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Microbial Fuel Cell: Optimizing Graphene-Sponge Anode Thickness and Chamber pH Using Taguchi Experimental Method

Emilio Steven C. Navarro*†^(D) and Melissa May M. Boado**^(D)

*Engineering Program, School of Advanced Studies, Saint Louis University, Lower Bonifacio St., Baguio City, 2600, Philippines

**Department of Chemical and Mining Engineering, School of Engineering and Architecture, Saint Louis University, Lower Bonifacio St., Baguio City, 2600, Philippines

[†]Corresponding author: Emilio Steven C. Navarro; ecnavarro1@alum.up.edu.ph

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INTRODUCTION

ABSTRACT

The rapid consumption of fossil fuels has led to calls to switch from non-renewable to renewable energy sources. Microbial fuel cells are a promising technology that simultaneously treats wastewater and produces power. This study used the Taguchi Experimental method to optimize anode thickness and pH to obtain the maximum power density of an air-cathode microbial fuel cell (ACMFC). The graphene-sponge (G-S) anode thickness and chamber pH were selected as operating parameters, with their corresponding levels. The L₉ orthogonal array was chosen for the experimental design. According to the Taguchi Method, the optimum G-S anode thickness and chamber pH were determined to be 1.0 cm and 8.0, respectively. A confirmatory run was performed under these optimum conditions, and the maximum power density observed was 707.75 mW·m⁻³. Analysis of variance (ANOVA) was conducted to identify the percentage contributions of the operating parameters to the process, which were found to be 30.66% for pH and 69.34% for anode thickness.

air-cathode microbial fuel cell (ACMFC) follows a fourelectron pathway (Bajracharya et al. 2016), expressed as:

 $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ $E^{\circ\prime} = 0.82V$ vs. SHE at pH 7

...(1)

The reduction of overpotential boosts the performance of MFCs. One way to achieve a lower overpotential is through an optimized selection of materials and configurations, which can provide more microbial enrichment and efficient electron transfer sites (Huang et al. 2021, Ji et al. 2020). Carbonbased electrodes, non-toxic by nature, offer significantly higher specific surface area and adsorption characteristics, which are important for biocompatibility and biocatalyst immobilization (Chen et al. 2020, Herkendell 2021, Logan et al. 2019, Taskan & Hasar, 2015). Several modern carbonbased electrodes, such as 3D-printed porous carbon anodes (Bian et al. 2018) and electrospun carbon fibers (Sanchez et al. 2021), have been proven to replace traditional graphite rods in wastewater-based electricity generation. The use of nanomaterials can significantly improve the electrochemical performance of MFCs by enhancing biofilm adhesion and facilitating fast direct electron transfer (Liu et al. 2020, Wei et al. 2011). Due to their promising capabilities, MFCs with nanomaterial-based electrodes have become popular

The rapid depletion of fossil fuel resources and elevated greenhouse gas levels are intensifying the call to switch to less environmentally damaging alternative fuels with the same efficiencies as conventional fuels (Chandrasekar et al. 2022). Microbial fuel cells (MFCs) provide a clean energy alternative due to their capability to treat organic and inorganic waste while simultaneously generating power (Tan et al. 2021, Kumar et al. 2015, Dewan et al. 2008). MFCs directly transform chemical energy into electrical energy by incorporating microbial and electrochemical cycles to oxidize organic matter into simpler fragments that are precursors to form water (Prathiba et al. 2022, Zhang et al. 2016).

The different mechanisms occurring within a singlechamber MFC are depicted in Fig. 1. In the anode section, respiratory bacteria attach to the electrode surface, feed on, and oxidize the organic compounds to produce electrons and protons (H^+). The electrons pass through an external circuit, and the protons travel through a proton exchange membrane (PEM) towards the cathode section, where oxygen reduction reaction (ORR) occurs, subsequently producing water (Yaqoob et al. 2020). The typical ORR in an



Fig. 1: Graphical illustration of the overall mechanism of a typical ACMFC.

in bioelectrochemical research. Among the pioneer studies, Nimje et al. (2011) have found that carbon materials with layered architecture and small-fiber diameter promote biofilm growth with high cell density. Mink & Hussain (2013) use multi-walled carbon nanotubes (MWCNTs) as an MFC, comparing gold and nickel as anode materials. Results show that MWCNTs produced current and power density at 880 mA·m⁻² and 19 mW·m⁻², respectively, outperforming gold and nickel anodes by 6 to 20 times. To increase the extracellular electron transfer efficiency, Zhao et al. (2019) used the chemical vapor deposition method to grow carbon nanotubes on carbon fibers and employed pyrrole via in situ polymerization on the exterior and interior of the carbon nanotubes. The study obtained a maximum power density of 1876.62 mW·m⁻², which is 2.63 times higher than a traditional carbon brush anode.

The modification mentioned above is limited to a small scale due to high production costs. One of the most promising techniques to address economic constraints to date is using macroporous sponges coated with carbon-based nanomaterials. This technique offers an easy-to-synthesize method, a continuous three-dimensional carbon nanotube surface, controllable and tunable pore size, and excellent mechanical properties. The same approach has been employed in a study by Xie et al. (2012), which holds the highest maximum volumetric power density (182 W·m⁻³). The authors found that voltage drop and power losses are

insignificant in a graphene-sponge-stainless steel (G-S-SS) anode up to 1 m conduction length, with 0.015 m graphene-sponge (G-S) thickness and 0.01 m stainless steel (SS) thickness.

Anode material and its configuration are key factors in reducing losses such as activation, concentration, and ohmic losses. To further enhance the electrode performance, determining the optimum chamber pH in ACMFC operation is essential in minimizing power losses by providing a favorable environment for microbial communities and electron transfer sites. Appropriate pH condition favors the proliferation of exoenergens on the anode surface, reducing the activation losses. Maintaining pH in the electrode environment is necessary for proton transfer from the anode to the cathode via a membrane to reduce concentration losses. With a constant pH in the chamber, hydrogen ions (H^{+}) being produced from anoxygenic conditions can freely travel from the anode to the cathode, preventing the accumulation of protons (Kim et al. 2007, Kumar & Mungray 2017). Puig et al. (2010) reported that continuous MFC operation at pH level 9.5 for 30 days improved the power generation up to 1.8 W \cdot m⁻³. The same study has also concluded a 70% removal rate of organic matter.

Meanwhile, Martin et al. (2010) demonstrated promising ACMFC performance with a maximum power density of 9.8 $W \cdot m^{-3}$ at an anolyte pH of 6.5 and organic matter reduction efficiency at 91.2% COD removal rate. In an optimization



study for a membrane-less MFC, Azmi et al. (2021) found that pH 8.0 is the most suitable pH condition for *Bacillus subtilis* to proliferate alongside a voltage generation of 807 mV, biomass concentration of 15.45 mg.L⁻¹, and power density of 373.3 mW·m⁻². To date, mixed culture microorganisms in an MFC can produce a decent amount of power at pH 10.0 (He et al. 2008).

Most studies on MFC focus on individual components of a microbial fuel cell which mostly are processes associated with it. Only a few studies involve a simultaneous variation of two or more components (Carmona-Martínez et al. 2021, Kumar & Mungray 2017). With this, the researcher has sought to conduct a study involving engineering design (Graphene-sponge Anode Thickness) and operational conditions (chamber pH). A simulation study by Xie et al. (2012) shows no significant voltage drop in the G-S anode having a thickness of up to 0.015 m. However, Xie et al. (2012) did not compare the performance of G-S experimentally at different thickness levels to confirm the extent of the effect of G-S thickness on the overall performance of the microbial fuel cell.

Varying operational pH could also alter the performance of anode materials. Several studies have observed favorable electricity generation and wastewater treatment performances at pH ranging from 6.5 to 9.5. However, no studies combining G-S anode thickness and pH of anolyte show results without significant voltage drop. This predicament of researchers may be due to studies that involve multidisciplinary approaches requiring too many experiments to develop reliable results. Hence, Genichi Taguchi developed the Taguchi Experimental Method to address experimental engineering problems by addressing factor biases and proposing less time-consuming and costly experimental runs compared to a normal full factorial design of the experiment. Besides its economic benefits, the Taguchi method has been widely used in applied industries for its uniform, decentralized, orderly, and contrasted orthogonal experiments (Cheng et al. 2010).

This study aims to optimize the system's design (anode thickness) and operating condition (pH) to get a suitable response. This response indicates the ACMFC chamber's performance in terms of power density and wastewater treatment efficiency expressed as percentage removal of the chemical oxygen demand (COD). By varying the G-S anode thickness (0.5, 1.0, 1.5 in cm) and the pH (6.5, 8.0, 9.5) of the chamber simultaneously, power density and COD removal will be determined in every parameter combination. As per Taguchi Method, the L₉ orthogonal array is suitable when two (2) factors in three (3) levels are to be analyzed. The relation between factors and response from the signal-to-noise (S/N) ratio is determined, and accordingly, optimum

parameters are deduced from the data. The results are also analyzed to find out which of the parameters greatly affects the response. Finally, a confirmatory run is performed on the experimental MFC set-up to check the optimized condition.

Several studies focusing on cell configuration (Estrada-Arriaga et al. 2017), electrode materials and design (Dong et al. 2012, Logan et al. 2007), enhanced operational conditions (Erable et al. 2009, He et al. 2008, Patil et al. 2011), microbial exoelectrogenic communities (Butler et al. 2010, Islam et al. 2017), etc., have been conducted to improve power density generated in MFCs. This technology can potentially treat wastewater on a large scale while generating power. However, its viability as an independent external power source is yet to be achieved (Jadhav et al. 2021a, 2021b). It is essential to conduct thorough research on the individual and synergism of different factors to gain a deeper understanding of scaling up the technology and achieving optimum power production and wastewater treatment capability at a minimal cost (D'Angelo et al. 2017, Wang et al. 2018).

The main framework of the study stems from treating wastewater while producing energy. The study commenced with a preliminary experiment to confirm and/or determine the different levels of independent variables: G-S anode thickness and chamber pH. In contrast, the dependent variables, namely power generation and percentage COD removal rate, were recorded simultaneously using a DC voltage data logger (Extech SD910) and a colorimeter (HACH). The data generated from the experiment were processed using experimental design software, which is expected to identify the optimum combination of anode thickness and chamber pH to maximize power production and COD reduction.

MATERIALS AND METHODS

Materials and Data Gathering Tools

Graphene-sponge with stainless steel current collector (Anode): A synthetic polyurethane (PU) sponge purchased from a local grocery store was used as the main material for the fabrication of the graphene-sponge anode electrode. The PU sponge was cut into three smaller sponges, each with the same width and length (3 cm by 5 cm) but with different thicknesses (0.5 cm, 1 cm, and 1.5 cm). These sponges were then cut in half to form a graphene sponge with stainless steel as a current collector placed between the halved sponges.

Graphene sponges were fabricated by preparing the sponge, graphene oxide (GO) solution, and L-Ascorbic Acid (LLA) reducing agent. From the concentrated solution, 10 mL of 10% GO dispersion (rGO 1% dispersion, William Blythe Ltd.) was dispensed using a pipette and diluted to

1 mg·mL⁻¹ by adding distilled water to form the soaking solution. The diluted solution was mixed thoroughly using a stirring rod. Afterward, 100 mg of LLA-reducing agent was added and dissolved in the 100 mL diluted GO solution for 15 min to ensure a homogeneous mixture. Subsequently, the precut PU sponges were submerged in the graphene oxideascorbic acid solution. Fig. 2 illustrates the step-by-step fabrication process of the graphene-sponge anode using the hydrothermal reaction method.

The sponges were soaked for 15 min and simultaneously pressed about five times to ensure that all the sponge strands were wetted with the solution. The sponges submerged in the GO solution were ultrasonicated for 15 min to further ensure the graphene oxide adhered to the scaffold surfaces of the sponge. Afterward, the sponges soaked in the GO solution were transferred to a sealed media bottle for a hydrothermal reaction at 95°C for 3 h. The hydrothermal reaction initiates and completes the conversion of graphene oxide to form a reduced graphene oxide-sponge (rGO) composite (Hu et al. 2010). The graphene-coated sponges were washed with an ethanol-water solution and then dried at 40°C for 24 h. The composite's durability was assessed using adhesive tape and water flashing to check whether the graphene had sufficiently adhered to the sponge. Stainless steel (SS) wire mesh, serving as a current collector, was placed and assembled between two graphene sponges (G-S) using carbon conductive glue (Ted Pella Inc.). Fig. 3 shows both the schematic of the G-S anode composite and how the stainless steel mesh aids the transfer of electrons from the G-S sponge to the other side of the MFC.

Air-cathode electrode: The air-cathode electrode consists of a conductive gas diffusion layer (CGDL) and a catalyst layer (CL). The CGDL comprises 60% carbon black and 40% polytetrafluoroethylene (PTFE), while the CL comprises 60% activated carbon (AC) and 40% PTFE. In addition, an updated fabrication technique by Dong et al. (2012) was also adopted for the catalyst layer by not subjecting it to heat treatment under the furnace to further improve the performance of the air-cathode electrode.



Note. (a) soaking and pressing of sponge in the GO solution, (b) sonication of sponge in the GO solution for 15 min, (c) sealed media bottle with the sponge soaked in GO solution, (d) hydrothermal reaction in an oven at 95°C for 3 h, (e) and (f) post-hydrothermal reaction: reduced graphene oxide clumped on the surface of the sponge, (g) dried graphene-sponge

Fig. 2: Step-by-step fabrication of graphene-sponge anode via hydrothermal reaction.





Note. Fig. 3 shows a G-S with a stainless steel (SS) collector (G-S-SS). (a) Schematic of the G-S-SS composite electrode (right) vs. the G-S composite electrode (left). (b) Schematic showing the electron pathways in the G-S electrode with (right) and without (left) SS current collector (Xie et al. 2012).

Fig. 3: Schematic of anode composite and electron transfer.



Note. Fig. 4 shows the (left) graphical representation of the experimental setup and (right) actual experimental setup in the study.

Fig. 4: Experimental set-up for optimization of graphene-sponge thickness and chamber pH of ACMFC.

...(2)

Experimental Set-Up: Experimental runs with duplicates were performed simultaneously within 24 h. Only duplicate reactors were used in the study, as noted by Logan (2012), as having two trials for a single run is sufficient as long as the results are in good agreement. The second trial was performed 24 h after the first trial. Based on the data, the generated currents are relatively similar to each other.

The prefabricated ACMFC reactor with a total working volume of 250 mL was purchased from Laborxing (China). G-S as the anode, is used in the ACMFC reactor. The external electric voltage was monitored and recorded every 10 min for 24 h using a DC voltage data logger (Extech SD910). Chemical oxygen demand (COD) in influents and effluents was measured using a HACH COD digestion reactor and HACH Colorimeter. COD Digestion Vials were purchased from Dynalab. Percentage COD removal (%) is calculated as:

 $Percentage \ COD \ removal = [(COD_0 - COD_t) \ / \ COD_0 \] \times \ 100.$

Where COD_0 is the influent COD concentration

 $(mg L^{-1})$, COD_t is the effluent COD concentration $(mg L^{-1})$, and t is time (h)

A digital handheld pH meter (HM Digital PH200) was used to measure the pH of the wastewater during adjustment and variation of the initial substrate pH (Eaton & Franson 2006).

Voltage and current were measured using a DC voltage data logger (Extech SD910) and an analog multimeter, respectively. The current measurements were varied by changing the external resistance from 10,000 Ω to 23 Ω (Logan et al. 2007) at all pH conditions. Moreover, voltage and current were measured every 10 min to stabilize the cells from being interrupted due to the effect of the measuring devices. This was done to minimize errors and to approximate the actual values of measured values. Every 24 h, current density, and power density were calculated using Equations 3 and 4, respectively (Logan et al. 2006a).

$$Current \ Density = \frac{l}{V_{wastewater}} \qquad \dots (3)$$

1350

Where the current density is in milliamperes per volume of the wastewater in cubic meters (m^3)

Power Density = *Voltage* × *Current Density*

...(4)

where voltage is in millivolts, and power density is in milliwatts per cubic meter

Activities and procedures: The wastewater used in the entire experiment was collected from the Baguio Sewerage Treatment Plant (BSTP) (Fig. 5). This wastewater served as a source of microorganisms and substrate during the operation of the ACMFC reactor. It was assumed that the wastewater contains naturally occurring exoenergetic microorganisms. The three anodes with varying thicknesses were assembled in their respective reactors, and the desired pH of the electrolytes was set by gradually adding 0.5 M NaOH and 0.5 M HCl solutions, depending on the target pH. Anode inoculation may take up to two weeks. In this study, it took eight days to achieve stable voltage generation and another two days to stabilize. Hence, ten days were allotted to acclimate the ACMFC reactors in preparation for the actual experimental runs.

For every experimental run, the pH of the wastewater feed was adjusted by dropping appropriate amounts of acid and base and measured using a handheld pH meter (HM Digital PH200). Pretreated wastewater was transferred into the ACMFC reactor using a syringe. The preliminary experiment was repeated thrice for a period of 24 hours to determine the expected stabilization duration of the biofilm. The COD, open circuit voltage (OCV), and current were measured after the completion of a single experimental run.

Treatment and Analysis of Data

Taguchi method: The experimental parameters and their levels were determined from studies conducted by Kumar & Mungray (2017) and Xie et al. (2012). Preliminary experiments were done to confirm these value levels. Based on the rules of the Taguchi Method, the L_o orthogonal array (9 experimental runs) is acceptable as a basis for the experimental procedure for two parameters of three levels each. The performance of the ACMFC could be influenced by several factors, such as controllable (thickness of anode and pH of the chamber) and uncontrollable (noise), which can be evaluated through its performance in terms of power density and COD removal rate. In the engineering system, the S/N ratio is defined as the ratio of the expected output to the unexpected output or variabilities around the mean. The S/N equation is used to optimize the system where a higher S/N ratio is desired. The two outputs are both desirable at a higher value. Hence, they fit into the larger-the-better characteristics described by Equation 5.

$$\frac{s}{N}(\eta) = -10 \times \log_{10} \left[\frac{1}{r} \sum_{i=1}^{r} \frac{1}{y_i^2} \right] \qquad \dots (5)$$

where *r* denotes the number of tests in a trial (number of repetitions regardless of noise levels), y_i indicates the measurement results, and subscript *i* points out the number of simulation design factors arranged in the experiments of the orthogonal array (OA) table. The S/N response Table and response graph are constructed by the S/N ratio, which then enables the robust design effect of the factor to be applied.

The data generated from the experiment were processed using the Minitab experimental design software, which located the optimum combination of the thickness of the



Note. Images of the (left) Primary clarifier and (right) sampling station in BSTP Fig. 5: BSTP Facility located in Sanitary Camp, Baguio City, Benguet.





Note. This photo of Hitachi TM4000Plus was taken inside the Testing Laboratory at the Philippine Science High School Ilocos Region Campus.

Fig. 6: Table-top Scanning Electron Microscope.

anode and chamber pH with the optimum amount of power produced.

Analysis of Means (ANOM): ANOM is used to identify the optimal combination of the design factors. It is important to determine the effects of each factor after calculating the S/N ratios in keeping with experimental results. The effect of a factor level signifies the difference it causes to the overall mean. The effects of each factor can illustrate the influence rank of every factor on quality parameters and locate the optimum combination of the factor levels from the response table to the response graph.

Analysis of Variance (ANOVA): The design and process parameters, which greatly influence the performance of ACMFC in terms of power density and COD removal, were determined using ANOVA. Minitab software is used to analyze the data collected. ANOVA is used as an aid in determining the effect of parameters and their contributions to the performance of the ACMFC (Gandhi et al. 2012, Douglas Montogomery 2005).

Morphology and characteristics of the submicronstructured anode: To determine the submicron structure of the graphene-sponge anode, the morphology is investigated using a scanning electron microscope (Hitachi TM4000Plus) (Fig. 6), and results are compared to other literature for reference.

RESULTS AND DISCUSSION

General Observations

The wastewater sample from the BSTP had an initial pH of 6.8, which is slightly acidic. This observation conforms to the study conducted by Odjadjare & Okoh (2010), in which the pH in municipal wastewater ranges from 6 to 8.

The inoculation period lasted for eight (8) days, with an additional two (2) days allotted to ensure maximum and stable voltage generation (e.g., 650 mV for all reactors) was observed. Among the reactors, the chamber with a G-S anode thickness of 1.0 cm has the fastest rate (reached 600 mV on Day 6) of attaining its high and stable voltage. The other two set-ups, with 0.5cm and 1.5cm anode thicknesses, had slower rates (reached 600 mV on Day 9) compared to the earlier one and almost had the same behavior in the early days in terms of voltage generation.

From the results, polarization curves and power curves have been constructed and are shown in Fig. 7, 8, and 9. The Figures depict a typical fuel cell behavior where an increase in external resistance corresponds to a decrease in the current generation.

Fig. 7a, 8a, and 9a present the changes in voltage produced in the ACMFC. Across all combinations of parameters, the voltage drop was abrupt when external resistance was very minimal while stable at a higher resistance. Moreover, Fig. 7a, 8a, and 9a show a slightly sharper voltage drop in all pH conditions at very low resistances (initial stages of the graphs) that can be attributed to activation overpotential. After a sharp fall, a straight line with a lesser slope is noticeable in every graph, which is due to the combined effects of mass transport efficiency and ohmic losses, collectively known as internal resistances in ACMFC.

Table 1 presents the comparison of the performances (current and power densities) of ACMFC based on different G-S anode thicknesses and chamber pH. The internal resistances were calculated using Equation 6 (Logan et al. 2006b), as shown in the 5th column.

$$E_{cell} = OCV - IR_{int} \qquad \dots (6)$$

Where E_{cell} is the actual voltage measurement in



Note. Polarization curves generated at pH level 6.5 with respect to the change of current density depicting (a) the behavior of voltage output and (b) the behavior of power density output (right).



Fig. 7: Polarization curves at pH level 6.5.

Note. Polarization curves generated at pH level 8 with respect to the change of current density depicting (a) the behavior of voltage output and (b) the behavior of power density output.

Fig. 8. Polarization curves at pH level 8.0.



Note. Polarization curves generated at pH level 9.5 with respect to the change of current density depicting (a) the behavior of voltage output and (b) the behavior of power density output.

Fig. 9. Polarization curves at pH level 9.5.

Chamber pH	G-S Thickness [cm]	Current Density [mA·m ⁻³]	Power Density [mW·m ⁻³]	Internal Resistance [Ω]
6.5	0.5	2060	370.51	1096.77
6.5	1	2320	550.29	809.64
6.5	1.5	1860	407.52	1053.17
8	0.5	2280	495.11	906.49
8	1	2650	654.02	763.84
8	1.5	2250	510.73	914.84
9.5	0.5	2260	448.06	733.72
9.5	1	3000	692.22	644.58
9.5	1.5	2380	511.20	760.56

Table 1: Internal resistance in L₉ orthogonal experiment.

millivolts, OCV is in millivolts, I is the current flowing at a certain external resistance, and R_{int} is the internal resistance.

Effect of Chamber pH on Polarization Curves

Bacteria react with varying internal and external pH by adapting the activity and synthesis of proteins accompanying various processes such as proton translocation, amino acid degradation, adjusting to acidic or basic conditions, and virulence. Electrolyte pH has a significant contribution to power generation. Researchers have found that acidic electrolyte lowers power output (Hernández-Fernández et al. 2015, Jung & Pandit 2019) by inhibiting the growth of both methanogens and electrons on the anode, while slightly alkaline electrolyte promotes microbial metabolism and leads to a more negative anode potential, hence improving MFC performance (Kumar & Mungray 2017). Meanwhile, highly alkaline conditions in the cathodic portion could reduce O_2 potential (Munoz-Cupa et al. 2021).

In the experimental runs, it was noticed that all reactors almost had the same OCV values initially but more evident differences at higher pH values. There was a noticeable increase in voltage generation and a faster stabilization rate when pH levels were adjusted to 8.0. Meanwhile, the difference in maximum voltage was most evident when pH was adjusted to 9.5. Furthermore, increasing pH levels caused a decrease in internal resistances across all G-S anode thicknesses. Polarization curves showed that power density initially increases up to a certain resistance and then begins to decrease after the internal resistance value approaches the external resistance value (where maximum power density is approximated).

Chamber pH at 9.5 gave the highest current density and power density, mainly due to relatively low internal resistance, as shown in Table 1. Meanwhile, pH 8.0 exhibited the highest OCV at 707.5 mV, however, it produced a lesser power density and current density than that of pH 9.5, probably due to higher internal resistance. In addition, performances observed in pH 6.5 were consistently lower than the other two (2) pH levels, which only peaked at 667 mV, 2320 mA·m⁻³, and 550 mW·m⁻³ for OCV, current density, and power density, respectively. The lowest performance was observed at pH 6.5, manifested by having the highest internal resistances across all G-S anode thickness levels.

Several studies show similar results as the present study, in which differences in performances are attributed to the effect of pH on microbial activity and kinetics, and electrochemical resistances. Behera et al. (2010) conducted a MFC study on rice mill wastewater treatment using a proton exchange membrane and earthen pot. They varied the operational pH and found that maximum power densities generated from Earthen pot and PEM MFCs were observed in reactors that were fed with wastewater at pH 8.0. They attributed their findings to the fact that extracellular electron transfer in alkaline conditions is more effective, and electrogenic bacterial growth is favored in this pH microenvironment. The morphological characterization of the anode done by Margaria et al. (2017) presented a more diverse microbial morphology in alkaline MFCs than in neutral MFCs. Moreover, the microbial community attached to the electrodes from the alkaline MFCs was more compact and tightly connected. This may be attributed to exopolymeric substances that thrive when the pH increases.

Meanwhile, Ge et al. (2013) reported that at pH 5.48 \pm 0.43, the current generation significantly dropped. The acidophilic condition, a condition that is attributed to concurrent reactions from the acidification process and proton accumulation, was observed after a buffer-less experiment. This inversely affected the growth of electrochemically active bacteria. In a study conducted by Zhang et al. (2011) on the influence of initial pH on the performance of anodic microbes, an increase in turbidity during MFC operation at pH 4.0 and 5.0 was observed, which was noted as a result of detached anodic biofilms. Detached biofilms are an indication of weakened or even dead microorganisms. The negative charges on the exopolysaccharide, an important component in biofilm formation, were neutralized at high H⁺ concentrations due to acidification. The neutralization reaction destroyed the electrostatic actions, resulting in reduced biomass and biofilm thickness. Hence, maintaining an alkaline condition near the anode, which means having an elevated OH, is of great importance to counter the accumulation of H⁺ ions.

To understand the electrochemical properties of twochamber MFCs at varying pH conditions, Yuan et al. (2011) analyzed the anode impedances using electrochemical impedance spectroscopy at different pH levels (9.0, 7.0, and 5.0). Their measurements showed slightly different ohmic resistances (7.8 Ω , 12.9 Ω , and 11.4 Ω for pH levels 5.0, 7.0, and 9.0, respectively) across pH levels but significantly variated in charge transfer resistances (256.2 Ω , 82.1 Ω and 9.1 Ω for pH levels 5.0, 7.0, 9.0 respectively). Karthikeyan et al. (2009) and Park et al. (2009) analyzed the electrochemical kinetics of biofilm using the Tafel plots in terms of exchange current density (i_0) and anodic Tafel slope (ba). The better anodic reaction is facilitated when ba is minimized, and i_o is maximized. Results of their studies revealed pH 9.0 had the lowest *b*a (200 mV/decade) and highest i_0 (4.4 μ A m⁻²), which means anodic reactions are most favorable in such microenvironment pH conditions.

The results of this study also agreed with other works (Margaria et al. 2017, Zhang et al. 2011) that pH can be considered a limiting agent in a single-chamber MFC since acidification and alkalinization could severely affect both the electrodes of an ACMFC. Hence, proper identification of pH improves the performance of ACMFC. Some studies, however, claim that high alkaline (pH level beyond 8.0) condition slows down bacterial activity but can still favor an effective extracellular electron transfer (Cui et al. 2019). This phenomenon could explain why almost the same power output of pH levels 8.0 and 9.5 have been observed while having different percentages of COD removal (pH 8.0 has a higher percentage of COD removal than pH 9.5).

Effect of Graphene-Sponge Anode Thickness on **Polarization Curves**

During the 8-day biofilm inoculation period, all G-S anode thicknesses have almost the same maximum OCV generated. However, they differed mainly in the rate at which their maximum voltage was reached. The reactor with a G-S anode thickness of 1.0 cm was the first to reach its maximum voltage after the refill of wastewater. Meanwhile, reactors with anode thicknesses of 0.5 cm and 1.5 cm had almost the same performance as an anode thickness of 1.0. This observation may be attributed to the time it took for biofilms to form on the surface area of the G-S sponges. Lesser biofilm may be formed at 0.5 cm and slower to form at 1.5 cm.

The experimental runs with a pH level of 6.5 showed relatively identical OCV across all G-S anode thicknesses. Among the three thickness levels, G-S anode thickness of 1.0 cm consistently showed to have the highest OCV, current density, and power density at 707.5 mV, 3000 mA·m⁻³, and 692.22 mW·m⁻³, respectively, and the lowest internal resistances across all pH levels.

Generally, increasing anode thickness also increases power generation which is in agreement with the result of this study comparing the power generation between the G-S thicknesses 0.5 cm and 1.0 cm. This can be associated with graphene-sponge anodes providing high surface area favorable for microbial immobilization (e.g., E. coli) and high porosity for more efficient mass transport suitable for biocatalyst proliferation (Chou et al. 2014). The high porosity of graphene-based anode is due to the tendency of graphene to form layer-like structures, which are caused by $\pi - \pi$ stacking between graphene sheets (Zhao et al. 2021). Hence, the thicker the sponge anode, the thicker biofilms are formed and the microorganisms consume the more organic compounds.

In addition, the G-S electrode's inherent biocompatibility can facilitate the direct electron transfer rate and growth of electroactive bacteria. A study on the electrochemical behavior of graphene-induced carbon cloth anode by Liu et al. (2012) reported high peak current and lower peak separation. Furthermore, their study revealed that grapheneinduced carbon cloth has a stronger impact on direct electron transfer than the mediated electron process, which is indicative of graphene's excellent enhancing property on the interfacial electron transfer rate for electrochemical reactions.

On the contrary, Cai et al. (2020) noted that current and power density, to some extent, decreases with increasing electrode thickness due to the blockage of biofilm from microbial propagation to the pore-like structure. This report was also observed in the current study where the G-S anode with a thickness of 1.5 cm has lesser power generated than with a thickness of 1.0 cm. Chou et al. (2014) reported that microbial growth not only within the porous area blocks the sponge but also contributes to the weakening of electron transfer due to the inherent insulating property of biofouling caused by a build-up of waste from secretions and metabolism. In effect, biofilm may be inactivated. Chen et al. (2012) also had similar claims on anode thickness, in spite of reticulated carbon foam from pomelo peel (RCF-PP) being a promising anode material, MFCs having porous-based anodes suffered mass transfer limitations, which increase as the electrode thickness also increases.

Increasing electrode thickness is also a primary consideration that affects the transport of waste and nutrients within the porous anode. An insufficient nutrient delivery negatively affects the growth of electrogenic bacteria, which may have happened in this study with the G-S anode sponge of 1.5 cm thickness. This phenomenon may lead to dormancy. Dormancy (lag time) is generally induced when bacterial colonies are subjected to stresses such as starvation, osmotic pressure, and temperature fluctuation. In this state,



bacteria tend to consume fewer organic compounds. Hence, microorganisms under this condition may inadequately act as biocatalysts for electrochemical oxidation (Chou et al. 2014).

The current study revealed a noteworthy trend in the performance of MFC when varying the anode thickness. Specifically, it was observed that increasing the anode thickness from 0.5 cm to 1.0 cm resulted in a significant improvement in MFC performance. However, further increasing the thickness to 1.5 cm led to a decline in performance. These findings align with a simulation study conducted by Xie et al. (2012), which demonstrated a similar graphical representation in Fig. 10. In their study, they observed that, at a fixed SS current collector thickness, the voltage drop and power loss exhibited an exponential increase with greater G-S thickness when the conduction length was less than 1 m.

Based on the results, it can be inferred that increasing the anode thickness from 0.5 cm to 1.0 cm creates a favorable electrochemical environment for both voltage and power generation, with losses not being dominant in this particular configuration. However, surpassing the thickness of 1.5 cm may have resulted in excessive energy overpotentials, thereby decreasing the overall performance of the MFC compared to the earlier anode thicknesses.

The power density achieved by Xie et al. (2012) was considerably higher at 392 W.m⁻³, as compared to the power density generated in the current study. This significant difference may be attributed to the variations in other components of the MFC, such as the carbon cloth electrode coated with Pt catalyst as the cathode, in contrast

to the simpler construction of the Nafion- and Platinumfree carbon black-activated carbon air cathode used in this study.

Effect of pH and G-S Anode Thickness on Percentage COD Removal

Table 2 shows the COD removal efficiency based on the chamber pH and corresponding G-S anode thickness. From the table, it can be seen that the peak percentage of COD removal was achieved at pH 8.0. On the other hand, the percentage of COD removal did not significantly change across all G-S anode thicknesses used. Both pH levels 6.5 and 9.5 have noticeably lower COD removal efficiency than pH 8.0, this might be due to reduced bacterial activity at highly acidic and alkaline conditions. Lower COD consumption levels (35% in acidic MFCs and 10% in pH greater than 10) were also observed by Margaria et al. (2017) while determining the effects of pH variation on anodic marine consortia in a dual chamber MFC. Hence, COD consumption might not be directly associated with electrochemical performance. As observed by Nimje et al. (2011), COD degradation could be for the direct growth and sustenance of bacteria.

At high solution pH, the sorption rate is reduced due to electrostatic repulsion between deprotonated acid functional groups, which may lead to lower organic removal (N. Cai et al. 2015). Whereas, at neutral to slightly alkaline conditions, microbial metabolism and methanogenesis coexist, which have an additive impact on the degradation of organic compounds, hence, high COD removal (Martin et al. 2010).



Note: Fig. 10 shows the simulation results for G-S electrodes in large-scale applications for voltage drop and power loss in different G-S thicknesses. Graphenesponges as high-performance, low-cost anodes for microbial fuel cells Xie et al. (2012).

Fig. 10: Voltage drop and Power loss v.s. G-S thickness.

Graphene-based materials have a high surface area, making them a popular adsorbing material for various chemicals such as oil (Wang et al. 2019) and toxic organic pollutants (Baig et al. 2019). Based on Table 2, the change in percentage COD removal with respect to varying G-S sponge anode thickness is statistically insignificant, with a P-value (0.4001) greater than 0.05. It can be inferred that graphene provides a sufficient effective surface area in which even the lowest anode thickness removes the same amount of COD concentration from wastewater as the thicker ones by adsorption. The same results were observed by di Lorenzo et al. (2010) using packed beds of irregular graphite granules, which are known to have a high surface area, as anode material. Varying layers of granules (e.g., 0.3 cm and 1 cm) were observed. However, the study showed no significant difference in percentage COD removal between 0.3 cm layer and 1 cm layer (35% and 41%, respectively.).

In addition, the large surface area of the graphene material may have been inhabited by both non-isoenergetic bacteria, which can also contribute to COD removal. Katuri et al. (2011) had similar observations where COD removal did not correlate with coulombic efficiency, which is attributed to different microbial communities coinhabited in the anode surface area, dominated by non-electrogenic microorganisms.

In this study, it can be said that pH (with a *P*-value less than 0.05) is the percentage COD removal limiting factor. Whereas varying anode thickness did not significantly contribute to the wastewater treatment performance, as supported by ANOVA in Table 3.

Optimization Study

In MFC studies, the higher the power density produced, the better. Hence, larger-the-better performance characteristics

Table 2: Percentage COD Removal.

	Chamber pH		
Anode Thickness	6.5	8.0	9.5
0.5	35.99	76.43	54.81
1.0	58.68	72.93	59.85
1.5	44.82	74.73	62.26

Note. The table shows the different percentages of COD removal of the different combinations of parametric conditions.

Table 3: ANOVA on the effect of varying Chamber pH and anode thickness on Percentage COD Removal.

	Degree of Freedom	Sum of square	<i>F</i> -value	P-value*
pН	2	27.6213	8.9374	0.0334
Thickness	2	3.5906	1.1618	0.4001

 $p^* < 0.05$ indicates the parameter significantly contributes to the output

were used for calculating the S/N ratio using the Taguchi Experimental Method. Table 4 gives the average power density and internal resistance values of the first and second runs at the same operating conditions. In addition, Table 5 presents the response table of the S/N ratio. In this table, the total variation in the S/N ratio is calculated using Equation 7.

$$S_T = \left[\sum_{i=1}^n \left(\frac{s}{N}\right)_t^2\right] - \left[\frac{\sum_{i=1}^n \left(\frac{s}{N}\right)_i}{n}\right]^2 \qquad \dots (7)$$

where *n* is the total number of experiments, *t* is the total sum of the S/N ratio, and *i* is the S/N ratio at a particular pH and thickness condition

Fig. 11 is a graph to show the impact of the parameters on the performance characteristics using the data presented in Table 4. The optimum values of parameters for obtaining the maximum power density are selected corresponding to the highest S/N ratio value calculated using Equation 5 (Table 3). Meanwhile, the best conditions can be observed in the graphical representation, e.g., Fig. 11, by locating the peak points in both graphs.

Based on the L_9 experimental analysis, the G-S anode thickness of 1.0 and pH 9.5 produced a maximum power density among all nine experiments. Nonetheless, the Taguchi method selects the conditions with the highest values of mean S/N ratios, which happened to be G-S anode

Table 4: Lo orthogonal array, power density, and S/N ratio for thickness and pH effects.

Exp. No.	Chamber pH	G-S Thickness	Power Density	S/N
1	6.5	0.5	370.51	51.38
2	6.5	1.0	550.29	54.81
3	6.5	1.5	407.52	52.20
4	8.0	0.5	495.11	53.89
5	8.0	1.0	654.02	56.31
6	8.0	1.5	510.73	54.16
7	9.5	0.5	448.06	53.03
8	9.5	1.0	692.22	56.80
9	9.5	1.5	511.20	54.17

Note. The table was generated using the Minitab software.

Table 5: Response table for S/N ratio according to the larger-the-better condition of Taguchi Experimental Method.

Level	Chamber pH, A	G-S Thickness, B		
1	52.80	52.77		
2	54.79	55.98		
3	54.67	53.51		
Delta	1.99	3.21		

Note. The table was generated using the Minitab software.





Note. The highest peaks of the graphs correspond to the optimum value of both (a) chamber pH and (b) anode thickness

Fig. 11: Optimization of parameters using the main effects plot for S/N ratios.

thickness 1.0 and pH 8.0 as the optimized condition. These optimum values are illustrated on the peak points both in pH and thickness in Fig. 11.

Analysis of Variance

The *F*-value for both anode thickness and chamber pH is defined as the ratio of the mean of the square error. The larger the *F*-value of a parameter, the greater its impact in producing maximum power density. The optimal combination of anode thickness and chamber pH can be approximated using performance characteristics and ANOVA. The results of the ANOVA for the experiments are presented in Table 6.

The degree of freedom for the factors is calculated by the number of levels (k) minus 1. Meanwhile, the *P*-value is calculated to either accept or reject the null hypothesis at 95% confidence. If the P-value is less than 0.05, then the null hypothesis can be rejected, and it can be concluded that the G-S thickness and chamber pH bear a significant impact on the process. As presented in Table 6, both the parameters have *P*-values less than 0.05, however, have different percentage contributions to the maximum power density of the experiment. G-S anode thickness has a larger percentage contribution, which means that it has a larger impact on the production of maximum power density. Meanwhile, chamber pH although it bears a smaller impact, still contributes a significant effect on the response.

Confirmatory Experiment

Based on the optimized conditions, i.e., G-S thickness of 1.0 and chamber pH of 8, the researcher conducted a confirmatory experiment with two (2) trials. The reactors were fed with wastewater with an adjusted pH of 8 and left to stabilize for three (3) days for better acclimatization. Optimal pH was maintained for the same number of days. The mean values of the two trials were plotted in Fig. 12, which gave similar results from the previous experiment with a high-power density of up

Table 6: ANOVA for the maximum power density value of the experiment.

	Degree of Freedom	Sum of square	Average of squares	F-value	P-value*	Percentage contribution
pН	2	7.4868	3.7434	29.10	0.004	30.66
Thickness	2	16.9340	8.4670	65.82	0.001	69.34
Residual Error	4	0.5145	0.1286			
Total	8	24.9353		94.92		

*p < 0.05 indicates the parameter significantly contributes to the output

^a not provided by Minitab, calculated separately.



Note. Polarization curves generated from the confirmatory experiment at optimized conditions resulting from the Taguchi Experimental Method. Fig. 12: Polarization Curves for the optimal levels of G-S anode thickness and chamber pH.



Note. SEM images at x30 and x 500 magnification of (a, b) plain polyurethane sponge, (c, d) G-S anode without biofilm, and (e, f) G-S with biofilm after experimental runs.

Fig. 13: Sub-microstructure images of different sponges generated under a SEM.



Note. Comparison of pore size area (in mm²) of (a) biofilm-less GS and (b) biofilm-rich GS

Fig. 14: Estimation of pore size using ImageJ software.

to 707.75 mW·m⁻³. The slightly higher power generation is due to the acclimatization time, which promotes better biofilm performance and proper proton transfer from the anode to the cathode (Kumar & Mungray 2017).

Morphological Characterization using Scanning Electron Microscopy

The SEM images of different forms in different magnifications (x30 and x500) are shown in Fig. 13, which clearly shows the intensity of shades among different conditions. Fig. 14a and 14b are images of a plain polyurethane sponge. It can be seen that compared to the latter images, plain polyurethane is less distinct and lighter. Meanwhile, Fig. 14c and 14d are images of the G-S anode that are more distinct than the plain polyurethane sponge with the presence of graphene. It is also evident that graphene conductive coating conforms to the morphology of the plain sponge without blocking the open porous structure. This conformity and stability of graphene wrapping around the sponge fibers are attributed to the flexibility of its 2D structure and strong van der Waals forces (Xie et al. 2012). The strong intermolecular forces are manifested by the anode's excellent performance during the scotch tape test and water flushing, where the graphene was neither removed nor flushed away.

Fig. 13e and 13f illustrate the condition of the G-S anode after several experimental runs. In Fig. 13f, it is very clear that thick biofilms have attached to the surfaces of the anode, which can be due to the excellent biocompatibility of the fabrication process and the synthesized material itself.

Furthermore, the ImageJ software was used to analyze the size of the pores. Fig. 14 illustrates a comparison of the average pore size, measured in terms of area (in mm²), between the biofilm-rich and biofilm-less GS. The results indicate that the mean pore size in biofilm-rich GS is slightly smaller (131.319 mm²) than in biofilm-less GS (140.479 mm²). Although there is a difference in the average pore size, the presence of biofilm did not significantly obstruct microbial growth between the scaffolds of the GS. However, the study did not provide a detailed characterization of the graphene structure, which could have helped to further investigate the growth of biofilm within the graphene structures.

CONCLUSIONS

Improving the engineering design and operating conditions of emerging technologies is crucial. The Taguchi method offers a cost-effective experimental design and reliable results through the L_9 orthogonal array. It is suitable for optimizing G-S anode thickness and chamber pH to maximize power density. ANOM and ANOVA were employed to evaluate the parameters, assess their impact on the output response, and determine their significance. The summarized results are as follows:

- 1. The optimal parameter values for G-S anode thickness and chamber pH are 1.0 cm and 8.0, respectively. A verification experiment using these values yielded a power density of 707.75 mW·m⁻³.
- 2. Both G-S anode thickness and chamber pH significantly contribute to the power generation of ACMFCs, as indicated by ANOVA. ANOM analysis further confirmed that anode thickness has a greater impact on the output.
- 3. Chamber pH plays a significant role in wastewater treatment capability, whereas varying anode thickness did not lead to significant differences in COD removal.

Anode thickness plays a vital role in power generation. Increasing the thickness provides a larger surface area for biofilm formation, resulting in higher current density. However, thicker biofilms can lead to pore blockage due to waste accumulation from microbial activity, affecting mass transport efficiency and electron transfer.

Chamber pH influences the internal resistance of the system. Slightly alkaline pH reduces internal resistance, thereby enhancing MFC performance in terms of power generation. This is attributed to the promotion of exopolymeric substances, which support extracellular electron transport. Conversely, pH acts as a limiting factor for COD removal since it affects bacterial growth kinetics. Lower pH slows down microbial activity, as evident in ACMFC chambers with a pH of 6.5, leading to lower current density output. It's important to note that good electrochemical performance does not necessarily translate to high COD removal, as the microbial degradation of compounds is influenced by pH, which depends on the specific microbial culture present in the wastewater.

Recommendations

To enhance the study, it would be beneficial to increase the level of parameter variation. By including other parameters, the findings of this study could apply to larger-scale MFC operations. Additionally, identifying the specific bacteria present could provide further insights into the optimal conditions for their growth and culture kinetics.

Future studies could explore other aspects of MFC operation as well. Investigating techniques such as continuous operation and wastewater recirculation using a G-S anode could help mitigate mass transfer limitations.

Furthermore, in the fabrication process of the G-S anode, a significant amount of excess reduced graphene oxide (rGO) is typically disposed of after the hydrothermal reaction. To minimize costs, it would be advisable to optimize the amount of graphene oxide (GO) solution used in relation to the volume of the sponge that needs to be coated. This optimization process could help reduce waste and improve cost-efficiency.

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ORCID DETAILS OF THE AUTHORS

Emilio Steven C. Navarro: https://orcid.org/0000-0003-1023-2617 Melissa May M. Boado: https://orcid.org/0000-0001-9596-5574

