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Kinetic Modelling of Microbial Fuel Cell Voltage Data From Market Fruit Wastes In Nairobi, Kenya

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ABSTRACT

Indiscriminate dumping of organic waste is a global problem rapidly being recognized as a health hazard since it attracts rodents and pests that risk human life. As a result, investigation of ways to manage it, which, among other things, involves investigation of the kinetics of its degradation by microorganisms, is crucial. Generally, mathematical and computational models assist in building a connection between input and output variables. This study examines the use of Monod, Haldane, and Han-Levenspiel models to model growth curves obtained from electricity obtained from microbial fuel cells (MFCs) loaded with fruit waste from Kenyan markets. The accuracy of the fitted model was assessed using JMP statistical analysis to provide a test of significance for each market fruit waste collected for the study. Kinetic constants of each model were determined as follows: the constant for the Monod model ranged from 99.57mgL^{-1}to 99.63mgL^{-1}, the constant for the Andrew Haldane model ranged from 50.09 mgL^{-1} to 50.18 mgL^{-1} and that of Han-Levenspiel model ranged from 91.09 mgL^{-1} to 100.75mgL^{-1}. Multivariate data analysis of market fruit waste (treatment) against both Voltage and current indicated a significant difference in fruit waste mixture and the banana waste, while fruit waste versus Power showed no statistical significant difference in output ($P<0.05$) in all the treatments. The findings show that extensive use of mathematical models can give a new understanding of how degradation inhibits the bacterium's electricity production, thus leading to new insights into predicting the progress of organic waste reduction in bioremediation analyses. The current study suggests that bacteria consortia have great potential in biodegrading market fruit waste in a fuel cell hence generating electricity. As a result, a successful scale-up process should include material and design optimization that allows for a cost and energy-efficient technology, more lab-based

and field-based research work to develop this technique for large-scale applications.

Keywords : Fruit Waste, Inhibitor, Microorganism, Treatment, Segregation, Kinetic Models

I. INTRODUCTION

Agriculture-related renewable and sustainable energy has become increasingly important in recent years. Agriculture generates massive amounts of waste, and how this waste is used could be a key element in energy production. Furthermore, the demand for energy in industry and everyday life is continually increasing, necessitating further study to solve massive waste generated. The solution to this issue is developing appropriate technologies for high-efficiency and cost-effective energy production and the use of second-generation energy sources, such as agricultural waste [1]. Due to rising costs of non-renewable energy and environmental effects, biological hydrogen production catalyzed by microorganisms is a possible alternative [2]. Bio-hydrogen is a novel renewable energy carrier that is more environmentally friendly than other renewable energy carriers such as wind, hydropower, biofuel, solar, and geothermal energy. Light-dependent activities and dark fermentation are the two main strategies to produce hydrogen via biological conversion pathways [3]. A microbial fuel cell (MFC) is a device that converts chemical energy to electrical energy using microorganisms as catalysts. For energy generation, a microbial fuel cell uses the electron-extracting characteristics of the bacteria that are connected to the Anode. Organic material is oxidized by these bacteria and releases carbon dioxide and protons into the anode chamber solution. Electrons are transmitted to the Anode, then to the cathode, consumed to reduce oxygen via an external electrical conductor. Meanwhile, protons pass via a membrane into the cathodic chamber. As a result of the flow of electrons, a current is generated [4]. In order to generate more energy, the standard technique employed by researchers is to stack several MFCs [5]. Furthermore, the incorporation of a better design MFC modules, leads to a more efficient electrochemical treatment and higher levels of usable electricity, which can be used to power indoor lighting [6]. As a result, this study aims to examine the kinetics of reducing fruit waste by bacteria in the MFCs and assess the model's limitations using statistical analysis. The findings could be used in secondary modeling studies, such as those looking at the effects of pH, temperature, and external resistance on reduction kinetics. As a result, new data and results should emerge, potentially spurring and revealing new knowledge and improvements to previous work [6].

1.1. Microbial growth phases

A previous study has found that voltage outputs are proportional to the pace of microbial growth, which is divided into phases [7]. Microorganisms in the lag phase slowly adapt to their surroundings, grow slowly, and once adapted, quickly metabolize the organic stuff available and reproduce, increasing their number numerically. The Log phase is a stage in which cell growth slows and strikes a balance with dying cells. The microbial population in the stationary phase remains stable. Finally, the Death stage occurs when all organic substance in the environment has been depleted, and all cells begin to die, as shown in **Figure 1,** [8].

Microbial Growth Curve

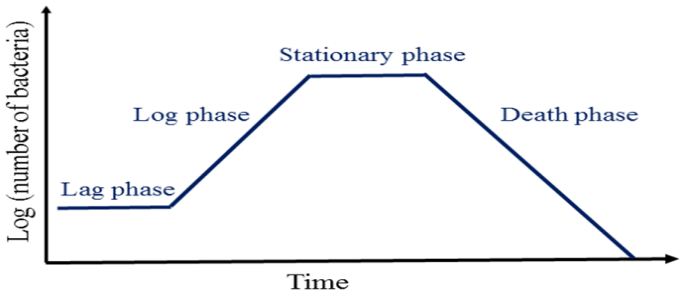

Figure 1: A typical bacterial growth curve [9].

II. MATERIALS AND METHODS

2.1. Study area and sample collection

The samples used in this study include Rumen fluid from a slaughterhouse in Huruma Estate (1˚15'16. 4" S 36˚52'42.4"E), fruit wastes from Ngara Fig-tree (1°16'27.9" S 36°49'20.6" E), Muthurwa (1˚17'13.3"S 36˚49'56.2"E), Kangemi (1˚15'52.0"S 36˚44'54.4"E), and City park markets (1˚15'42.1"S 36˚49'33.6"E) all located in Nairobi County Kenya as shown in **Figure 2.**

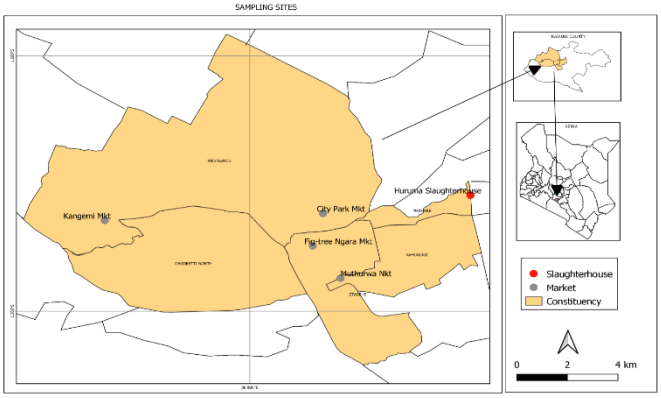

Figure 2 : The map of Nairobi County in Kenya and the sampling sites for fruit waste and Goat rumen fluid.

2.2. Statistical analysis

The quality of fit to the same experimental data was statistically tested using JMP methods to determine whether there is a statistically significant difference between distinct fruit waste and the varied parameters.

2.3. Simulation design

2.3.1: Models of Substrate utilization and Current density at the Anode

The kinetics of anode respiration bacteria (ARB) are closely linked to energy generation and substrate use. Several models have been devised to determine the kinetic parameters of ARB due to the participation of the anode biofilm [11]. The gradient in substrate concentration and the potential difference between the terminal electron acceptor and the Anode are two aspects to consider when determining kinetic parameters [12]. The Monod model (Equation 2.1), in which a single substrate limits the bacteria's growth, was the most utilized in prior studies [12-14]. Despite this, Haldane Andrew's kinetics model was devised to account for this shortcoming, which includes the substrate inhibition effect (Equation 2.2). Intense Osmotic pressure or substrate toxicity may cause substrate inhibition [15]. The Han-Levenspiel model (Equation 2.3) was used to characterize microbial growth ceasing entirely when a threshold inhibitor concentration ($\mathbf{S_m}$) was achieved, and so accounted for competitive, uncompetitive, and noncompetitive inhibition [16].

Monod Model:

$$r = r_{max} * \frac{S}{(K_S + S)} \qquad (2.1)$$

Andrew's Kinetic Model:

$$r = r_{max} * \frac{S}{\left(K_S + S + \frac{S^2}{K_{IH}}\right)} \qquad (2.2)$$

Han-Levenspiel Model:

$$r = r_{max} * \frac{S\left(1 - \frac{S}{\mathbf{S_m}}\right)^n}{S + K_S * \left(1 - \frac{S}{\mathbf{S_m}}\right)^m} \qquad (2.3)$$

Where:

r - Is the substrate utilization rate (g/L. d), the maximum output current density (mA/m^2) or Maximum power density (mW/m^2) or Voltage (mV) at each substrate concentration.

$\mathbf{r_{max}}$ – Is the substrate utilization rate (g/L. d), the maximum output current density (mA/m^2) or maximum power density (mW/m^2) , or Voltage (mV) among all range of substrate concentration., **S**– Is the substrate concentrations (mgL^{-1}). , $\mathbf{K_S}$**–** Is the half-saturation coefficient (mgL^{-1})., $\mathbf{K_{IH}}$ – Is the self-inhibition coefficient (mgL^{-1})., $\mathbf{S_m}$ **–** Is the critical inhibitory concentration above which growth stops (mgL^{-1})., **n/m**– Are the empirical constants used to account for different types of inhibition.

2.4. Microbial Fuel Cells Construction

The anodic and cathodic chambers were prepared in two 2-liter containers. Through two small holes punched in the tops of the containers, a wire was introduced. One end of the copper wire was attached to a 5.7cm long graphite rod electrode with a 0.7cm diameter. 2.5 liters of 1M NaCl, 3% agarose solution, and lamp wicks were used to make a salt bridge. The wicks were boiled in a NaCl and 3% agarose solution for 15 minutes before being frozen at -4°C. The solidified salt bridge was transferred through PVC pipes and then sealed with Araldite adhesive between the two chambers, making it leak-proof. (**Figure 3**).

Figure 3: Double chamber microbial fuel cell setup and digital multimeter DT9205A-(Output readings).

2.5. Sample collection of fruit wastes and Rumen fluid

Waste samples were collected from selected markets in Nairobi city, as shown in **Figure 2**. The wastes were segregated and left to stand in the laboratory for three days to decompose naturally. After that, the various fruit wastes were blended separately and kept under refrigerated conditions for use. Fresh Rumen fluid and cow dung were collected from *Huruma* slaughterhouse in Nairobi County in 5-liter cooler box containers and sampling bags, sealed, and transported to the Microbiology laboratory at the College of Agriculture and Veterinary Sciences, the University of Nairobi for bacterial studies.

2.6. Bacteria Total Count, Culture, Isolation, and Identification

The Standard Plate Count (SPC) method [17-18] gave the total bacteria in the rumen fluid and cow dung samples. The samples were plated in semi-solid nutrient media and then incubated for 48 hours at 30°C to promote bacterial growth. All bacterial plate counts were expressed as the number of colony-forming units (CFU) per milliliter (ml).

III. RESULTS AND DISCUSSIONS

In this study, microorganisms were used to generate Voltage in Microbial fuel cells utilizing the fruit waste. The Voltage was monitored for 22 days using a digital multimeter. Results indicated voltage generation and average daily Voltage reported.

3.1. Modelling of Substrate utilization and Simulation Approach

The modeling studies were used to determine whether the chemical reactions in the MFC chambers were proceeding as expected of the microbial activities in different substrates (market fruit wastes) for 22 days.

3.1.1 Model assumptions

In mathematical modelling the influence of different operating conditions and design parameters on the performance of the MFC system is reflected [19]. Bioelectrochemical being a complex system, there are a number of assumptions which needs to be considered for developing a mathematical or numerical model of a complex system (BES) into a simple and linear form.

(i) In the cathodic chamber, a non-limiting reaction rate is considered [20]

(ii) The pH, temperature, and other operational variables are supposed to be fully controlled in order to limit their impact on the microenvironment of MFC [21].

(iii) The majority of modeling approaches are built with one consideration (e.g., electrochemical or biological) in mind while keeping other parameters constant/controlled. etc.

The voltage, current and power obtained when various market fruits wastes were used as goat rumen fluid used as catalyst in MFC for 22 days plots are shown in **Fig 4, Fig 5** & **Fig 6** respectively.

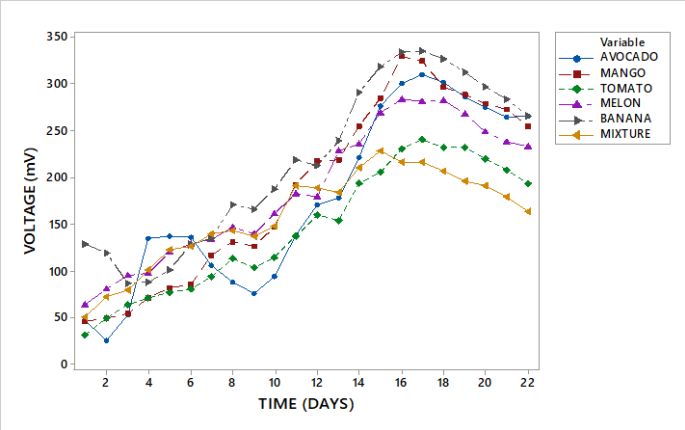

Figure 4. Graphs of voltage generated from fruit waste against time (days)

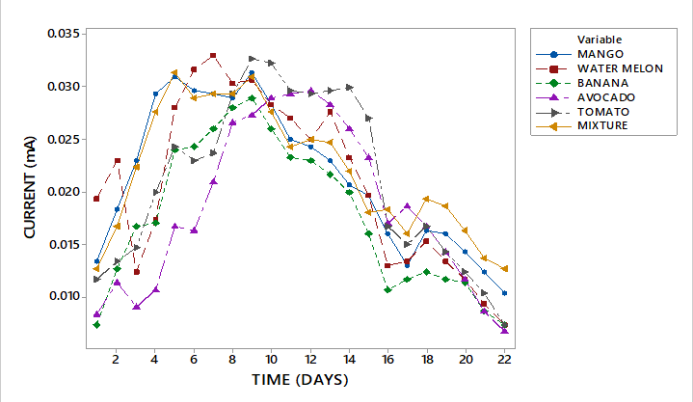

Figure 5. Graphs of current generated from fruit waste against time (days)

Highest voltage, current and power was obtained from banana fruit waste as shown in the figures indicating availability of enough carbon in the substrate for microbes to feed on.

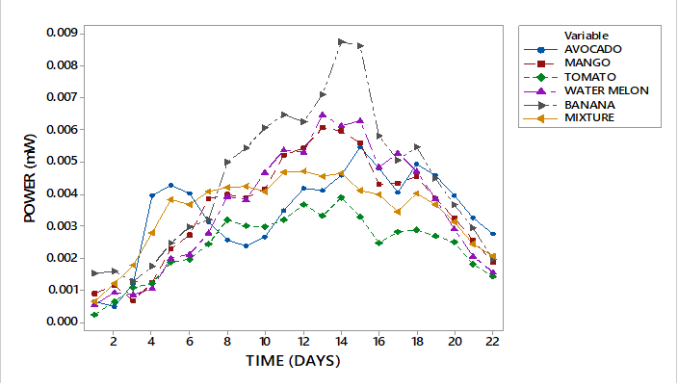

Figure 6. Graphs of power from fruit waste against time (days)

3.1.2. Estimation of anode respiration Kinetic parameters

3.1.2.1. Bacterial growth

The Monod model (Equation 3.0) was used to calculate the kinetic parameters for bacterial growth in the batch MFC because it describes the unstructured and unsegregated phenomenon in the cell. The half-saturation coefficient (K_S), maximum growth rate (μ_{max}), and growth yield coefficient (μ), of bacteria in the various fruit waste-fed MFC were recorded after 408hours (17 days) of microbial activities, calculated using Equation (3.0 – 3.3).

Monod Model:

$$r = r_{max} * \frac{S}{(K_S + S)} \quad (3.0)$$

Taking the reciprocal of Monod Equation: (y = mx + c). Note: (r = μ)

$$\frac{1}{\mu} = \frac{K_S + S}{\mu_{max} * S} = \left(\frac{K_S}{\mu_{max}}\right) * \left(\frac{1}{S}\right) + \left(\frac{1}{\mu_{max}}\right) \quad (3.1)$$

$$\mu = \frac{\text{amount of biomass produced}}{\text{time taken}} \quad (3.2)$$

$$\mu_{max} = \frac{\text{amount of biomass produced}}{\text{initial biomass x time taken}} \quad (3.3)$$

Where: **S**-Is substrate concentration, **μ**-Is growth yield coefficient. μ_{max} –Is maximum growth rate. **Ks** -Is half-saturation coefficient

Taking an example of a Banana fruit, the maximum voltage produced = 336 mV, and Time taken = 408 hours (17 days)

μ = 0.82

Specific growth rate(μ) = $0.82h^{-1}$ $\mu_{max} = 0.003$

Maximum specific growth rate (μ_{max}) = $0.003h^{-1}$

Using **Monod** equation: (K_S)-half-saturation coefficient for Banana fruit was calculated:

$$\frac{1}{\mu} = \frac{K_S + S}{\mu_{max} . S} = \left(\frac{K_S}{\mu_{max}}\right).\left(\frac{1}{S}\right) + \left(\frac{1}{\mu_{max}}\right)$$

$$K_S = 99.63 \pm 0.03 mgL^{-1}$$

Using the same equations (3.0-3.3) above, the half-saturation coefficient (K_S), growth yield coefficient (μ) and maximum growth rate (μ_{max}) results for all the fruit waste samples collected from selected markets in Nairobi County, Kenya, were calculated and tabulated as in (**Table 1**).

Table 1: half-saturation coefficient, growth yield coefficient, and maximum growth rate values for vegetable waste-fed MFCs

No	Sample	μ	μ_{max}	K_S
1	AVOCADO	$0.76h^{-1}$	$0.003h^{-1}$	$99.61\pm0.03mgL^{-1}$
2	MANGO	$0.80h^{-1}$	$0.003h^{-1}$	$99.63\pm0.03mgL^{-1}$
3	TOMATO	$0.59h^{-1}$	$0.002h^{-1}$	$99.66\pm0.03mgL^{-1}$
4	BANANA	$0.82h^{-1}$	$0.003h^{-1}$	$99.63\pm0.03mgL^{-1}$
5	WATER MELON	$0.69h^{-1}$	$0.003h^{-1}$	$99.57\pm0.03mgL^{-1}$
6	MIXTURE	$0.53h^{-1}$	$0.002h^{-1}$	$99.62\pm0.03mgL^{-1}$

μ- Growth yield coefficient. **μ_{max}**– Maximum growth rate. **K_S**. - Half-saturation coefficient

From the results obtained (**Table 1**) in this study, banana fruit waste gave the highest growth yield $0.82h^{-1}$ and a high half-saturation coefficient of $99.66\pm0.03\ mgL^{-1}$, attributed to the availability of carbon sources in bananas fruit waste. The lowest output in growth yield coefficient was obtained by the mixture of fruits sample of $0.53h^{-1}$, and the lowest value obtained for the half-saturation coefficient was the one obtained for watermelon fruit waste at $99.57\pm0.03\ mgL^{-1}$ which can be attributed to competing reactions among the substrates, thus lowering the bacteria's growth yield, hence inhibiting the substrate's degradation process. Studies by [22-23] reported that toxic effects from other vegetables might

inhibit the degradation process at high substrate concentrations. As a result, limiting the bioavailability of a degradable substrate could either improve the overall dynamics of degradation by lowering microbial access to the substrate or slow down biodegradation by mitigating the contaminant toxicity effects on microorganisms. According to [24], biodegradation kinetics of a self-inhibitive substrate are primarily concerned with bacteria's physiological reactions to substrate concentration levels. However, using numerical solutions, this study has theoretically investigated the role and interconnections between self-inhibition and mass transfer constraints, resulting in a simple model that could be useful in practice. Although both self-inhibition and mass transfer have negative impacts on biodegradation, their combined action may increase biodegradation rates over a concentration threshold by boosting overall degradation dynamics and mitigating the pollutant toxicity effects on microorganisms.

Monod model only explained microbial growth and activities but not the effect of self-inhibition. This study used other models (Haldane Andrew's Kinetic and Han-Lavenspiel Models) to observe substrate consumption and bacteria growth and estimate substrate degradation's kinetic parameters.

3.1.2.2 Substrate self-inhibitory effect

Haldane Andrew's Kinetic Model (Equation 3.4) was used to calculate the K_{IH} self-inhibitory effect coefficient of the substrate (banana fruit waste). Substrate-fed MFC was found to be $50.18 \pm 0.04 mgL^{-1}$ after 408hours (17 days) of microbial activities using Equation (3.0 – 3.3) and (3.4 – 3.5), indicating that the inhibitory effect was weak since the K_{IH} value was high.

Haldane Andrew's Kinetic Model

$$r = r_{max} * \frac{S}{\left(K_S + S + \frac{S^2}{K_{IH}}\right)} \quad (3.4)$$

Note: (r = μ)

Taking the reciprocal of Haldane Andrew's Kinetic Equation: (y = mx + c)

$$\frac{1}{\mu} = \frac{K_S + \frac{S^2}{K_{IH}}}{\mu_{max}} * \frac{1}{S} + \frac{1}{\mu_{max}} \quad (3.5)$$

Where: **S**-Is substrate concentration. **μ**-Is growth yield coefficient. μ_{max} –Is maximum growth rate. $\boldsymbol{K_S}$ -Is a half-saturation coefficient. $\boldsymbol{K_{IH}}$ -Is inhibitory effect coefficient

Given: μ = 0.82h^{-1}, μ_{max} = 0.003h^{-1}, S = 100mgL^{-1}, K_S = 99.63mgL^{-1}

Using **Haldane Andrew's** equation 3.5: the ($\boldsymbol{K_{IH}}$) - inhibitory effect coefficient for Banana fruit waste was calculated:

$$\frac{1}{\mu} = \frac{K_S + \frac{S^2}{K_{IH}}}{\mu_{max}} * \frac{1}{S} + \frac{1}{\mu_{max}}$$

$$\boldsymbol{K_{IH}} = 50.18 \pm 0.04 mgL^{-1}$$

Using the same equations (3.4-3.5) above, the self-inhibitory effect coefficient (K_{IH}) results for all the fruit waste samples collected from markets in Nairobi County, Kenya, were calculated and tabulated in **Table 2** below.

Table 2 : Self-inhibitory effect coefficient for all the

No	Sample	K_{IH}
1	AVOCADO	50.09±0.04mgL^{-1}
2	MANGO	50.09±0.04mgL^{-1}
3	TOMATO	50.09±0.04mgL^{-1}
4	BANANA	50.18±0.04mgL^{-1}
5	WATER MELON	50.11±0.04mgL^{-1}
6	MIXTURE	50.09±0.04mgL^{-1}

In this study, banana fruit waste gave the highest inhibitory coefficient output than a mixture of fruit waste. All the market fruit wastes sampled gave self-

inhibitory effect coefficient (K_{IH}) output in a range of $50.18 \pm 0.04 mgL^{-1} - 50.09 \pm 0.04 mgL^{-1}, a$ clear indication that the inhibitory effect was weak; when (K_{IH}) value is high, the inhibitory effect is weak. When (K_{IH}) value is small, the inhibitor is tightly bound, and the number of active enzymes present will be limited; hence the inhibitory effect will be substantial [25]. Therefore in this study, the inhibitors were loosely attached to the microbes. The number of active microbes present was high enough to bio-degrade the available substrate in a given experimental period of 22 days, as was earlier indicated by the Monod model on microbial growth and substrate degradation. Non-competitive inhibition is when the inhibitor does not bind to the active sites of the substrate, giving the substrate space to attach to the bacteria and form a complex that decreases the activation energy of a chemical process. A study by [26] observed that when the K_{IH} value was high ($K_{IH} > 10 mgL^{-1}$), the inhibitory effect was weak and that when K_{IH} value was small ($K_{IH} < 10 mgL^{-1}$) meant the inhibitor was tightly bound. The amount of active enzyme present was small, indicating that the inhibitory effect was substantial. The K_{IH} values obtained in this study compared well to ($K_{IH} = 67 mgL^{-1}$) in the previous study by [27].

3.1.2.3 Critical inhibitor concentration

Han-Lavenspiel model (Equation 3.6) was used to calculate the critical inhibitor concentration coefficient ($\boldsymbol{S_m}$) of the substrate (banana fruit waste). The amount of substrate degraded by the microbes was up to $100.75 \pm 3.71 mgL^{-1}$ after 408hours (17 days) of microbial activities using Equation 3.0 – 3.3, 3.4 – 3.5, and 3.6, suggesting that after consumption of the 100 mgL^{-1} substrate and the 0.75 mgL^{-1} substrate initially in the rumen fluid, the chemical reaction completely stopped and the production of electrons and protons, indicating the microbes' death and the end of a chemical reaction.

Han-Levenspiel Model : Note: ($r = \mu$) and ($n = m = 1$)

$$r = r_{max} * \frac{S\left(1 - \frac{S}{S_m}\right)^n}{S + K_S * \left(1 - \frac{S}{S_m}\right)^m} \quad (3.6)$$

Where: **S**-Is substrate concentration, **μ**-Is growth yield coefficient. μ_{max} – Is maximum growth rate. $\boldsymbol{K_S}$ – Is a half-saturation coefficient. $\boldsymbol{S_m}$-Is critical inhibitor concentration coefficient.

Given: $\mu = 0.82 h^{-1}$, $\mu_{max} = 0.003 h^{-1}$, $S = 100 mgL^{-1}$, $K_S = 99.63 mgL^{-1}$

Using the **Han-Levenspiel** equation 3.6: the ($\boldsymbol{S_m}$) - substrate concentration for Banana fruit waste was calculated:

$$\boldsymbol{S_m} = 100.75 \pm 3.67 mgL^{-1}$$

Using the same equations (3.6) above, the critical inhibitor concentration coefficient ($\boldsymbol{S_m}$) results for all the fruit waste samples collected from markets in Nairobi County, Kenya, for this study were calculated and tabulated in **Table 3** below.

Table 3: critical inhibitor concentration coefficient for all the fruit waste samples

No	Sample	$\boldsymbol{S_m}$
1	AVOCADO	99.72±3.68mgL^{-1}
2	MANGO	99.75±3.68mgL^{-1}
3	TOMATO	99.85±3.68mgL^{-1}
4	BANANA	100.75±3.71mgL^{-1}
5	WATER MELON	99.60±3.67mgL^{-1}
6	MIXTURE	91.09±3.36mgL^{-1}

It is evident in this study (**Table 3**) that all the experiments stopped after each fruit was biodegraded to up to individual fruit critical inhibitor concentration coefficient ($\boldsymbol{S_m}$) at different times. Banana fruit waste, for example, was exhaustively consumed by the microbes up to $100 mgL^{-1}$substrate concentration and the $0.75 mgL^{-1}$ substrate initially in the rumen fluid, which means the chemical reaction stopped 5 hours

after peaking at 408hours (17 days). The sample mixture stopped much earlier after peaking at 373hours, which could be attributed to the toxicity of individual fruits. Avocado stopped the reaction at 408.45hrs; mango fruit waste stopped at 408.49hrs; tomato stopped at 408.77hrs, and watermelon stopped the reaction at 408.12hrs which were all attributed to acid inhibition hence causing microbial death. They proposed competitive inhibition, in which a substrate-like inhibitor attached to the enzyme's active site and blocked the substrate from binding. Uncompetitive inhibition occurs when an inhibitor only binds to the enzyme-substrate complex rather than the free enzyme. Thus decline in microbial growth, facilitating an end of chemical reaction in a microbial fuel cell.

A study by [28] reported that microbial growth stopped when the critical inhibitor concentration (S_m) was reached, and they accounted for different inhibitions using the Han-Levenspiel model. Considered substrate inhibition by using four other models to estimate the kinetic parameters of substrate degradation based on the relationship between substrate concentration and the substrate degradation rate, power density, and output voltage in an anodic denitrified MFC (AD-MFC) [24]. Due to the flask's increasing partial pressure of hydrogen and simultaneous acid inhibition, their investigation found that high substrate concentrations initially indicated strong bio-hydrogen generation, but that this fell to lower levels [29]. This implies that during pilot-scale investigations and continuous bio-hydrogen generation, the ideal carbon source levels in bioreactors are crucial. Failure to do so may have an impact on the microorganism's rate of growth, special substrate usage speed, enzyme activity, and general process yield. As a result, the liquid phase substrate (glucose) concentration must be regulated at optimal levels to prevent the formation of volatile fatty acids and the emergence of substrate inhibitions [30].

Given that the mixture generated the lowest (S_m) value, the Critical inhibitor concentration (S_m) values showed that while setting up MFC chambers for optimal voltage output, it may be necessary to segregate solid waste. Bacteriological studies are necessary here to describe dominant anaerobic consortia responsible for bio-hydrogen formation, given the work's practicality. In general, kinetic models are used to investigate and evaluate the metabolic features of defined cultures.

3.2. Multivariate Data Analysis (ANOVA) for market fruit wastes

All the statistical analyses were performed with JMP 11 (SAS Institute Inc., NC, and USA). The Analysis of Variance (ANOVA) and student's t-test at a 95% confidence level was conducted, and statistical significance was defined as a value of $p<0.05$. Note: Treatment-Is a fruit sample (Fruit waste).

3.2.1. Fruit (Treatment) against Voltage output

The data were subjected to ANOVA to test the significant difference between the various market fruit wastes. Further, an inspection of the correlation probability in which the diagonal shows the correlation of each variable with itself, while the off-diagonal values show the correlation at the intersection and scatter plot matrix revealed the correlation coefficients between the variables obtained from the analysis. As shown in **Figure 7**, the differences in the mixture of fruits, banana, and tomato samples were significantly different, indicating that other factors such as temperature, pressure, substrate preparation, etc., were affecting the reaction.

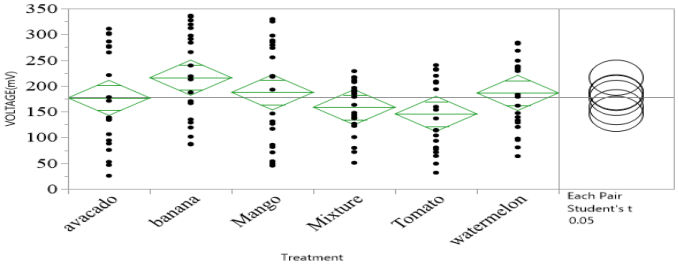

Figure 7 : Friut waste (Treatment) versus Voltage output

ANOVA findings in **Table 4** showed a significant difference in banana, tomato, and a mixture of fruits waste.

Table 4 : ANOVA test for market fruit wastes Voltage

Connecting Letters Report

Level			Mean (mV)
Banana	A		215.96±88.26
Mango	A	B	187.56±98.55
Watermelon	A	B	186.38±72.99
Avocado	A	B	176.78±95.16
Mixture		B	158.76±50.96
Tomato		B	145.75±68.72

Levels not connected by the same letter are significantly different.

3.2.2. Fruit (Treatment) against Current output

Figure 8 Illustrates dot-plot and confidence intervals for each fruit waste for current output for market fruit waste. The mixture and banana samples showed a significant difference in terms of the current production. The results illustrated that, just like in the kinetic models discussed above. The mixture sample generated low output, attributed to the toxicity of individual fruit waste in the mix.

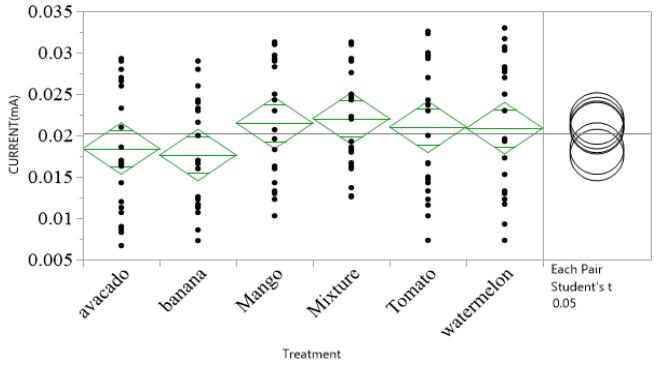

Figure 8 : Fruit waste (Treatment) against Current output

ANOVA findings in **Table 5** showed that the difference in fruit waste mixture and banana samples was significant, just like in voltage output, indicating that other factors such as temperature, pressure, etc., were affecting the reaction. The results clearly illustrated that segregation of fruit wastes before experimenting would be beneficial.

Table 5: ANOVA test for market fruit wastes Current

Connecting Letters Report

Level			Mean(mA)
Mixture	A		0.0221±0.006
Mango	A	B	0.0215±0.007
Tomato	A	B	0.0210±0.008
Watermelon	A	B	0.0209±0.008
Avocado	A	B	0.0184±0.008
Banana		B	0.0176±0.007

Levels not connected by the same letter are significantly different.

3.2.3. Fruit (Treatment) against Power output

A visualized dot-plot and confidence intervals for each treatment against power output for market fruit waste are shown in **Figure 9**.

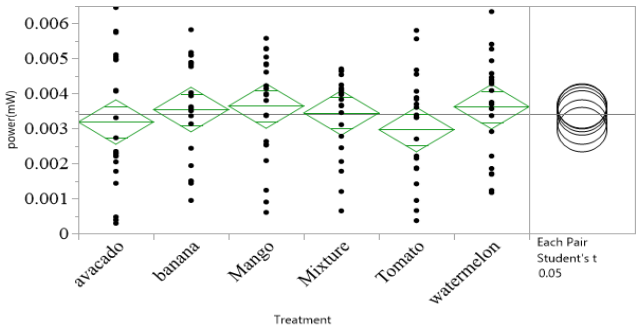

Figure 9: Fruit waste (Treatment) versus Power output

ANOVA findings in **Table 6** show all levels were connected by the same letter indicating there is no significant difference in all the fruit wastes in power output, therefore suggesting that there is a possibility of generating electricity from fruit waste.

Table 6: ANOVA test for market fruit wastes Power

Connecting Letters Report

Level		Mean (mW)
Mango	A	0.0036±0.001
Watermelon	A	0.0036±0.001
Banana	A	0.0035±0.001
Mixture	A	0.0034±0.001
Avocado	A	0.0032±0.002
Tomato	A	0.0029±0.002

Levels not connected by the same letter are significantly different.

Figure 10 below shows the Correlation probability and Scatterplot matrix for Banana fruit waste for Voltage, current, power, power density, and current densities output. Values are coloured according to the magnitude of their correlation; for correlation probability, diagonal values show each variable's correlation with itself, while the off diagonal values are correlated at the intersection of variables. The positive correlation values are bold black, and negative values are red, indicating that an increase in one variable is associated with a decrease in the other variable. The brown colour shows how close the values are to zero, a correlation with no linear association between the variables within the samples.

Correlation Probability

	DAYS	VOLTAGE(mV)	CURRENT(mA)	Current density(mA/m2)	power(mW)	power density (mW/m2)
DAYS	<.0001	<.0001	0.0499	0.0499	0.1037	0.1037
VOLTAGE(mV)	<.0001	<.0001	0.0357	0.0357	0.0256	0.0256
CURRENT(mA)	0.0499	0.0357	<.0001	<.0001	0.0102	0.0102
Current density(mA/m2)	0.0499	0.0357	<.0001	<.0001	0.0102	0.0102
power(mW)	0.1037	0.0256	0.0102	0.0102	<.0001	<.0001
power density (mW/m2)	0.1037	0.0256	0.0102	0.0102	<.0001	<.0001

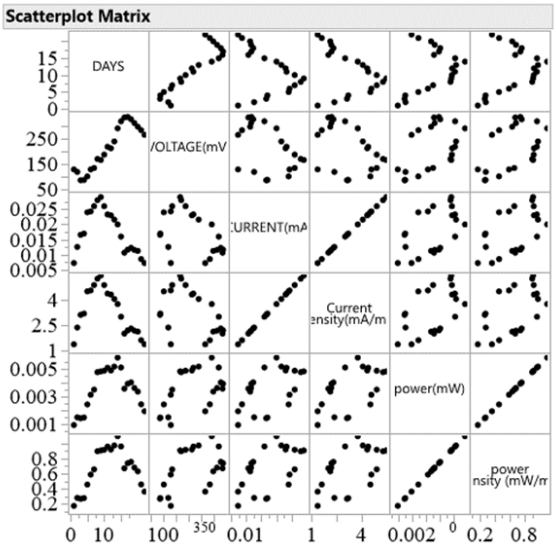

Figure 10 : Correlation and Scatterplot matrix for Banana fruit waste output

Despite statistical significance in some samples, Pearson correlation coefficients (r) were generally poor, indicating a limited predictive capacity of one variable versus another but a high possibility of linear correlations. The p-value in all the output in this study is higher than P<0.05, which is not significant relative to pure error, suggesting that the fitting model accurately describes the experimental data. Other fruit waste in this study, just like banana fruit waste, was computed the same way.

IV. CONCLUSION

Different electrochemical and microbial kinetic techniques can simplify the complexity of the MFC system in mathematical model form. Input variables (such as operating conditions or design variations) can be mathematically optimized, and the outcomes of that optimization can be validated using experimental data. The present study underlined the following finding: Bacteria's growth and reduction kinetics can be modelled using various models available in the literature. Literature survey has shown that multiple models have been found optimum in different systems for the same compound. Hence, a comprehensive modeling exercise was carried out on available

published works to demonstrate this observation. In this work, we demonstrated based on a kinetics model that the Han- Levenspiel model suggests after consumption of the $100mg.L^{-1}$ substrate together with the $0.75\,mg.L^{-1}$ substrate initially from the rumen fluid for banana and $91.09\pm3.36mg.L^{-1}$ for the mixture of fruit wastes, the chemical reaction stops, and the production of electrons and protons, indicating the microbes' activities are inactive. Findings strongly supported by statistical model; multivariate data analysis revealed a significant difference in banana and a mixture of waste, though showing no significant difference in power outputs in all the collected market fruit waste (treatments).

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Recommendation

Optimization of inoculum, process parameters, substrates, evaluation of performance, and economics of continuous bio-hydrogen generation systems should be the focus of future research in this subject (bioreactors).

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Ethics statement. This manuscript uses unpublished data from microbial fuel cells studies. This research work did not include any animal testing. As a result, there was no need for ethics approval.

Data accessibility. None.

Authors' contributions. Conceptualization, I.K.O., M.J.K., M.D.N. and K.P.K.; methodology, M.J.K., I.K.O. and M.D.N.; software, M.J.K. and K.A.P; validation, O.J.O. and M.D.N.; formal analysis, I.K.O.; writing—original draft preparation, I.K.O.; writing—review and editing, I.K.O., M.J.K., K.P.K., M.D.N., and O.J.O.; supervision, M.D.N. and O.J.O.; project administration, I.K.O. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

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

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