OPTIMIZATION AND PROCESS MODELLING OF BACTERIO-ALGAL FUEL CELL

Thesis

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DECLARATION OF ORIGINALITY AND COMPLIANCE WITH ACADEMIC ETHICS

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ABSTRACT

Bacterio-algal Fuel Cells (BAFCs) offer a sustainable bio-energy solution by converting organic waste into electricity, treating wastewater, and capturing CO2 emissions, addressing the urgent need for renewable energy sources. This study focused on measuring the voltage, current, and power output in a bacterio-algal fuel cell. For the analysis, multiple dual-chamber bacterial fuel cells were employed, using glucose and lactic acid as substrates. The anode chamber was inoculated with a pure culture of the microorganism *Shewanella putrefaciens*, while *Leptolyngbya subtilis* was used as the cathode organism. The microbe facilitated the oxidation of the substrate, and the resultant energy produced was assessed. A study on the growth kinetics of the microorganism *Shewanella putrefaciens MTCC 8104* was conducted using Glucose and lactic acid separately as substrate. This aimed to ascertain the maximum specific growth rate (μ_{max}) and the substrate saturation constant (k_s), both are crucial kinetic parameters of the system. Lactic acid displayed inhibition, prompting the determination of the substrate inhibition constant, K_I**.** The highest power density achieved from the BAFC setups was 396 mW/m^2 for lactic acid, 121.98 mW/m^2 for glucose, and 74.82 mW/m^2 for the dual substrate (lactic acid & glucose) configuration**.** The BAFC was mathematically modeled using MATLAB 2017b sofware in order to theoretically examine the operations and efficiency of the bacterio-algal fuel cell**.** Response surface analysis was conducted utilizing Design Expert 13 software to anticipate a model of power density under optimal conditions.

Chapter 1
INTRODUCTION

1.1Background

The rapid exhaustion of traditional non-renewable energy reservoirs, such as fossil fuels, has ignited a pressing need for alternative solutions, prompting a surge of interest among researchers towards renewable energy technologies. At the forefront of this pursuit lies the innovative domain of Bacterio-algal Fuel Cells (BAFCs), which represent a promising avenue for sustainable bio-energy generation.

BAFCs operate as sophisticated bio-electrochemical systems, ingeniously engineered to convert the chemical energy harbored within biodegradable organic waste into usable electrical energy. Remarkably, these systems not only facilitate energy production but also concurrently serve as effective tools for wastewater treatment, alongside the crucial function of capturing and sequestering CO2 emissions.

By harmonizing the symbiotic relationship between bacteria and algae, BAFCs emerge as comprehensive solutions to contemporary environmental challenges. Through their integration of nature's own processes, these pioneering technologies exemplify a steadfast commitment to fostering a cleaner, greener energy landscape, thereby paving the way towards a more sustainable future.

1.2 Bacterio-algal Fuel Cell

A microbial fuel cell is a bio-electrochemical system that exploits several key processes: (I) the capability of bacteria to metabolize various biodegradable organic substrates present in wastewater under anaerobic conditions in the anode chamber; (II) the transportation of electrons ($e-$) generated through microbial reactions to the anode and subsequently through an external circuit to the cathode; (III) the transfer of protons (H+) produced through microbial reactions in the anode to the cathode chamber via a proton exchange membrane (PEM); and (IV) the reaction of $H+$, $e-$, and electron acceptor, i.e., oxygen present/supplied in the cathode chamber ($2H+ + 0.5O2 + 2e- \rightarrow H2O$), at the cathode to generate water. For each $e-$ generated through the biochemical reaction, the theoretical amount of charge generated is equal to Faraday's constant (=96485 Coulombs).

On the other hand, a bacterio-algal fuel cell is a modification of traditional Microbial Fuel Cells, where algae are grown in the cathode chamber and serve as the supplier of oxygen. Consequently, the replenishment of synthetic reagents, e.g., potassium ferrocyanide, etc., can be avoided. It also ensures the capture of CO2 generated in the anode chamber by algae. A Bacterio-algal fuel cell system typically consists of two chambers: an anode chamber and a cathode chamber separated by a proton exchange membrane, as illustrated in Figure 1.

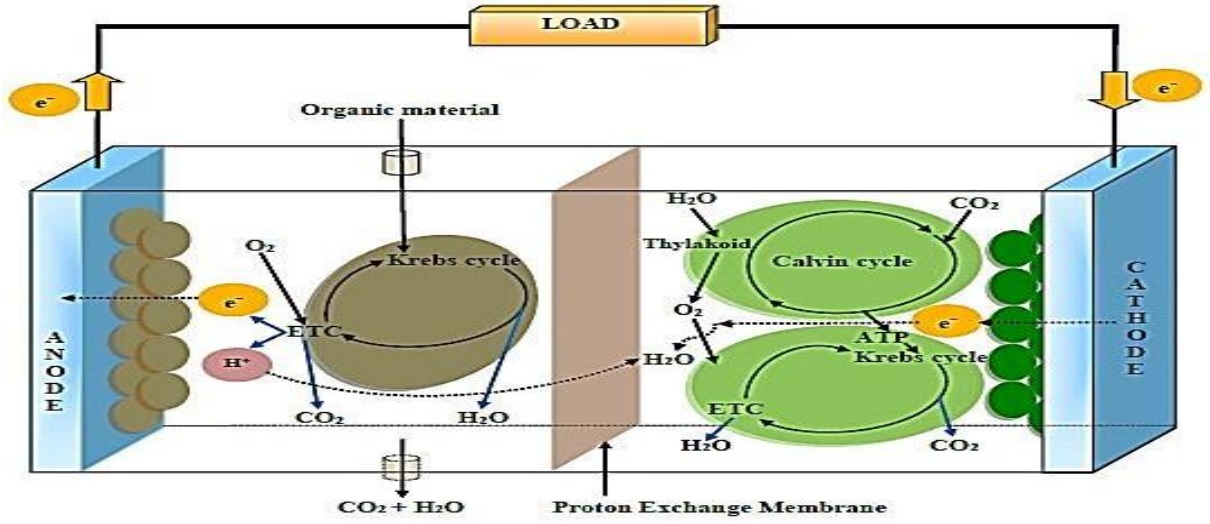


Figure 1. Schematic representation of Bacterio-algal Fuel Cell

In the anode chamber, bacteria and the substrate to be metabolized are added. The electrons generated due to oxidation of the substrates in the anode are transferred through the anode and via the external circuit to the electron acceptor (i.e., oxygen) produced by algae in the cathode chamber. The proton exchange membrane (PEM) transfers the protons generated by bacterial substrate oxidation from the anode chamber to the cathode chamber, while acting as a barrier to the transfer of electron acceptor from the cathode chamber to the anode chamber. Impermeable to oxygen (or any electron acceptor), the PEM ensures that no electron acceptor can reach the anode chamber to accept electrons there, enabling the generation of electricity. The algae generate oxygen by taking up carbon dioxide, with which the proton and electron react to produce water. Thus, a Bacterio-algal Fuel Cell simultaneously treats wastewater and produces electricity from wastewater streams.

1.3.Classification of a Bacterio-algal Fuel Cell

Bacterio-algal Fuel Cells are mainly of two types based on the number of chambers present in the system i.e, Single Chambered Bacterio-algal Fuel Cells (SC-BAFC) and Dual Chambered

Bacterio-algal Fuel Cells (DBAFC).

1.3.1Single Chambered Bacterio-algal Fuel Cell

A bacterial-algal fuel cell with a single chamber primarily includes an anode chamber. Unlike conventional designs, it does not have a separate cathode chamber; instead, it employs an open-air cathode[1]. A proton exchange membrane separates this open-air cathode from the anode chamber. Both electrodes, the anode, and the open-air cathode, are linked by a copper wire to complete the circuit[2]. Below is a depiction of a Single Chambered Bacterio-algal Fuel Cell.

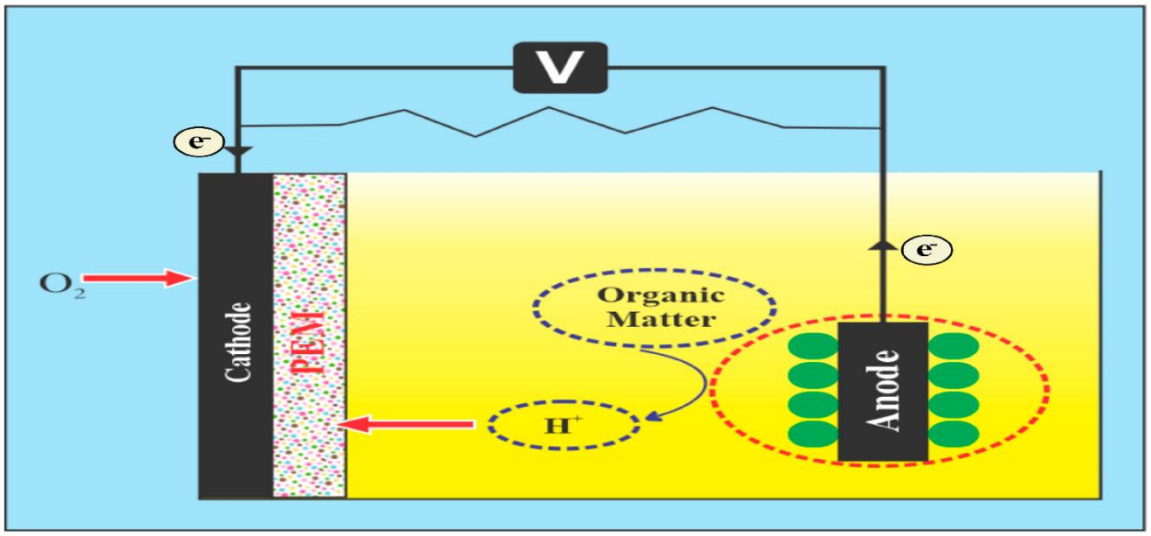


Figure 2. Schematic representation of single chamber Bacterio-algal Fuel Cell

A single-chamber bacteria-algal fuel cell (SCBAFC) is a type of microbial fuel cell (MFC) that integrates both bacteria and algae to produce electrical energy from organic matter. Here's how it generally works:

1. Bacteria are used to break down organic matter through a process called anaerobic respiration. As they consume organic compounds, they release electrons as waste.
2. Algae are photosynthetic organisms that can convert sunlight into chemical energy through photosynthesis. This process produces oxygen and organic compounds.

3. In the SCBAFC, the electrons released by bacteria during the breakdown of organic matter are transferred to an electrode surface, generating an electric current. This current can then be

harnessed for various applications.

4. Algae in the system serve multiple purposes. They can provide additional organic matter for bacterial metabolism, leading to increased electron production. Also, the oxygen produced during photosynthesis helps maintain an aerobic environment within the cell, which is crucial for the bacterial metabolism.

5. The electrical output of the SCBAFC depends on various factors such as the types of bacteria and algae used, the organic compounds available for metabolism, and the efficiency of electron transfer to the electrode.

SCBAFCs are of interest due to their potential for sustainable energy production using renewable resources and their ability to treat wastewater simultaneously. They represent an innovative approach at the intersection of microbiology, bioenergy, and environmental engineering.

1.3.2 Dual Chambered Bacterio-algal Fuel Cell

A dual-chambered bacterio-algal fuel cell (DBFC) is a type of microbial fuel cell (MFC) that utilizes both bacteria and algae to generate electricity through the breakdown of organic matter.

A Bacterio-algal fuel cell, with its dual chambers, presents a distinct configuration where each chamber serves a specific function. In this setup, one chamber accommodates the cathode, while the other houses the anode. Within the anode chamber, electro-active species coexist with the substrate, the electron donor, and the positive electrode (anode), forming an essential ensemble. Conversely, the cathode chamber hosts the negative electrode (cathode) alongside the algal culture, which may exist in suspended or attached form, or even both, depending on the design. These chambers remain discreetly separated by a Proton Exchange Membrane (PEM), which not only partitions them but also acts as a conduit, facilitating the selective transport of H+ ions solely from the anode to the cathode chamber. Furthermore, the anode and cathode chambers establish connectivity through an external wired circuit, enabling the seamless flow of electrons from the anode to the cathode, thereby closing the circuit. Notably, any gas produced within the anode finds its way to the cathode, fostering optimal conditions for algal growth. For a comprehensive visual representation, please refer to Figure 3.

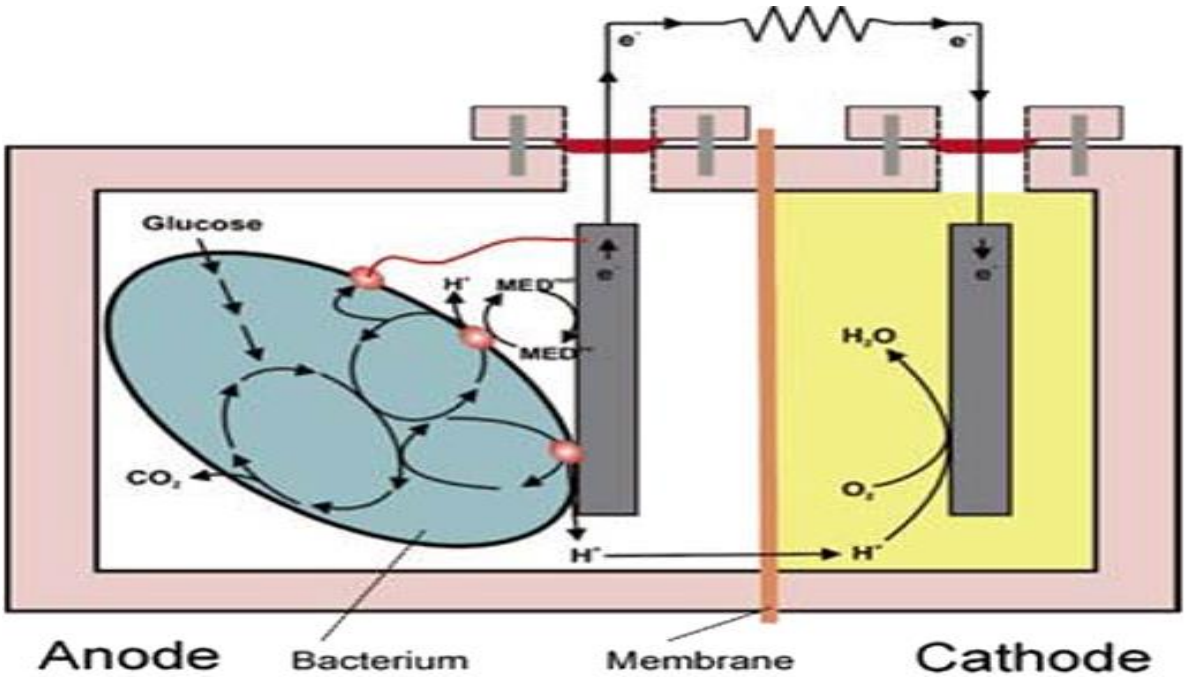


Figure 3. Schematic representation of single chamber Bacterio-algal Fuel Cell

1. Dual Chambers: As the name suggests, a DBFC consists of two chambers separated by a semi-permeable membrane. One chamber houses the bacteria, while the other contains the algae.

2. Bacteria Chamber: This chamber typically contains anaerobic bacteria. These bacteria break down organic matter into electrons and protons through a process called anaerobic respiration. The electrons produced during this process are transferred to an electrode, generating an electric current.

3. Algal Chamber: In the algae chamber, photosynthetic algae are cultured. These algae utilize light energy to convert carbon dioxide and water into organic compounds and oxygen through photosynthesis. The organic compounds produced by algae can be used as a food source for the bacteria in the other chamber. Additionally, the oxygen generated during photosynthesis helps to maintain aerobic conditions in the bacteria chamber, optimizing bacterial activity.

4. Electrodes: Each chamber contains an electrode. The electrode in the bacteria chamber collects electrons released during the bacterial metabolism, while the electrode in the algae chamber provides electrons for the photosynthetic process.

5. Electrochemical Reaction: The electrons collected at the electrodes flow through an external circuit, generating an electric current. This current can be utilized to power electronic devices or stored for later use.

Dual-chambered bacterio-algal fuel cells offer several advantages, including efficient energy production, wastewater treatment capabilities, and potential for use in remote or resource-limited areas. They represent an innovative approach to sustainable energy generation and environmental remediation.

1.4.Architecture Of BAFCs

BAFC can be differentiated according to size: macro-, meso- or micro scale. One of the largest BAFC systems to be reported is a modularized BAFC with a total volume of 1000 L, operated successfully over 12 months [3]. This system did not consist of a single large BAFC of 1000 L, but of 50 stacked BAFC modules instead, each with a volume of 20 L. One of the smallest MFCs to be reported is a microfluidic 1.5 μL anode chamber with a 4 μL cathode chamber [4]. The largest (20 L) and the smallest (totaling 5.5 μL) give more than a 7-log fold difference in size. Therefore, scale and size of the anodic compartment is by far the biggest difference reported in BAFCs and is therefore likely to have the largest effect on the formation and behavior of the biofilms that colonize electrode surfaces. It appears that small scale BAFCs are more energy dense than larger volume systems [4,5]. The three most critical components of an BAFC are the anode, cathode, and PEM. Several anode and cathode materials have been utilized to increase or alter the capacity and performance of BAFCs over time.

1.4.1.Anode

The selection of appropriate anode material is very significant for the performance and efficiency of BAFCs. In a polarized electrical device, the anode is the electrode via which current enters from the external circuit. Oxidation takes place at the anode. In the past decade, the design and fabrication of anode materials influencing the performance of BAFCs has attracted attention through several studies. The electrodes have some desirable characteristics for their optimum use. These are as follows:

- Good conduction of electricity and little resistance to flow:
- Good corrosion resistance and satisfactory chemical stability.
- Biocompatibility.
- Mechanical strength.
- Large surface area.
- Low cost and availability.

- Low or zero toxicity.

As a result, studies have been conducted to design anode materials considering the above factors to optimize the efficiency of BAFCs. Throughout time, numerous sorts of materials have been utilized to manufacture anodes. Different carbonaceous materials are widely applied as the material for the anode in an BAFC [6]. Additionally, some metals and metal oxides have also been used as the material for anodes. The anodes manufactured using different materials can vary in design, surface area, and other properties.

1.4.2.Cathode

In the cathode chamber of the Bacterio-algal Fuel Cell, the electrode present is known as the cathode. Electrons, generated through bacterial metabolism within the anode chamber, traverse an external circuit from the anode surface to reach the cathode. Concurrently, protons created within the anode chamber migrate to the cathode chamber through the proton exchange membrane. Within the cathode, electrons are received by the terminal electron acceptor produced by algae, namely oxygen, which combines with protons to create water. Ferricyanide (K3[Fe(CN)6]) is very popular as an experimental electron acceptor in microbial fuel cells [7]. The greatest advantage of ferricyanide is the low over potential using a plain carbon cathode, resulting in a cathode working potential close to its open circuit potential. The greatest disadvantage is the insufficient reoxidation by oxygen, which requires the catholyte to be regularly replaced [8]. In addition, the long term performance of the system can be affected by diffusion of ferricyanide across the PEM and into the anode chamber. Oxygen is the most suitable electron acceptor for an BAFC due to its high oxidation potential, availability, low cost (it is free), sustainability, and the lack of a chemical waste product (water is formed as the only end product). The choice of the cathode material greatly affects performance, and is varied based on application.

In terms of the exact biochemical reactions that occur in the fuel cells, they are as follows

Oxidation at the anode

Organic molecules [composition: $(CH_2O)_n$] get oxidized by microorganisms through hydrolysis to produce CO_2, H^+ and electrons. Oxidation may either occur through bacterial communities present in the wastewater (bacterio-algal fuel cells) using up

available substrates or through the consumption of the substrates by algae when grown in both, the anodic and cathodic chamber.

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

CO_2 transport and photosynthesis

In some systems where algae are introduced in both the anode and cathode chambers, CO_2 may be supplied to promote better growth and photosynthesis of the organisms and also improve carbon fixation. The overall CO_2 in the system is the sum of the externally added CO_2 and the CO_2 formed as a result of the oxidation of organic matter. The transport of CO_2 after oxidation of organic matter occurs from the liquid through the algal cell membrane with the controlling step being the resistance of the liquid film (two-film theory). (Zhang et al., 2019). This CO_2 transferred to the cell in the presence of light is converted into organic matter by the algae.

$$nCO_2 + nH_2O \rightarrow (CH_2O)_n + nO_2 \text{ (in the presence of light and algae)}$$

The produced matter is used in the algal cell for cell growth, cell maintenance and bioproduct formations .

Reduction at the cathode

In most studies of AFCs, the algae are introduced into the cathodic chamber only At the cathode, the electrons that were transported from the anode combine with the H+ ions that make their way through the PEM to produce water in the presence of oxygen. This oxygen is a direct product of photosynthesis by the algae. In some cases, to ensure availability of a steady oxygen concentration, spargers can be used to bubble O_2 through the column (Kakarla et al., 2015). In the case of SCMFCs, cathodes exposed to the air are used to increase oxygen availability at the cathode biofilm .

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

1.4.3. Proton Exchange Membrane.

Proton Exchange Membranes (PEM)s are the components that physically divide the cathodic and

anodic chambers. Membranes are one of the most crucial elements in the development of BAFCs. Membranes allow the proton to transfer and stop the diffusion of oxygen. The membranes also help in chemical and ionic conjugation. The current density of a single-chambered BAFC without a membrane is greater. However, oxygen and substrate diffusivity increase due to the absence of membranes, allowing the reduction in coulombic efficiency (CE). Thus, a membrane contributes to the long-term efficiency of BAFCs. However, the challenges associated with membranes are their expense, fouling, and increase in internal resistance. Throughout the duration of BAFC research, scientists attempted to determine the optimal membrane separator for BAFCs. The membrane for an BAFC should have properties such as lower cost, internal resistance, selectivity, chemical and mechanical stability, and fouling resistance [11].

The most commonly used PEM is Nafion, Nafion 112, Nafion 117,CNF/Nafion, ACNF/Nafion are some of the examples [12]. Alternatives to Nafion, such as Ultrex CMI-7000 , GO-SPEEK(Graphene oxide coated self-fabricated sulfonated poly ether ether ketone membrane)also are well suited for BAFC applications. All of these membranes have great performance but are associated with high cost , substrate loss, oxygen leakage etc [13]. As per literature review, alternative like clay is being attempted. Clay wall of the earthen pots, made of local red soil consisting of a varieties of mineral compounds, has been reported to serve as a cost effective and efficient alternative to the traditionally used PEMs[13].

1.5. Working materials of bacterial fuel cell

1.5.1. Microorganism:

Living cells are inoculated in the anode compartment of a bacterial fuel cell to feed on the substrate and carry out its oxidation. Numerous studies have been conducted over the years to examine bacterial metabolism in the anode chamber. Earlier research primarily focused on *Escherichia coli* and *P. vulgaris* as the bacterial species in the anode chamber. More recently, bacterial cultures of *Shewanella putrefaciens, Shewanella oneidensis,* and *Geobacter sulfurreducens* have also been used in many studies. Notably, *Shewanella spp.* can produce external electron mediators that facilitate electron transfer. They have also been found to have electrically conductive protein structures which help in the process of electron transfer.

1.5.2.Algae

Algae are photosynthetic organisms that utilize carbon dioxide in the presence of light energy, ultimately producing oxygen. They can be used in the cathode chamber of a bacterio-algal fuel cell to generate oxygen as an electron acceptor. This eliminates the need for synthetic electron acceptors, which is costly due to their regular replenishment. Commonly used algal strains is *Chlamydomonas reinhardtii, Chlorella vulgaris, and Laminaria.*Algae exhibit phototrophic growth in the presence of sunlight and atmospheric or sparged CO2.

1.6. Mechanisms of electron transfers

In earlier studies, artificial electron shuttles (neutral red, methyl viologen, etc.) were used in BAFCs for electron transfer from inside the cells to electrodes, and many exoelectrogens showed the ability to transfer electrons to electrode. But only a finite number of exoelectrogens are known for electron transfer without the use of artificial electron shuttles. Direct interspecies electron transfer (DIET) has been reported in some bacterial communities.

Direct Electron Transfer

Till now, only a few genera and species have been shown to be electrochemically active by means of direct conductive mechanisms; these include Shewanella, Geobacter, Rhodoferax ferrireducens, Pelotomaculum thermopropionicum, Geothrix and Geoalkalibacter. Of these, Geobacter and Shewanella have been studied the most. The first group to report this phenomenon were using Shewanella [14], later named as a cable bacterium [15]. A summary of important components in the electron transport mechanism from cells to the anode in Geobacter MFCs has been provided by Lovley [16]. Metabolism of electron rich (reduced) substrate such as acetate or lactate drives the production of reduced NADH from NAD+ within the cell. In order for the cell to maintain reducing power, it must re-oxidise NAD+ by abstracting electrons. Further transfer of electrons, even within multilayers of cells may also occur, via a dense network of appendages with metal-like conductivity called bacterial nanowires [17]. The transfer may occur cell by cell over distances of more than 1 cm, until electrons are donated to the electrodes [18].

1.7 Overview of Thesis

The present research focuses on the experimental studies on BAFC using a suitable bacterial strain in the anode and an algal strain in the cathode using multiple carbon sources in the anode. The reaction kinetic data of growth kinetics of the bacterial and algal strain a mathematical model will be developed to predict the performance of the BAFC, run on two carbon sources in the anode. Optimization of the performance of the multi-substrate BAFC will also be performed. The thesis has been presented in seven following chapters with the following lay-out: Chapter 2: Literature Review; Chapter 3: Aims and Objectives; 4: Theoretical Analysis; Chapter 5: Materials and Methods; Chapter 6: Results and Discussions; Chapter 7: Conclusions and Future Scope of Research.

Chapter 2
LITERATURE REVIEW

As stated in Chapter 1, the present research focuses on the experimental studies on BAFC using multiple substrates and suitable bacterial and algal strains in the anode and cathode chambers respectively. The development of mathematical model and optimization of the performance of the BAFC are also planned. Therefore, extensive literature review has been performed in this direction.

2.1. Review on details of Bacterio-Algal Fuel Cell

Table 1 Bacterio-Algal Fuel Cell

Title Of Article	**Substrate & Microorganism Used**	**Reactor Configuration**	**Performance**	**Reference**
Bioelectricity Production From kitchen waste water using MFC	Substrate: Lactic acid, Fatty acids, Sodium carbonate Algae: *Synechococcus sp. & Chloroccum Sp.*	Dual chamber MFC which used graphite plate as electrode.	Power density 41.5 mW/m2	Samsudeen et al. (2016)
Bioelectricity production from Textile waste water using SCMFC	Substrate: Sodium sulfide, Sodium carbonate, Sulfuric paint Algae: *C. vulgaris*	An air exposed single chamber MFC which used microalgal biocathodes.	Power density 123.2 mW/m2	Washington et al. (2017)
MFC with an algae-assisted cathode	Substrate: Activated sludge and synthetic wastewater(Glucose, Fructose) in a 3:1 ratio. Algae: *Chlorella Vulgaris*	Dual Chamber MFC . Electrodes were built of Toray Carbon cloths.	Power density 13.5 mW/m2	Campo et al. (2013)

Effect of nanowire conductive transfer on the performance of batch-MFC	Substrate: sodium lactate Microorganism: *Shewanella oneidensis* and *Shewanella putrefacient*	Dual chamber MFC which used Graphite rod as electrode.	Dual chamber MFC which used Graphite rod as electrode.	Wang et al. (2021)

Research Gap:

Studies on bacterio-algal fuel cells are gaining popularity due to inherent assimilation of CO2 in the system and for the exclusion of any external oxygen supply in the cathode. More studies are, however needed to use new combinations of substrates appearing in waste streams. Application of new algal strains other than *Chlorella Vulgaris, Synechococcus sp.,Chloroccum Sp.* etc. should also be attempted.

2.2. Optimization

In the optimization studies available in the literature, various parameters of MFCs have been optimized under certain conditions. However, the understanding of the fact that some parameters have a higher impact on the performance of an MFC is crucial in paving its way to the industrial sector. A study conducted by Zeng et al. [31] revealed a list of parameters to which MFC's power density output is most sensitive.

Table 2: Comparison of various works on Optimization of Bacterial fuel cell

Title	Parameters	Condition (reactor configuration/ operational)	Reference
Optimization of MFC process using a novel consortium for aromatic hydrocarbon bioremediation and bioelectricity generation.	• Biofilm • Length of salt bridge • Substrate Concentration • External resistance	Mediatorless H-type Double chamber, Sodium benzoate as a substrate, Electrode- Graphite plate, Temp- 30˚C PH-8.6 , COD conc -1440 mg/L	Mukherjee et al. (2021)

Response surface optimization of microalgae microbial fuel cell enhanced by yeast immobilization for bioelectricity production .	• Power Density • COD removal	Dual chamber MMFC, Electrode- Graphite Algae- Spirulina platensis, Temp- 25◦C, pH- 3.92 , COD- 1286 mg/L	Hadiyanto et al. (2022)
Optimal biofilm formation and power generation in a micro-sized microbial fuel cell (MFC)	• Biofilm • Power Density	PEM- naflon 117, External resistance - 150Ω Substrate- Acetate, Electrode- gold plate	Choi et al. (2013)
Biodegradation of reactive red 120 in microbial fuel cell by Staphylococcus equoruma : Statistical modelling and process optimization	• PH • Current Density	Double Chamber, PEM- nafion 117, External resistance - 1000Ω, PH -7	Shahi et al. (2021)

Research Gap:

There is a scarcity of data on optimization studies of BAFC.

2.3. Mathematical Modelling

Considering the complexity associated with bio-electrochemical processes, the performance of a microbial fuel cell (MFC) is governed by input operating parameters. For scaled-up applications, an MFC system needs to be modeled from engineering perspectives in terms of optimum operating conditions to get higher performance and energy recovery. Several conceptual numerical models to advanced computational simulation approaches have been developed to represent simple-form of a complex MFC system. Application of mathematical and computation models are explored to establish the relationship between operating input-variables and power output. The review discusses about the complexity of system, modeling strategies used and reality of such modeling for scaling-up applications of MFCs. Additionally, the selection of an appropriate mathematical model reduces the computational duration and provides better understanding of the system process. It also explores the possibility and progress towards commercialization of MFCs and thus the need of development of model-based optimization and process-control approaches.

Table 3: Modelling of bacterial fuel cell

Title	Modeling method	MFC variables	Major findings	References
Effect of operating parameters on the performance of sediment microbial fuel cell treating aquaculture water.	Statistical method	Electrode spacing, pH, external resistor	Low pH 6.5 high COD removal	Sajana et al., 2014
Neural network and neuro-fuzzy modeling for the power density and Columbic efficiency of microbial fuel cell.	Black box model	Ionic strength, pH, nitrogen concentration, and temperature	R^2 (0.98) correlation between power and input variables.	Esfandyari et al., 2016
Mathematical modeling of two-chamber batch microbial fuel cell with pure culture of Shewanella.	Dynamic model	Chamber working Volume, Substrate concentration.	There was a good agreement between the experimental data and the results of modeling.	Esfandyari et al., 2017
Modeling of MFC power generation using machine learning-based super learner algorithms.	Super Linear Model	membrane thickness, external resistance, anode area, power generation	High R^2 (greater than 0.99) validated the stability and reliability of the models.	Hossain et al, 2023

Research Gap:

- Incorporation of Microbial Community Dynamics: While some MFC models consider microbial activity, they often simplify microbial dynamics or focus on a single microbial species. However, MFC performance is heavily influenced by complex microbial community interactions, including competition, cooperation, and syntrophy. Zhang et al. (2019) emphasized the need for dynamic models that capture the dynamics of microbial

communities and their impact on MFC performance, underscoring a critical research gap in this domain.

- Validation and Experimental Calibration: Many MFC models rely on theoretical assumptions and parameters obtained from laboratory experiments or literature, leading to uncertainties in model predictions. There is a lack of comprehensive validation and experimental calibration of MFC models using real-world data. Yang et al. (2021) discussed the importance of model validation and parameter estimation based on experimental data to improve the accuracy and reliability of MFC models, indicating a significant research gap in this regard.

- Prediction of Long-Term Performance and Stability: Most existing MFC models focus on short-term performance prediction under steady-state conditions, overlooking long-term dynamics and stability issues. However, MFC performance can be affected by factors such as biofilm growth, substrate depletion, and electrode fouling over extended operation periods. Wang et al. (2020) highlighted the need for dynamic models that can predict MFC performance and stability over long-term operation, indicating a critical research gap in this area.

- Mathematical models on BAFC have been rarely reported.

Chapter 3
AIMS AND OBJECTIVES

AIM 1: Determination of tolerance limit of *Shewanella Putrefaciens* MTCC 8104

Objective

1. Determination of tolerance limit of *Shewanella Putrefaciens* MTCC 8104 towards waste water of Sugar industries (Glucose) and Dairies (whey).

AIM 2: Development of BAFC using *S. Putrefaciens* MTCC 8104 in anode and *Leptolyngbya subtilis* JUCHE1 in cathode.

Objective

1. Construction of BAFC and characterization of performance using *S. Putrefaciens* MTCC 8104 and *L. subtilis JUCHE1* in suspension.

AIM 3: Development of mathematical models for BAFC

Objectives

1. Development of mathematical model for BAFCs using *S. Putrefaciens MTCC 8104* and *L. subtilisJUCHE1* in suspension.

AIM 4: Optimization of BAFC

Objectives:

1. Optimization of the performance of the operation of the multi-substrate (lactic acid and glucose) BAFC

Chapter 4
THEORETICAL ANALYSIS

4.1 Determination of growth kinetic parameters for *Shewanella putrefaciens* MTCC 8104

The effects of organic acids on microbial growth vary based on their ionic or dissociated form (Suganya et al., 2020). Growth kinetics of *Shewanella putrefaciens* MTCC 8104 were examined in substrate-augmented M9 medium. Inoculum was obtained from cultures adapted to artificial waste medium with glucose (0.1gm/L) and lactic acid (2500ppm) to determine initial specific growth rates for testing in growth models.

Similarly, growth kinetics were studied in glucose and DW-augmented M9 medium individually. Specific growth rates (μ) were determined using a specified equation.

$$\mu = \frac{1}{C_x} * \frac{dC_x}{dt} \quad (1)$$

Where, C_x = biomass concentration, $\frac{dC_x}{dt}$ = rate of change of biomass concentration with respect to time. Linearized form of Monod equation was used to calculate the desired value of growth kinetic parameters of glucose as there is no inhibition. On other hand as lactic acid showed inhibition so, Haldane model considered. The Monod equation can be written as:

$$\mu = \frac{\mu_{max}\, C_s}{K_s + C_s} \quad (2)$$

$$\mu = \frac{\mu_{max}\, C_s}{K_s + C_s + \frac{C_s^2}{K_I}} \quad (3)$$

Now by taking reciprocal on both sides of the Monod equation we get-

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}} \times \frac{1}{C_s} + \frac{1}{\mu_{max}} \quad (4)$$

It can be clearly seen that the above equation is in the form of a straight line i.e. y = mx+c where $\frac{K_s}{\mu_{max}}$ is the slope which gives us the value of Ks and $\frac{1}{\mu_{max}}$ is the intercept which gives the value

ofμ_{max}.

For Haldane model The value of inhibition constant, (K_I) is given as follows:

$$K_I = \frac{C_{s\ crit}}{K_s} \quad (5)$$

Where, $C_{s\ crit}$is the substrate concentration (mg/L) corresponding to the maximum value of growth rate.

4.2. Bacterio-Algal Fuel Cell Model

4.2.1Model assumptions

- The Bacterial fuel cell (BFC) is operated in batch mode.
- Temperature and pressure of the system is maintained constant.
- A constant pH is maintained throughout the system.

4.2.2Reactions occurring in the BAFC

The overall reactions in the system have been assumed as follows:

Half reaction in the anode:

$C_6H_{12}O_6 + 6H_2O = 6CO_2 + 24H^+ + 24e^-$ (Glucose decomposition)

$C_3H_6O_3 + 2H_2O = 3CO_2 + 12H^+ + 12e^-$ (Lactic acid decomposition)

Half reaction in the cathode:

$2O_2 + 4H^+ + 4e^- = 2H_2O$

4.2.3. Mass balance in anode chamber:

The general mass balance equation is again written as:

Input – output + generation through reaction– consumption through reaction = accumulation.

As, this is a batch study hence, input = output = 0

For any substrate the differential mole balance over t to t+Δt gives,

$$V_L C_{S(t+dt)} - V_L C_{S_t} = -(\frac{1}{Y_{X/S}} \mu C_x V_L)\Delta t \quad (6)$$

Where, V_L is the total volume of liquid in anode chamber, Yx/s is substrate to biomass yield coefficient, $C_{S(t+dt)}$ is final substrate concentration.

Dividing equation (6) by Δt and limiting $\Delta t \rightarrow 0$, equation 6 reduces to,

$$\frac{dC_s}{dt} = -\frac{1}{Y_{X/S}}\mu C_x \quad (7)$$

Substituting the value of μ from equation (2) in the above equation we get:

$$\frac{dC_s}{dt} = -\frac{1}{Y_{X/S}}\frac{\mu_{max}\, C_s}{K_s + C_s} C_x \quad (8)$$

For the substrates the μ value can be written as follows:

Glucose

$$\mu_G = \frac{\mu_{max,G}\ C_{C_6H_{12}O_6}}{K_{S,G} + C_{C_6H_{12}O_6}} \quad (9)$$

Lactic acid

$$\mu_L = \frac{\mu_{max,L}\ C_{C_3H_6O_3}}{K_{S,L} + C_{C_3H_6O_3} + \frac{{C_{C_3H_6O_3}}^2}{K_{I,L}}} \quad (10)$$

In this mathematical modeling a summative approach for μ is adopted.

$$\mu = \frac{1}{C_x}\frac{dC_x}{dt} = \mu_L + \mu_G \quad (11)$$

Or, $\frac{dC_x}{dt} = (\mu_L + \mu_G) \times C_x$

After putting the value of μ_L and μ_G

$$\frac{dC_x}{dt} = \left(\frac{\mu_{max,L} \times C_{C_3H_6O_3}}{K_{S,L} + C_{C_3H_6O_3} + \frac{{C_{C_3H_6O_3}}^2}{K_{I,L}}} + \frac{\mu_{max,G} \times C_{C_6H_{12}O_6}}{K_{S,G} + C_{C_6H_{12}O_6}} \right) C_x \quad (12)$$

The mole balance equation for substrate consuming lactic acid and glucose will be as follows:

$$\frac{dC_{C_3H_6O_3}}{dt} = -\frac{1}{Y_L}\left(\frac{\mu_{max,L} \times C_{C_3H_6O_3}}{K_{S,L} + C_{C_3H_6O_3} + \frac{{C_{C_3H_6O_3}}^2}{K_{I,L}}} \right) C_x \quad (13)$$

$$\frac{dC_{C_6H_{12}O_6}}{dt} = -\frac{1}{Y_G}\left(\frac{\mu_{max,G} \times C_{C_6H_{12}O_6}}{K_{S,G} + C_{C_6H_{12}O_6}} \right) C_x \quad (14)$$

In the anode chamber, the comprehensive assessment of the total CO2 mass takes into account both the CO2 production and the dynamic process of mass transfer, wherein CO2 shifts from its dissolved state to the gaseous phase.

The mass balance of total CO2 in the anode chamber written as:

$$\frac{dCO_2}{dt} = \left((-\frac{1}{Y_L}) * \left(\frac{3*\mu_{max,L}\times C_{C_3H_6O_3}}{k_{S,L}+C_{C_3H_6O_3}+\frac{C_{C_3H_6O_3}{}^2}{k_{I,L}}}\right) * C_x\right) + \left((-\frac{1}{Y_G}) * \left(\frac{6*\mu_{max,G}*C_{C_6H_{12}O_6}}{k_{s,G}+C_{C_6H_{12}O_6}}\right) * C_x\right) - k_L aCO_2(C_{CO_{2_L}} - C^*_{CO_2}) \quad (15)$$

Where, '$k_L a_{CO_2}$' is overall volumetric CO_2 mass transfer coefficient (h^{-1}), '$C^*_{CO_2}$' is the equilibrium concentration of the dissolved CO_2 (g m−3).

Overall mass balance for hydrogen ion will be:

$$\frac{dH^+}{dt} = \left((-\frac{1}{Y_L}) * \left(\frac{12*\mu_{max,L}\times C_{C_3H_6O_3}}{k_{S,L}+C_{C_3H_6O_3}+\frac{C_{C_3H_6O_3}{}^2}{k_{I,L}}}\right) * C_x\right) + \left((-\frac{1}{Y_G}) * \left(\frac{24*\mu_{max,G}*C_{C_6H_{12}O_6}}{k_{s,G}+C_{C_6H_{12}O_6}}\right) * C_x\right) \quad (16)$$

4.2.4. Mole balance in cathode chamber

To study the growth kinetics of *Leptolyngbya Subtilis JUCHE01* Monod growth kinetics is considered. According to Monod model, the growth rate of algae can be written as:

$$\frac{dC_{x_a}}{dt} = \frac{\mu_{max_a} \times C_{CO_{2_L}}}{K_{CO_2} + C_{CO_{2_L}}} * C_{x_a} \quad (17)$$

Considering the consumption of CO_2 due to presence of algae and CO_2 coming from the anode chamber and dissolving in the catholyte, the mass balance of dissolved CO_2 in cathode chamber written as:

$$\frac{dCO_2}{dt} = \left(-\frac{1}{Y_{x_a/_{CO_2}}}\right) * \left(\frac{\mu_{max_a}\times C_{CO_{2_L}}}{K_{CO_2}+C_{CO_{2_L}}} * C_{x_a}\right) + k_L a_{CO_2} * \left(\mathrm{H}_{CO_2} * C_{CO_{2_L}} - C^*_{CO_2}\right) \quad (18)$$

Where, 'H_{CO_2}' is the Henrey's constant for CO_2, '$k_L a_{CO_2}$' is overall volumetric CO_2 mass transfer coefficient (h^{-1}), '$C^*_{CO_2}$' is the equilibrium or saturation concentration of the dissolved CO_2 (g m−3).

At cathode chamber, continuous CO_2 sequestration due to presence of algae, continuously replenishes the electron acceptor O_2, but the production of oxygen only occurs on day time due to presence of sunlight as no external light source is being used here. So, the mole balance of dissolved oxygen in cathode chamber can be written as:

$$\frac{dO_2}{dt} = \left(\frac{1}{Y_{xa}/_{CO_2}}\right) * \left(\frac{\mu_{max_a} \times C_{CO_{2_L}}}{K_{CO_2} + C_{CO_{2_L}}} * C_{x_a}\right) - k_L a_{O_2} * \left(C^*_{O_2} - C_{O_{2_L}}\right) \quad (19)$$

With production of oxygen due to consumption of CO_2, mass transfer of oxygen occurs according to the following equation:

$$\frac{dO_2}{dt} = -k_L \mathrm{a} O_2 * \left(C^*_{O_2} - C_{O_{2_L}}\right) \text{(in night time)} \quad (20)$$

4.2.5. Overall mass balance of carbon-di-oxide:

Continuous sequestration of carbon di oxide making the BAFC more advanced and advantageous over a conventional MFC. The production of CO_2 due to anaerobic digestion, the mass transfer of CO_2 at the anode chamber, the presence of CO_2 in the cathode headspace and also dissolved CO_2 in the catholyte occurring in the BAFC reactor simultaneously. Taking assumption that whatever CO_2 is producing due to bacterial anaerobic digestion is completely moving across the anode chamber to the cathode chamber, the overall mass balance of CO_2 can be written as:

$$\frac{dCO_2}{dt} = k_L a_{CO_2} * \left(\frac{V_{l_A}}{V_g}\right) * \left(C_{CO_{2_A}} - C^*_{CO_2}\right) - k_L a_{CO_2} * \left(\frac{V_{l_C}}{V_g}\right) * \left(\mathrm{H}_{CO_2} * C_{CO_{2_g}} - C_{CO_{2_C}}\right) \quad (21)$$

Where, 'V_{l_A}'is the total volume of anolyte present in anode chamber (L), 'V_g' is the total volume of headspace including anode and cathode chamber (L), '$C_{CO_{2_A}}$' is the concentration of CO_2 present in the headspace due to production from the anaerobic digestion in the anode chamber (g/L), '$C^*_{CO_2}$' is the CO_2 concentration already existing in the headspace of total anode chamber

case of purging in the cathode chamber headspace (g/L), ' V_{l_C}' is the total volume of catholyte present in cathode chamber (L), '$C_{CO_{2_g}}$' is the total concentration of CO_2 present in the headspace or in ' V_g' (g/L), '$C_{CO_{2_C}}$' is the total concentration of CO_2 dissolved in the catholyte (g/L).

4.2.6. Electrochemical model

Electro-active bacteria (EAB) are able to transfer electrons by oxidizing substrates to a solid electrode. In this work, Nernst equation used for describing the electrochemical behavior of the system and the equation is:

$$E_{cell} = E^0_{cell} - \frac{R*T}{n*F} ln(Q_a) \quad (22)$$

Where, reaction quotient

$$Q_a = \frac{concentration\ of\ products}{concemtration\ of\ reactants}$$

For anode half-cell the equation followed by Nernst equation can be written as:

$$E_{anode} = E^0_{anode} - \frac{R*T}{n1*F} ln(Q_{anode}) \quad (23)$$

As, there are two substrate in the anode compartment so, the Q_{anode} will be:

$$Q_{anode} = Q_{LC} \times Q_{GLc} \quad (24)$$

Where, the value of $Q_{AC}$and $Q_{Lc}$are:

$$Q_{LC} = \left(\frac{[C_{CO_2}]^3 * [C_{H^+}]^{12}}{[C_{C_3H_6O_3}]}\right) \quad (25)$$

Q_{LC} = Reaction quotient for Lactic acid based Whey

$$Q_{GLc} = \left(\frac{[C_{CO_2}]^6 * [C_{H^+}]^{24}}{[C_6H_{12}O_6]}\right) \quad (26)$$

Q_{GLc} = Reaction quotient for Glucose in lignocellulosic hydrolysate

Putting the Value of Q_{LC} and Q_{GLc} in equation no (23) and after rearranging the equation:

$$E_{anode} = E^0_{anode} - \frac{R*T}{n*F} ln\left(\frac{[C_{CO_2}]^6 * [C_{H^+}]^{24}}{[C_6H_{12}O_6]} \times \frac{[C_{CO_2}]^3 * [C_{H^+}]^{12}}{[C_{C_3H_6O_3}]}\right) \quad (27)$$

In the same manner, the potential of cathode chamber can be calculated as:

$$E_{cathode} = E^0_{cathode} - \frac{R * T}{n2 * F} ln(Q_{cathode}) \quad (28)$$

Where, the value of

$$Q_{Cathode} = \left(\frac{[C_{H_2O}]}{[C_{H^+}] * [C_{O2}]}\right)$$

So, rearranging equation 28 we get:

$$E_{cathode} = E^0_{cathode} - \frac{R * T}{n2 * F} ln\left(\frac{[C_{H_2O}]}{[C_{H^+}] * [C_{O2}]}\right) \quad (29)$$

The potential of the MFC can then be calculated as,

$$E_{BAFC} = E_{cathode} - E_{anode} \quad (30)$$

4.2.7. Equations calculating electric current, current density and power density

From the value of the resistance attached to the circuit and the EMF or voltage generated (determined from the Nernst equation) in the cell we can find the value of electric current flowing through the circuit by using Ohm's law:

$$I_{cell} = \frac{E_{anode}}{R} \quad (31)$$

Dividing the I_{cell} by the anode surface area we can find the current density:

$$I_d = \frac{I_{cell}}{A} \quad (32)$$

Finally, the power density of the cell can be calculated from the equation:

$$P_d = \frac{I_{cell} \times E_{anode}}{A}$$

(33)

Nomenclature:

μ	Specific growth rate (hr-1).
μ_L	Specific growth rate lactic acid (hr-1).
μ_G	Specific growth rate glucose (hr-1).
μ_{max}	Maximum specific growth rate (hr-1)
$\mu_{max,G}$	Maximum specific growth rate of *Shewanella putrefaciens* in Glucose(hr-1).
$\mu_{max,L}$	Maximum specific growth rate of *Shewanella putrefaciens* in lactic acid(hr-1).
k_S	Substrate saturation constant (mg/l).
$k_{S,G}$	Substrate saturation constant of *Shewanella putrefaciens* in Glucose substrate(mg/l).
$k_{S,L}$	Substrate saturation constant of *Shewanella putrefaciens* in Lactic acid substrate(mg/l).
C_x	Concentration of bacteria (mg/l).
C_{x0}	Initial concentration of bacteria (mg/l).
$C_{S,}$	Concentration of Substrate (mg/l).
$C_{S,2}$	Concentration of Lactic acid(mg/l).
V_L	Total volume of liquid in anode chamber
$C_{S(t+dt)}$	Final substrate concentration
KI	Substrate Inhibition constant (mg/l)
$Y_{(x/S),}$	Substrate to biomass yield
Y_G	Yield of bacteria in presence of Glucose.
Y_L	Yield of bacteria in presence of Lactic acid.
$k_L a_{CO_2}$	Overall volumetric CO_2 mass transfer coefficient (h^{-1})
$C^*_{CO_2}$	Equilibrium concentration of the dissolved CO_2 (g m−3).
H_{CO_2}	The henrey's constant
$k_L a_{O_2}$	Overall volumetric oxygen mass transfer coefficient
$C_{O_{2_L}}$	Dissolved oxygen concentration,
$C^*_{O_2}$	Equilibrium or saturation concentration of the dissolved oxygen.
V_{l_A}	Total volume of anolyte present in anode chamber (L)

V_g	Total volume of headspace including anode and cathode chamber (L)
V_{l_C}	Total volume of catholyte present in cathode chamber (L)
$C_{CO_{2_A}}$	Concentration of CO_2 present in the headspace in the anode chamber (gm/L)
$C_{CO_{2_C}}$	Total concentration of CO_2 dissolved in the catholyte (gm/L).
$C_{CO_{2_g}}$	Total concentration of CO_2 present in the headspace (gm/L)
E_{cell}	E.M.F of a cell (V).
$E^0{}_{cell}$	Standard EMF of a cell (V).
E_{Anode}	E.M.F of the anodic half-cell of the BFC (V).
E^0_{anode}	Standard E.M.F of anodic half-cell of the BFC (V).
Q_{anode}	Reaction quotient of the reaction occurring at the anode.
Q_{AC}	Reaction quotient of acetic acid reaction.
Q_{LC}	Reaction quotient of lactic acid reaction.
n	Number of electrons.
R	Universal gas constant.
F	Number of faradays.
T	Temperature (°C).
C_{CO_2}	Concentration of CO2 (mg/l).
C_{H^+}	Concentration of H+ ions (mg/l)
$C_{C_6H_{12}O_6}$	Concentration of Glucose (mg/l).
$C_{C_3H_6O_3}$	Concentration of lactic acid (mg/l).
I_{Cell}	Current generated in a cell (A).
I_d	Current density (A/cm2).
P_d	Power density (W/m2).

Chapter 5
MATERIALS AND METHODS

5.1 Materials

5.1.1. *Shewanella putrafeciens* MTCC8104 revival and culture

Requirements for culture

- LB media with Agar
- Conical flask(100ml)
- Petri dish
- Nichrome wire inoculating loop
- Non-absorbent Cotton plug
- Aluminium foil paper.
- Stock culture of *Shewanella putrefaciens MTCC8104.*

Composition of LB media

- Peptone-10g/L
- Yeast Extract -5g/L
- Beef Extract - 5g/L
- Sodium Chloride(NaCl)–5g/L
- Agar – 15g/L

Instruments used

- Digital weighing machine
- Autoclave
- B.O.D incubator filled with CFL lights
- Laminar air flow bench.

5.1.2. Culture of *Leptolyngbya subtilis* JUCHE1

- 250 Ml conical flask
- CO_2 from gas cylinder

- Catholyte media(M-18)
- Non-absorbent cotton plug
- Aluminium foil.
- Paraffin wax film.
- Stock culture of *Leptolyngbya subtilis JUCHE1*

Instruments used

- B.O.D incubator with CFL lights
- Digital weighing machine
- Autoclave

5.1.3. Preparation of Anolyte and Catholyte

Anolyte(M-9media)

Before Autoclave:

- Di-Potassium hydrogen phosphate(K_2HPO_4)–75g/l
- Potassium di-hydrogen phosphate(KH_2PO_4)–30g/l
- Potassium chloride(NaCl)–5g/l
- Ammonium chloride(NH_4Cl)- 5g/l

After autoclave:

- Magnesium sulfate($MgSO_4$)–10g/l
- Calcium Chloride($CaCl_2$)–10g/l.

Catholyte(M-18media)

- Sodium nitrate($NaNO_3$)–1.5g/l
- Magnesium sulphate heptahydrate($MgSO_4.7H_2O$)-0.38g/l
- Di-Potassium hydrogen phosphate(K_2HPO_4)–0.12g/l
- Calcium chloride dihydrate($CaCl_2.2H_2O$)–0.11g/l
- Sodium chloride(NaCl) –0.07g/l
- Ferric sulphate tetrahydrate($Fe_2(SO4)_3.4H2O$)–0.01g/l
- Boric acid powder(H_3BO_3)–0.003g/l
- Manganese sulphate tetraHydrate($MnSO_4.4H_2O$)–0.002g/l

- Zinc sulphate heptahydrate($ZnSO_4.7H_2O$)–0.002g/l
- Copper sulphate crystals($CuSO_4.5H_2O$)–0.00008g/l
- Chloride crystals($CoCl_2.6H_2O$)–0.00004g/l.

Instruments used

- Digital weighing machine
- Autoclave

5.1.4.Materials required for BAFC setup

- PET vessel as cathodic chamber (Volume – 1 L).
- Earthen column as anodic chamber (Volume – 400 mL).
- Ceramic material of the earthen column's wall as proton exchange membrane.
- Electrical wires for making the connection between cathode and the anode with an external Resistance (variable).
- *Shewanella putrefaciens MTCC 8104* preformed Electrochemically active bacteria (EAB).
- *Leptolyngbya subtilis JUCHE1* preformed as cathodic electron acceptor producer.
- Carbon cloth (10cm*4cm) as electrodes in both chamber.
- PET pipe (Length – 100 mm, internal diameter – 2 mm) for CO2 flow from anodic chamber to cathodic chamber.
- Anolyte as Anode media (M-9).
- Catholyte as Cathode media (M-18).
- Substrates present in wastewater – Glucose (simulate wastewater containing Glucose sugar industry) and Lactic Acid (to simulate wastewater containing cheese whey from food industry).
- Measuring Cylinder.
- Micro pipette.
- Micro tips.
- Paraffin wax film.

5.2.Methods

5.2.1.Culture of *Shewanella putrefaciens* MTCC 8104

1. LB media was prepared with agar and autoclaved.
2. The autoclaved LB media containing agar was taken to the laminar air flow bench and unplugged under sterile condition.
3. The liquid phase LB media poured into petri dish (sterilize under UV ray for 15 min) and cooled down for 30 minutes , after cooling it became harden.
4. An inoculating loop made of nichrome wire was burned in flame of the spirit lamp and cooled down for a minute.
5. Now with the help of the loop a distinct colony of viable bacteria from previously plated bacterial culture plate was added to the agar LB media which was platted in petri dish .
6. Next the petri dish sealed with the help of paraffin wax film .
7. Finally the petri dish was taken out of the LAFB and since the bacteria used is mesophilic so it is incubated at 37ºC for almost 24 hours and then transferred to the refrigerator after significant growth in colony form was observed.

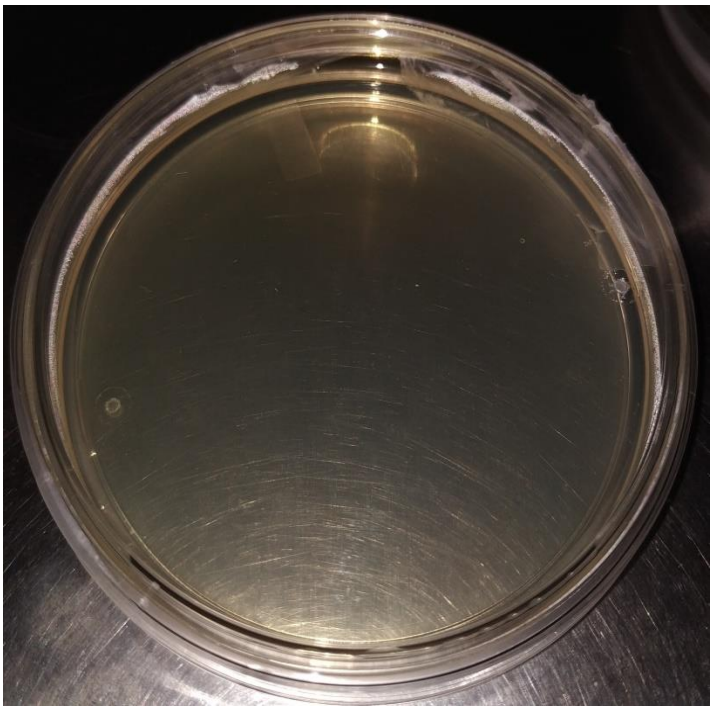


Figure 4: Showing plate with lb broth poured into it without bacteria.

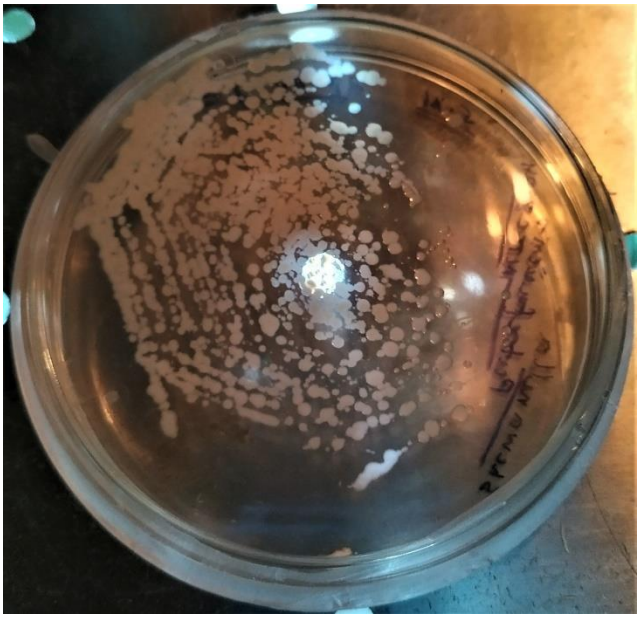


Figure5: Showing Plate with bacterial colony viz., *Shewanella putrafeciens*.

5.2.2.Experimental procedure for growth kinetic batch study of *Shewanella putrefaciens* with variations of Glucose as substrate

1. The batch study was conducted for 48 hours .
2. Total 25 conical flasks of 50 ml volume each were taken and filled with 10 ml of anolyte media.
3. One conical was considered as control and was not inoculated.
4. To the rest of the conical varying concentrations of glucose viz., 0.45mg/l, 0.9mg/l, 1.8 mg/l, 3.6mg/l and 7.224mg/l were added.
5. Then each of the conical flasks were inoculated with 1 mL of bacterial culture (*Shewanella putrefaciens)* with the help of millipipette and sterilised tip. The inoculation was done from a preadapted culture of the bacteria in the same media with glucose as a substrate i.e., anolyte.
6. After inoculation, the flasks were sealed with the help of parafilm and then purged N_2 in order to maintain anaerobic condition.
7. After purging the flasks were sealed with the help of parafilm and foil paper.
8. Starting from 0^{th} hour the samples for each concentration was analyzed for every 2^{th} , 4^{th} , 6^{th}, 24^{th} , 48^{th} hour interval.
9. For each run of the experiment the culture for each concentration was taken out in a 3 ml

glass cuvette and analyzed in a UV Spectrometer to measure the absorbance of the sample at a wavelength of 600nm.

10. A 1.5 mL Eppendorf tube was taken and it was weighed using a weighing machine. The collected sample was then poured in the pre-weighed Eppendorf tube and then centrifuged for 10 min at 10000 rpm.
11. After the centrifuge, the Eppendorf tube with the biomass was weighed in the weighing machine and subtracted from the weight of the empty Eppendorf tube. Thus, the amount of biomass present in 1.5 mL of the sample and the biomass concentration can now be calculated.

Growth curves for cell concentration vs time was plotted for every biomass concentration for the corresponding glucose concentration. The kinetic parameters i.e., μ_{max} and k_S were calculated by using the Monod equation.

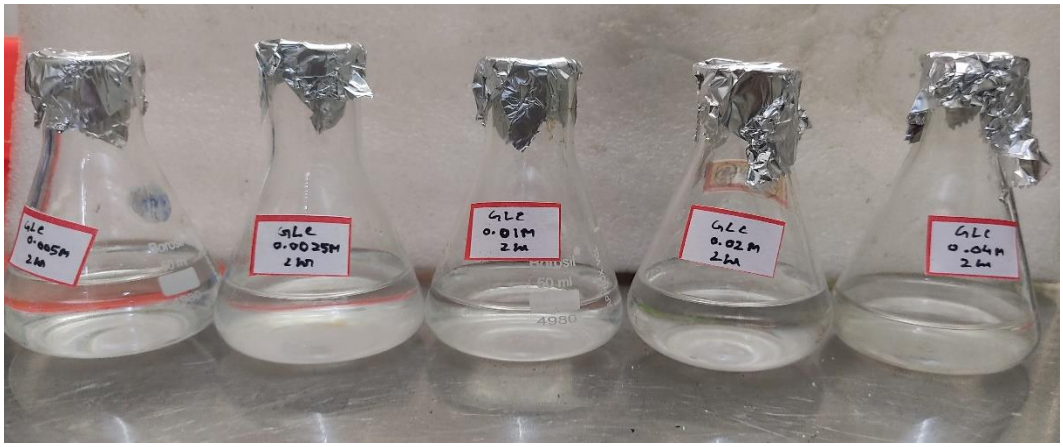


Figure 6: batch study of *Shewanella putrefaciens* in Glucose substrate.

5.2.3.Experimental procedure for growth kinetic batch study of *Shewanella putrefaciens* with variations of Lactic acid as substrate

1. The batch study was conducted for 48 hours .
2. Total 12 conical flasks of 50 ml volume each were taken and filled with 25 ml of anolyte media.
3. One conical was considered as control and was not inoculated.
4. To the rest of the conical varying concentrations of lactic acid viz., 625 ppm , 1250 ppm, 2500 ppm, 5000 ppm were added.

5. Then each of the conical flasks were inoculated with 1 mL of bacterial culture (*Shewanella putrefaciens)* with the help of millipipette and sterilised tip. The inoculation was done from a preadapted culture of the bacteria in the same media with lactic acid as a substrate .
6. After inoculation, the flasks were sealed with the help of parafilm and then purged N_2 in order to maintain anaerobic condition.
7. After purging the flasks were sealed with the help of parafilm and foil paper.
8. Starting from 0^{th} hour the samples for each concentration was analyzed for every 4^{th} , 24^{th} , 48^{th} hour interval.
9. For each run of the experiment the culture for each concentration was taken out in a 3 ml glass cuvette and analyzed in a UV Spectrometer to measure the absorbance of the sample at a wavelength of 600nm.
10. A 1.5 mL Eppendorf tube was taken and it was weighed using a weighing machine. The collected sample was then poured in the pre-weighed Eppendorf tube and then centrifuged for 10 min at 10000 rpm.
11. After the centrifuge, the Eppendorf tube with the biomass was weighed in the weighing machine and subtracted from the weight of the empty Eppendorf tube. Thus, the amount of biomass present in 1.5 mL of the sample and the biomass concentration can now be calculated.

Growth curves for cell concentration vs time was plotted for every biomass concentration for the corresponding glucose concentration. The kinetic parameters i.e., μ_{max} and k_S were calculated by using the Monod equation.

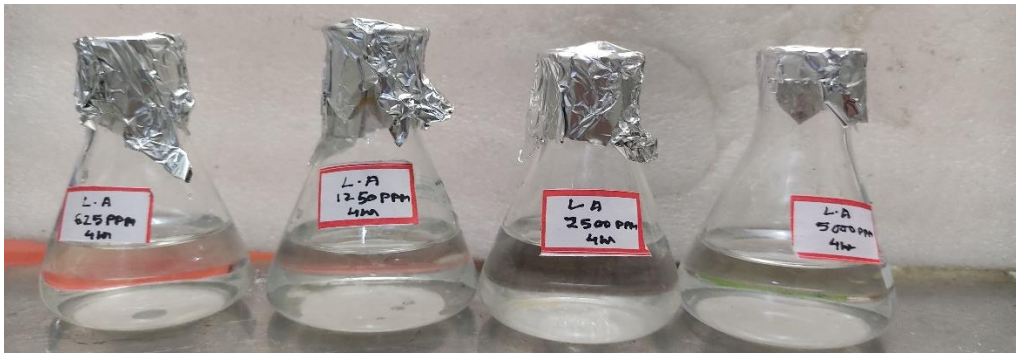


Figure 7: batch study of *Shewanella putrefaciens* in Lactic acid substrate.

5.2.4.Spectrophotometric analysis of Glucose in order to determine the yield of *Shewanella putrefaciens* in glucose substrate

Procedure to make DNS reagent

1. One 250 ml conical flask was taken and a 20 ml of 2M NaOH solution was prepared.
2. Dissolved 1 g of DNS (Dinitrosalicylic acid) into the NaOH solution.
3. Slowly 30 g potassium tartarate added into the solution.
4. Make up to 100 ml with distilled water.



Figure 8: DNS Reagent

5.2.4.1. Calibration curve construction

1. Four different concentrations of glucose were taken. They were 0.5 g/l , 0.25 g/l, 0.125 g/l , 0.0625 g/l glucose.
2. From each glucose solution 2 ml of solution taken out using a mili pipet to a test tube.
3. Then 2 ml of DNS reagent was added to each test tube.
4. The reference solution chosen for this analysis was 2 mL distilled water with 2ml of DNS of reagent
5. Then all the test tube containing solutions along with reference solution heated with boil water for 2-3 minutes for color change.
6. the colour change that happened in the solutions were analysed using a UV spectrophotometer (540 nm).
7. The construction of calibration plot was done by using an excel spread sheet.

5.2.4.2. Determination of Glucose in bacterial sample

1. A 50 mL conical flask was taken and it was filled with anolyte and then 0.5 g/l Glucose substrate was added to it. The sample was then inoculated with bacteria (*Shewanella*

putrefaciens) and kept in the incubator for 24 hour duration..

2. After 24 h the culture was taken out in a 1.5 mL eppendrof it was centrifuged to separate the bacterial liquid form the cells.
3. The supernatant which was left was taken out using a pipette .
4. The supernatant was then diluted to its original concentration.
5. Glucose present was then determined by spectrophotometric method.
6. Then concentration of the glucose was determined from the calibration curve constructed.

5.2.4.3. Determination of yield

1. From the process mentioned in section 5.2.4.2 concentration glucose was measured after 24 h and the initial concentration of glucose at zero hour was known (0.5 g/l Glucose).
2. We had determined previously the biomass concentration at zero hour and biomass concentration at 24 hour.
3. The yield of the bacteria (*Shewanella putrefaciens*) in glucose was measured by the formula mentioned below:

$$Y = \frac{Biomass\ concentration\ at\ 24\ hour - biomass\ concentration\ at\ 0\ hours}{Substrate\ concentration\ at\ 24\ hour - Substrate\ concentration\ at\ 0\ hour}$$

5.2.5.Spectrophotometric analysis of lactic acid in order to determine the yield of *Shewanella putrefaciens* in lactic acid substrate

5.2.5.1. Calibration curve construction

1. A 0.2% solution of Ferric chloride ($FeCl_3$) was prepared in a 100 mL volumetric flask.
2. The above mentioned solution was stirred continuously till the complete dissolution of the salt.
3. The solution was prepared at a room temperature of 25 ºC.
4. Five different concentrations of lactic acid were taken. They were 14000 ppm, 12000 ppm, 10000 ppm, 8000 ppm, 6000 ppm lactic acid.
5. The lactic acid solutions were then added to 0.2% Ferric chloride solution (50µL in 2 mL of $FeCl_3$) and the colour change that happened in the solutions were analysed using a UV spectrophotometer (390 nm).

6. The reference solution chosen for this analysis was 2 mL of 0.2% Ferric Chloride solution.
7. The construction of calibration plot was done by using an excel spread sheet.

5.2.5.2. Determination of lactic acid in bacterial sample

1. A 50 mL conical flask was taken and it was filled with anolyte and then 2500 ppm lactic acid substrate was added to it. The sample was then inoculated with bacteria (*Shewanella putrefaciens*) and kept in the incubator for 24hour duration.
2. After 24 h the culture was taken out in a 1.5 mL eppendrof it was centrifuged to separate the bacterial liquid form the cells.
3. The supernatant which was left was taken out using a pipette .
4. The supernatant was then diluted to its original concentration.
5. Lactic acid present was then determined by spectrophotometric method.
6. Then concentration of the lactic acid was determined from the calibration curve constructed.

5.2.5.3.Determination of yield

1. From the process mentioned in section 5.2.5.2 concentration lactic acid was measured after 24 h and the initial concentration of lactic acid at zero hour was known (2500 ppm lactic acid).
2. We had determined previously the biomass concentration at zero hour and biomass concentration at 24 hour.
3. The yield of the bacteria (*Shewanella putrefaciens*) in lactic acid was measured by the formula mentioned below:

$$Y = \frac{Biomass\ concentration\ at\ 24\ hour - biomass\ concentration\ at\ 0\ hours}{Substrate\ concentration\ at\ 24\ hour - Substrate\ concentration\ at\ 0\ hour}$$

5.2.6.Bacterio-algal Fuel Cell Set-up

1. PET vessel used as cathodic chamber was filled with 200 mL of catholyte media.
2. The earthen column used as anode chamber was filled with 200 mL of anolyte media.
3. Two 10 mm × 4 cm of carbon cloth were cut and electrical wires were connected to it. These two served as two separate electrodes, anode and the cathode respectively.
4. The anode was dipped in the anode chamber and the cathode in the cathode chamber.

5. In the anode chamber substrate was added and after that 1 ml of preadapted bacteria (*Shewanella putrefaciens)* in the substrate was given using a pipet.
6. The catholyte in cathode chamber was purged by CO_2 gas for 15 minutes.
7. One PET pipe was taken. One end of the pipe was inserted in the anode chamber and the other end in the cathode chamber for flow of carbon di oxide, which acted as a gas passage for the CO_2 produced in the anode chamber to the catholyte.
8. The anode chamber was purged with nitrogen gas in order to maintain anaerobic condition then the anode chamber was tightly sealed using foil paper and paraffin film.
9. In cathode chamber algae was added and the PET vessel then closed with lid.
10. The wires from the electrodes are hung up outside the cathode chamber before closing lid of the cathode chamber.
11. The anode and the cathode were connected through an external resistance of 10 kΩ and hence a stable connection was established.
12. The voltage generated and the current produced was measured using a multimeter.

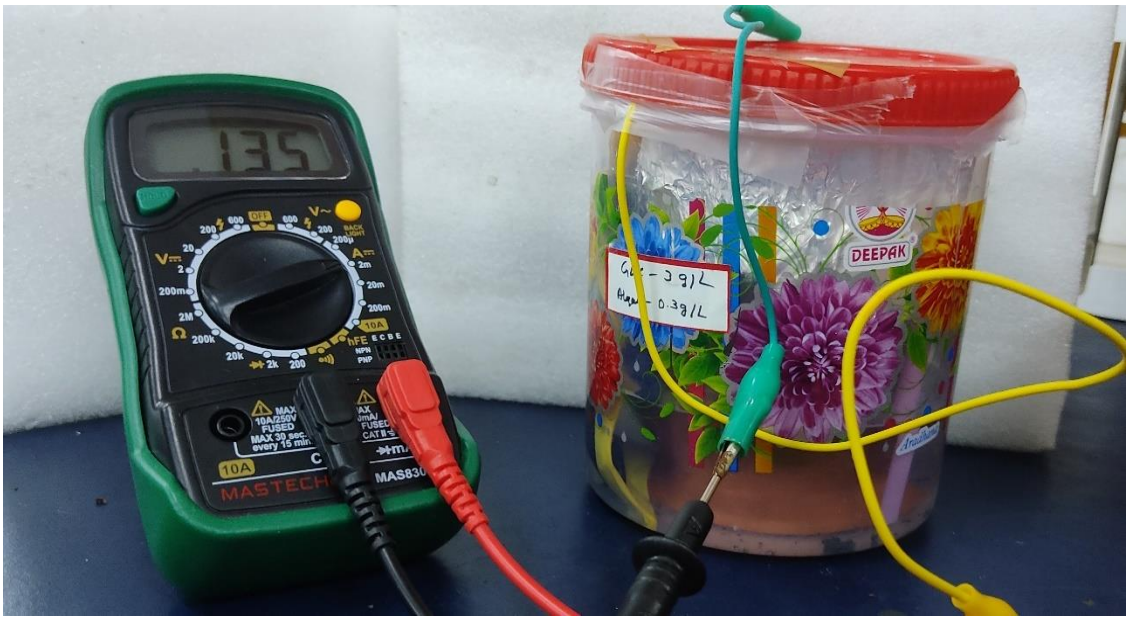


Figure 9: Bacterio-Algal Fuel cell set up

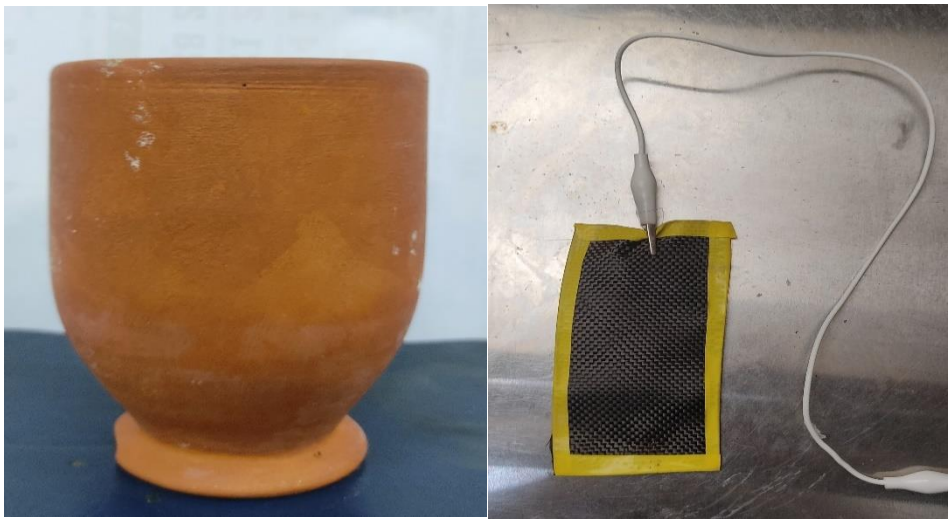


Figure 10: Showing Anode chamber and Electrode.



Figure 11: Showing Cathode chamber and Algae culture(*Leptolyngbya subtilis*).

5.2.7.Cyclic Voltammetry (CV)

Cyclic voltammetry, a method of electrochemical characterization utilized in Bacterial Fuel Cell analysis, employs a potentiostat. This technique employs a three-electrode system to gauge the current within a circuit and the resultant voltage, visualized through a voltage vs. current graph. The cyclic voltammogram presents two distinct phases: one for oxidation and the other for reduction reactions. Each of the three electrodes in a CV setup holds specific significance: the working electrode, the counter electrode, and the reference electrode. The primary chemical reactions of interest occur at the working electrode, while the counter electrode complements the cell's operation. The applied potential is measured between the working and counter electrodes, determining the current at either electrode. Beyond completing the circuit, the counter electrode

stabilizes the interfacial potential at the working electrode, independent of current flow. Conversely, the reference electrode serves to benchmark and regulate the potential of the working electrode without engaging in current transfer. In our study, cyclic voltammetry was employed to ascertain the current output of a BAFC.

The BAFC setup is made following the procedure illustrated in section 5.2.6.

1. The setup is kept for 2 days in the incubator at a temperature of 37 °C to allow the bacteria present in the anode chamber to grow.
2. On 2th day the setup is taken out for cyclic voltammetry study.
3. Carbon cloth electrodes used for anode and cathode are used as working and the counter electrode respectively. Saturated calomel electrode (SCE) is chosen as reference electrode for the experiment.

The peak currents were noted in order to calculate the current and the voltage at the oxidation peak and the reduction peak.



Figure 12: A BAFC set-up undergoing Cyclic Voltammetry analysis using potentiostat.

5.2.8. Response Surface Methodology

Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes. It is particularly useful in situations where multiple input variables potentially influence some performance measure or quality characteristic of the process.

Objective:

- To model and analyze problems in which a response of interest is influenced by several variables.
- To optimize this response (output) by finding the optimal levels of the input variables.

Process:

- Input variables(substrate concentration, time and algae concentration)
- Response variable(power density)
- Employing the fitted model to find the optimal conditions for the input variables to achieve the desired response.

Steps in RSM:

- Screening: Identify the significant factors affecting the response.
- Experimentation: Design experiments to explore the effects of these factors.
- Modeling: Fit a response surface model to the experimental data.
- Optimization: Determine the optimum levels of the input factors by analyzing the fitted model.

Overall, RSM is a powerful tool looking to improve processes and products by optimizing the variables that influence the desired outcomes.

5.2.9. Mathematical modeling using MATLAB

The mathematical modeling of BAFC was done using MATLAB 2017b software using ODE45 which is based on Runge Kutta method. Using the Monod parameters obtained from the experiment the i.e., maximum specific growth rate and substrate saturation constant and also the yield obtained from spectrophotometric assay glucose and lactic acid, mass balance equations for the substrates and products involved in the system, the unknown parameters related to our experiment could be derived theoretically. Validating our model by comparing its predictions to experimental data or other sources of information. This will help us to ensure that how our model accurately captures the behavior of the bacteria-algal fuel cell system.

MATLAB provides a powerful platform for building and simulating mathematical models due to its extensive mathematical and numerical computing capabilities.

Chapter 6
RESULTS AND DISCUSSIONS

6.1. Evaluation of growth kinetics parameters μ_{max} and k_S

6.1.1 Evaluation of growth kinetics parameters for *Shewanella putrefaciens* in various concentration of Glucose substrate in anolyte.

Growth kinetics parameters for the bacteria *Shewanella putrefaciens* has been obtained by varying the glucose concentration which is 0.0625 g/l to 0.5 g/l (0.0025 M to 0.04 M).

Table 4: Data of optical density(600 nm) and the corresponding time for glucose concentration

Time(hr)	0.0025M	0.005M	0.01M	0.02M	0.04M
0	0.023	0.017	0.02	0.016	0.016
2	0.027	0.021	0.023	0.02	0.019
4	0.018	0.016	0.025	0.017	0.025
6	0.033	0.022	0.03	0.014	0.018
24	0.038	0.032	0.038	0.034	0.064
48	0.095	0.086	0.129	0.107	0.125

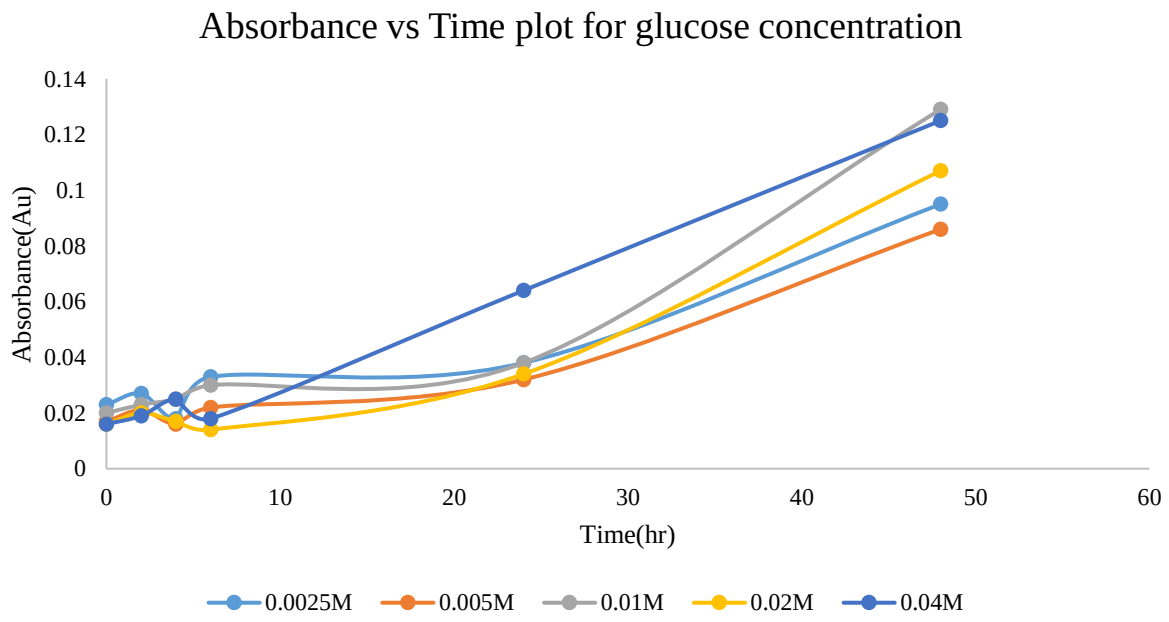


Figure 13: Plot on Absorbance and corresponding time for glucose concentration.

Table 5: Biomass concentration data of *Shewanella putrefaciens* at different glucose concentrations and corresponding times.

Time(hr)	0.0025M	0.005M	0.01M	0.02M	0.04M
0	0.058	0.01	0.025	0.058	0.011
2	0.067	0.014	0.033	0.065	0.017
4	0.068	0.042	0.088	0.078	0.063
6	0.071	0.091	0.097	0.098	0.104
24	0.084	0.101	0.136	0.149	0.167
48	0.128	0.132	0.229	0.232	0.242

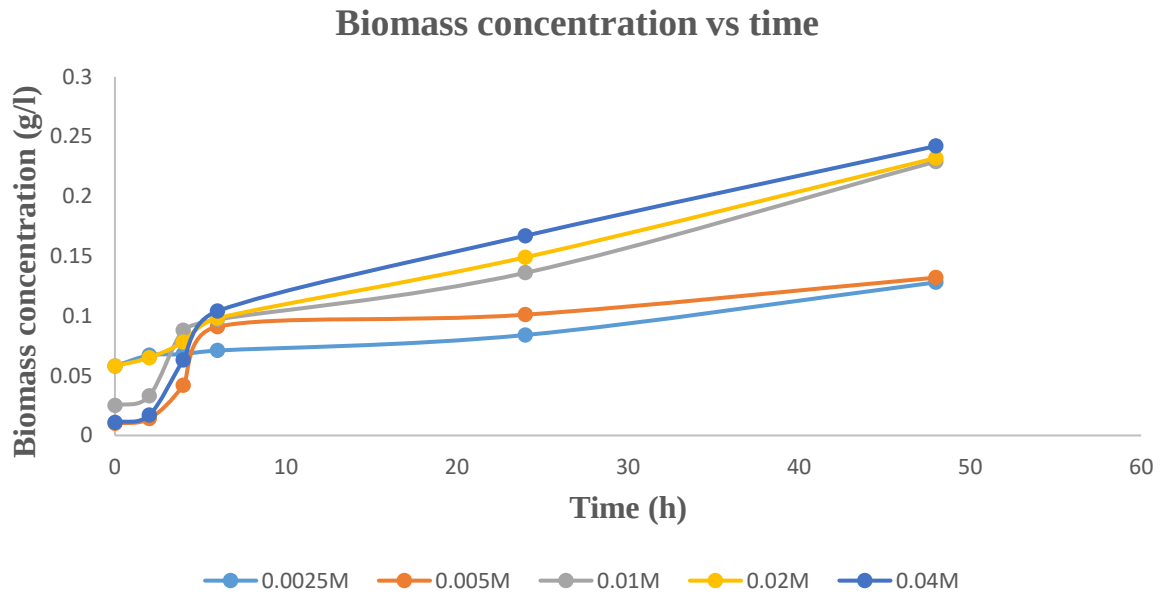


Figure 14: Biomass concentration plot of *Shewanella putrefaciens* at different glucose concentrations and corresponding times.

It can be referred from the above growth curve that the growth curve of *Shewanella putrefaciens* typically follows a sigmoidal curve. Additionally the following inferences can be drawn from the above curves:

- The culture was adapted for 24 h in the substrate prior to use and therefore the lag phase is not there in the above growth curves.
- The exponential phase continues till 48 h.

Best growth of the bacteria was obtained at 0.04M glucose (0.5 g/l) , so it's shows there is no inhibition in glucose as substrate.

To obtain the value of μ_{max} and k_S $\ln(X/X_0)$ was plotted against the corresponding value of time for different glucose concentrations . The slope of the graph gave us the value of specific growth rate of the substrate.

Table 6: Biomass logarithmic concentration data of *Shewanella putrefaciens* at different glucose concentrations and corresponding times.

Time(hr)	0.0025M	0.005M	0.01M	0.02M	0.04M
0	0	0	0	0	0
2	0.14425	0.336472	0.277632	0.113944	0.435318
4	0.159065	1.435085	1.258461	0.296266	1.745239
6	0.202237	2.208274	1.355835	0.524524	2.246496
24	0.370374	2.312535	1.693779	0.943503	2.720099
48	0.791587	2.580217	2.214846	1.386294	3.091042

Table 7: Data of $\ln(X/X_0)$ and corresponding time for 0.0025M glucose

Time(hr)	$\ln(X/X_0)$
0	0
2	0.14425
4	0.159065
6	0.202237
24	0.370374
48	0.791587

The slope of the curve gives us the value of specific growth rate i.e., μ of *Shewanella putrefaciens* in 0.0025M glucose. The value of μ from the graph is estimated to be 0.0167 hr^{-1}.

Table 8: Data of $\ln(X/X_0)$ and corresponding time for 0.005M glucose.

Time(hr)	$\ln(X/X_0)$
0	0
2	0.336472
4	1.435085
6	2.208274
24	2.312535
48	2.580217

The slope of the curve gives us the value of specific growth rate i.e., μ of *Shewanella putrefaciens* in 0.005M glucose. The value of μ from the graph is estimated to be 0.3511 hr^{-1}.

Table 9: Data of $ln(X/X_0)$ and corresponding time for 0.01M glucose.

Time(hr)	$ln(X/X_0)$
0	0
2	0.277632
4	1.258461
6	1.355835
24	1.693779
48	2.214846

The slope of the curve gives us the value of specific growth rate i.e., μ of *Shewanella putrefaciens* in 0.01M glucose. The value of μ from the graph is estimated to be 0.2451 hr^{-1}.

Table 10: Data of $ln(X/X_0)$ and corresponding time for 0.02M glucose.

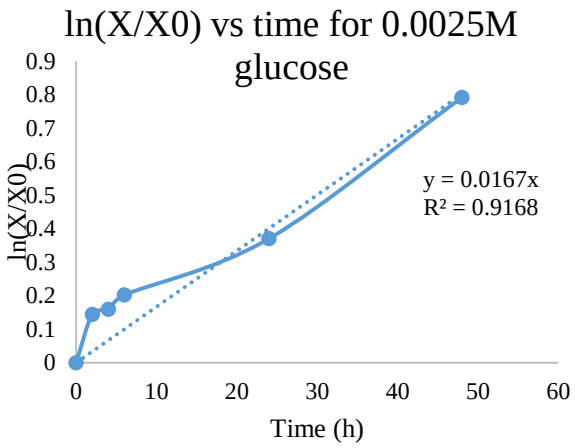
Time (h)	$ln(X/X_0)$
0	0
2	0.113944
4	0.296266
6	0.524524
24	0.943503
48	1.386294

The slope of the curve gives us the value of specific growth rate i.e., μ of *Shewanella putrefaciens* in 0.02M glucose. The value of μ from the graph is estimated to be 0.0814 hr^{-1}.

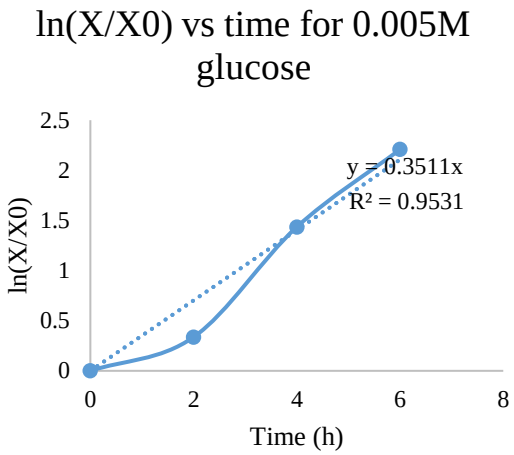
Table 11: Data of $ln(X/X_0)$ and corresponding time for 0.04M glucose.

Time (h)	$ln(X/X_0)$
0	0
2	0.435318
4	1.745239
6	2.246496
24	2.720099
48	3.091042

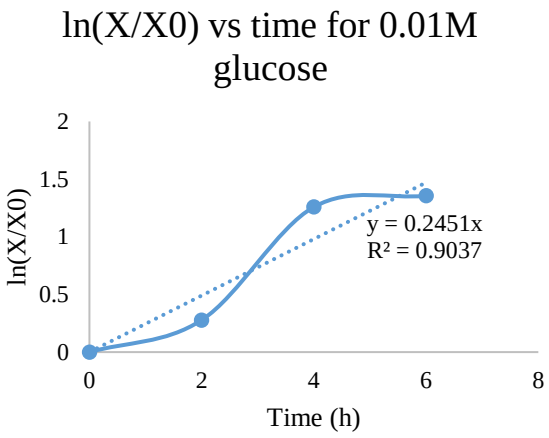
The slope of the curve gives us the value of specific growth rate i.e., μ of *Shewanella putrefaciens* in 0.04M glucose. The value of μ from the graph is estimated to be 0.3809 hr^{-1}.

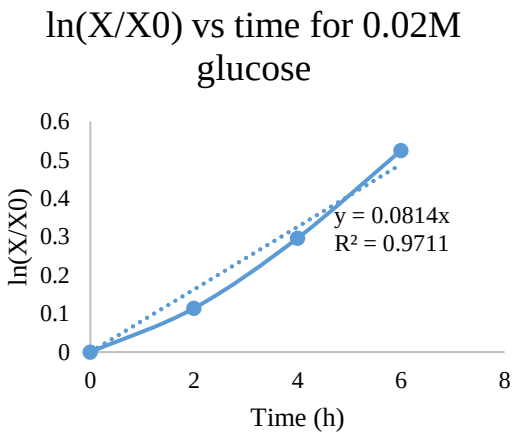


(a)



(b)





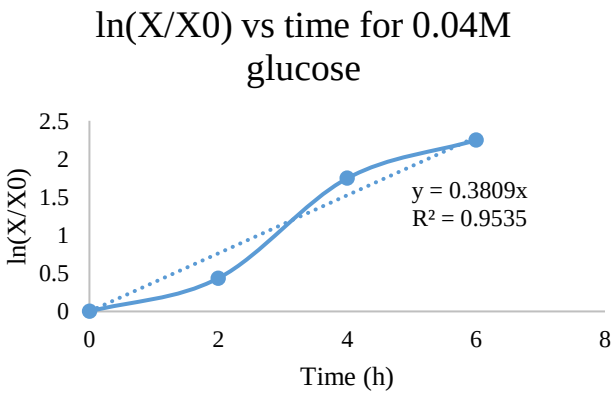


Figure 15: Plot of $\ln(X/X_0)$ and corresponding time for glucose.

Table 12: Data of μ (specific growth rate of bacteria *Shewanella putrefaciens*) and corresponding C_S (substrate concentration).

Cs(M)	μ(hr^{-1})
0.0025	0.0167
0.005	0.3511
0.01	0.2451
0.02	0.0814
0.04	0.3809

The inverse of specific growth rate (μ) was then plotted against inverse of different substrate concentrations(C_S). It gave us a straight line plot having a slope and an intercept. From the slope and intercept of the said plot the value of maximum specific growth rate i.e., μ_{max} and saturation constant i.e., k_S was obtained.

Table 13: Data of 1/μ and corresponding 1/C_S.

1/Cs	1/μ
400	59.88024
200	2.848191
100	4.079967
50	12.28501
25	2.625361

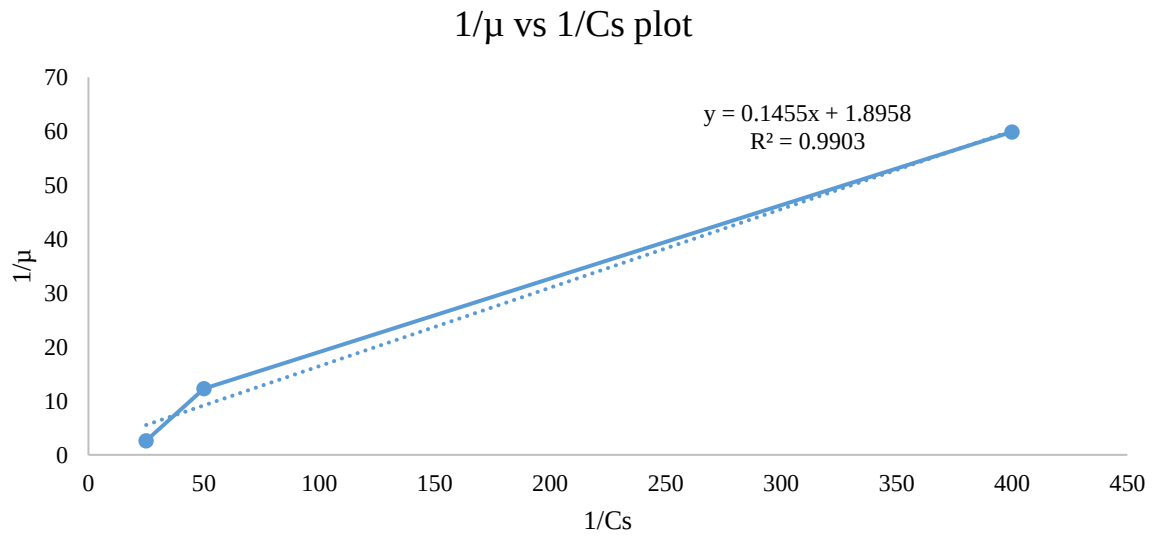


Figure 16: Plot of 1/μ and corresponding 1/C_S.

The above curve represents the Monod equation for glucose concentration. From the above curve we calculate μ_{max} and k_S as follows.

The intercept of the curve represents 1/ μ_{max} and so:

μ_{max} = 1/1.8958

or, μ_{max} **= 0.527 hr^{-1}**.

The slope of the curve gives the value of k_S/ μ_{max} and so:

k_S/ μ_{max} = 0.1455

or, k_S = 0.1455 * 0.527 or, k_S = 0.0766 g/l.

Thus, **k_S = 76.6 mg/l**.

6.1.2. Evaluation of growth kinetics parameters for *Shewanella putrefaciens* in various concentration of Lactic Acid substrate in anolyte.

Growth kinetics parameters for the bacteria *Shewanella putrefaciens* has been obtained by varying the lactic acid concentration which is 625 ppm to 5000 ppm (0.755 g/l to 6.044 g/l)

Table 14: Data of optical density(600 nm) and the corresponding time for lactic acid concentration

Time(hr)	625 PPM	1250 PPM	2500 PPM	5000 PPM
0	0.008	0.011	0.009	0.01
4	0.014	0.029	0.014	0.009
24	0.028	0.034	0.033	0.03
48	0.8	0.128	0.109	0.094

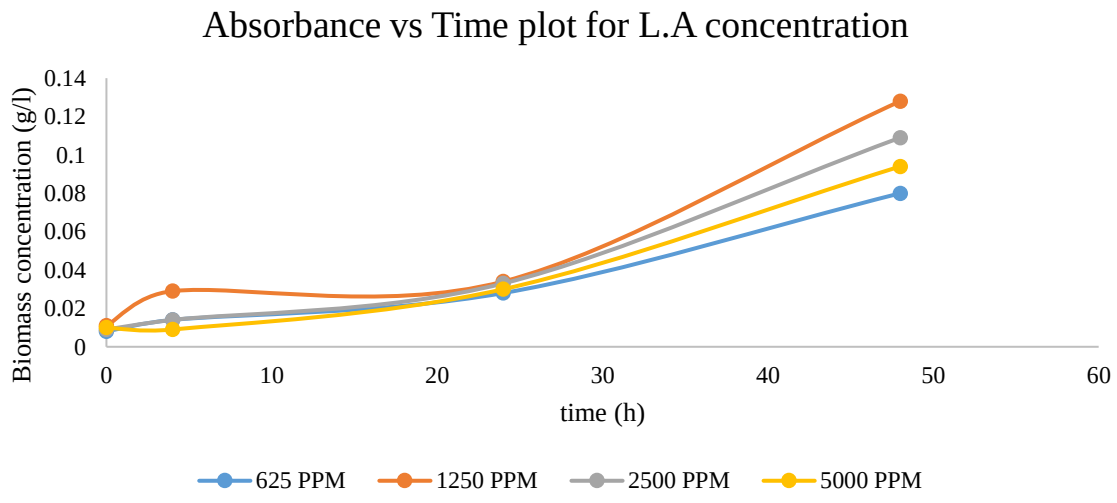


Figure 17: Plot on Absorbance and corresponding time for lactic acid concentration.

Table 15: Biomass concentration data of *Shewanella putrefaciens* at different lactic acid concentrations and corresponding times.

Time(hr)	625 PPM	1250 PPM	2500 PPM	5000 PPM
0	0.059	0.058	0.055	0.051
4	0.0651	0.0676	0.062	0.0658
24	0.082	0.1022	0.0991	0.104
48	0.08714	0.1048	0.1307	0.1115

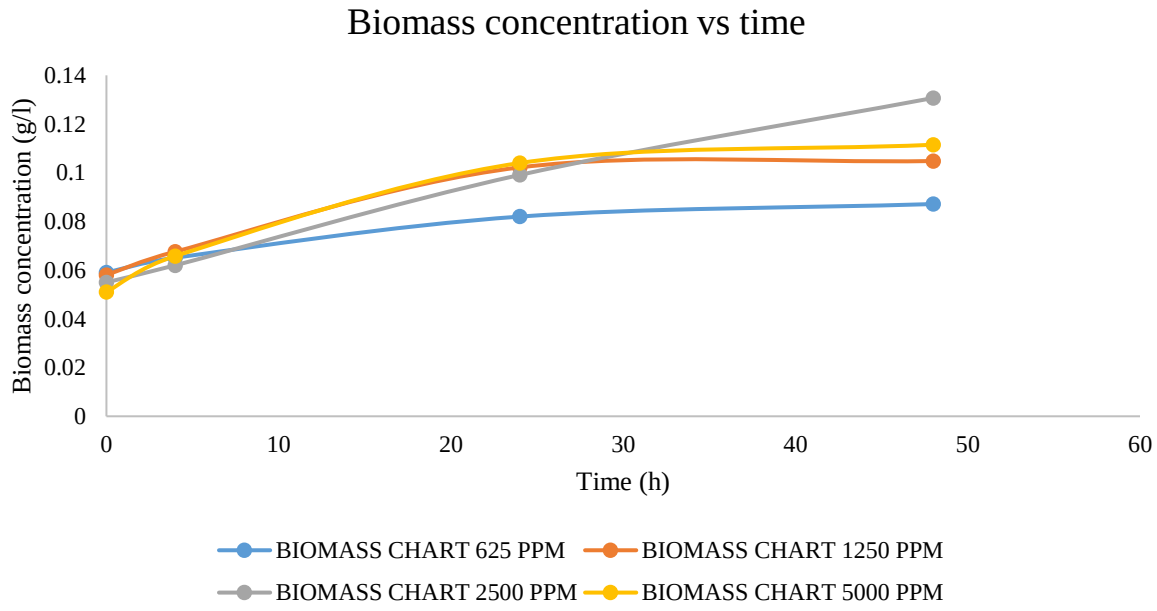


Figure 18: Biomass concentration plot of *Shewanella putrefaciens* at different lactic acid concentrations and corresponding times.

It can be referred from the above growth curve that the growth curve of *Shewanella putrefaciens* typically follows a sigmoidal curve. Additionally the following inferences can be drawn from the above curves:

- The culture was adapted for 24 h in the substrate prior to use and therefore the lag phase is not there in the above growth curves.
- The exponential phase continues till 48 h.

Best growth of the bacteria was obtained at 2500 ppm lactic acid(3.022 g/l) , so it's shows there is a inhibition effect in lactic acid as substrate.

To obtain the value of μ_{max} and k_S , $\ln(X/X_0)$ was plotted against the corresponding value of time for different lactic acid concentrations . The slope of the graph gave us the value of specific growth rate of the substrate and then there after at the critical concentration which is 2500 ppm lactic acid K_I will be obtained.

Table 16: Biomass logarithmic concentration data of *Shewanella putrefaciens* at different lactic acid concentrations and corresponding times.

Time(hr)	625 PPM	1250 PPM	2500 PPM	5000 PPM
0	0	0	0	0
4	0.098387	0.153165	0.119801	0.254794
24	0.329182	0.566489	0.588796	0.712565
48	0.389979	0.591611	0.865571	0.782199

Table 17: Data of $\ln(X/X_0)$ and corresponding time for 625 ppm lactic acid.

Time(hr)	$\ln(X/X_0)$
0	0
4	0.098387
24	0.329182
48	0.389979

The slope of the curve gives us the value of specific growth rate i.e., μ of *Shewanella putrefaciens* in 625 ppm lactic acid. The value of μ from the graph is estimated to be 0.0082 hr^{-1}.

Table 18: Data of $\ln(X/X_0)$ and corresponding time for 1250 ppm lactic acid.

Time(hr)	$\ln(X/X_0)$
0	0
4	0.153165
24	0.566489
48	0.591611

The slope of the curve gives us the value of specific growth rate i.e., μ of *Shewanella putrefaciens* in 1250 ppm lactic acid. Value of μ from the graph is estimated to be 0.024 hr^{-1}.

Table 19: Data of $\ln(X/X_0)$ and corresponding time for 2500 ppm lactic acid.

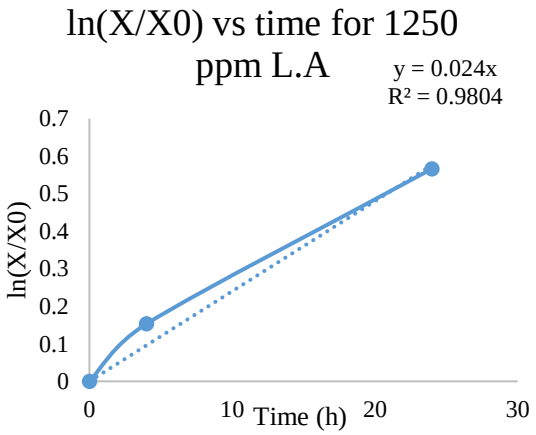
Time(hr)	$\ln(X/X_0)$
4	0.119801
24	0.588796
48	0.865571

The slope of the curve gives us the value of specific growth rate i.e., μ of *Shewanella putrefaciens* in 2500 ppm lactic acid. Value of μ from the graph is estimated to be 0.0194 hr^{-1}.

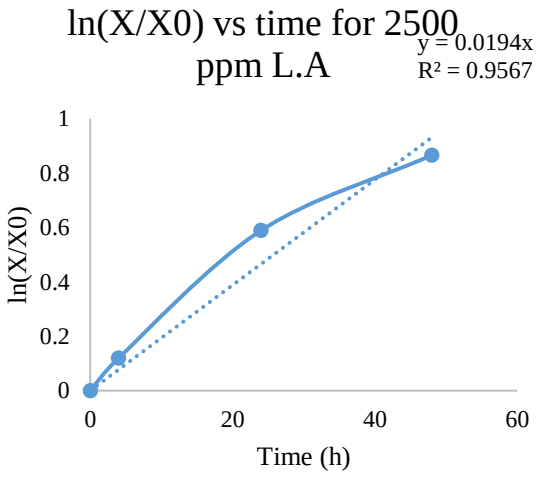
Table 20: Data of $\ln(X/X_0)$ and corresponding time for 5000 ppm lactic acid.

Time(hr)	$\ln(X/X_0)$
0	0
4	0.254794
24	0.712565
48	0.782199

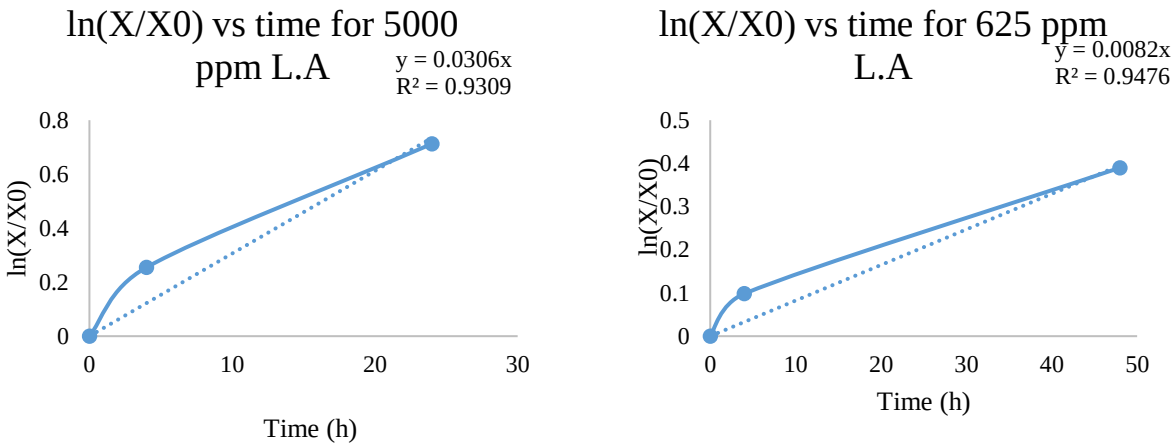
The slope of the curve gives us the value of specific growth rate i.e., μ of *Shewanella putrefaciens* in 5000 ppm lactic acid. Value of μ from the graph is estimated to be 0.0306 hr^{-1}.



(a)



(b)



(c) (d)

Figure 19: Plot of $\ln(X/X_0)$ and corresponding time lactic acid.

Table 21: Data of μ (specific growth rate of bacteria *Shewanella putrefaciens*) and corresponding C_S (substrate concentration).

Cs	μ
0.755	0.0082
1.51	0.024
3.022	0.0194
6.044	0.0306

The inverse of specific growth rate (μ) was then plotted against inverse of different substrate concentrations(C_S). It gave us a straight line plot having a slope and an intercept. From the slope and intercept of the said plot the value of maximum specific growth rate i.e., μ_{max} and saturation constant i.e., k_S was obtained.

Table 22: Data of $1/\mu$ and corresponding $1/C_S$.

1/Cs	1/μ
1.324503	121.9512
0.662252	41.66667
0.330907	51.54639
0.165453	32.67974

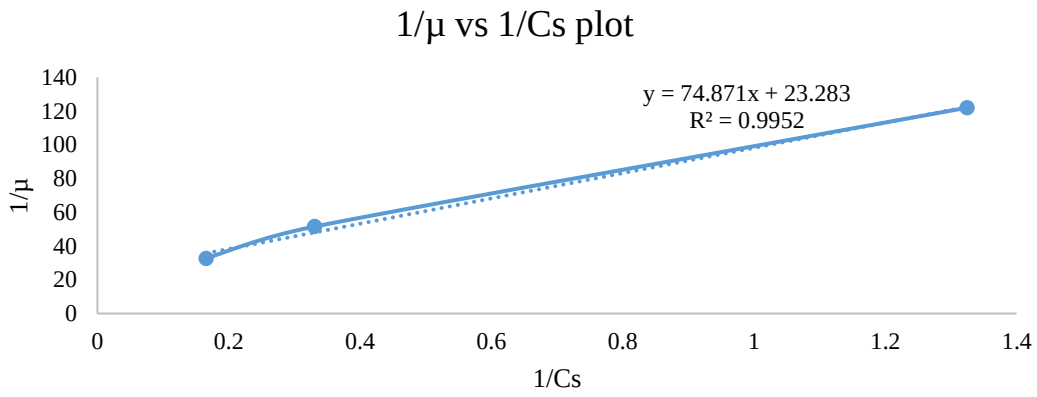


Figure 20: Plot of 1/μ and corresponding $1/C_S$.

From the above curve we calculate μ_{max} and k_S as follows.

The intercept of the curve represents $1/\mu_{max}$ and so:

$\mu_{max} = 1/23.283$

or, $\boldsymbol{\mu_{max} = 0.0353\ hr^{-1}}$.

The slope of the curve gives the value of k_S/μ_{max} and so:

$k_S/\mu_{max} = 74.871$

or, $k_S = 74.871 * 0.0353$ or, $k_S = 2.647$ g/l.

Thus, $\boldsymbol{k_S = 2647\ mg/l}$.

The specific growth rate of inhibition is determined by,

$$K_I = \frac{C^2_{s\ crit}}{K_s}$$

Where, $C_{s\ crit}$ is 2500 ppm as observed from rate (μ) vs substrate concentration (Cs) plot.

So, $K_I = 3.45$

6.2.Determination of the yield by Spectroscopic analysis

6.2.1.Lactic Acid yield determination:

Calibration curve and data related to yield determination

Table 23: Data of absorbance & corresponding concentration for the calibration curve.

Concentration (ppm)	Absorbance
6000	0.143
8000	0.166
10000	0.168

12000	0.175
14000	0.19

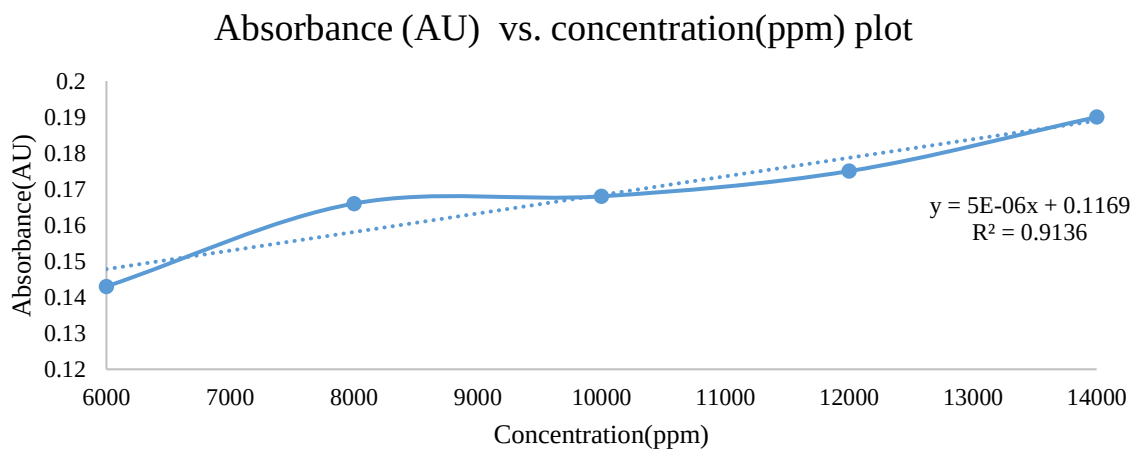


Figure 21: Plot of absorbance at 390 nm and corresponding concentration for lactic acid.

Calculation of Yield of *Shewanella putrefaciens* in Lactic acid substrate

OD of test sample calculated was found to be 0.128 AU.

Now in the above plot where y-axis represents absorbance and the x-axis represents the concentration, we get:

Y = $5\times 10^{-6}x + 0.1169$

So, we can say concentration of the sample at 0.128 AU as,

Concentration = $\frac{0.129-0.1169}{5\times10^{-6}}$ =2328ppm or 2.814 g/l

Initial concentration of the substrate was 2500 ppm or 3.022 g/l.

Table 24: Showing biomass concentration for 2500 ppm lactic acid for 0 hour and 24 hour.

Time (h)	Biomass concentration (g/l)
0	0.05
24	0.169

So, now we can calculate the yield

$$Y_{X/S} = \frac{0.169 - 0.05}{3.022 - 2.814} = 0.572$$

Thus, Yield of *Shewanella putrefaciens* in lactic acid substrate is calculated to be 0.572.

6.2.2.Glucose yield determination:

Calibration curve and data related to yield determination

Table 25: Data of absorbance and corresponding concentration for the construction of calibration curve.

Glucose Concentration (gm/L)	Absorbance(AU)
0.5	0.921
0.25	0.623
0.125	0.52
0.0625	0.226

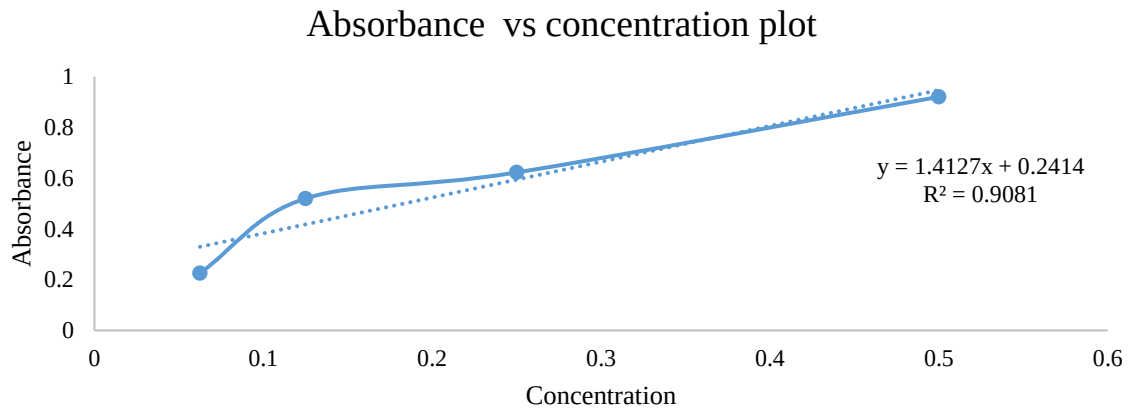


Figure 22: Plot of absorbance at 540 nm and corresponding concentration for Glucose.

Calculation of Yield of *Shewanella putrefaciens* in Glucose substrate

OD of test sample calculated was found to be 0.822 AU.

Now in the above plot where y-axis represents absorbance and the x-axis represents the concentration, we get:

$Y = 1.4127x + 0.2414$

So, we can say concentration of the sample at 0.822 AU as,

Concentration = $\frac{0.822-0.2414}{1.4127}$ =0.411 g/l

Initial concentration of the substrate was 0.5 g/l.

Table 25: Showing biomass concentration 0.5 g/l glucose for 0 hour and 24 hour.

Time (h)	Biomass concentration (g/l)
0	0.01
24	0.101

So, now we can calculate the yield

$$Y_{X/S} = \frac{0.5 - 0.411}{0.101 - 0.01} = 0.944$$

Thus, Yield of *Shewanella putrefaciens* in glucose substrate is calculated to be 0.944.

6.3.Determination of performance of BAFC using suspended culture of *Shewanella putrefaciensMTCC8104* in anode and *Leptolyngbya subtilisJUCHE1* in the cathode chamber.

A double chambered Bacterio-algal Fuel cell using suspended culture of *Shewanella putrefaciensMTCC 8104* in anode and *Leptolyngbya subtilisJUCHE1in* the cathode chambers respectively was developed. A mixture of Glucose and lactic acid in the anode chamber and CO_2 inthe cathode chamber are used as the carbon substrates. The performance of the developed DC-BAFC is determined with respect to the electrical characteristics.

6.3.1.BAFC I (Using Lactic Acid as substrate):

A double chambered Bacterio-algal Fuel cell (DC-BAFC) using suspended culture of Shewanella putrefaciensMTCC 8104 in anode and Leptolyngbya subtilisJUCHE1in the cathode chambers respectively was developed. Lactic acid in the anode chamber and CO2 in the cathode chamber are used as the carbon substrates. The performance of the developed DC-BAFC is determined with respect to the electrical characteristics and with respect to the variable lactic acid concentration.

A total time period of 5 days the setup been observed for proper understanding of the performance. Below table and figure shows the result of the BAFC 1's output voltage, current and power density respectively which utilizes glucose as only substrate. From the result it is observed that the highest produced voltage from the BAFC1 setup produced by the LA 3.022+0.45gm/L ALG setup which is 947 mV reached at 2nd day of setting up the cell and maintained a steady state for another 3 days and then a decrease of voltage occurred. Current output also reached its maximum value on 2nd day of setting up which valued 940 µA.

Time (Days)	Lactic acid 1.81gm/L			Lactic acid 2.41gm/L			Lactic acid 3.022gm/L		
	algae 0.15g/l	algae 0.3g/l	algae 0.45g/l	algae 0.15g/l	algae 0.3g/l	algae 0.45g/l	algae 0.15g/l	algae 0.3g/l	algae 0.45g/l
1	189	41.4	79	110	150	62	500	875	610
2	237	297	300	433	707	584	860	897	947
3	231	194	213	381	658	478	872	821	707
4	158	132	148	256	620	357	789	758	853
5	131	123	102	145	541	258	557	612	475

Table 27: Voltage (mV) vs. time

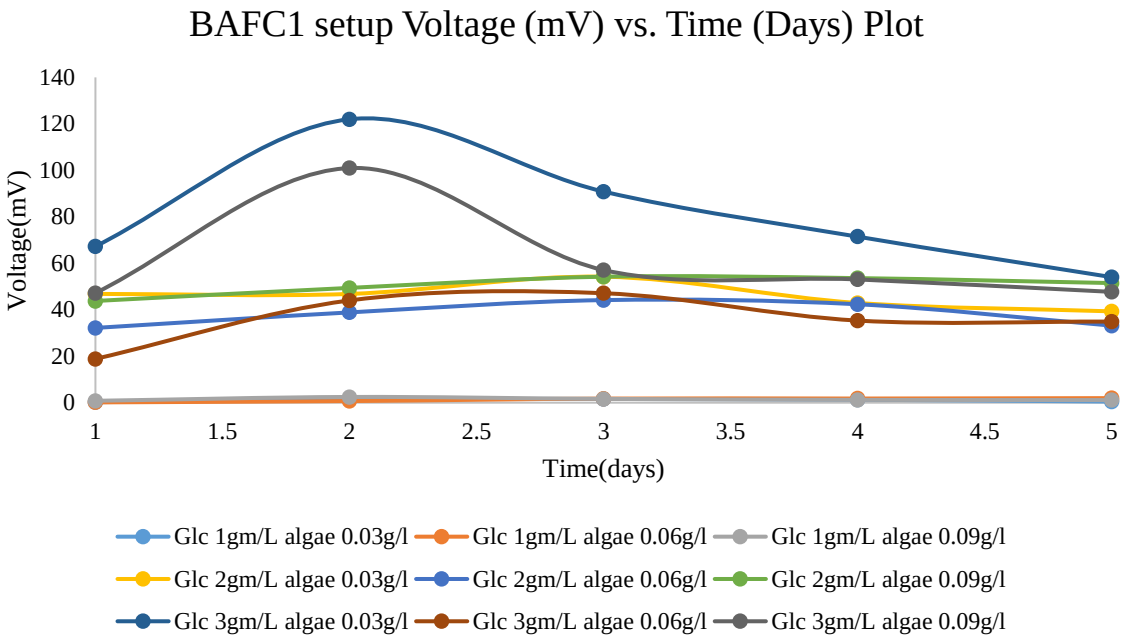


Figure 23: BAFC1 Setups Voltage (V) vs. Time (Days) plot

Table 28: Current (mA) vs. time

Time (Days)	Lactic acid 1.81gm/L			Lactic acid 2.41gm/L			Lactic acid 3.022gm/L		
	algae 0.15g/l	algae 0.3g/l	algae 0.45g/l	algae 0.15g/l	algae 0.3g/l	algae 0.45g/l	algae 0.15g/l	algae 0.3g/l	algae 0.45g/l
1	0.23	0.25	0.3	0.45	0.69	0.21	0.83	0.81	0.67
2	0.31	0.34	0.27	0.47	0.89	0.28	1.65	0.91	0.94
3	0.24	0.36	0.25	0.32	0.31	0.15	0.85	0.84	0.74
4	0.21	0.21	0.28	0.36	0.16	0.12	0.72	0.79	0.81
5	0.11	0.19	0.21	0.16	0.18	0.09	0.41	0.68	0.31

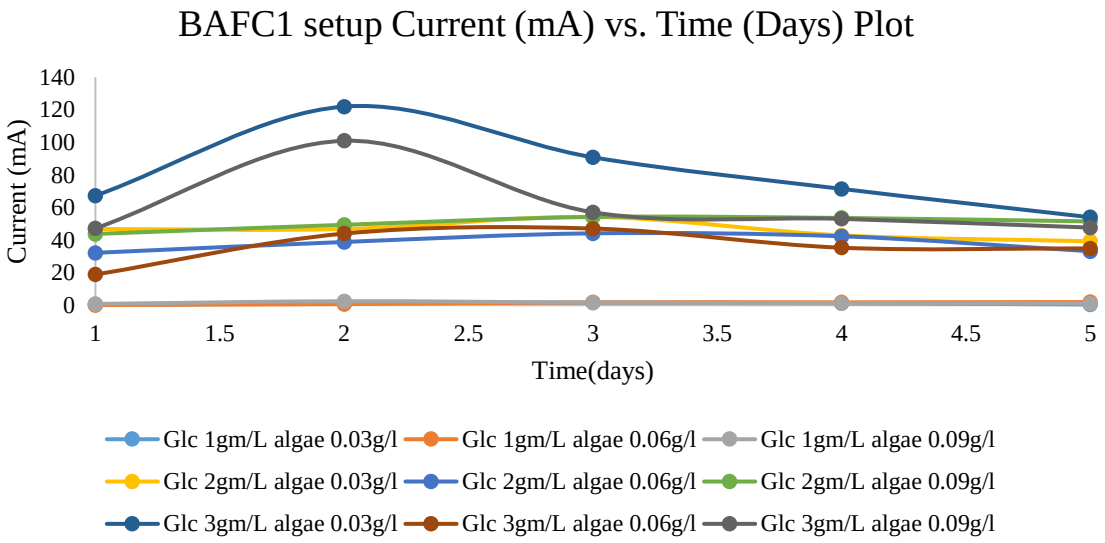


Figure 24: BAFC1 Setups Current (mA) vs. Time (Days) plot

Table 29: Power density (mW/m2) vs. time

Time (Days)	Lactic acid 1.81gm/L			Lactic acid 2.41gm/L			Lactic acid 3.022gm/L		
	algae 0.15g/l	algae 0.3g/l	algae 0.45g/l	algae 0.15g/l	algae 0.3g/l	algae 0.45g/l	algae 0.15g/l	algae 0.3g/l	algae 0.45g/l
1	10.867	2.587	5.925	12.375	33.75	3.255	103.75	177.187	102.175
2	18.367	25.24	20.25	50.877	157.307	40.88	396	249.567	222.545
3	13.86	17.46	13.312	30.48	50.995	17.925	185.3	172.41	130.795
4	8.295	6.93	10.36	23.04	24.8	10.71	142.02	149.705	172.732
5	3.6025	5.842	5.355	5.8	24.345	58.05	57.092	104.04	36.8125

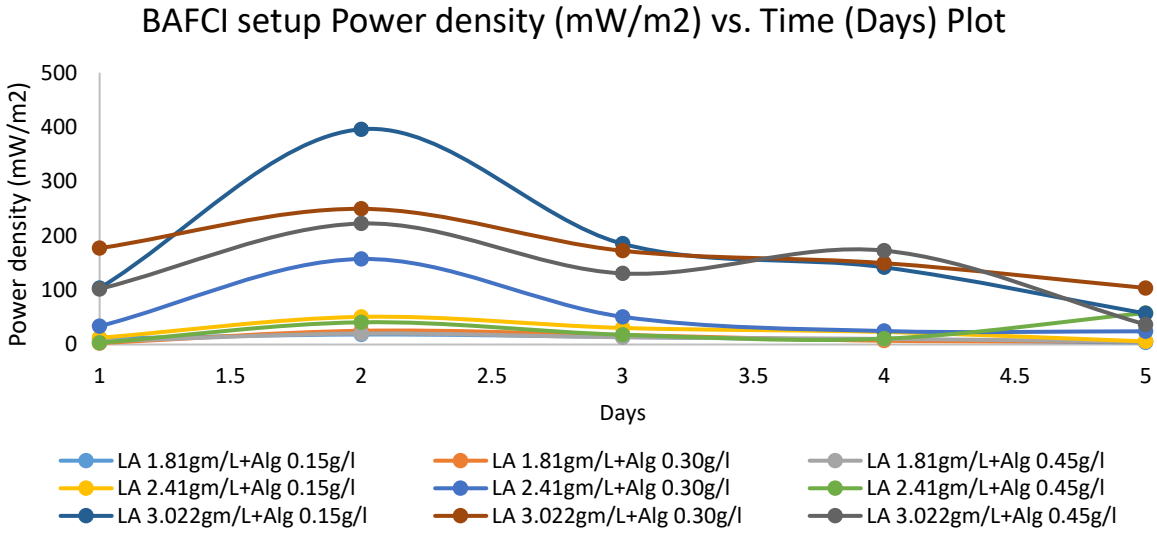


Figure 25: BAFC1 Setups power density (mW/m2) vs. Time (Days) plot

6.3.2.BAFC II (Using Glucose as substrate):

A double chambered Bacterio-algal Fuel cell is developed using attached growth of Shewanella putrefaciensMTCC8104 on anode and suspended growth of Leptolyngbya subtilisJUCHE1 in the cathode chamber and the electrical performance is studied with glucose as substrate.

A total time period of 5 days the setup been observed for proper understanding of the performance. Below table and figure shows the result of the BAFC 1's output voltage, current and power density respectively which utilizes glucose as only substrate. From the result it is observed that the highest produced voltage from the BAFC1 setup produced by the 3gm/L GLC+0.15gm/L ALG setup which is 642 mV reached at 2nd day of setting up the cell and maintained a steady state for another 3 days and then a decrease of voltage occurred. Current output also reached its maximum value on 2nd day of setting up which valued 760 µA.

Table 30: Voltage (mV) vs. time

Time (Days)	**Glucose 1gm/L**			**Glucose 2gm/L**			**Glucose 3gm/L**		
	algae 0.03g/l	**algae 0.06g/l**	**algae 0.09g/l**	**algae 0.03g/l**	**algae 0.06g/l**	**algae 0.09g/l**	**algae 0.03g/l**	**algae 0.06g/l**	**algae 0.09g/l**
1	280	127	180	307	330	287	390	260	320
2	297	148	197	360	370	380	642	440	612
3	256	243	156	398	389	398	617	415	587
4	243	239	143	402	362	342	631	384	601
5	128	212	128	395	341	295	597	359	527
6	112	231	112	371	351	271	561	315	431
7	103	243	103	385	358	285	530	326	430

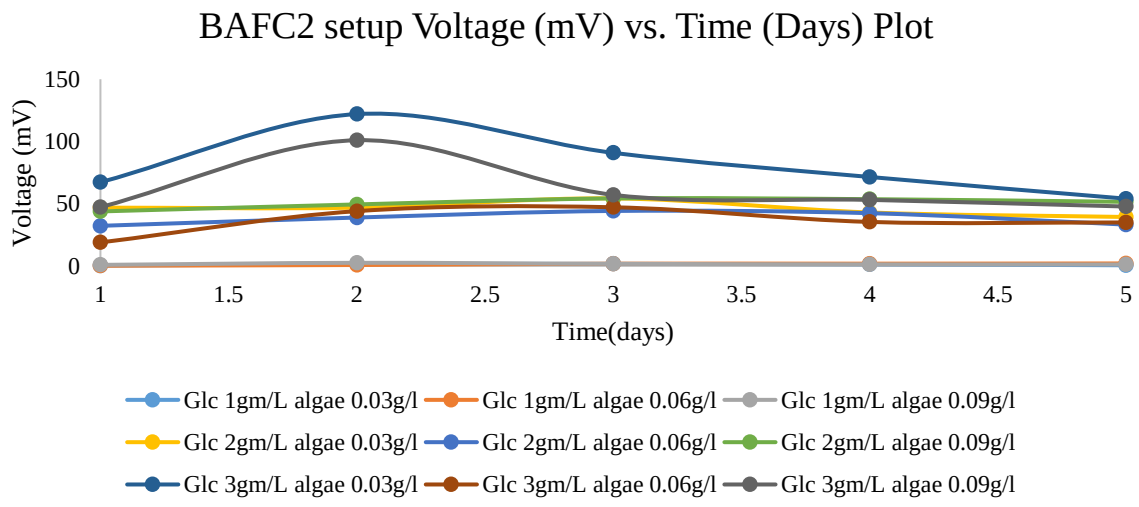


Figure 26: BAFCII Setups Voltage (mV)vs. Time (Days) plot

Table 31: Current (µA) vs. time

Time (Days)	Glucose 1gm/L			Glucose 2gm/L			Glucose 3gm/L		
	algae 0.03g/l	algae 0.06g/l	algae 0.09g/l	algae 0.03g/l	algae 0.06g/l	algae 0.09g/l	algae 0.03g/l	algae 0.06g/l	algae 0.09g/l
1	3	4	18	610	390	610	690	290	590
2	18	19	50	520	420	520	760	400	660
3	25	29	42	545	454	545	589	454	389
4	21	30	34	427	468	627	453	368	353
5	15	37	35	398	389	698	362	389	362
6	13	33	16	249	264	749	242	264	242
7	12	35	12	290	240	890	210	140	110

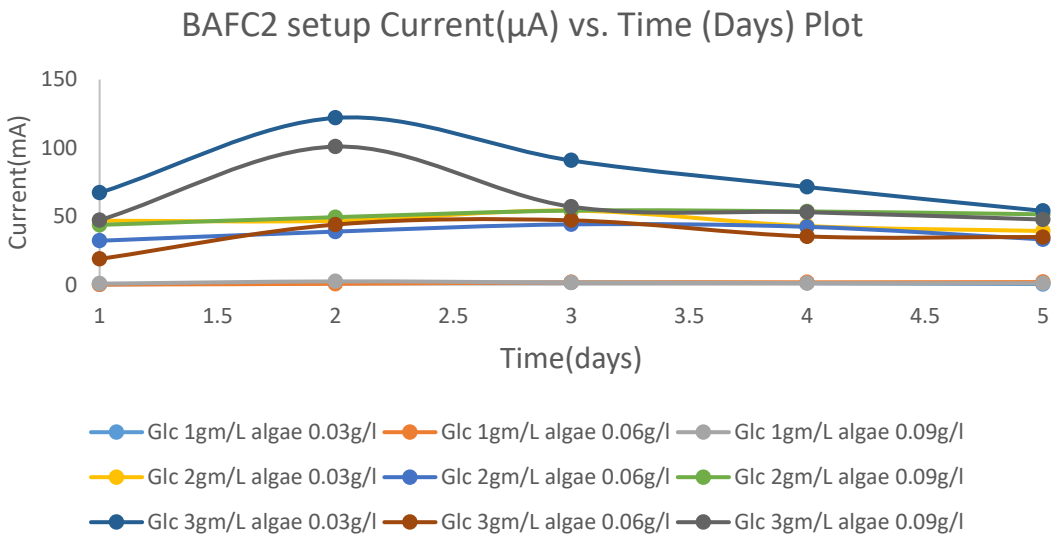


Figure 27: BAFCII Setups Current (µA)vs. Time (Days) plot

Table 32: Power density (mW/m2) vs. time

Time (Days)	Glucose 1gm/L			Glucose 2gm/L			Glucose 3gm/L		
	algae 0.03g/l	algae 0.06g/l	algae 0.09g/l	algae 0.03g/l	algae 0.06g/l	algae 0.09g/l	algae 0.03g/l	algae 0.06g/l	algae 0.09g/l
1	0.21	0.127	0.81	46.817	32.175	43.7675	67.275	18.85	47.2
2	1.33	0.703	2.4625	46.8	38.85	49.4	121.98	44	100.98
3	1.6	1.765	1.638	54.227	44.1515	54.2275	90.8532	47.1025	57.0857
4	1.27	1.7925	1.2155	42.913	42.354	53.6085	71.4607	35.328	53.0382
5	0.48	1.961	1.12	39.302	33.1622	51.4775	54.0285	34.91275	47.6935
6	0.364	1.9057	0.448	23.094	23.166	50.7447	33.9405	20.79	26.0755
7	0.309	2.1262	0.309	27.9125	21.48	63.4125	27.825	11.41	11.825

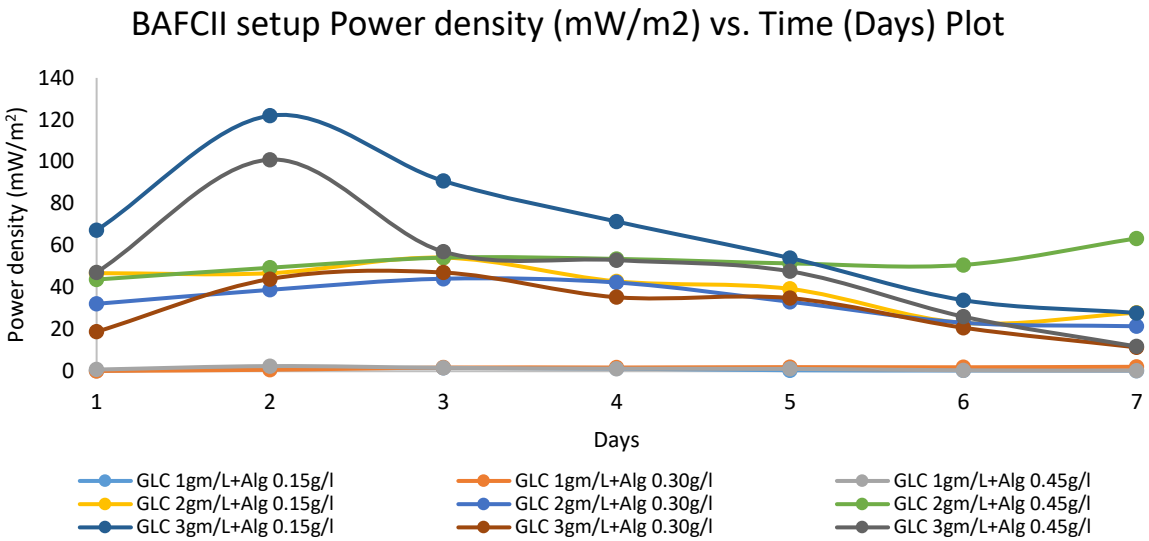


Figure 28: BAFCII Setups Power density (mW/m2) vs. Time (Days) plot

6.4.Mathematical Modelling of the BAFC using MATLAB

BAFC model is simulated through MATLAB 2023 software with varying substrate concentration and varying algal concentration and after simulating the data dual substrate response model is being used in the optimization part. The E_0 cell voltages are tabulated below.

Table 33: E0 (V) potential chart

System	E_0 potential(V)	
	E_{0a}	E_{0c}
LA BAFC I	0.19	0.81
GLC BAFC II	0.43	0.81
GLC+LA BAFC III	0.62	0.81

6.4.1. Mathematical Modelling of the BAFC using Lactic acid as substrate

6.4.1.1. Codes in MATLAB for lactic acid substrate

Function for substrate.

```
function dxdt = substrate_L(t,x)
%glucose growth kinetic parameters;
uml = 0.0353;
```

```
ksl = 2.6472;
ksi = 3.4455;
Yl = 0.575;
dxdt = zeros(4,1);
% anode mass balance;
dxdt(1) = ((uml*x(2)/(ksl+x(2)+((x(2)^2)/ksi)))*x(1));
dxdt(2) = (1/(-Yl))*(uml*x(2)/(ksl+x(2)+((x(2)^2)/ksi)));
dxdt(3) = (((1/Yl)*((uml*x(2)/(ksl+x(2)+((x(2)^2)/ksi))))));
dxdt(4) = ((1/Yl)*(5*(uml*x(2)/(ksl+x(2)+((x(2)^2)/ksi)))));
end
```

Substrate main code

```
tspan = [0 2 4 6 12:12:96];
X_in = [0.05 3.022 0 0];
xalg = 0.45;
E0a = 0.19;
E0c = 0.81;
R = 8.314;
T = 298;
F = 96500;
n = 4;
A = 0.004;
Res = 1000;
n1 = 4;
vg = 0.45;
vl= 0.2;
umal = 0.0215;
ksal = 1.650;
Yal = 0.106;
Cco2l = 0.0037;
Cco2g = 0.196;
```

```
Cco2c= 0.04;
Cco2s = 0.1368;
H = 1.364*10^-2;
klac = 0.263;
klao = 0.291;
Co2s = 8.42;
[tsol,xsol] = ode45(@substrate_L,tspan,X_in);
count = numel(tspan);
for i = 1:count
e1(i) = power(xsol(i,3),1);
e2(i) = power(xsol(i,4),5);
e3(i) = power(xsol(i,4),4);
% carbon di oxide mass balance equation;
e4(i) = (xsol(i,3)-(klac*(xsol(i,3)-Cco2s)));
if xsol(i,3) >= Cco2s
    e5(i) = (klac*(vl/vg)*(e4(i)-Cco2s))- (klac*(Cco2g*H - Cco2c)*(vl/vg));
else
   e5(i) =  (klac*(Cco2g*H - Cco2c)*(vl/vg));
end
% cathode chaber mass balance;
f = @(t,x)[((umal*e5(i)*xalg)/(ksal+e5(i)));(((1/(-Yal))*(umal*e5(i)*xalg))/(ksal+e5(i)))];
[t,x] = ode45(f,tspan,[xalg,e5(i)]);
e6(i) = e5(i)+(klac*(H*Cco2l-Cco2c));
% oxygen producing in day time;
e7(i) = -x(i,2)-(klao*(Co2s-(-x(i,2))));
% oxygen producing in night time;
e8(i) = -(klao*(Co2s-(-x(i,2))));
% reaction quotient equation of anode chamber;
Q(i) = log((e1(i)*e2(i))/(xsol(i,2)));
E_a(i) = E0a - ((R*T*Q(i))/(n*F));
% reaction quotient equation in cathode chamber;
```

```
bd(i) = log(1/(e7(i)*e3(i)));
bn(i)= log(1/(e8(i)*e3(i)));
b(i) = bd(i)+bn(i);
E_c(i) = E0c - ((R*T*b(i))/(n1*F));
%total currentvoltage,power density and current denisty equations;
E(i) = E_c(i) - E_a(i);
curr(i) = E(i)/Res;
Currd(i) = curr(i)/A;
p(i) = (curr(i)*E(i));
Pd(i) = (curr(i)*E(i))/A;
End

subplot(3,3,1);
plot(tsol,xsol(:,1),'r', tsol,xsol(:,2),'g');
xlabel('time(days)');
ylabel('concentration (g/l');
subplot(3,3,2);
plot(tsol,E);
xlabel('time(days)');
ylabel('voltage(V)');
subplot(3,3,3);
plot(tsol,Pd);
xlabel('time(days)');
ylabel('Power density(W/m2)');
subplot(3,3,4);
plot(tsol,Currd);
xlabel('time(days)');
ylabel('current density(A/m2)');
subplot(3,3,5);
plot(t,x(:,1),'r');
xlabel('time(days)');
```

```
ylabel('algae concentration (g/L)');

%vg= total volume of gas in headspace of cathode+anode chember;
%vl= total volume of liquid in cathode or in anode chamber;
%Cco2l= dissolved carbon di oxide in bulk liquid in anode chamber;
%Cco2= saturated carbon di oxide concentration in catholyte;
%klac= volumetric mass transfer co-efficient of carbon di oxide;
%klao= volumetric mass transfer co-efficient of oxygen;
%dCo2= saturated dissolved oxygen concentration;
```

6.4.1.2.Data set generated from MATLAB

The table below shows the data set that was generated from MATLAB for the BAFC using Lactic acid as substrate.

Table 34: Concentration Data set of biomass and the 1.81 g/l lactic acid and algae generated from MATLAB.

Time(hr)	Biomass	lactic acid	Algae(0.15 g/l)	Algae(0.3 g/l)	Algae(0.45 g/l)
0	0.05	1.81	0.15	0.3	0.45
2	0.051191	1.769055	0.150418	0.300837	0.451255
4	0.052401	1.7284172	0.150837	0.301673	0.45251
6	0.05363	1.6881013	0.151255	0.30251	0.453765
12	0.057424	1.5692332	0.15251	0.30502	0.45753
24	0.065432	1.3421866	0.15502	0.31004	0.46506
36	0.07384	1.1319491	0.15753	0.31506	0.472589
48	0.0824	0.9411892	0.16004	0.32008	0.480119
60	0.090829	0.7718118	0.16255	0.325099	0.487649
72	0.098846	0.6247057	0.16506	0.330119	0.495179
84	0.106215	0.4996619	0.16757	0.335139	0.502709
96	0.112772	0.3954824	0.17008	0.340159	0.510239

Table 35: Concentration Data set of biomass and the 2.41 g/l lactic acid and algae generated from MATLAB.

Time(hr)	Biomass	lactic acid	Algae(0.15 g/l)	Algae(0.3 g/l)	Algae(0.45 g/l)
0	0.05	2.41	0.15	0.3	0.45
2	0.051276	2.3661747	0.150512	0.301024	0.451536
4	0.052581	2.322474	0.151024	0.302048	0.453072
6	0.053915	2.2789091	0.151536	0.303072	0.454608
12	0.058091	2.1491442	0.153072	0.306144	0.459215
24	0.067235	1.8949156	0.156144	0.312287	0.468431
36	0.077394	1.6501885	0.159215	0.318431	0.477646
48	0.088444	1.4180917	0.162287	0.324574	0.486861
60	0.100158	1.2017768	0.165359	0.330718	0.496077
72	0.112215	1.0040966	0.168431	0.336861	0.505292
84	0.124226	0.827252	0.171502	0.343005	0.514507
96	0.135787	0.6724982	0.174574	0.349148	0.523723

Table 36: Concentration Data set of biomass and the 3.022 g/l lactic acid and algae generated from MATLAB.

Time(hr)	Biomass	lactic acid	Algae(0.15 g/l)	Algae(0.3 g/l)	Algae(0.45 g/l)
0	0.05	3.022	0.15	0.3	0.45
2	0.051299	2.9774023	0.150575	0.30115	0.451725
4	0.052631	2.9328108	0.15115	0.3023	0.45345
6	0.053998	2.8882319	0.151725	0.30345	0.455176
12	0.058309	2.7546417	0.15345	0.306901	0.460351
24	0.067938	2.48883	0.156901	0.313801	0.470702
36	0.078999	2.2264965	0.160351	0.320702	0.481053
48	0.091553	1.9700029	0.163801	0.327603	0.491404
60	0.105579	1.7221177	0.167252	0.334504	0.501756
72	0.120937	1.4859132	0.170702	0.341404	0.512107
84	0.137352	1.2645646	0.174153	0.348305	0.522458
96	0.154403	1.0610509	0.177603	0.355206	0.532809

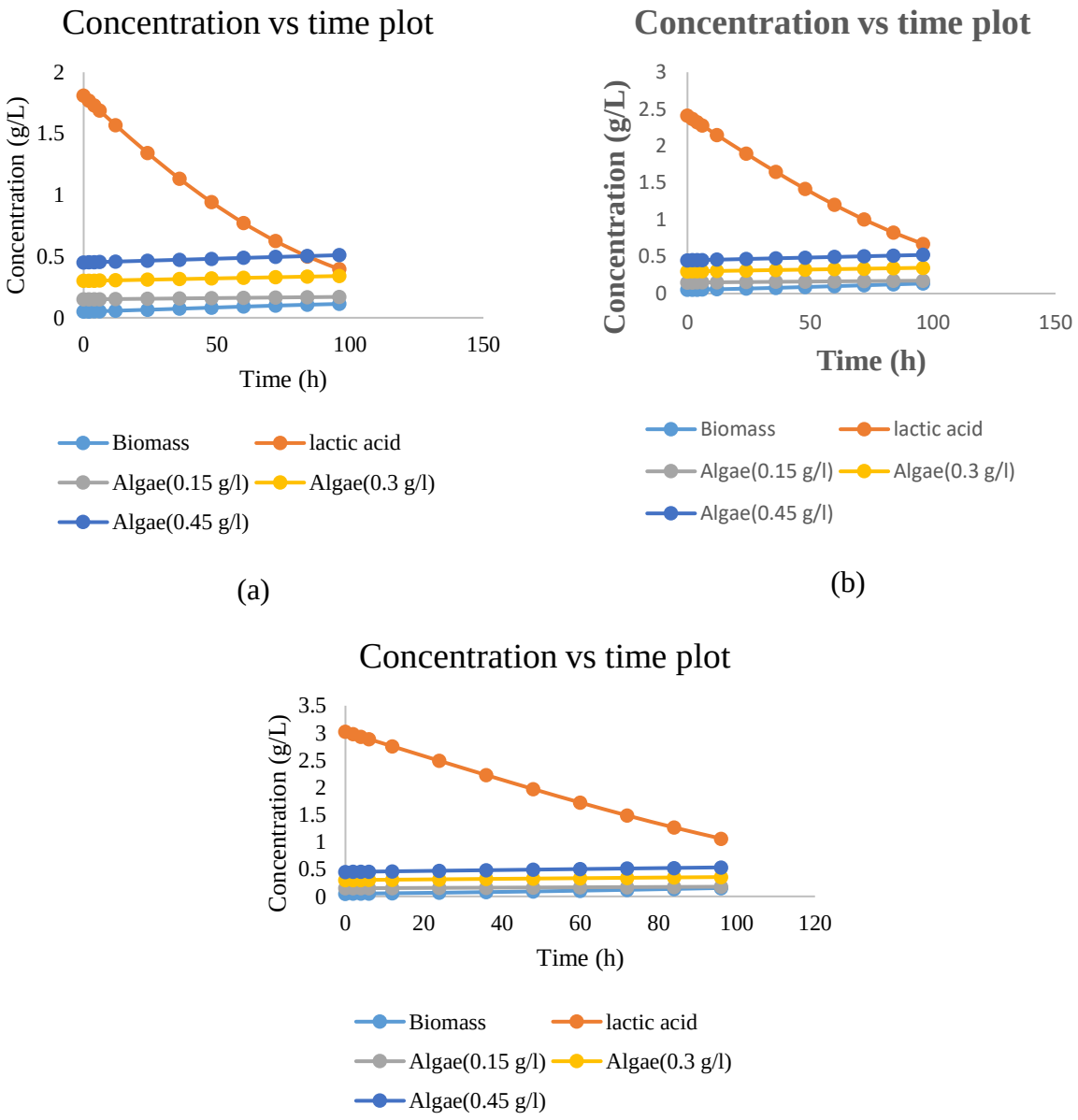


Figure 29: Concentration vs time plot for biomass ,(a) substrate-1.81 g/l, (b)substrate-2.41 g/l (c)substrate3.022 g/l and algae concentrations.

Simulated results from model:

Using MATLAB 2017b these differential equations are solved using ODE45 solver.

The predictions of the proposed model, the voltage and current of the MFC are measured with time. The predicted current , voltage, power density, current density shown below.

Table 37 : Simulated current (mA) data

Time (h)	Lactic Acid 1.81 g/L			Lactic Acid 2.41g/L			Lactic Acid 3.022g/L		
	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L
0	0.6200	0.6200	0.6200	0.6200	0.6200	0.6200	0.6200	0.6200	0.6200
2	0.4750	0.4750	0.4750	0.4792	0.4792	0.4792	0.4793	0.4793	0.4793
4	0.5371	0.5371	0.5371	0.5415	0.5415	0.5415	0.5417	0.5417	0.5417
6	0.5733	0.5733	0.5733	0.5779	0.5779	0.5779	0.5782	0.5782	0.5782
12	0.6350	0.6350	0.6350	0.6402	0.6402	0.6402	0.6408	0.6408	0.6408
24	0.6957	0.6957	0.6956	0.7022	0.7021	0.7020	0.7035	0.7035	0.7034
36	0.7302	0.7300	0.7299	0.7380	0.7378	0.7377	0.7402	0.7400	0.7399
48	0.7536	0.7534	0.7531	0.7629	0.7626	0.7623	0.7661	0.7658	0.7655
60	0.7708	0.7704	0.7701	0.7816	0.7812	0.7807	0.7859	0.7854	0.7849
72	0.7840	0.7835	0.7829	0.7963	0.7957	0.7950	0.8017	0.8010	0.8003
84	0.7943	0.7937	0.7929	0.8080	0.8072	0.8063	0.8147	0.8138	0.8128
96	0.8026	0.8017	0.8008	0.8176	0.8165	0.8153	0.8255	0.8243	0.8229

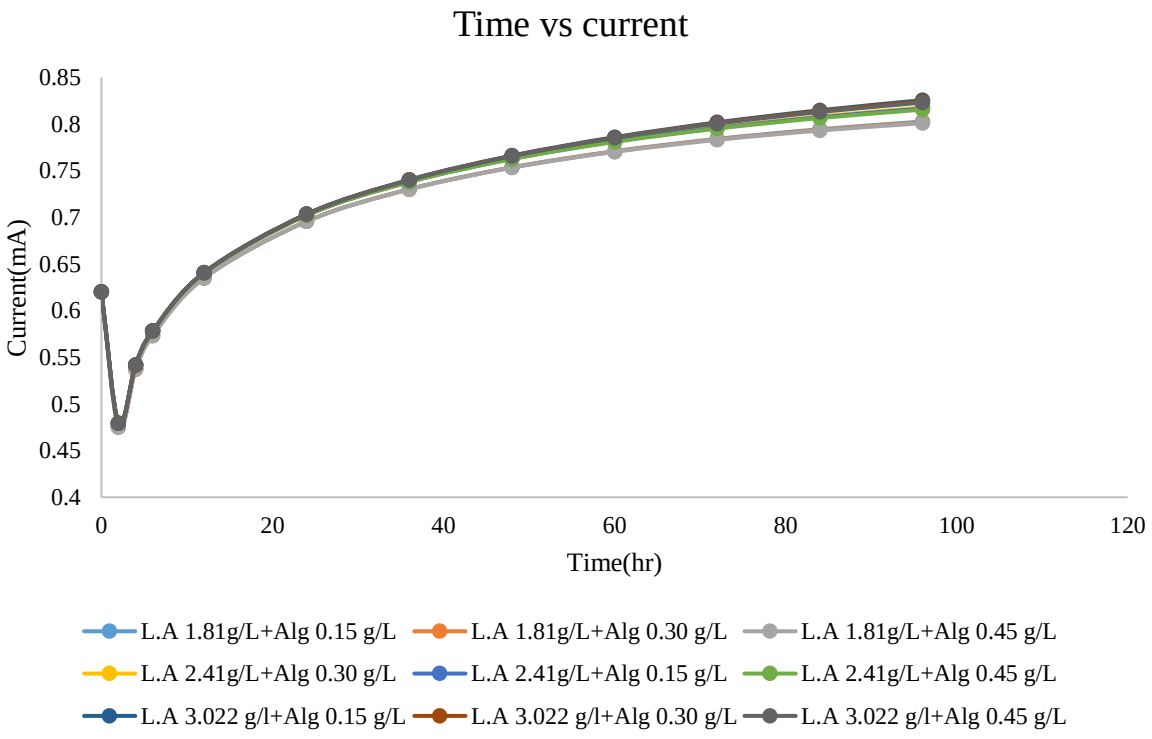


Figure 30 : Simulated current (mA) vs. time (h) Plot

Table 38 : Simulated Voltage(mV) data

Time (h)	Lactic Acid 1.81 g/L			Lactic Acid 2.41g/L			Lactic Acid 3.022g/L		
	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L
0	620.00	620.00	620.00	620.00	620.00	620.00	620.00	620.00	620.00
2	474.97	474.97	474.97	479.21	479.21	479.21	479.31	479.31	479.31
4	537.07	537.07	537.07	541.49	541.49	541.49	541.68	541.69	541.69
6	573.31	573.31	573.31	577.91	577.91	577.92	578.21	578.21	578.21
12	635.00	634.98	634.97	640.18	640.17	640.16	640.80	640.79	640.78
24	695.72	695.66	695.60	702.17	702.10	702.03	703.52	703.45	703.38
36	730.16	730.03	729.89	737.99	737.83	737.68	740.19	740.03	739.87
48	753.59	753.35	753.11	762.88	762.60	762.32	766.06	765.76	765.46
60	770.81	770.44	770.06	781.60	781.17	780.72	785.86	785.40	784.91
72	783.99	783.48	782.94	796.27	795.66	795.00	801.71	801.03	800.31
84	794.34	793.66	792.93	808.04	807.22	806.31	814.70	813.78	812.76
96	802.60	801.75	800.81	817.61	816.55	815.34	825.51	824.31	822.90

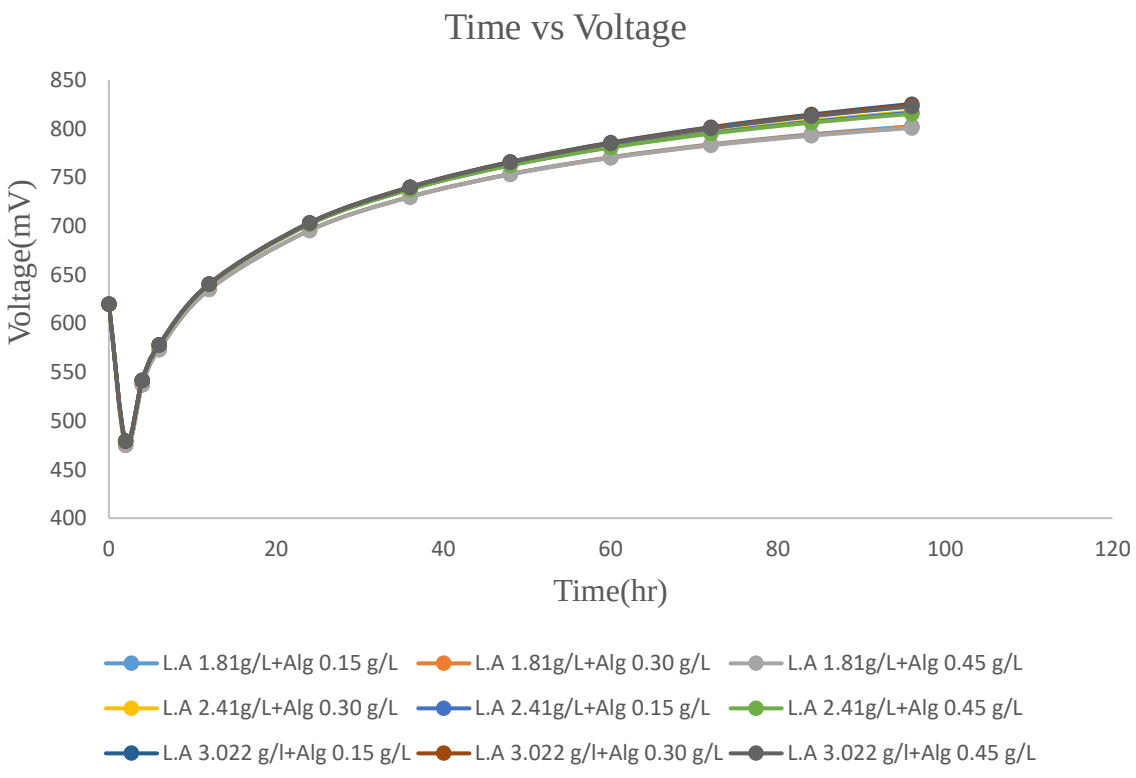


Figure 31: Simulated voltage (mV) vs. time (h) Plot

Table 39 : Simulated Current density(mA/m^2)data

Time (h)	Lactic Acid 1.81 g/L			Lactic Acid 2.41g/L			Lactic Acid 3.022g/L		
	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L
0	0.1550	0.1550	0.1550	0.1550	0.1550	0.1550	0.1550	0.1550	0.1550
2	0.1187	0.1187	0.1187	0.1198	0.1198	0.1198	0.1198	0.1198	0.1198
4	0.1343	0.1343	0.1343	0.1354	0.1354	0.1354	0.1354	0.1354	0.1354
6	0.1433	0.1433	0.1433	0.1445	0.1445	0.1445	0.1446	0.1446	0.1446
12	0.1587	0.1587	0.1587	0.1600	0.1600	0.1600	0.1602	0.1602	0.1602
24	0.1739	0.1739	0.1739	0.1755	0.1755	0.1755	0.1759	0.1759	0.1758
36	0.1825	0.1825	0.1825	0.1845	0.1845	0.1844	0.1850	0.1850	0.1850
48	0.1884	0.1883	0.1883	0.1907	0.1907	0.1906	0.1915	0.1914	0.1914
60	0.1927	0.1926	0.1925	0.1954	0.1953	0.1952	0.1965	0.1963	0.1962
72	0.1960	0.1959	0.1957	0.1991	0.1989	0.1988	0.2004	0.2003	0.2001
84	0.1986	0.1984	0.1982	0.2020	0.2018	0.2016	0.2037	0.2034	0.2032
96	0.2006	0.2004	0.2002	0.2044	0.2041	0.2038	0.2064	0.2061	0.2057

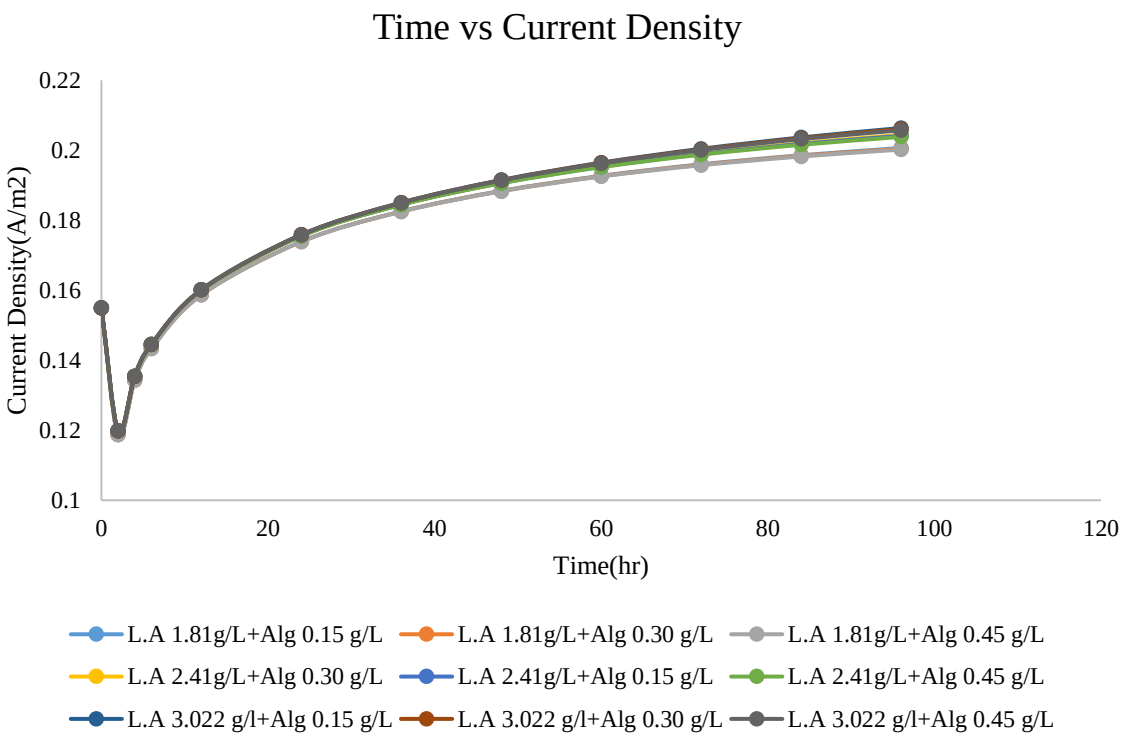


Figure 32: Simulated Current density(mA/m^2) vs. time (h) Plot

Table 40 : Simulated Power Density(W/m^2) data

Time (h)	Lactic Acid 1.81 g/L			Lactic Acid 2.41g/L			Lactic Acid 3.022g/L		
	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L
0	0.0961	0.0961	0.0961	0.0961	0.0961	0.0961	0.0961	0.0961	0.0961
2	0.0560	0.0560	0.0560	0.0570	0.0570	0.0570	0.0570	0.0570	0.0570
4	0.0717	0.0717	0.0717	0.0729	0.0729	0.0729	0.0729	0.0729	0.0729
6	0.0818	0.0818	0.0818	0.0831	0.0831	0.0831	0.0832	0.0832	0.0832
12	0.1004	0.1004	0.1004	0.1021	0.1020	0.1020	0.1023	0.1022	0.1022
24	0.1206	0.1206	0.1206	0.1229	0.1228	0.1228	0.1233	0.1233	0.1233
36	0.1329	0.1328	0.1328	0.1357	0.1357	0.1356	0.1366	0.1365	0.1364
48	0.1416	0.1415	0.1414	0.1451	0.1450	0.1449	0.1463	0.1462	0.1461
60	0.1481	0.1480	0.1478	0.1523	0.1521	0.1520	0.1540	0.1538	0.1536
72	0.1533	0.1531	0.1528	0.1581	0.1579	0.1576	0.1603	0.1600	0.1597
84	0.1573	0.1571	0.1568	0.1628	0.1625	0.1621	0.1655	0.1652	0.1647
96	0.1606	0.1603	0.1599	0.1667	0.1663	0.1658	0.1700	0.1695	0.1689

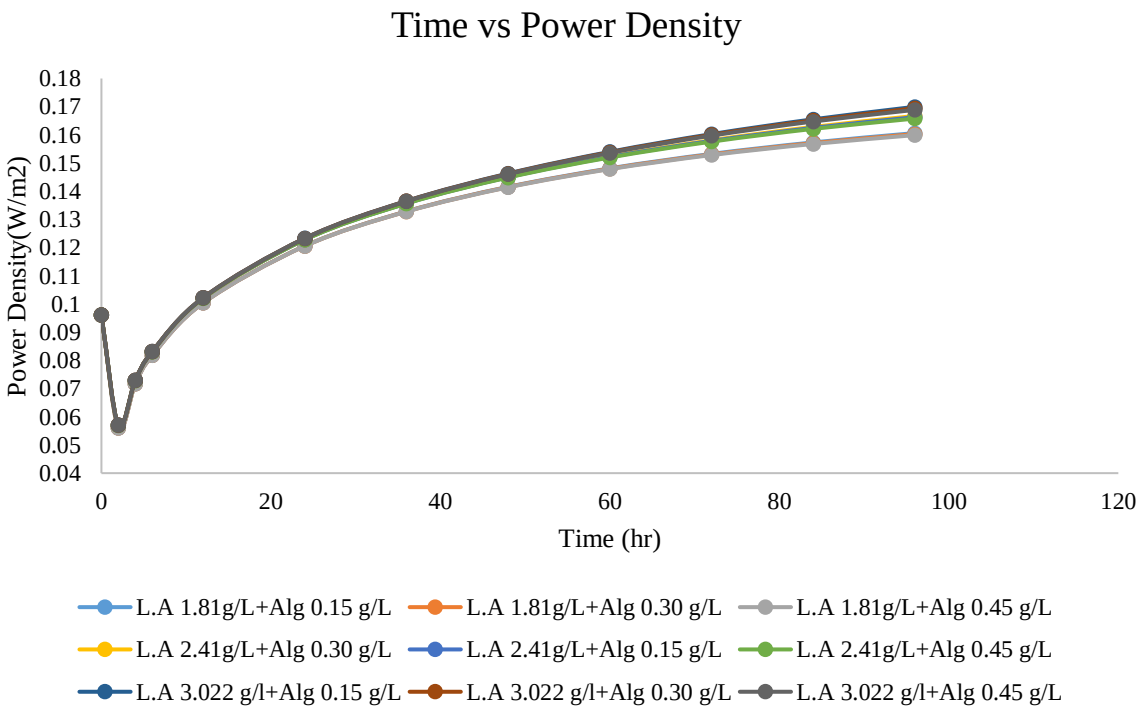


Figure 33: Simulated Power density(W/m^2) vs. time (h) Plot

6.4.2. Mathematical Modelling of the BAFC using Glucose as substrate

6.4.2.1. Codes in MATLAB for glucose substrate

Function for substrate.

```
function dxdt = substrate_G(t,x)
%glucose growth kinetic parameters;
ksg = 0.077;
umg = 0.597;
Yg = 0.944;
dxdt = zeros(4,1);
% anode mass balance;
dxdt(1) = (((umg*x(2)/(ksg+x(2))))*x(1));
dxdt(2) = (1/(-Yg))*(umg*x(2)*x(1)/(ksg+x(2)));
dxdt(3) = ((1/Yg)*(6*umg*x(2)*x(1)/(ksg+x(2))));
dxdt(4) = ((1/Yg)*(24*umg*x(2)*x(1)/(ksg+x(2))));
 end
```

Substrate main code

```
tspan = [0 2 4 6 12:12:96];
X_in = [0.05 3 0 0];
xalg = 0.45;
E0a = 0.43;
E0c = 0.81;
R = 8.314;
T = 298;
F = 96500;
n = 24;
A = 0.004;
Res = 1000;
n1 = 4;
vg = 0.45;
vl= 0.2;
umal = 0.0215;
```

```
ksal = 1.650;
Yal = 0.106;
Cco2l = 0.0037;
Cco2g = 0.196;
Cco2c= 0.04;
Cco2s = 0.1368;
H = 1.364*10^-2;
klac = 0.263;
klao = 0.291;
Co2s = 8.42;
[tsol,xsol] = ode45(@substrate_G,tspan,X_in);
count = numel(tspan);
for i = 1:count
e1(i) = power(xsol(i,3),6);
e2(i) = power(xsol(i,4),24);
e3(i) = power(xsol(i,4),4);
% carbon di oxide mass balance equation;
e4(i) = (xsol(i,3)-(klac*(xsol(i,3)-Cco2s)));
if xsol(i,3) >= Cco2s
    e5(i) = (klac*(vl/vg)*(e4(i)-Cco2s))- (klac*(Cco2g*H - Cco2c)*(vl/vg));
else
   e5(i) =  (klac*(Cco2g*H - Cco2c)*(vl/vg));
end
% cathode chaber mass balance;
f = @(t,x)[((umal*e5(i)*xalg)/(ksal+e5(i)));(((1/(-Yal))*(umal*e5(i)*xalg))/(ksal+e5(i)))];
[t,x] = ode45(f,tspan,[xalg,e5(i)]);
e6(i) = e5(i)+(klac*(H*Cco2l-Cco2c));

% oxygen producing in day time;
e7(i) = -x(i,2)-(klao*(Co2s-(-x(i,2))));
% oxygen producing in night time;
```

```
e8(i) = -(klao*(Co2s-(-x(i,2))));
% reaction quotient equation of anode chamber;
Q(i) = log((e1(i)*e2(i))/(xsol(i,2)));
E_a(i) = E0a - ((R*T*Q(i))/(n*F));
% reaction quotient equation in cathode chamber;
bd(i) = log(1/(e7(i)*e3(i)));
bn(i)= log(1/(e8(i)*e3(i)));
b(i) = bd(i)+bn(i);
E_c(i) = E0c - ((R*T*b(i))/(n1*F));
%total currentvoltage,power density and current denisty equations;
E(i) = E_c(i) - E_a(i);
curr(i) = E(i)/Res;
Currd(i) = curr(i)/A;
p(i) = (curr(i)*E(i));
Pd(i) = (curr(i)*E(i))/A;
end
subplot(3,3,1);
plot(tsol,xsol(:,1),'r', tsol,xsol(:,2),'g');
xlabel('time(days)');
ylabel('concentration (mg/l');
subplot(3,3,2);
plot(tsol,E);
xlabel('time(days)');
ylabel('voltage(V)');
subplot(3,3,3);
plot(tsol,Pd);
xlabel('time(days)');
ylabel('Power density(W/m2)');
subplot(3,3,4);
plot(tsol,Currd);
xlabel('time(days)');
```

```
ylabel('current density(A/m2)');
subplot(3,3,5);
plot(t,x);
xlabel('time(days)');
ylabel('algae concentration (mg/L)');
%vg= total volume of gas in headspace of cathode+anode chember;
%vl= total volume of liquid in cathode or in anode chamber;
%Cco2l= dissolved carbon di oxide in bulk liquid in anode chamber;
%Cco2= saturated carbon di oxide concentration in catholyte;
%klac= volumetric mass transfer co-efficient of carbon di oxide;
%klao= volumetric mass transfer co-efficient of oxygen;
%dCo2= saturated dissolved oxygen concentration;
```

6.4.2.2.Data set generated from MATLAB

The table below shows the data set that was generated from MATLAB for the BAFC using Glucose as substrate.

Table 41: Concentration Data set of biomass and the 1 g/l glucose and algae generated from MATLAB.

Time(hr)	Biomass	Glucose	Algae(0.15 g/l)	Algae(0.3 g/l)	Algae(0.45 g/l)
0	0.05	1	0.15	0.3	0.45
2	0.150954	0.8930568	0.151522	0.303043	0.454565
4	0.446123	0.5803784	0.153043	0.306087	0.45913
6	0.988745	0.0055671	0.154565	0.30913	0.463695
12	0.994	6.12E-08	0.15913	0.31826	0.47739
24	0.994	1.79E-07	0.16826	0.33652	0.504781
36	0.994001	5.94E-07	0.17739	0.354781	0.532171
48	0.994	1.65E-07	0.18652	0.373041	0.559561
60	0.994	3.25E-09	0.195651	0.391301	0.586952
72	0.994	3.66E-07	0.204781	0.409561	0.614342
84	0.994	7.18E-08	0.213911	0.427822	0.641732
96	0.994	2.49E-07	0.223041	0.446082	0.669123

Table 42: Concentration Data set of biomass and the 2 g/l glucose and algae generated from MATLAB.

Time(hr)	Biomass	Glucose	Algae(0.15 g/l)	Algae(0.3 g/l)	Algae(0.45 g/l)
0	0.05	2	0.15	0.3	0.45
2	0.157709	1.8859019	0.152473	0.304947	0.45742
4	0.494733	1.5288844	0.154947	0.309894	0.464841
6	1.49808	0.4660168	0.15742	0.314841	0.472261
12	1.938	6.25E-08	0.164841	0.329682	0.494523
24	1.938	4.02E-08	0.179682	0.359364	0.539046
36	1.938	1.35E-07	0.194523	0.389046	0.583569
48	1.938	2.97E-08	0.209364	0.418728	0.628091
60	1.938	2.35E-07	0.224205	0.44841	0.672614
72	1.938	1.29E-08	0.239046	0.478091	0.717137
84	1.938	4.38E-07	0.253887	0.507773	0.76166
96	1.938	6.49E-08	0.268728	0.537455	0.806183

Table 43: Concentration Data set of biomass and the 3 g/l glucose and algae generated from MATLAB.

Time(hr)	Biomass	Glucose	Algae(0.15 g/l)	Algae(0.3 g/l)	Algae(0.45 g/l)
0	0.05	3	0.15	0.3	0.45
2	0.160082	2.8833879	0.153117	0.306234	0.459351
4	0.511283	2.5113524	0.156234	0.312469	0.468703
6	1.613652	1.3435885	0.159351	0.318703	0.478054
12	2.882	5.29E-07	0.168703	0.337406	0.506109
24	2.882	1.51E-07	0.187406	0.374812	0.562218
36	2.881999	8.98E-07	0.206109	0.412218	0.618327
48	2.882	1.08E-07	0.224812	0.449624	0.674436
60	2.882	1.93E-07	0.243515	0.48703	0.730545
72	2.882	9.39E-08	0.262218	0.524436	0.786654
84	2.882	2.94E-07	0.280921	0.561842	0.842763
96	2.882	4.73E-07	0.299624	0.599248	0.898872

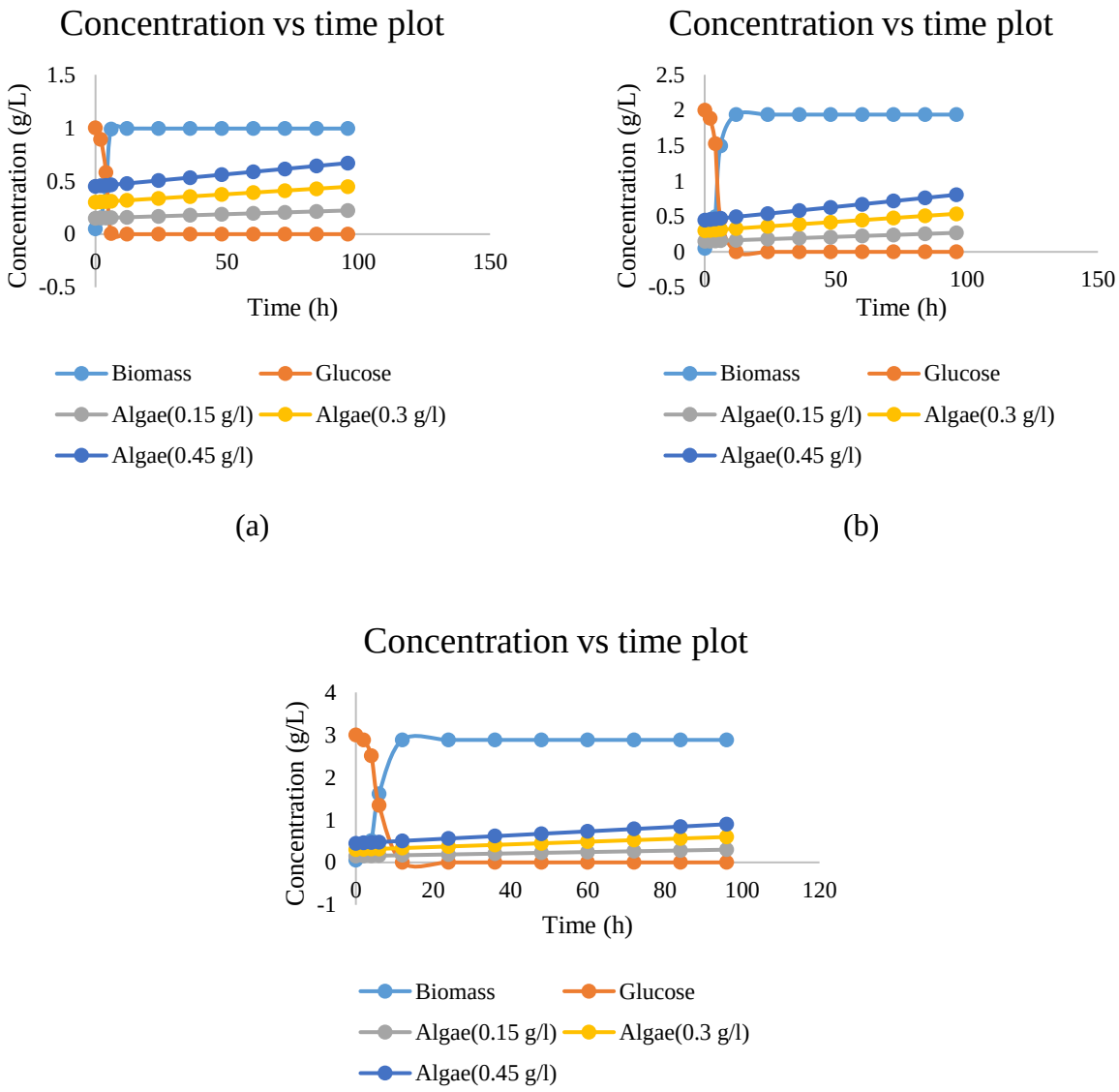


Figure 34: Concentration vs time plot for biomass , substrate -1 g/l, substrate -2 g/l , substrate-3 g/l and algae concentrations.

Simulated results from model:

Using MATLAB 2017b these differential equations are solved using ODE45 solver.

The predictions of the proposed model, the voltage and current of the MFC are measured with time. The predicted current , voltage, power density, current density shown below.

Table 44 : Simulated current (mA) data

Time (h)	Glucose 1g/L			Glucose 2g/L			Glucose 3g/L		
	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L
0	0.3800	0.3800	0.3800	0.3800	0.3800	0.3800	0.3800	0.3800	0.3800
2	0.4616	0.4616	0.4616	0.4662	0.4662	0.4662	0.4676	0.4675	0.4675
4	0.5767	0.5766	0.5766	0.5854	0.5853	0.5853	0.5880	0.5879	0.5878
6	0.6546	0.6545	0.6543	0.6869	0.6867	0.6865	0.6924	0.6922	0.6920
12	0.6672	0.6669	0.6665	0.7265	0.7260	0.7256	0.7594	0.7589	0.7584
24	0.6657	0.6651	0.6644	0.7265	0.7256	0.7246	0.7602	0.7592	0.7581
36	0.6641	0.6631	0.6620	0.7248	0.7233	0.7215	0.7578	0.7562	0.7542
48	0.6652	0.6637	0.6620	0.7259	0.7238	0.7209	0.7596	0.7571	0.7539
60	0.6690	0.6671	0.6647	0.7232	0.7203	0.7157	0.7584	0.7550	0.7495
72	0.6636	0.6612	0.6577	0.7258	0.7218	0.7139	0.7586	0.7540	0.7428
84	0.6650	0.6619	0.6569	0.7215	0.7162	0.6889	0.7567	0.7505	0.7410
96	0.6633	0.6594	0.6517	0.7229	0.7158	0.7118	0.7556	0.7468	0.7470

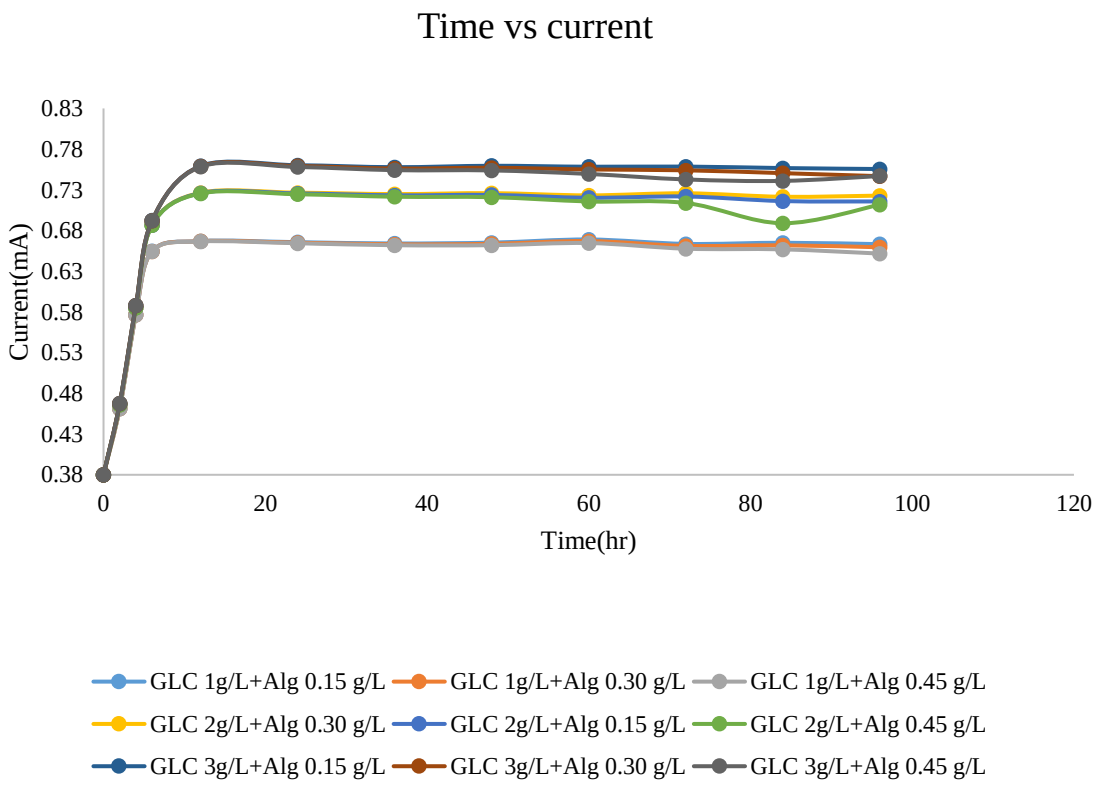


Figure 35 : Simulated current (mA) vs. time (h) Plot

Table 45 : Simulated Voltage (mV) data

Time (h)	Glucose 1g/L			Glucose 2g/L			Glucose 3g/L		
	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L
0	380.00	380.00	380.00	380.00	380.00	380.00	380.00	380.00	380.00
2	461.57	461.56	461.55	466.19	466.18	466.17	467.56	467.55	467.54
4	576.69	576.63	576.58	585.40	585.35	585.29	587.95	587.89	587.84
6	654.62	654.47	654.32	686.92	686.73	686.54	692.38	692.19	691.99
12	667.16	666.86	666.54	726.47	726.04	725.60	759.37	758.89	758.40
24	665.71	665.07	664.38	726.52	725.60	724.58	760.23	759.21	758.08
36	664.11	663.09	661.95	724.77	723.29	721.52	757.83	756.17	754.17
48	665.15	663.72	662.01	725.92	723.77	720.94	759.57	757.15	753.85
60	669.02	667.11	664.66	723.21	720.25	715.72	758.40	755.03	749.51
72	663.61	661.16	657.69	725.80	721.83	713.87	758.59	754.00	742.75
84	664.99	661.90	656.90	721.48	716.19	688.95	756.74	750.50	740.99
96	663.28	659.43	651.71	722.93	715.78	711.80	755.57	746.78	746.97

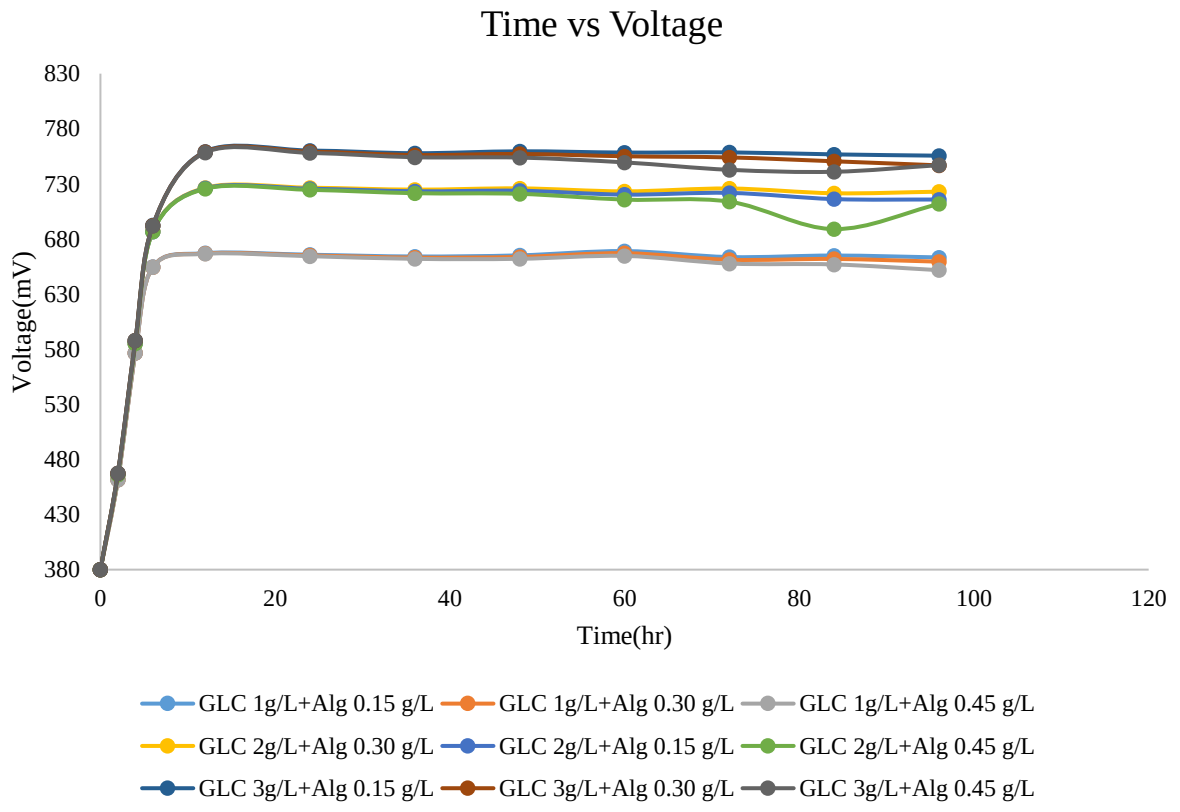


Figure 36 : Simulated voltage (mV) vs. time (h) Plot

Table 46 : Simulated Current Density(A/m^2) data

Time (h)	Glucose 1g/L			Glucose 2g/L			Glucose 3g/L		
	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L
0	0.0950	0.0950	0.0950	0.0950	0.0950	0.0950	0.0950	0.0950	0.0950
2	0.1154	0.1154	0.1154	0.1165	0.1165	0.1165	0.1169	0.1169	0.1169
4	0.1442	0.1442	0.1441	0.1464	0.1463	0.1463	0.1470	0.1470	0.1470
6	0.1637	0.1636	0.1636	0.1717	0.1717	0.1716	0.1731	0.1730	0.1730
12	0.1668	0.1667	0.1666	0.1816	0.1815	0.1814	0.1898	0.1897	0.1896
24	0.1664	0.1663	0.1661	0.1816	0.1814	0.1811	0.1901	0.1898	0.1895
36	0.1660	0.1658	0.1655	0.1812	0.1808	0.1804	0.1895	0.1890	0.1885
48	0.1663	0.1659	0.1655	0.1815	0.1809	0.1802	0.1899	0.1893	0.1885
60	0.1673	0.1668	0.1662	0.1808	0.1801	0.1789	0.1896	0.1888	0.1874
72	0.1659	0.1653	0.1644	0.1814	0.1805	0.1785	0.1896	0.1885	0.1857
84	0.1662	0.1655	0.1642	0.1804	0.1790	0.1722	0.1892	0.1876	0.1852
96	0.1658	0.1649	0.1629	0.1807	0.1789	0.1780	0.1889	0.1867	0.1867

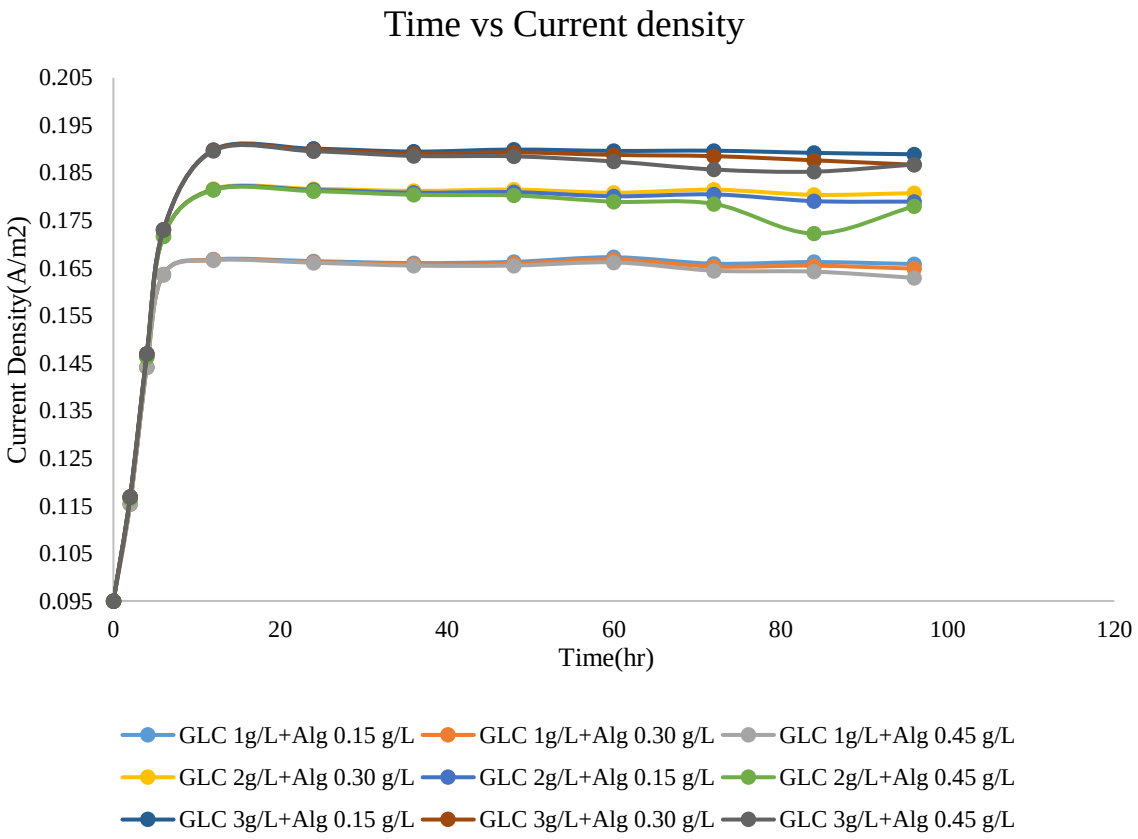


Figure 37: Simulated Current density(A/m^2) vs. time (h) Plot

Table 47 : Simulated Power Density(W/m^2) data

Time (h)	Glucose 1g/L			Glucose 2g/L			Glucose 3g/L		
	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L
0	0.0361	0.0361	0.0361	0.0361	0.0361	0.0361	0.0361	0.0361	0.0361
2	0.0529	0.0529	0.0529	0.0539	0.0539	0.0539	0.0542	0.0542	0.0542
4	0.0827	0.0827	0.0827	0.0853	0.0853	0.0852	0.0860	0.0860	0.0860
6	0.1067	0.1067	0.1066	0.1176	0.1175	0.1174	0.1194	0.1194	0.1193
12	0.1109	0.1108	0.1107	0.1315	0.1314	0.1312	0.1438	0.1436	0.1434
24	0.1104	0.1102	0.1099	0.1316	0.1313	0.1309	0.1441	0.1438	0.1433
36	0.1099	0.1096	0.1092	0.1309	0.1304	0.1297	0.1432	0.1425	0.1418
48	0.1102	0.1097	0.1092	0.1314	0.1306	0.1296	0.1438	0.1429	0.1417
60	0.1116	0.1109	0.1101	0.1304	0.1293	0.1277	0.1435	0.1422	0.1401
72	0.1097	0.1089	0.1077	0.1313	0.1299	0.1270	0.1435	0.1417	0.1375
84	0.1101	0.1091	0.1075	0.1297	0.1278	0.1186	0.1428	0.1405	0.1372
96	0.1096	0.1083	0.1058	0.1303	0.1277	0.1266	0.1423	0.1390	0.1394

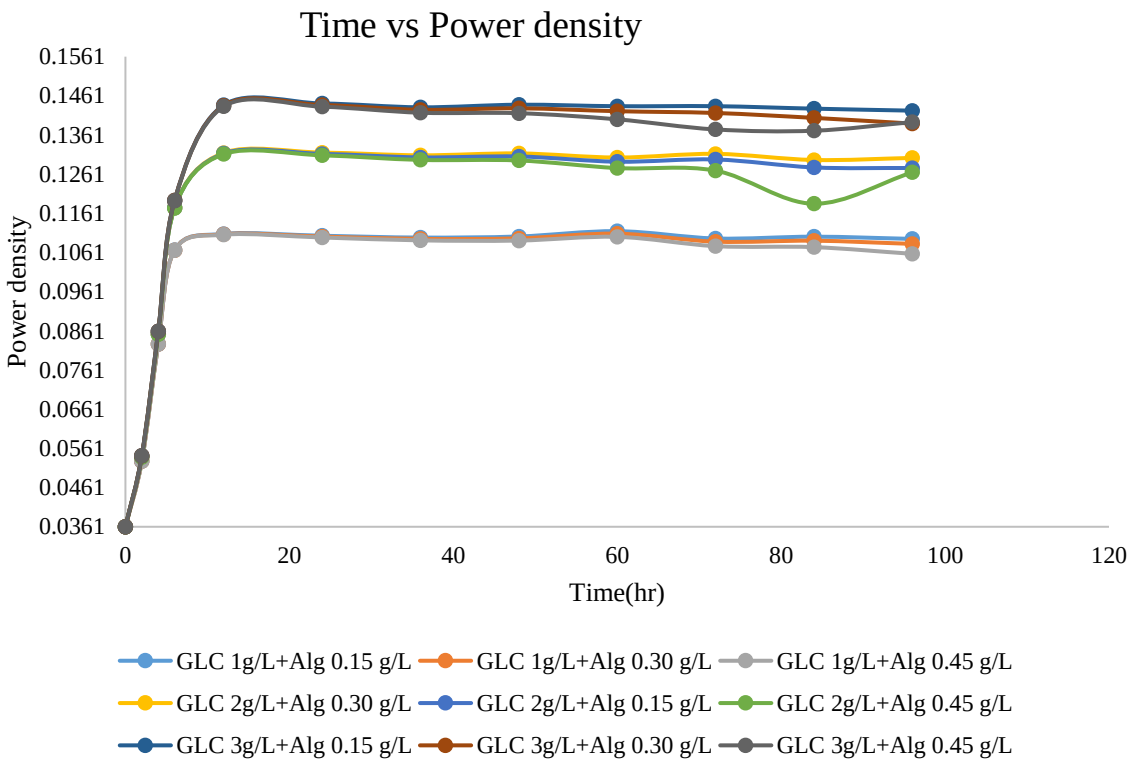


Figure 38 : Simulated Current density(A/m^2) vs. time (h) Plot

6.4.3. Mathematical Modelling of the BAFC using Lactic acid + glucose as substrate

6.4.3.1. Codes in MATLAB for Dual substrate (Lactic Acid & Glucose)

Function code:

```
function dxdt = substrate_1(t,x)
%lactic acid growth kinetic parameters;
uml = 0.0353;
ksl = 2.6472;
ksi = 3.4455;
Yl = 0.575;
%glucose growth kinetic parameters;
ksg = 0.077;
umg = 0.529;
Yg = 0.944;
dxdt = zeros(5,1);
% anode mass balance;
dxdt(1) = (((uml*x(2)/(ksl+x(2)+((x(2)^2)/ksi))+(umg*x(3)/(ksg+x(3))))*x(1)));
dxdt(2) = (1/(-Yl))*(uml*x(2)/(ksl+x(2)+((x(2)^2)/ksi)));
dxdt(3) = (1/(-Yg))*(umg*x(3)*x(1)/(ksg+x(3)));
dxdt(4) =
(((1/Yl)*((uml*x(2)/(ksl+x(2)+((x(2)^2)/ksi))))+((1/Yg)*(6*umg*x(3)*x(1)/(ksg+x(3))))));
dxdt(5) =
(((1/Yl)*(5*(uml*x(2)/(ksl+x(2)+((x(2)^2)/ksi))))+((1/Yg)*(24*umg*x(3)*x(1)/(ksg+x(3))))));
end
% x(1) bacterial biomass concentration;
% x(2) lactic acid concentration;
% x(3) glucose concentration;
% x(4) dissolved carbon di oxide concentration produced in anode chamber;
% x(5) dissolved hydrogen ion concentration produced in anode chamber;
% x(6) algae biomass concentration;
% x(7) dissolved carbon di oxide concentration consuming in cathode chamber;
% x(8) dissolved oxygen concentration producing in cathode chamber;
```

Main Code:

```
tspan = [0 2 4 6 12:12:96];
X_in = [0.05 3.022 2 0 0];
xalg = 0.45;
E0a = 0.62;
E0c = 0.81;
R = 8.314;
T = 298;
F = 96500;
n =28;
A = 0.004;
Res = 1000;
n1 = 4;
vg = 0.45;
vl= 0.2;
umal = 0.0215;
ksal = 1.650;
Yal = 0.106;
Cco2l = 0.0037;
Cco2g = 0.196;
Cco2c= 0.04;
Cco2s = 0.1368;
H = 1.364*10^-2;
klac = 0.263;
klao = 0.291;
Co2s = 8.42;
[tsol,xsol] = ode45(@substrate_1,tspan,X_in);
count = numel(tspan);
for i = 1:count
e1(i) = power(xsol(i,4),7);
e2(i) = power(xsol(i,5),28);
```

```
e3(i) = power(xsol(i,5),4);
% carbon di oxide mass balance equation;
e4(i) = (xsol(i,4)-(klac*(xsol(i,4)-Cco2s)));
if xsol(i,4) >= Cco2s
    e5(i) = (klac*(vl/vg)*(e4(i)-Cco2s))- (klac*(Cco2g*H - Cco2c)*(vl/vg));
else
   e5(i) =  (klac*(Cco2g*H - Cco2c)*(vl/vg));
end
% cathode chaber mass balance;
f = @(t,x)[((umal*e5(i)*xalg)/(ksal+e5(i)));(((1/(-Yal))*(umal*e5(i)*xalg))/(ksal+e5(i)))];
[t,x] = ode45(f,tspan,[xalg,e5(i)]);
e6(i) = e5(i)+(klac*(H*Cco2l-Cco2c));
% oxygen producing in day time;
e7(i) = -x(i,2)-(klao*(Co2s-(-x(i,2))));
% oxygen producing in night time;
e8(i) = -(klao*(Co2s-(-x(i,2))));
% reaction quotient equation of anode chamber;
Q(i) = log((e1(i)*e2(i))/(xsol(i,2)));
E_a(i) = E0a - ((R*T*Q(i))/(n*F));
% reaction quotient equation in cathode chamber;
bd(i) = log(1/(e7(i)*e3(i)));
bn(i)= log(1/(e8(i)*e3(i)));
b(i) = bd(i)+bn(i);
E_c(i) = E0c - ((R*T*b(i))/(n1*F));
%total currentvoltage,power density and current denisty equations;
E(i) = E_c(i) - E_a(i);
curr(i) = E(i)/Res;
Currd(i) = curr(i)/A;
p(i) = (curr(i)*E(i));
Pd(i) = (curr(i)*E(i))/A;
End
```

```
subplot(3,3,1);
plot(tsol,xsol(:,1),'r', tsol,xsol(:,2),'g' , tsol,xsol(:,3),'b');
xlabel('time(days)');
ylabel('concentration (mg/l');
subplot(3,3,2);
plot(tsol,E);
xlabel('time(days)');
ylabel('voltage(V)');
subplot(3,3,3);
plot(tsol,Pd);
xlabel('time(days)');
ylabel('Power density(W/m2)');
subplot(3,3,4);
plot(tsol,Currd);
xlabel('time(days)');
ylabel('current density(A/m2)');
subplot(3,3,5);
plot(t,x);
xlabel('time(days)');
ylabel('algae concentration (mg/L)');
%vg= total volume of gas in headspace of cathode+anode chember;
%vl= total volume of liquid in cathode or in anode chamber;
%Cco2l= dissolved carbon di oxide in bulk liquid in anode chamber;
%Cco2= saturated carbon di oxide concentration in catholyte;
%klac= volumetric mass transfer co-efficient of carbon di oxide;
%klao= volumetric mass transfer co-efficient of oxygen;
%dCo2= saturated dissolved oxygen concentration;
```

6.4.3.2.Data set generated from MATLAB

The table below shows the data set that was generated from MATLAB for the BAFC

Table 48: Concentration Data set of biomass ,Glucose 1g/l, lactic acid 3.022 g/l lactic acid and algae generated from MATLAB.

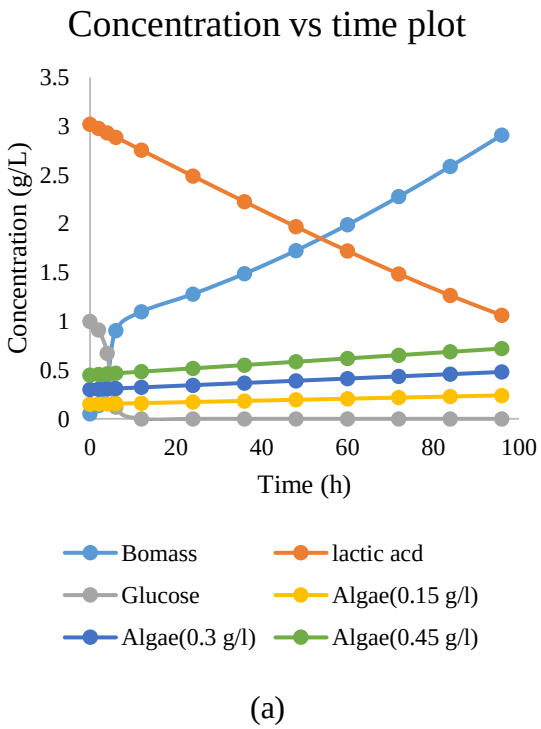
Time(hr)	Biomass	lactic acid	Glucose	Algae(0.15 g/l)	Algae(0.3 g/l)	Algae(0.45 g/l)
0	0.05	3.022	1	0.15	0.3	0.45
2	0.136631	2.9774023	0.910573	0.151879	0.303758	0.455638
4	0.368226	2.9328108	0.671596	0.153758	0.307517	0.461275
6	0.903515	2.8882319	0.121087	0.155638	0.311275	0.466913
12	1.09868	2.7555678	1.43E-07	0.161275	0.322551	0.483826
24	1.280116	2.4911267	3.93E-08	0.172551	0.345102	0.517653
36	1.488535	2.2333457	2.63E-07	0.183826	0.367653	0.551479
48	1.725085	1.9775432	3.55E-08	0.195102	0.390204	0.585306
60	1.989354	1.7246679	5.63E-08	0.206377	0.412755	0.619132
72	2.278751	1.4922407	8.98E-07	0.217653	0.435306	0.652959
84	2.588045	1.2665443	9.46E-07	0.228928	0.457857	0.686785
96	2.909332	1.0609887	2.16E-07	0.240204	0.480408	0.720612

Table 49: Concentration Data set of biomass and ,Glucose 2g/l, lactic acid 3.022 g/l and algae generated from MATLAB.

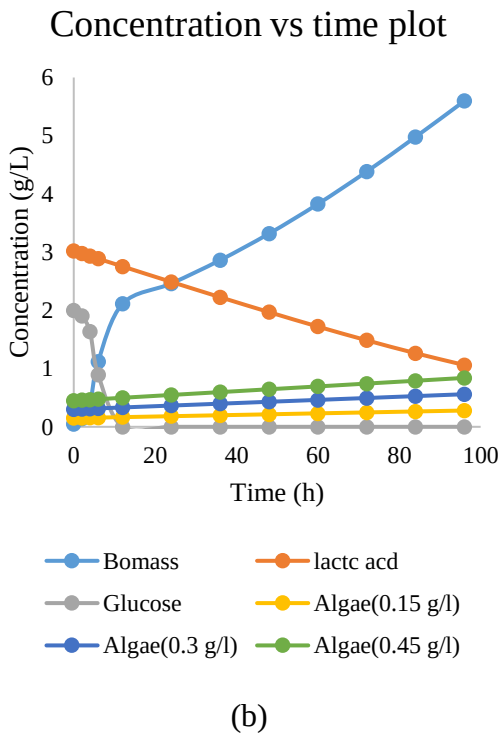
Time(hr)	Biomass	lactic acid	Glucose	Algae(0.15 g/l)	Algae(0.3 g/l)	Algae(0.45 g/l)
0	0.05	3.022	2	0.15	0.3	0.45
2	0.141981	2.9774023	1.904957	0.15271	0.305419	0.458129
4	0.401699	2.9328108	1.636618	0.155419	0.310838	0.466258
6	1.120222	2.8882319	0.894535	0.158129	0.316258	0.474386
12	2.113724	2.7555678	5.89E-07	0.166258	0.332515	0.498773
24	2.462786	2.4911267	8.05E-07	0.182515	0.36503	0.547545
36	2.863759	2.2333457	1.35E-07	0.198773	0.397545	0.596318
48	3.318852	1.9775432	7.53E-08	0.21503	0.43006	0.64509
60	3.827275	1.7246679	1.62E-07	0.231288	0.462575	0.693863
72	4.384039	1.4922407	4.53E-07	0.247545	0.49509	0.742635
84	4.979085	1.2665443	3.11E-07	0.263803	0.527605	0.791408
96	5.5972	1.0609887	4.95E-08	0.28006	0.56012	0.84018

Table 50: Concentration Data set of biomass , Glucose 3g/l, 3.022 g/l lactic acid and algae generated from MATLAB.

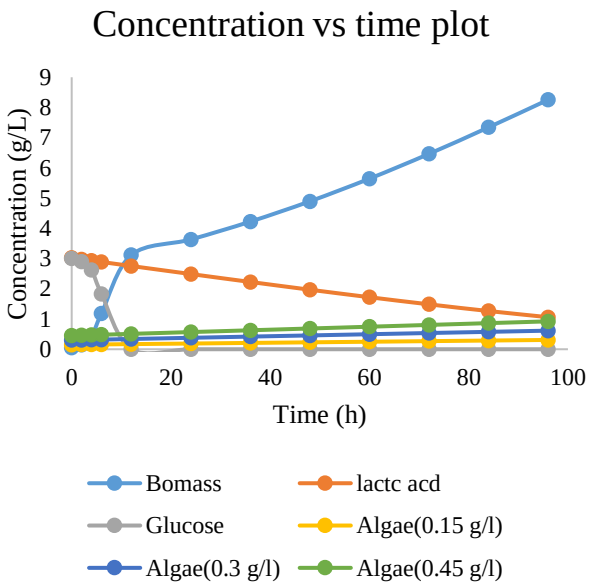
Time(hr)	Biomass	lactic acid	Glucose	Algae(0.15 g/l)	Algae(0.3 g/l)	Algae(0.45 g/l)
0	0.05	3.022	3	0.15	0.3	0.45
2	0.143862	2.9774023	2.902983	0.153285	0.306569	0.459854
4	0.413234	2.9328108	2.624566	0.156569	0.313138	0.469708
6	1.179833	2.8882319	1.83235	0.159854	0.319708	0.479562
12	3.117091	2.7555678	1.20E-07	0.169708	0.339415	0.509123
24	3.631849	2.4911267	2.48E-07	0.189415	0.378831	0.568246
36	4.223161	2.2333457	1.09E-06	0.209123	0.418246	0.62737
48	4.894282	1.9775432	6.93E-07	0.228831	0.457662	0.686493
60	5.644048	1.7246679	1.30E-07	0.248539	0.497077	0.745616
72	6.465104	1.4922407	1.33E-07	0.268246	0.536493	0.804739
84	7.342611	1.2665443	2.64E-07	0.287954	0.575908	0.863862
96	8.25414	1.0609887	4.49E-08	0.307662	0.615324	0.922985



(a)



(b)



(C)

Figure 39: Concentration vs time plot for biomass ,substrates (a) Glc – 1g/l, (b) Glc-2g/l, (c) Glc-3 g/l , L.A- 3.022 g/l and algae concentrations.

Simulated results from model:

Using MATLAB 2017b these differential equations are solved using ODE45 solver.

The predictions of the proposed model, the voltage and current of the MFC are measured with time. The predicted current , voltage, power density, current density shown below.

Table 51 : Simulated current (mA) data

Time (h)	**Lactic Acid 3.022g/L**								
	Glucose 1g/L			**Glucose 2g/L**			**Glucose 3g/L**		
	Algae 0.15g/L	**Algae 0.30g/L**	**Algae 0.45g/L**	**Algae 0.15g/L**	**Algae 0.30g/L**	**Algae 0.45g/L**	**Algae 0.15g/L**	**Algae 0.30g/L**	**Algae 0.45g/L**
0	0.1900	0.1900	0.1900	0.1900	0.1900	0.1900	0.1900	0.1900	0.1900
2	0.2636	0.2636	0.2636	0.2683	0.2683	0.2682	0.2698	0.2698	0.2698
4	0.3690	0.3690	0.3690	0.3771	0.3771	0.3770	0.3797	0.3797	0.3797
6	0.4502	0.4501	0.4499	0.4692	0.4690	0.4689	0.4738	0.4736	0.4734
12	0.4630	0.4627	0.4624	0.5201	0.5197	0.5192	0.5545	0.5541	0.5536
24	0.4670	0.4664	0.4656	0.5220	0.5211	0.5201	0.5557	0.5547	0.5535
36	0.4708	0.4697	0.4684	0.5238	0.5223	0.5205	0.5568	0.5551	0.5531
48	0.4743	0.4727	0.4707	0.5255	0.5233	0.5203	0.5578	0.5554	0.5520
60	0.4775	0.4753	0.4723	0.5271	0.5240	0.5191	0.5587	0.5553	0.5497
72	0.4804	0.4774	0.4729	0.5285	0.5243	0.5152	0.5595	0.5549	0.5433

84	0.4830	0.4791	0.4714	0.5297	0.5240	0.5096	0.5602	0.5539	0.5448
96	0.4852	0.4801	0.4561	0.5307	0.5228	0.5211	0.5608	0.5518	0.5522

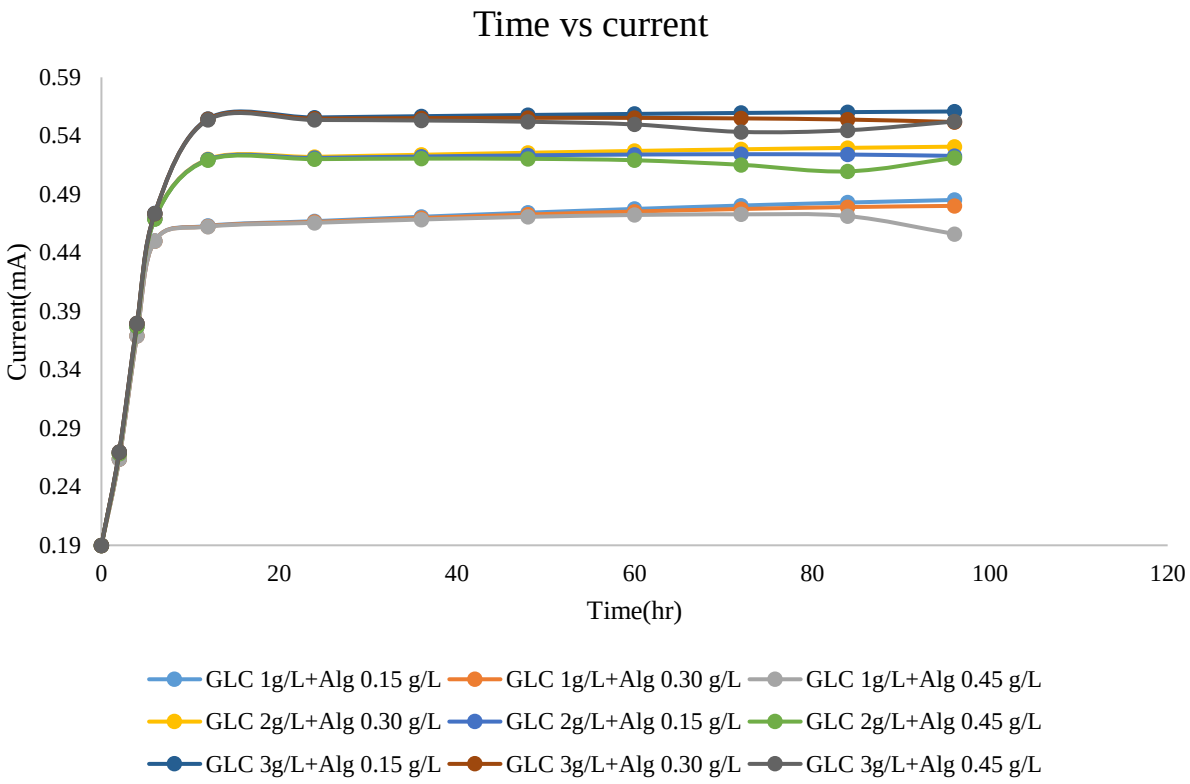


Figure 40 : Simulated current (mA) vs. time (h) Plot

Table 52 : Simulated Voltage(mV) data

Time (h)	**Lactic Acid 3.022g/L**								
	Glucose 1g/L			**Glucose 2g/L**			**Glucose 3g/L**		
	Algae 0.15g/L	**Algae 0.30g/L**	**Algae 0.45g/L**	**Algae 0.15g/L**	**Algae 0.30g/L**	**Algae 0.45g/L**	**Algae 0.15g/L**	**Algae 0.30g/L**	**Algae 0.45g/L**
0	190.000	190.000	190.000	190.000	190.000	190.000	190.000	190.000	190.000
2	263.627	263.621	263.615	268.262	268.255	268.248	269.832	269.825	269.818
4	369.047	369.003	368.958	377.133	377.085	377.036	379.749	379.700	379.650
6	450.202	450.062	449.920	469.195	469.034	468.872	473.754	473.590	473.422
12	462.991	462.677	462.353	520.119	519.687	519.235	554.544	554.070	553.572
24	467.036	466.358	465.627	522.024	521.093	520.061	555.694	554.671	553.530
36	470.809	469.705	468.449	523.829	522.309	520.494	556.782	555.114	553.094
48	474.301	472.694	470.734	525.516	523.291	520.336	557.796	555.352	552.024
60	477.499	475.290	472.322	527.069	523.977	519.131	558.721	555.317	549.716
72	480.390	477.445	472.880	528.468	524.269	515.226	559.540	554.898	543.340
84	482.957	479.092	471.402	529.694	524.003	509.628	560.235	553.894	544.764
96	485.188	480.126	456.095	530.726	522.835	521.089	560.788	551.834	552.222

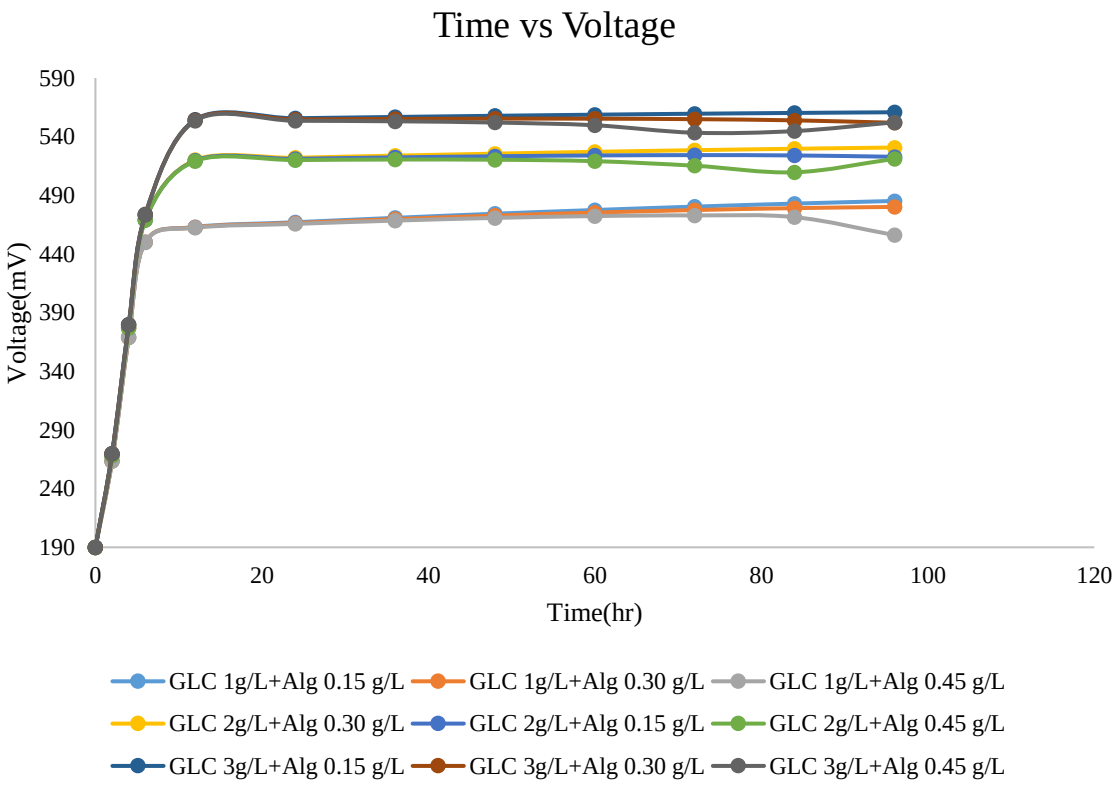


Figure 41 : Simulated voltage (mV) vs. time (h) Plot

Table 53 : Simulated Current density(A/m^2)data

Time (h)	Lactic Acid 3.022g/L								
	Glucose 1g/L			Glucose 2g/L			Glucose 3g/L		
	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L	Algae 0.15g/L	Algae 0.30g/L	Algae 0.45g/L
0	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048
2	0.066	0.066	0.066	0.067	0.067	0.067	0.067	0.067	0.067
4	0.092	0.092	0.092	0.094	0.094	0.094	0.095	0.095	0.095
6	0.113	0.113	0.112	0.117	0.117	0.117	0.118	0.118	0.118
12	0.116	0.116	0.116	0.130	0.130	0.130	0.139	0.139	0.138
24	0.117	0.117	0.116	0.131	0.130	0.130	0.139	0.139	0.138
36	0.118	0.117	0.117	0.131	0.131	0.130	0.139	0.139	0.138
48	0.119	0.118	0.118	0.131	0.131	0.130	0.139	0.139	0.138
60	0.119	0.119	0.118	0.132	0.131	0.130	0.140	0.139	0.137
72	0.120	0.119	0.118	0.132	0.131	0.129	0.140	0.139	0.136
84	0.121	0.120	0.118	0.132	0.131	0.127	0.140	0.138	0.136
96	0.121	0.120	0.114	0.133	0.131	0.130	0.140	0.138	0.138

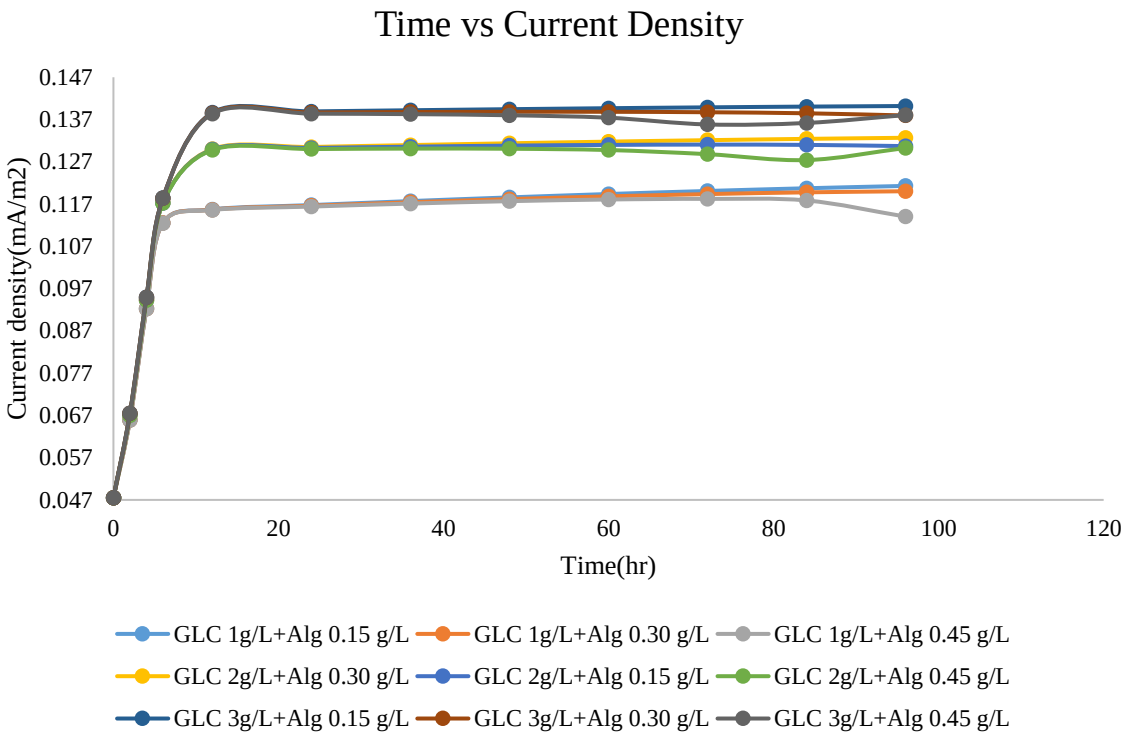


Figure 42 : Simulated Current density(A/m^2) vs. time (h) Plot

Table 54 : Simulated Power Density(W/m^2) data

Time (h)	**Lactic Acid 3.022g/L**								
	Glucose 1g/L			**Glucose 2g/L**			**Glucose 3g/L**		
	Algae 0.15g/L	**Algae 0.30g/L**	**Algae 0.45g/L**	**Algae 0.15g/L**	**Algae 0.30g/L**	**Algae 0.45g/L**	**Algae 0.15g/L**	**Algae 0.30g/L**	**Algae 0.45g/L**
0	0.0090	0.0090	0.0090	0.0090	0.0090	0.0090	0.0090	0.0090	0.0090
2	0.0170	0.0170	0.0170	0.0176	0.0176	0.0176	0.0178	0.0178	0.0178
4	0.0336	0.0336	0.0336	0.0352	0.0351	0.0351	0.0356	0.0356	0.0356
6	0.0503	0.0502	0.0502	0.0546	0.0546	0.0546	0.0557	0.0557	0.0556
12	0.0532	0.0531	0.0530	0.0672	0.0671	0.0670	0.0765	0.0763	0.0762
24	0.0541	0.0540	0.0538	0.0677	0.0675	0.0672	0.0768	0.0765	0.0762
36	0.0550	0.0547	0.0545	0.0682	0.0678	0.0673	0.0771	0.0766	0.0761
48	0.0558	0.0555	0.0550	0.0686	0.0681	0.0673	0.0774	0.0767	0.0758
60	0.0566	0.0561	0.0554	0.0690	0.0682	0.0670	0.0776	0.0767	0.0751
72	0.0573	0.0566	0.0555	0.0694	0.0683	0.0660	0.0779	0.0766	0.0734
84	0.0579	0.0570	0.0551	0.0697	0.0682	0.0648	0.0781	0.0763	0.0741
96	0.0584	0.0572	0.0516	0.0700	0.0679	0.0678	0.0782	0.0757	0.0761

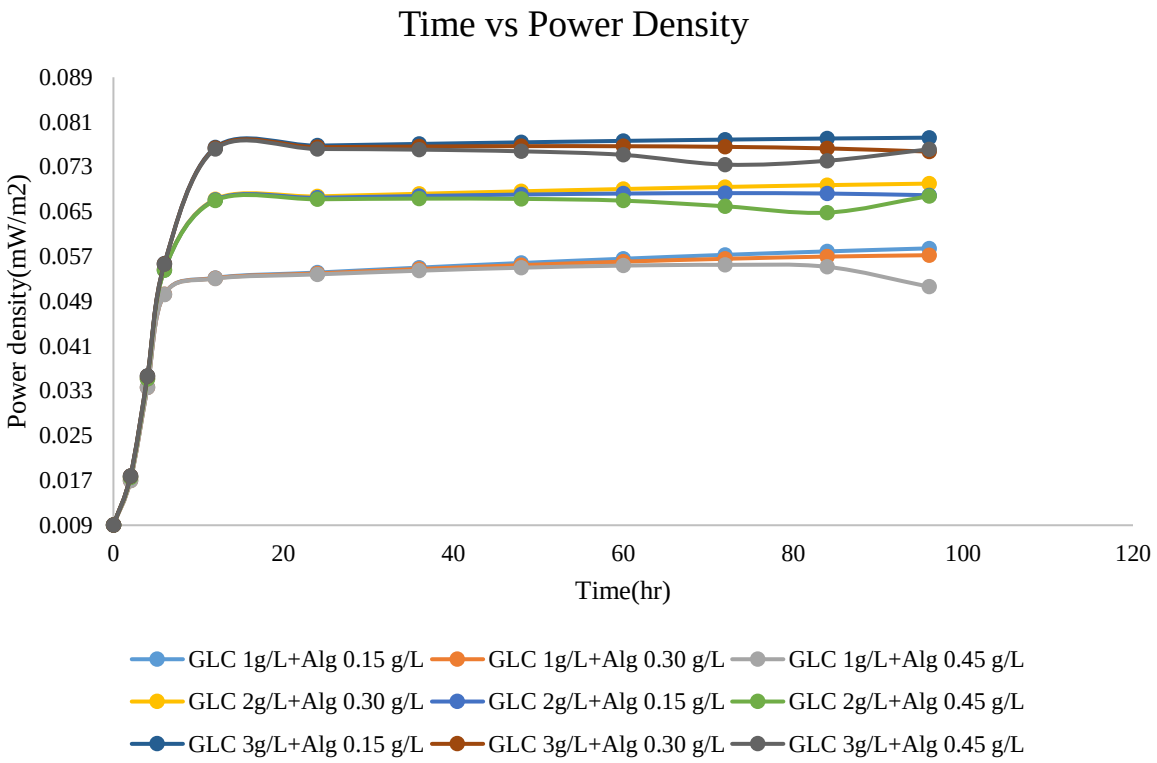


Figure 43 : Simulated Power density(W/m^2) vs. time (h) Plot

6.5.Response surface Analysis:

Using Design expert 13 software the optimization is done. In design expert RSM analysis with box-behnken model the three parameters being observed and the optimum points are recorded. A three-level, 3-factor Box-Behnken statistical design in response surface methodology (RSM) has been used.

The input factor in the software are tabulated bellow:

Table 55: variables with coded level used for experimental run

Input variables	Unit	Coded variable level			Model Response
		-1	0	1	
Glucose concentration (A)	g/L	1	2	3	Power density (mW/m^2)
Algae concentration (B)	g/L	0.15	0.3	0.45	
Time (C)	h	2	49	96	

Table 55a: Operating parameters

Run	A: Glucose concentration (g/L)	B: Algae concentration (g/L)	C: Time (h)	Power density (mW/m^2)
1	1	0.3	2	16
2	1	0.15	49	55.5
3	3	0.3	2	19.2
4	2	0.15	96	70.01
5	2	0.15	2	17.2
6	2	0.45	96	67.78
7	3	0.3	96	75
8	2	0.3	49	68.05
9	2	0.3	49	68.05
10	1	0.3	96	57.2
11	2	0.3	49	68.05
12	3	0.45	49	75.78
13	2	0.3	49	68.05
14	3	0.15	49	77.34
15	2	0.3	49	68.05
16	1	0.45	49	55.4
17	2	0.45	2	17.1

Table 56: ANOVA table

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	8178.04	9	908.67	103.25	< 0.0001	significant
A-Glucose concentration	499.60	1	499.60	56.77	0.0001	
B-Algae concentration	1.99	1	1.99	0.2261	0.6489	
C-Time	5024.53	1	5024.53	570.90	< 0.0001	
AB	0.5329	1	0.5329	0.0605	0.8127	
AC	53.29	1	53.29	6.05	0.0434	
BC	1.13	1	1.13	0.1289	0.7302	
A^2	10.90	1	10.90	1.24	0.3026	
B^2	0.8013	1	0.8013	0.0910	0.7716	
C^2	2546.23	1	2546.23	289.31	< 0.0001	
Residual	61.61	7	8.80			
Lack of Fit	61.61	3	20.54			
Pure Error	0.0000	4	0.0000			
Cor Total	8239.65	16				

(R^2: 0.9925; Adjusted R^2: 0.9827; Predicted R^2:0.8804)

Correlation of power density with three variables

$$Power\ density = 68.05 + 7.9025A - 0.49875B + 25.0613C - 0.365AB + 3.65AC - 0.525BC - 1.60875A^2 - 0.43625B^2 - 24.5912C^2$$

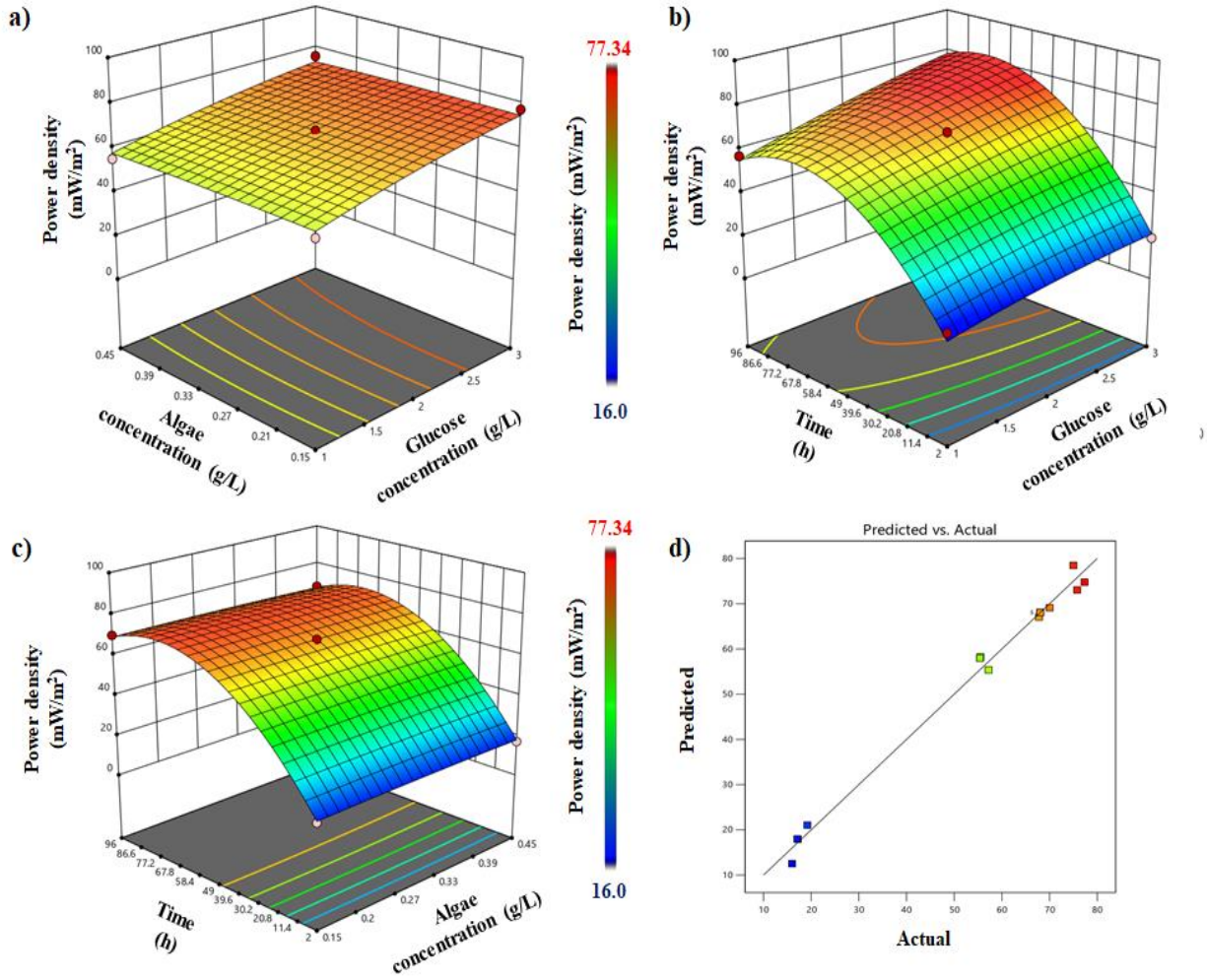


Figure 44: Response surface plots for optimization of power density

Table 57: Model predicted value of the power density under optimum condition

Run	A: Glucose concentration (g/L)	B: Algae concentration (g/L)	C: Time (h)	Power density (mW/m^2)
Model Predicted	2.833	0.417	40.150	66.536

6.6. Validation of mathematical model

To validate the predictions of the proposed model, the voltage and current of the BAFC are measured with time. The experimental set up of BAFC III which is using dual substrate (Glucose + Lactic acid) is developed on the basis of optimization. As in the lactic acid growth kinetics an inhibition effect was seen, so the critical concentration of lactic acid (2500 ppm, 3.022 g/l) is used in all the set up.The glucose concentration and algae concentration varied from 1 g/l to 3 g/l and 0.15 g/l to 0.45 g/l respectively. The predicted and measured current, voltage and power density of maximum producing set up are compered and shown below respectively and the data are given in table .

Table 58: Simulated vs. Experimental current (mA) data for 1 g/l glucose concentration.

Time (hr)	**Experimental**			**Simulated**		
	Alg 0.15 g/l	**Alg 0.30 g/l**	**Alg 0.45 g/l**	**Alg 0.15g/l**	**Alg 0.30 g/l**	**Alg 0.45 g/l**
4	0.18	0.235	0.26	0.369	0.369	0.369
26	0.196	0.27	0.23	0.467	0.466	0.466
48	0.39	0.43	0.46	0.474	0.473	0.471

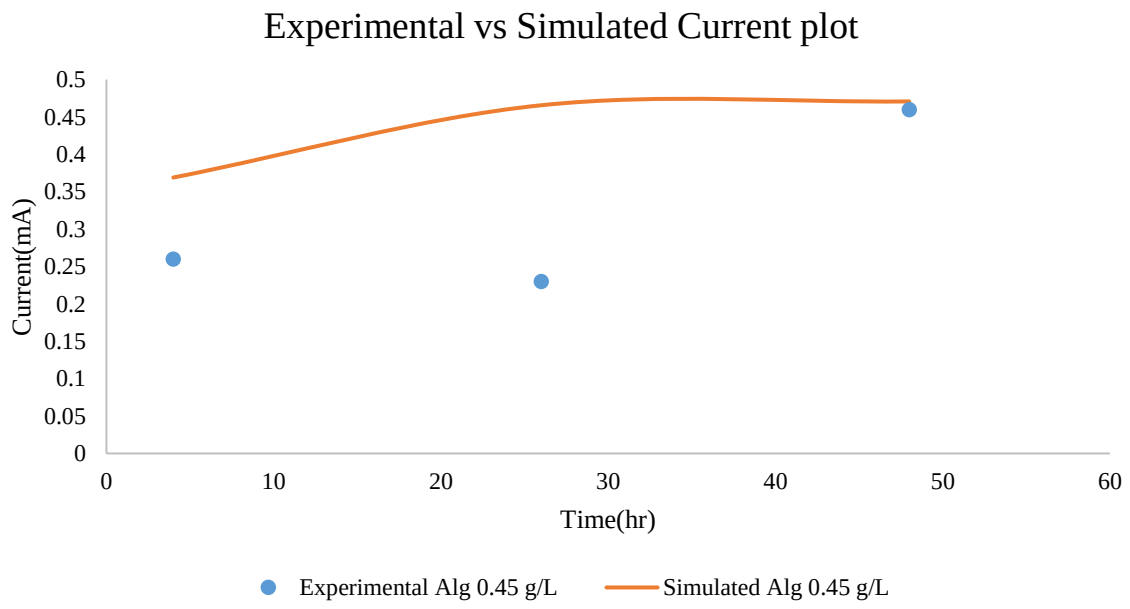


Figure 45: Simulated and experimental values of full cell current (mA) vs. time (h) for 1 g/l glucose and 0.45 g/l algae concentration.

Table 59: Simulated vs. Experimental current (mA) data for 2 g/l glucose concentration.

Time (hr)	Experimental			Simulated		
	Alg 0.15 g/l	Alg 0.30 g/l	Alg 0.45g/l	Alg 0.15g/l	Alg 0.30 g/l	Alg 0.45 g/l
4	0.185	0.21	0.35	0.377	0.377	0.377
26	0.32	0.247	0.42	0.522	0.521	0.520
48	0.352	0.41	0.48	0.526	0.523	0.520

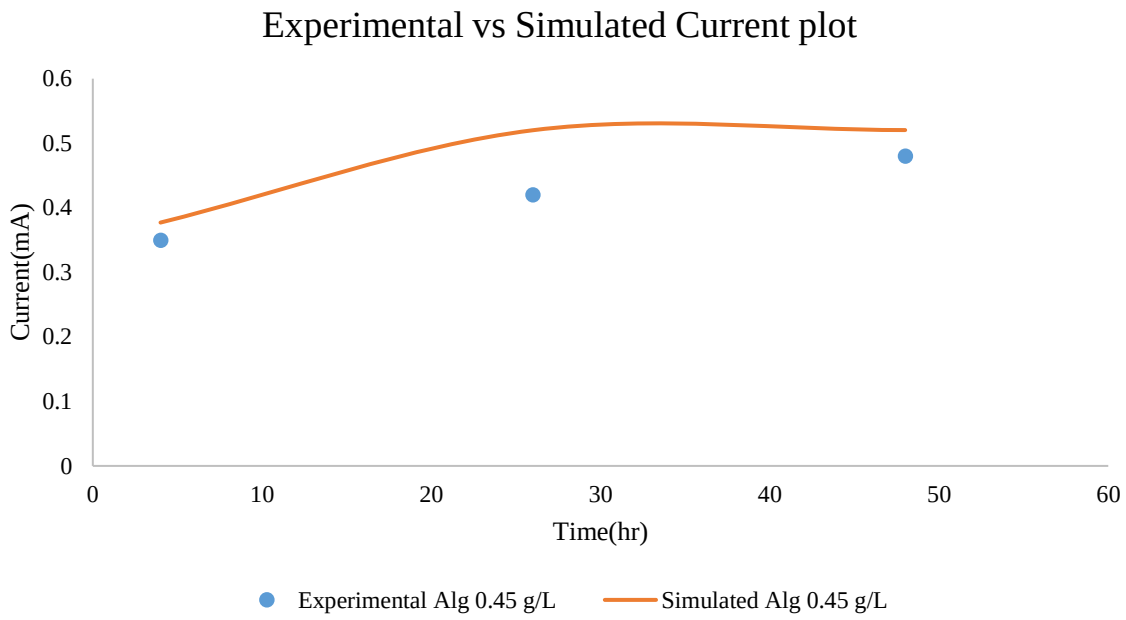


Figure 46: Simulated and experimental values of full cell current (mA) vs. time (h) for 2 g/l glucose concentration and 0.45 g/l algae concentration.

Table 60: Simulated vs. Experimental current (mA) data for 3 g/l glucose concentration.

Time (hr)	Experimental			Simulated		
	Alg 0.15 g/l	Alg 0.30 g/l	Alg 0.45 g/l	Alg 0.15g/l	Alg 0.30 g/l	Alg 0.45 g/l
4	0.278	0.29	0.31	0.380	0.380	0.380
26	0.48	0.55	0.54	0.556	0.555	0.554
48	0.52	0.51	0.58	0.558	0.555	0.552

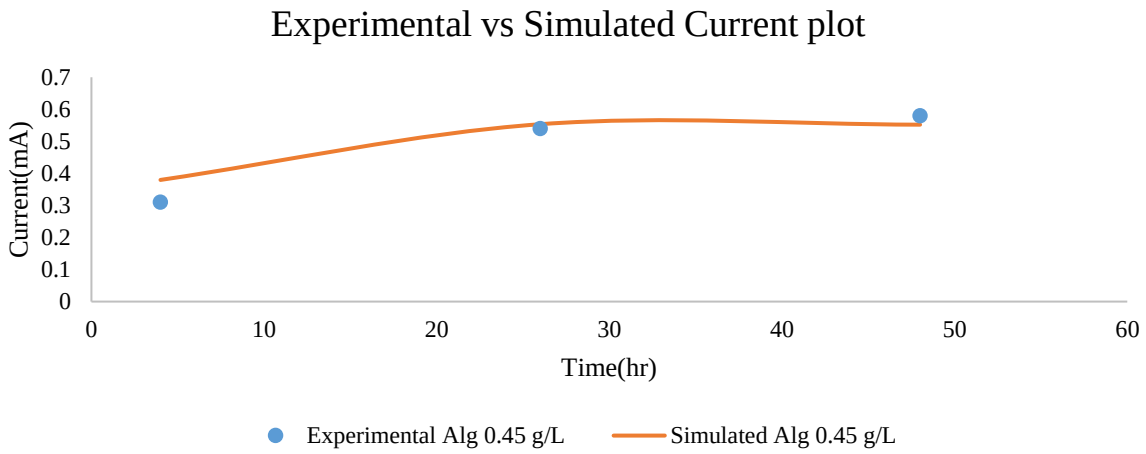


Figure 47: Simulated and experimental values of full cell current (mA) vs. time (h) for 3 g/l glucose concentration and 0.45 g/l algae concentration.

Table 61: Simulated vs. Experimental Voltage (mv) data for 1 g/l glucose concentration.

Time (hr)	Experimental			Simulated		
	Alg 0.15 g/l	Alg 0.30 g/l	Alg 0.45 g/l	Alg 0.15 g/l	Alg 0.30 g/l	Alg 0.45 g/l
4	170	208	228	369.05	369.00	368.96
26	186	231	390	467.04	466.36	465.63
48	210	189	415	474.30	472.69	470.73

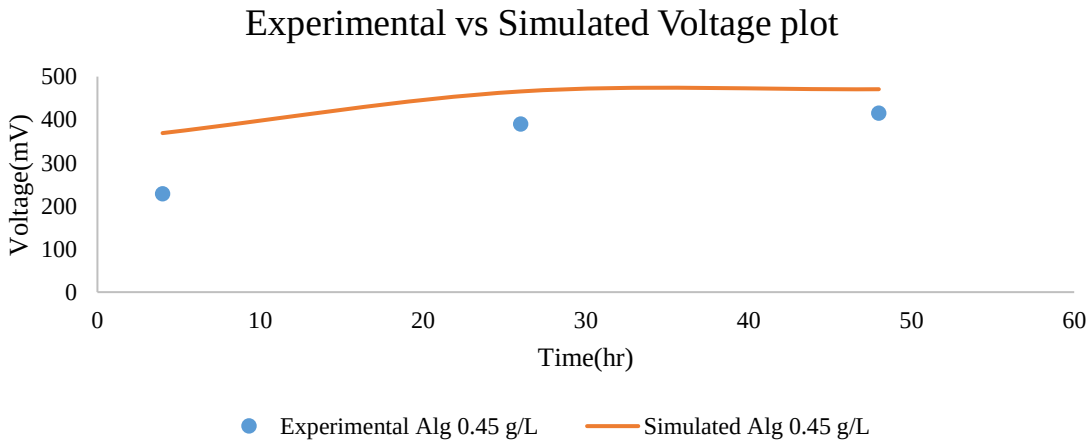


Figure 48: Simulated and experimental values of full cell Voltage (mV) vs. time (h) for 1 g/l glucose and 0.45 g/l algae concentration.

Table 62: Simulated vs. Experimental Voltage (mv) data for 2 g/l glucose concentration.

Time (hr)	Experimental			Simulated		
	Alg 0.15 g/l	Alg 0.30 g/l	Alg 0.45 g/l	Alg 0.15 g/l	Alg 0.30 g/l	Alg 0.45 g/l
4	215	260	385	377.13	377.08	377.04
26	258	425	490	522.02	521.09	520.06
48	245	474	511	525.52	523.29	520.34

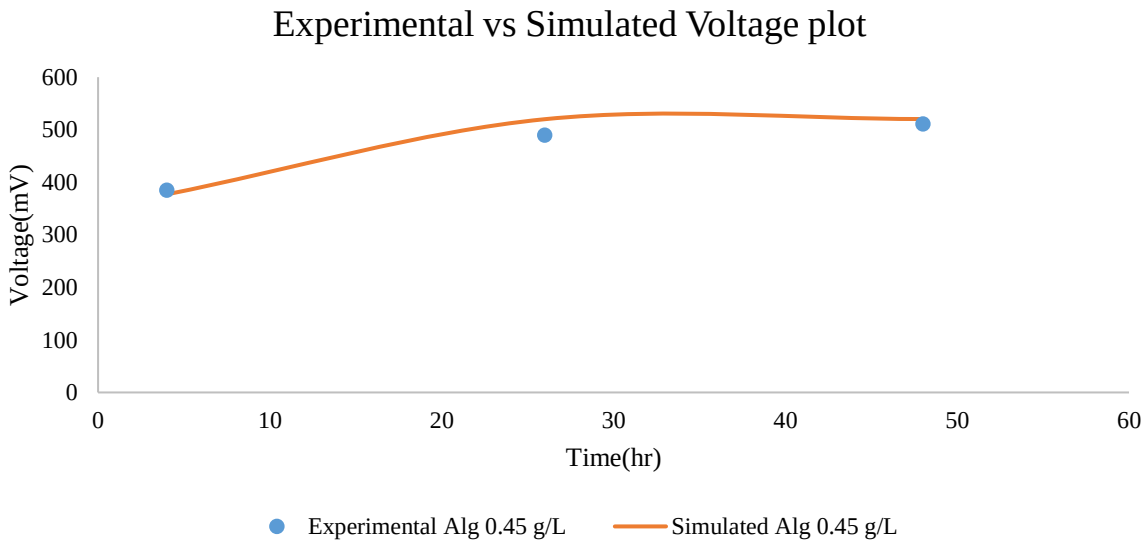


Figure 49: Simulated and experimental values of full cell Voltage (mV) vs. time (h) for 2 g/l glucose and 0.45 g/l algae concentration.

Table 63: Simulated vs. Experimental Voltage (mv) data for 3 g/l glucose concentration.

Time (hr)	Experimental			Simulated		
	Alg 0.15 g/l	Alg 0.30 g/l	Alg 0.45 g/l	Alg 0.15 g/l	Alg 0.30 g/l	Alg 0.45 g/l
4	240	301	326	379.75	379.70	379.65
26	450	445	461	555.69	554.67	553.53
48	428	528	516	557.80	555.35	552.02

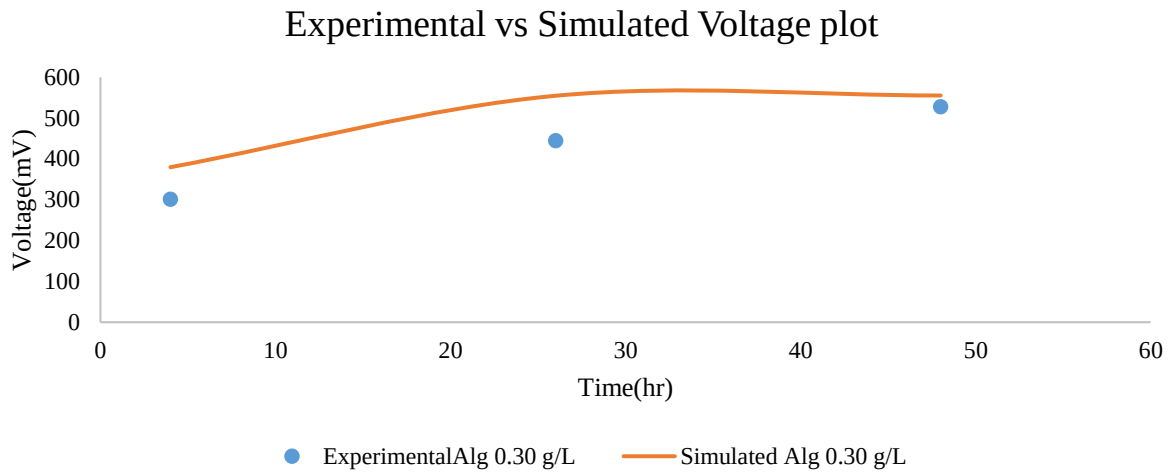


Figure 50: Simulated and experimental values of full cell Voltage (mV) vs. time (h) for 3 g/l glucose and 0.3 g/l algae concentration.

Table 64: Simulated vs. Experimental Power Density (mW/m^2) data for 1 g/l glucose concentration.

Time (hr)	Experimental			Simulated		
	Alg 0.15 g/l	Alg 0.30 g/l	Alg 0.45 g/l	Alg 0.15 g/l	Alg 0.30 g/l	Alg 0.45g/l
4	7.65	12.22	14.82	33.64	33.63	33.63
26	9.114	15.5925	22.425	54.12	53.97	53.80
48	20.475	20.3175	47.725	55.83	55.45	54.99

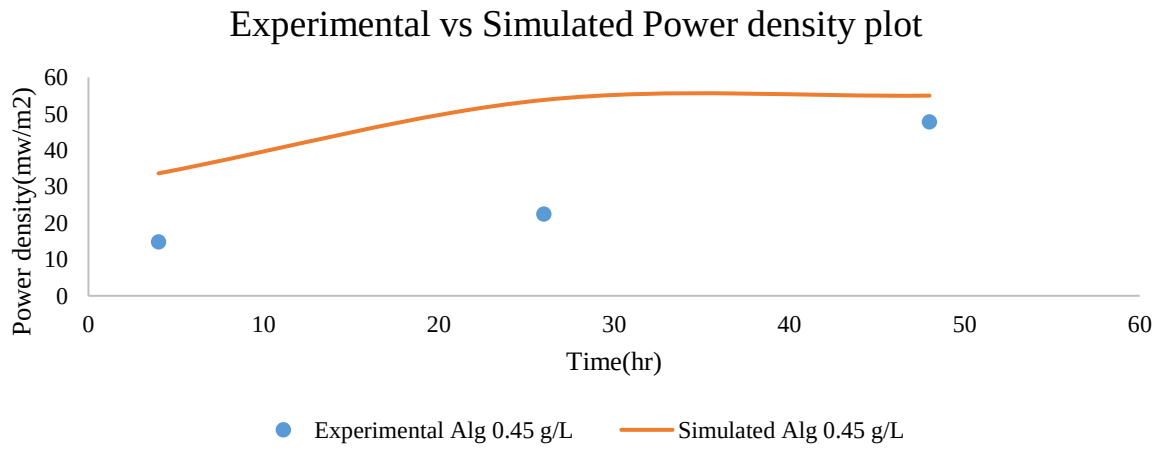


Figure 51: Simulated and experimental values of Power Density (mW/m^2) vs. time (h) for 1 g/l glucose and 0.45 g/l algae concentration.

Table 65: Simulated vs. Experimental Power Density(mW/m^2) data for 2 g/l glucose concentration.

Time (hr)	Experimental			Simulated		
	Alg 0.15 g/l	Alg 0.30 g/l	Alg 0.45 g/l	Alg 0.15 g/l	Alg 0.30 g/l	Alg 0.45 g/l
4	9.94375	13.65	33.6875	35.15	35.14	35.13
26	20.64	26.24375	51.45	67.72	67.48	67.21
48	21.56	48.585	61.32	68.64	68.05	67.28

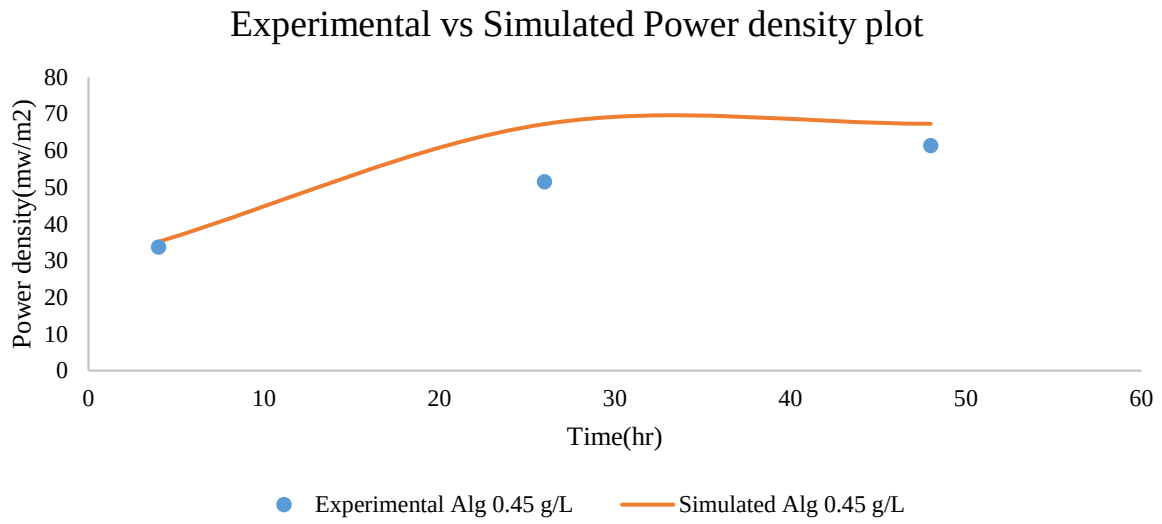


Figure 52: Simulated and experimental values of Power Density (mW/m^2) vs. time (h) for 2 g/l glucose and 0.45 g/l algae concentration.

Table 66: Simulated vs. Experimental Power Density (mW/m^2) data for 3 g/l glucose concentration.

Time (hr)	Experimental			Simulated		
	Alg 0.15 g/l	Alg 0.30 g/l	Alg 0.45 g/l	Alg 0.15 g/l	Alg0.30 g/l	Alg 0.45 g/l
4	16.68	21.8225	25.265	35.64	35.64	35.62
26	54	61.1875	62.235	76.80	76.50	76.10
48	55.64	67.32	74.82	77.37	76.79	75.60

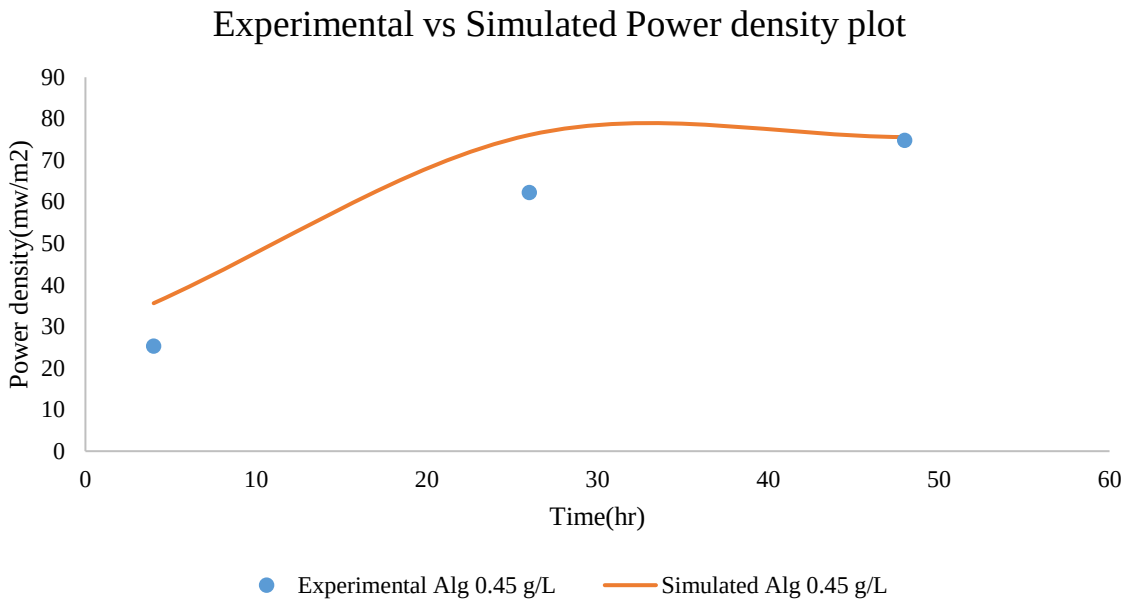


Figure 53: Simulated and experimental values of Power Density (mW/m^2) vs. time (h) for 3 g/l glucose and 0.45 g/l algae concentration.

The simulated power density chart are tabulated using lactic acid and glucose as dual substrate in anode chamber. From the result it is seen that the model is predicting the most power productive system containing algal concentration 0.45g/L at 48 h is similar to experimental system.

6.7. Cyclic voltammetry analysis of Bacterial algal fuel cell (BAFC):

Bacterio-Algal fuel cells were prepared by using the methodology mention in section 5.2.6. the BAFC was then taken for cyclic voltammetry (CV) analysis. According to specifications, the CV analysis was conducted to assess the peak voltage and peak current from oxidation at the anode and reduction at the cathode. In the anode chamber, oxidation of the substrates occurs, while in the cathode chamber, reduction of the oxygen takes place.

Table 67: Types of Electrodes for cyclic voltammetry (CV) analysis.

Electrode type	Name of electrode used	Placement in MFC
Working electrode	Carbon cloth	Anode
Counter electrode	Carbon cloth	Cathode
Reference electrode	Saturated calomel electrode (SCE)	Cathode

6.7.1.CV analysis of BAFC using Lactic Acid as substrate:

A Bacterio-Algal fuel cell was prepared by using Lactic acid as substrate which was taken for cyclic voltammetry (CV) analysis.

Table 68: The value of various parameters set to analyze the CV curve.

Parameters	**Value**
Sweep rate	25.000 mV/s
Minimum voltage	-2.000 V
Maximum Voltage	2.000 V
Start Voltage	-2.000 V
End voltage	-2.000 V
Cycles	1
Current limit	100 mA
Wait time	2.00 sec

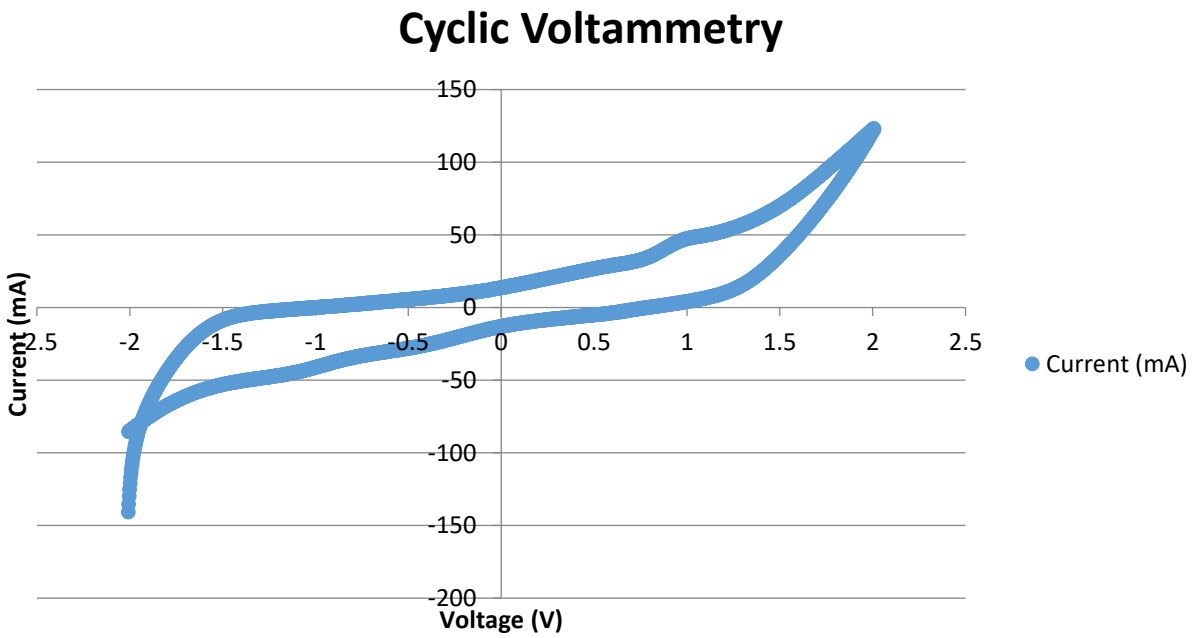


Figure 54: Plot of Current and corresponding voltage obtained from the cyclic voltammetry analysis.

The above curve of CV analysis shows one oxidation and one reduction peak. This analysis is evident that the oxidation occurs of substrate (Lactic acid) in anode chamber and the reduction of oxygen occurs in cathode. The peak current and voltage from the analysis are shown below.

Table 69: Data of peak voltage and current as obtained from the CV analysis.

Substrate	Voltage (V)	Current (mA)
Lactic acid	0.98	46.9
Oxygen	-1.08	-43.8

6.7.2.CV analysis of BAFC using Glucose as substrate:

A Bacterio-Algal fuel cell was prepared by using Glucose as substrate which was taken for cyclic voltammetry (CV) analysis.

Table 70: The value of various parameters set to analyze the CV curve.

Parameters	Value
Sweep rate	10.000 mV/s
Minimum voltage	-1.000 V
Maximum Voltage	1.000 V
Start Voltage	-1.000 V
End voltage	-1.000 V
Cycles	1
Current limit	10 mA
Wait time	2.00 sec

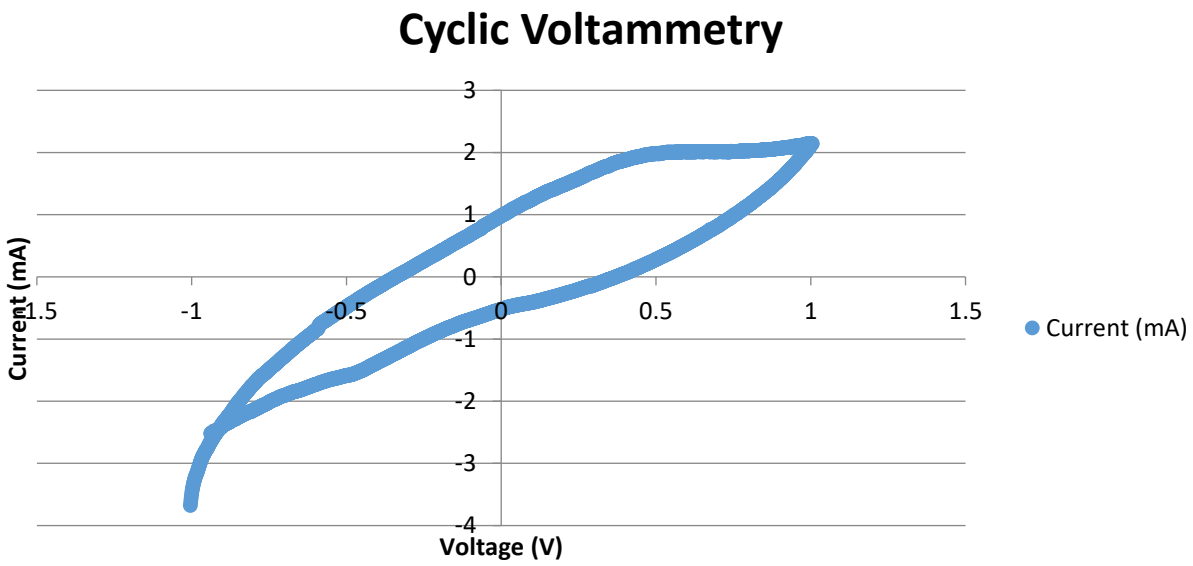


Figure 55: Plot of Current and corresponding voltage obtained from the cyclic voltammetry analysis.

The above curve of CV analysis shows one oxidation and one reduction peak. This analysis is evident that the oxidation occurs of substrate (Glucose) in anode chamber and the reduction of

oxygen occurs in cathode. The peak current and voltage from the analysis are shown below.

Table 71: Data of peak voltage and current as obtained from the CV analysis.

Substrate	Voltage (V)	Current (mA)
Glucose	0.465	1.958
Oxygen	-0.478	-1.534

6.7.3.CV analysis of BAFC using Dual substrate(Glucose & Lactic Acid):

A Bacterio-Algal fuel cell was prepared by using Glucose & Lactic acid as dual substrate which was taken for cyclic voltammetry (CV) analysis.

Table 72: The value of various parameters set to analyze the CV curve.

Parameters	Value
Sweep rate	10.000 mV/s
Minimum voltage	-0.800 V
Maximum Voltage	1.000 V
Start Voltage	-0.800 V
End voltage	-0.800 V
Cycles	1
Current limit	100 uA
Wait time	2.00 sec

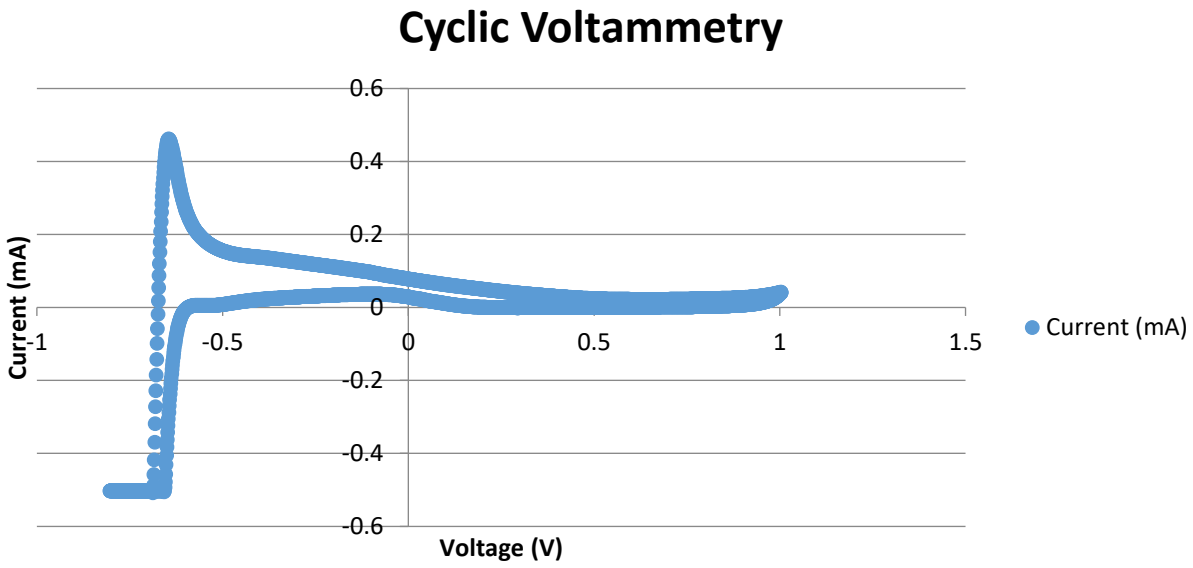


Figure 56: Plot of Current and corresponding voltage obtained from the cyclic voltammetry analysis.

The above curve of CV analysis shows one oxidation and one reduction peak. This analysis is evident that in the anode chamber of BAFC, lactic acid and glucose are oxidized, and oxygen

undergoes reduction. The peak current and voltage from the analysis are shown below.

Table 73: Data of peak voltage and current as obtained from the CV analysis.

Substrate	**Voltage (V)**	**Current (mA)**
Glucose	0.9934	0.0303
Lactic acid	0.4244	0.0289
Oxygen	-0.6542	-0.5018

Chapter 7
CONCLUSION and FUTURE SCOPE OF RESEARCH

- The growth kinetics of *Shewanella putrefaciens* using lactic acid as a substrate followed Monod kinetics up to a substrate concentration of 2500 ppm (3.022 g/L). Beyond this concentration, a steady decline in growth was observed. The specific rate of inhibition was then calculated using the Haldane model of growth inhibition.
- Monod type growth kinetics is followed when *Shewanella Putrefaciens* was grown on Glucose.
- The model equation determined through RSM agrees with the data (R^2= 0.9925). Glucose concentration, and time have been identified as the most significant parameters.
- Presence of Algae in cathode chamber does not significantly influence the energy outcome.
- The simulated power density data obtained using the model agrees well with the experimental ones particularly at 48h.

Future Scope of Research

1. Development of BAFC using biofilm for the same substrates and subsequent development of Mathematical model.
2. Development and designing of a control system which will be able to stabilize the output.
3. Energy and Environmental Analysis of the optimally operating Lab scale BAFC.
4. Development of BAFC set up for degradation of other substrates.

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