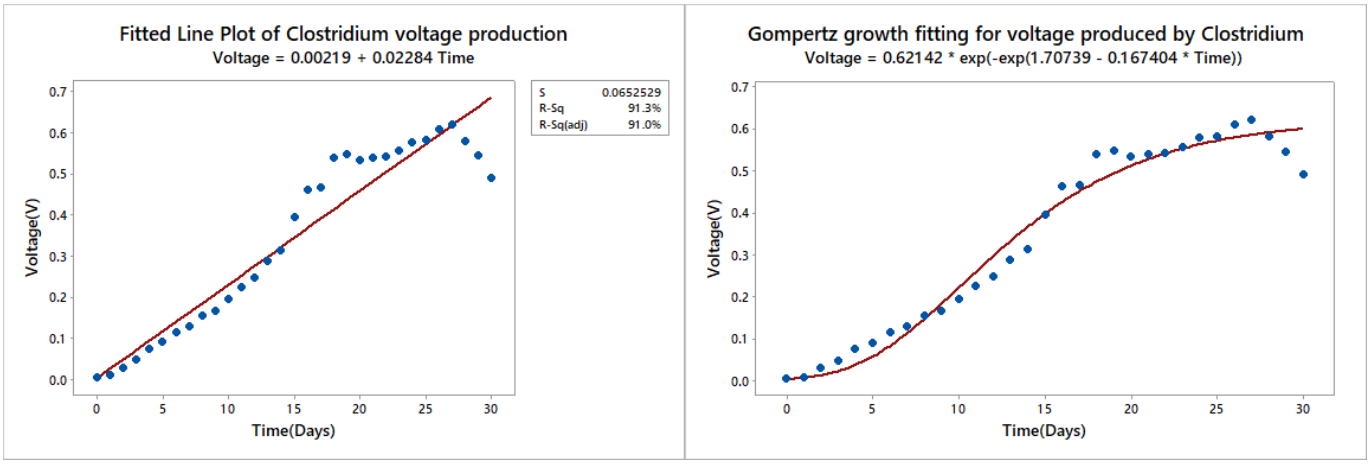

Figure 8: Fitted plots for voltage generation by *Clostridium spp* a) linear b) Gompertz .

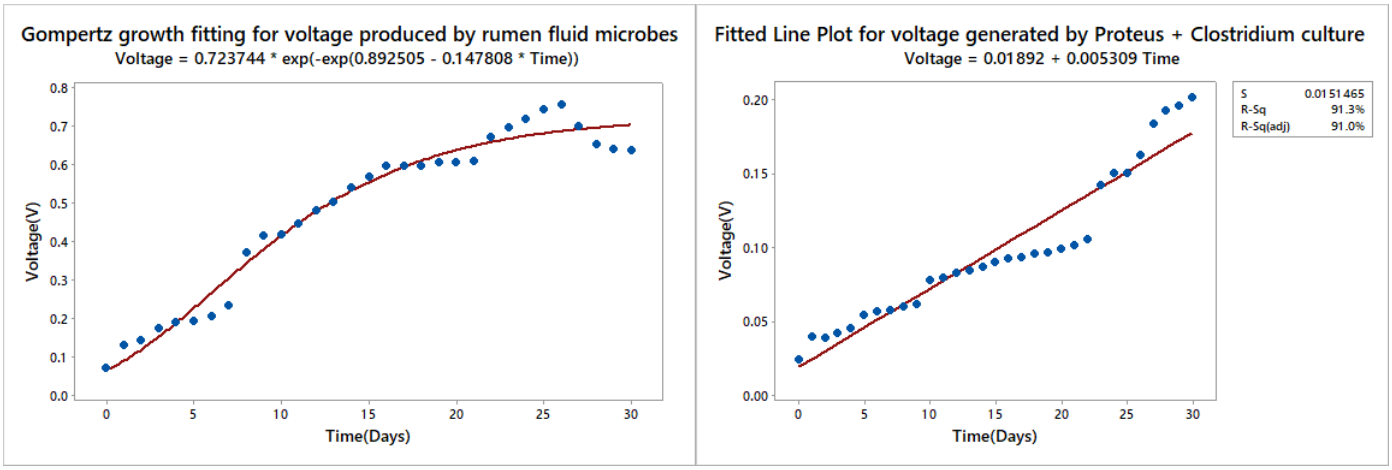

Figure 9: Gompertz and linear fitted plots for voltage generation by (a) rumen fluid and *Clostridium spp* + *Proteus* sps microbes.

Clostidium sps

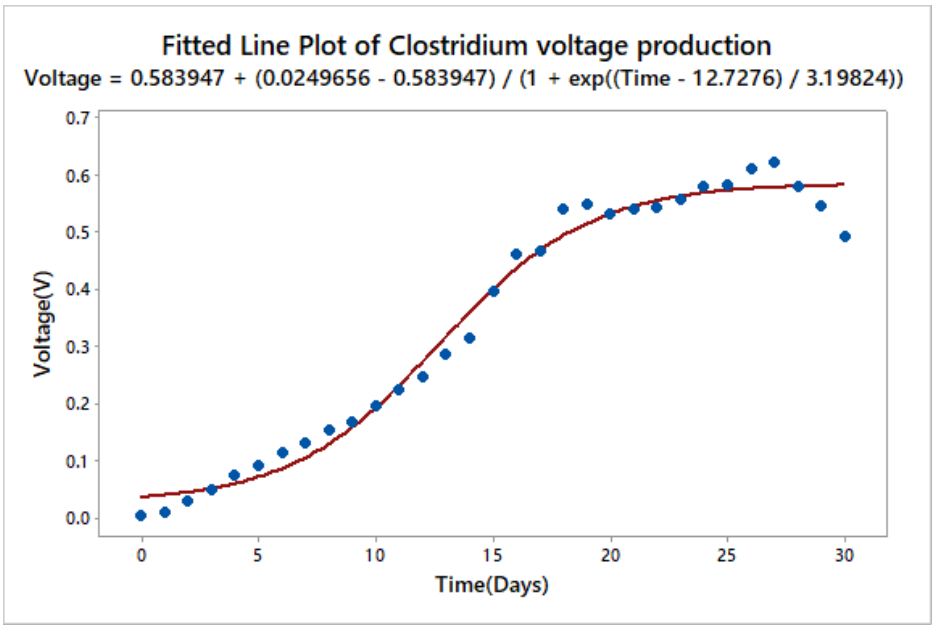

Figure 10: Voltage fitted onto modified Gompertz model.

CONCLUSION

The MFC for rumen fluid inoculate registered the highest current (0.074 mA) explained by a higher microbe's population resulting in a higher substrate breakdown rate. Low voltage was recorded in a mixed culture of *Clostridium spp*. and *Proteus spp*. compared to pure cultures. The Gompertz equation growth model was applicable, with regression values of 0.967 compared to 0.922 obtained in linear data fitting. The same was well reflected by the simulating growth model of *Clostridium spp*. Hence, the modified Gompertz model can be used to simulate voltage generation from different market wastes in MFC.

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