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Enhanced Anode Performance in Yeast Microbial Fuel Cells via Optimized Calcination of Eggshell Using Response Surface Method

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Abstract

This study aims to explore the potential of calcined eggshells as an economical and effective anode material in microbial fuel cells (MFCs). This research examines the enhancement of calcined eggshells as an anode material in MFCs by operating condition optimization using the Response Surface Method (RSM). The experimental findings underscore the substantial influence of temperature and the eggshell/NaOH ratio on voltage and maximum power density (MPD). Raising the calcination temperature from 550°C to 700°C improves both voltage and MPD, with peak performance seen at 700°C. Nonetheless, performance stabilizes above 850°C. The eggshell/NaOH ratio is significant, with enhancements seen at an optimum ratio of 4. ANOVA analysis indicates that the model accounts for 79.89% of the variability in voltage and 82.74% in MPD, while the modified R-squared values imply possible overfitting. Optimal calcination parameters (704.55°C and a ratio of 2.52) improve the microstructural characteristics of calcined eggshells and crystallinity, which are essential for electron transport and bacterial adhesion. SEM study indicates a morphological transition to a rough, porous structure, whilst XRD and FTIR investigations validate the conversion from calcium carbonate to calcium hydroxide, enhancing electrochemical characteristics. This study highlights the promise of optimized calcined eggshells as economical and effective materials for microbial fuel cells, advancing sustainable energy and materials science.

1. Introduction

Microbial fuel cells (MFCs) are a potential technology in renewable energy and wastewater remediation [1]. These MFCs systems use the metabolic activities of microorganisms to transform organic matter into electrical energy, providing a sustainable approach to energy generation and environmental stewardship [2, 3]. The anode's performance is a crucial determinant of MFC efficiency, acting as the locus for microbial colonization and electron transfer [4]. Improving anode materials and designs may significantly enhance the total power output and stability of MFCs. Yeast-based MFCs have attracted interest among the many kinds of MFCs owing to their durability, simplicity of culture, and capacity to function under diverse environmental circumstances [5]. Yeast cells, characterized by their elevated metabolic rates and robustness, serve as dependable and effective biocatalysts, making yeast-based microbial fuel cells a feasible choice for scalable and practical applications in energy production and wastewater treatment [6].

The performance of anodes in MFCs is often impeded by many obstacles, namely insufficient conductivity and inadequate biocompatibility of the used materials [7, 8]. These difficulties may markedly diminish the efficiency of electron transfer and microbial colonization, both of which are essential for good MFC performance. Material alterations, including the integration of conductive polymers, carbon-based nanomaterials, and metal oxides, have been used to enhance the electrical conductivity of anodes [9, 10]. Moreover, surface treatments such as chemical functionalization and the application of biocompatible coatings have been explored to improve microbial adherence and activity [11, 12]. These methodologies seek to provide an anode environment conducive to vigorous microbial proliferation and effective electron transfer, hence enhancing the overall performance and stability of MFCs.

An avant-garde methodology in MFC technology incorporates eggshell as an anode material. Eggshells, abundant in calcium carbonate and exhibiting natural porosity, provide a viable option for improving anode performance [13]. The elevated calcium concentration enhances the structural integrity and conductivity of the anode, while the intrinsic porosity promotes microbial colonization and electron transmission. The calcination procedure, which is heating eggshells at high temperatures, augments these capabilities by transforming calcium carbonate into calcium oxide [14]. This alteration enhances the surface area and conductivity of the material, consequently augmenting its efficacy as an anode in MFCs. Researchers want to use the distinctive characteristics of calcined eggshells to create more efficient and sustainable microbial fuel cells for renewable energy generation and wastewater treatment.

The Response Surface Method (RSM) is a statistical approach used to optimize experimental settings by modeling and evaluating the interactions among numerous variables and their responses [15]. Within the framework of MFCs, RSM may be pivotal in optimizing the calcination parameters of eggshell anodes to get peak performance. By methodically altering variables such as temperature, duration, and environment throughout the calcination process, RSM facilitates the identification of optimal conditions for improving the anode's conductivity and biocompatibility of MFCs [16]. Enhancing these parameters is essential for optimizing electron transfer efficiency and microbial activity, which are vital to the overall performance of MFCs. Prior research has effectively used RSM to enhance many facets of MFC components, demonstrating its efficacy in augmenting material characteristics and operational efficiency [17]. By using RSM, researchers may create more efficient and dependable MFCs, hence advancing renewable energy and wastewater treatment technologies [18].

The existing body of research on MFCs has extensively explored various optimization techniques to enhance performance. Previous studies have focused on optimizing MFC performance for wastewater treatment and sustainable energy generation using RSM, as seen in the work on performance optimization of MFCs for wastewater treatment [19]. Additionally, research has delved into the optimization of double-chamber MFCs through RSM, highlighting the importance of experimental design in improving MFC efficiency [20]. Other studies have concentrated on optimizing cathode operational parameters for the removal of copper ions from wastewater [21]. Enhancing bioelectricity production in microalgae MFCs through yeast immobilization using RSM demonstrates the diverse approaches to improving MFC performance [22].

However, a notable gap exists in the optimization of anode materials, particularly using calcined eggshells in yeast MFCs. While previous research has addressed various aspects of MFC optimization, the specific focus on the calcination process of eggshells and its impact on anode performance remains underexplored. This research aims to fill this gap by investigating the effects of calcination temperature and eggshell/NaOH ratio on the electrochemical performance of yeast MFCs. By employing RSM, this study systematically examines the influence of these parameters on voltage and maximum power density (MPD), providing a comprehensive understanding of the optimal conditions for anode material enhancement.

The main aim of this work is to improve anode performance in yeast-based MFCs by improving the calcination process of eggshells via RSM. The theory posits that optimizing calcination parameters, including temperature and time, would enhance the conductivity and biocompatibility of the resultant anode material, hence improving MFC efficiency. Initial study indicates that calcined eggshells may significantly enhance electron transfer rates and microbiological activity. The improved anode material is anticipated to improve the total power output and stability of yeast microbial fuel cells, hence facilitating more sustainable and efficient energy generation.

The novelty of this research lies in its innovative approach to enhancing anode performance in yeast MFCs through the optimized calcination of eggshells. Unlike previous studies that have focused on various aspects of MFC optimization, this research uniquely targets the calcination process of eggshells, a waste material, to improve their electrochemical properties. By systematically investigating the effects of calcination temperature and eggshell/NaOH ratio using RSM, this study provides new insights into the optimal conditions for anode material enhancement.

A primary challenge faced by this study is the optimization of calcination settings to get optimal performance of the anode material. The procedure requires the meticulous adjustment of factors like temperature, time, and the eggshell/NaOH ratio, demanding exacting control and comprehensive testing. A notable problem is guaranteeing the repeatability and scalability of the improved calcination process, since laboratory conditions may not consistently transfer to larger-scale applications. Moreover, it is essential to balance the enhancement of the anode's electrochemical capabilities with the preservation of its biocompatibility to ensure optimal microbial activity.

This research not only highlights the immediate advantages of MFC technology but also has significant implications for renewable energy and environmental applications, presenting an innovative method for waste valorization and resource recovery. This study utilizes waste materials such as eggshells to promote the development of sustainable technology in accordance with circular economy concepts.

2. Experimental

2.1. Eggshell Calcination Process

The eggshell calcination process followed the method described by Ahmad *et al.* [23]. Initially, the eggshells were washed with deionized (DI) water and then sun-dried for 24 hours. Once dried, the eggshells were ground into a fine powder using either a blender or a mortar. After weighing the eggshells and NaOH in ratios of 1:4, 1:1, and 4:1, 100 mL of DI water was added to the mixture. This mixture was stirred with a magnetic stirrer for 1 hour until it became homogeneous. It was then left at room temperature for 24 hours to complete the activation process. Following this, the mixture was filtered and thoroughly washed with DI water. The filtered solids were then subjected to calcination in a furnace at temperatures of 550, 700, or 850°C for 3 hours.

2.2. Physicochemical Analysis

To determine the crystalline morphology of the modified carbon Felt (CF) anodes, X-ray diffraction was performed using a Rigaku SmartLab SE (Tokyo, Japan) operating at 40 kV, 30 mA, and 5–80° (2θ) with CuK radiation (λ = 1.5406 nm). FTIR measurements were conducted using a Compact FT-IR Spectrometer ALPHA II (Massachusetts, United States). The morphology of the modified CF anode with a calcined eggshell surface was examined using an FE-SEM FEI Quanta 650 (Oregon, United States). To enhance the sample's conductivity, a double-sided conductive adhesive tape was applied. SEM images were taken before and after the modification process for comparison.

2.3. Full Cell Characterization

This study utilized an acrylic single-chamber MFC reactor with a 28 mL capacity [24]. The anode consisted of modified CF with calcined eggshell, while the cathode was plain CF. Both electrodes had a projected surface area of 7 cm^2 and were spaced approximately 10 cm apart. A Nafion 117 membrane separated the anode and cathode compartments. The anode compartment was filled with 28 mL of anolyte, which included a biocatalyst and a YPD medium containing 14 mg/mL of yeast *Saccharomyces cerevisiae*, 14 mg/mL of d-glucose, 5 mg/mL of yeast extract, and 2.5 mg/mL of peptone [25]. Meanwhile, the oxygen reduction reaction occurred at the cathode, which was exposed to air. Stainless steel wire served as the current collector during the incubation phase, and the anode and cathode were connected to an external resistance of 1000 Ω.

Voltage measurements were taken every three days over a 30-day incubation period, totaling 10 cycles. During the first three cycles, the anolyte was replaced with yeast and YPD medium. Subsequently, the anolyte was replaced with d-glucose, assuming a mature biofilm had formed over the previous cycles. The MPD was calculated from the polarization and power curves by multiplying the voltage by the current density for various external resistance values. After the 10th cycle, a resistor box (Elenco RS500 Resistance Substitution Box, Illinois, USA) was connected between the anode and cathode of the yeast MFC, with resistance values set from 10 MΩ to 10 Ω for 30 minutes at each value. During this time, the data collection system recorded the output voltage every 10 minutes.

2.4. Design of Experiment

The optimization study aimed to examine the impact of two key parameters: calcination temperature and the eggshell/NaOH ratio, both of which influence the anode's microstructure and electricity generation. These factors were tested at three levels: calcination temperatures of 550, 700, and 850°C, and eggshell/NaOH wt. ratios of 0.25, 1, and 4. These were treated as independent variables, while Closed Circuit Voltage (CCV) and MPD served as response variables to assess the combined effects of the inputs. The study's primary goal was to explore the relationship between calcination temperature and eggshell/NaOH ratio in yeast-MFC anodes using the RSM. RSM is a highly efficient technique for identifying optimal conditions with minimal experimentation.

The experimental design was based on the Box-Behnken methodology, and Minitab 17 software (Pennsylvania, USA) was utilized to model empirical equations and generate response surface plots. Analysis of Variance (ANOVA) was employed to analyze the statistical parameters, with One-way ANOVA specifically used to evaluate the response of a dependent continuous variable (CCV or MPD) from a set of unrelated and independent variables (calcination temperature and eggshell/NaOH ratio) in unique experiments. Consequently, a 32 design with single replication resulted in a total of 9 unique runs. The complete set of experimental runs is detailed in Table 1.

Table 1. Complete set of experimental runs

Run	Temperature (°C)	Eggshell/NaOH wt. ratio
1	550	0.25
2	550	1
3	550	4
4	700	0.25
5	700	1
6	700	4
7	850	0.25
8	850	1
9	850	4

3. Results and Discussion

3.1. Voltage and Maximum Power Density Result

The data reveals interesting trends when examining the relationship between temperature and voltage, as well as the eggshell/NaOH ratio and voltage. As the temperature increases from 550°C to 700°C, there is a noticeable rise in voltage across all eggshell/NaOH ratios. This suggests that higher temperatures generally enhance the voltage output, with the most significant increase observed at 700°C. However, at 850°C, the voltage does not follow a consistent upward trend; instead, it peaks at a ratio of 1 and then decreases at a ratio of 4. This indicates that while higher temperatures can boost voltage, there is a threshold beyond which the efficiency may drop.

Similarly, the eggshell/NaOH ratio plays a crucial role in determining the voltage. At a constant temperature, increasing the ratio from 0.25 to 4 typically results in higher voltage, particularly evident at 700°C, where the voltage jumps from 0.037 V to 0.165 V. However, at 850°C, the voltage initially increases but then declines, suggesting an optimal ratio exists for maximum voltage output. This complex interplay between temperature and eggshell/NaOH ratio highlights the importance of optimizing both parameters to achieve the best performance in voltage generation.

The data reveals significant trends in the relationship between temperature and MPD, as well as the eggshell/NaOH ratio and MPD. As the temperature increases from 550°C to 700°C, MPD rises markedly across all ratios, with the highest values observed at 700°C, indicating that elevated temperatures generally enhance power output. However, at 850°C, the power density does not follow a consistent upward trend; instead, it peaks at a ratio of 1 and then decreases at a ratio of 4. This suggests that while higher temperatures can boost power density, there is a threshold beyond which the efficiency may decline. Similarly, the eggshell/NaOH ratio significantly impacts the MPD.

Table 2. Experimental results of runs

Run	Temperature (°C)	Eggshell/NaOH wt. ratio	Voltage (V)	MPD (mW/m^2)
1	550	0.25	0.008	9.2
2	550	1	0.021	18
3	550	4	0.045	31
4	700	0.25	0.037	21
5	700	1	0.114	53
6	700	4	0.165	70
7	850	0.25	0.063	11
8	850	1	0.108	45
9	850	4	0.072	22

At a constant temperature, increasing the ratio from 0.25 to 4 typically results in higher power density, particularly evident at 700°C, where the power density increases from 21 mW/m^2 to 70 mW/m^2. However, at 850°C, the power density initially increases but then declines, indicating an optimal ratio for MPD. This complex interplay between temperature and eggshell/NaOH ratio underscores the importance of optimizing both parameters to achieve the best performance in power generation. The complete data was provided in Table 2.

3.2. ANOVA Analysis of Voltage

The ANOVA table and model summary provide useful insights into the influence of temperature and eggshell-NaOH ratio on a dependent variable (Table 3). The model explains 79.89% of the variability in the response variable. Nevertheless, the corrected R-squared value of 46.39% and the predicted R-squared value of 0% indicate that the model may be overfitting the data. None of the components, including linear, square, and interaction factors, show statistical significance at the 0.05 level, since all p-values are over this threshold. The temperature's square term has the highest F-value of 4.03, suggesting its potential relevance. Nevertheless, the p-value of 0.138 indicates that it lacks statistical significance. This study indicates that while the model adequately captures the current data, it may not effectively apply to fresh data, and the factors analyzed do not have a significant impact on the response variable.

The coded coefficients table provides a thorough understanding of how temperature and eggshell-NaOH ratio affect a response variable (Table 4). The constant term is statistically significant, shown by a coefficient of 0.1522 and a p-value of 0.034, which suggests the existence of a baseline effect. The temperature has a coefficient of 0.0522, indicating a positive relationship. However, the coefficient is not statistically significant, as shown by a p-value of 0.191. Similarly, the eggshell to NaOH ratio has a positive effect (0.0580), although it is not statistically significant (p-value: 0.151). The interaction terms, including the squared variables for temperature and eggshell-NaOH ratio, have detrimental effects. Out of these factors, the temperature square term has the highest t-value (-2.01). However, it does not reach statistical significance (p-value: 0.138). The variance inflation factors (VIF) for all variables are about 1, indicating the lack of any issues related to multicollinearity. The regression resulted from regression analysis as shown in Equation (1).

$$\text{Voltage (V)} = -1.268 + 0.00352\ \text{Temperature (°C)} + 0.1043\ \text{Eggshell-NaOH Ratio} - 0.000002\ \text{Temperature (°C)*Temperature (°C)} - 0.0148\ \text{Eggshell-NaOH Ratio*Eggshell-NaOH Ratio} - 0.000037\ \text{Temperature (°C)*Eggshell-NaOH Ratio} \quad (1)$$

$$\text{MPD } (mW/m^2) = -569 + 1.628\ \text{Temperature (°C)} + 56.9\ \text{Eggshell-NaOH Ratio} - 0.001124\ \text{Temperature (°C)*Temperature (°C)} - 8.66\ \text{Eggshell-NaOH Ratio*Eggshell-NaOH Ratio} - 0.0183\ \text{Temperature (°C)*Eggshell-NaOH Ratio} \quad (2)$$

Table 3. ANOVA results of temperature and eggshell/NaOH wt ratio on voltage

Source	DF	Adj SS	Adj MS	F-Value	p-value
Model	5	0.016311	0.003262	2.38	0.253
Linear	2	0.008924	0.004462	3.26	0.177
Temperature (°C)	1	0.003878	0.003878	2.83	0.191
Eggshell-NaOH ratio	1	0.005046	0.005046	3.69	0.151
Square	2	0.007505	0.003752	2.74	0.210
Temperature (°C)*Temperature (°C)	1	0.005513	0.005513	4.03	0.138
Eggshell-NaOH Ratio*Eggshell-NaOH Ratio	1	0.001992	0.001992	1.46	0.314
2-Way Interaction	1	0.000481	0.000481	0.35	0.595
Temperature (°C)*Eggshell-NaOH Ratio	1	0.000481	0.000481	0.35	0.595
Error	3	0.004105	0.001368		
Total	8	0.020416			

Table 4. Regression analysis of temperature and eggshell/NaOH wt ratio on voltage

Term	Effect	Coef	SE Coef	T-Value	p-value	VIF
Constant		0.1522	0.0411	3.70	0.034	
Temperature (°C)	0.0522	0.0261	0.0155	1.68	0.191	1.05
Eggshell-NaOH ratio	0.0580	0.0290	0.0151	1.92	0.151	1.12
Temperature (°C)*Temperature (°C)	-0.1050	-0.0525	0.0262	-2.01	0.138	1.00
Eggshell-NaOH Ratio*Eggshell-NaOH Ratio	-0.1044	-0.0522	0.0433	-1.21	0.314	1.12
Temperature (°C)*Eggshell-NaOH Ratio	-0.0207	-0.0104	0.0175	-0.59	0.595	1.05

Table 5. ANOVA results of temperature and eggshell/NaOH wt ratio on MPD

Source	DF	Adj SS	Adj MS	F-Value	p-value
Model	5	2834.60	566.92	2.88	0.207
Linear	2	1144.59	572.29	2.90	0.199
Temperature (°C)	1	29.38	29.38	0.15	0.725
Eggshell-NaOH ratio	1	1115.21	1115.21	5.66	0.098
Square	2	1957.81	978.90	4.97	0.112
Temperature (°C)*Temperature (°C)	1	1280.18	1280.18	6.49	0.084
Eggshell-NaOH Ratio*Eggshell-NaOH Ratio	1	677.63	677.63	3.44	0.161
2-Way Interaction	1	118.49	118.49	0.60	0.495
Temperature (°C)*Eggshell-NaOH Ratio	1	118.49	118.49	0.60	0.495
Error	3	591.48	197.16		
Total	8	3426.08			

Table 6. Regression analysis of temperature and eggshell/NaOH wt ratio on MPD

Term	Effect	Coef	SE Coef	T-Value	p-value	VIF
Constant		74.7	15.6	4.78	0.017	
Temperature (°C)	4.54	2.27	5.88	0.39	0.725	1.05
Eggshell-NaOH ratio	27.27	13.63	5.73	2.38	0.098	1.12
Temperature (°C)*Temperature (°C)	-50.60	-25.30	9.93	-2.55	0.084	1.00
Eggshell-NaOH Ratio*Eggshell-NaOH Ratio	-60.9	-30.4	16.4	-1.85	0.161	1.12
Temperature (°C)*Eggshell-NaOH Ratio	-10.29	-5.14	6.63	-0.78	0.495	1.05

3.3. ANOVA Analysis of MPD

The ANOVA table and model summary indicate that the model explains 82.74% of the variation in the response variable (Table 5). Nevertheless, the corrected R-squared value decreases to 53.96%, whereas the anticipated R-squared is 0%, indicating the presence of overfitting. The linear factors, namely temperature and eggshell-NaOH ratio, exhibit p-values of 0.725 and 0.098, respectively, showing that they are not statistically significant predictors. The square terms, namely the temperature square term, show more potential with an F- value of 6.49 and a p-value of 0.084, but they are still not statistically significant at the 0.05 level. The p-value of 0.495 for the interaction term between temperature and eggshell-NaOH ratio suggests that this relationship is not statistically significant. This implies that while the model accurately represents the existing data, it may not be effective in predicting outcomes with fresh data, and the variables examined have little influence on the response variable.

Table 6 presents a statistical study of how temperature and the eggshell-NaOH ratio affect a dependent variable. The constant term, with a p-value of 0.017, suggests a statistically significant baseline influence. While the temperature (°C) and its interaction with the eggshell-NaOH ratio have positive coefficients, their p-values (0.725 and 0.495, respectively) indicate that they are not statistically significant. The quadratic coefficients for temperature and the eggshell-NaOH ratio exhibit negative values, indicating declining returns. However, their p-values (0.084 and 0.161) also imply a lack of statistical significance.The eggshell-NaOH ratio has a comparatively low p-value of 0.098, indicating the possibility of an influence. The Variance Inflation Factor (VIF) values indicate that there is little multicollinearity among the predictors, since they are close to 1. In general, the model exhibits some patterns but does not have significant statistical significance for most variables. The regression resulted from the regression analysis shown in Equation (2).

3.4. The 2D and 3D Contour Analysis of Voltage

The contour and three-dimensional surface plots (Figure 1) depict the correlation between the ratio of eggshell to NaOH, the temperature during calcination, and their indirect impact on the voltage output when the calcined eggshell is used as an anode in MFCs. The ratio of eggshell to NaOH and the temperature have a substantial impact on the physical properties of the calcined eggshell, including porosity and crystallinity [26]. These properties are important for the effectiveness of electron transfer in MFCs [27]. Increased porosity leads to a larger surface area for microbial adhesion and electron exchange, hence improving the performance of the microbial fuel cell [28].

Crystallinity has an impact on conductivity, with more crystalline structures allowing for more efficient electron transport compared to amorphous materials [29]. The figures demonstrate that certain combinations of eggshell-NaOH ratio and temperature provide increased voltage outputs, as seen by the deeper green areas on the contour plot and the elevated regions on the z-axis of the surface plot. These findings indicate that when the ratio of eggshell to NaOH is modest and higher temperatures are used, the resulting calcined eggshells possess features that enhance the performance of MFCs. Optimal calcination conditions theoretically enhance the structural characteristics of the eggshell, hence increasing its efficacy as an anode material [26].

3.5. The 2D and 3D Contour Analysis of Maximum Power Density

When employed as an anode in MFCs, the eggshell-NaOH ratio and temperature during calcination have a complex connection that ultimately affects the MPD. This relationship is shown by the contour and three-dimensional graphs (Figure 2). The calcination conditions have a considerable impact on the microstructural features of the calcined eggshell, including porosity and crystallinity. These qualities are essential for improving the performance of the anode. The contour plot and three-dimensional graph highlight the optimal calcination settings, which are shown by the darker parts and higher areas, respectively. These conditions reveal a synergistic effect, where certain combinations of eggshell-NaOH ratio and temperature result in higher MPD values. The enhanced bacterial adherence and electron transfer efficiency inside the MFC may be linked to the development of a favorable microstructure [30]. In contrast, any deviations from these ideal circumstances are likely to lead to less than perfect microstructural properties, such as a decrease in surface area or poorer conductivity, which ultimately reduces the MPD [31].

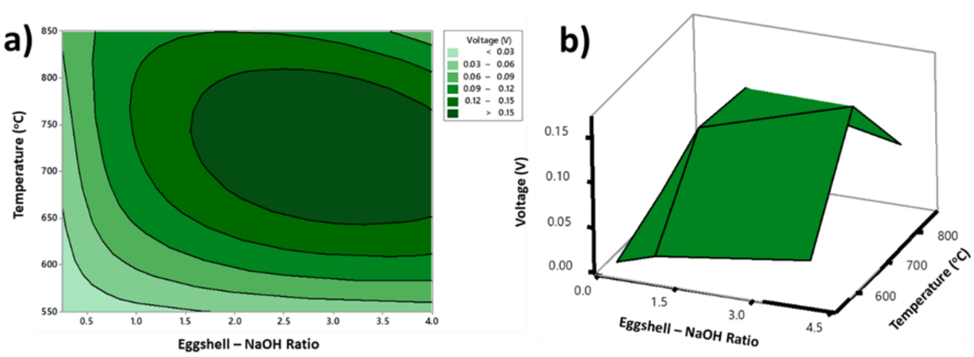

Figure 1. (a) Plot and (b) contour of temperature and eggshell/NaOH wt ratio on voltage

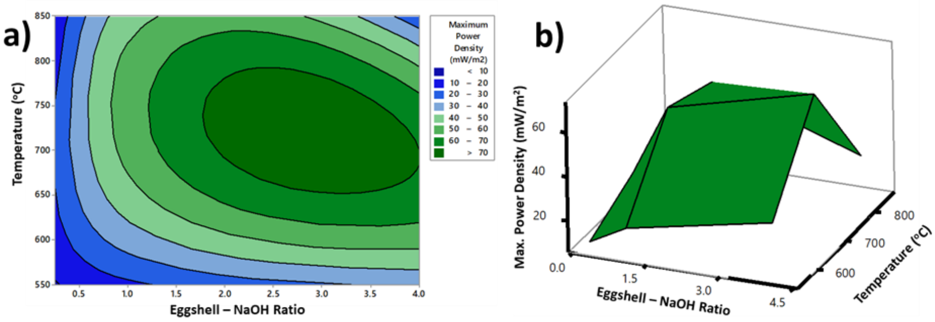

Figure 2. (a) Plot and (b) contour of temperature and eggshell/NaOH wt ratio on MPD

Higher temperatures enhance the crystallinity of the calcined eggshell, resulting in improved electron routes inside the anode material, thereby increasing efficiency [32]. Similarly, changes in the ratio of eggshell to NaOH affect the porosity of the calcined eggshell. Higher ratios are anticipated to increase the distribution of pore sizes, making it easier for bacteria to colonize and interact with the substrate inside the pores. The MPD results are indirectly determined by the microstructural changes that occur as a result of the calcination conditions. Theoretical justification for these discoveries may be derived from the principles of materials science, which highlight the significance of precise processing conditions in modifying the microstructural characteristics of materials to attain certain macroscopic qualities [31]. The significance of this connection highlights the need to meticulously optimize the calcination parameters in order to achieve the highest possible energy recovery from MFCs that use calcined eggshells as anodes.

3.6. Optimized Parameters

The optimization findings suggest that the most favorable conditions for attaining the MPD in MFCs using calcined eggshell as an anode are a temperature of 704.55°C and an eggshell-NaOH ratio of 2.52, as shown in Figure 3. Given these specific circumstances, the MPD is projected to be 76.21 mW/m², accompanied by a very favorable composite desirability rating of 1. These exact calcination conditions result in a calcined eggshell that has excellent microstructural features, including improved porosity and crystallinity. These qualities are essential for successful electron transfer and bacterial adhesion in MFCs.

The confidence intervals (95% CI) and prediction intervals (95% PI) represent the range of possible values for the findings, considering the inherent uncertainties in experimental circumstances and material attributes. The data may be explained by the concepts of materials science, which include using controlled calcination techniques to modify the microstructure of materials in order to improve their electrochemical performance [33]. Therefore, it is crucial to optimize the calcination settings accurately in order to maximize the energy recovery potential of MFCs that use calcined eggshells as anodes.

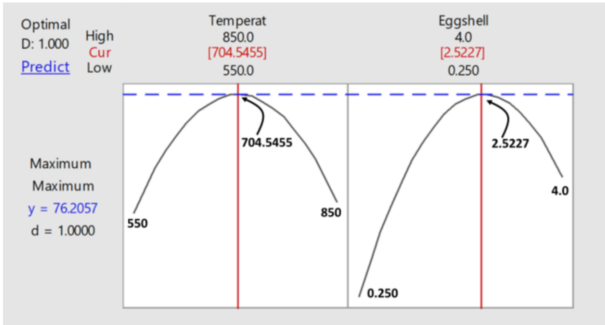

Figure 3. Statistical optimization of temperature and eggshell/NaOH wt ratio on voltage and MPD

3.7. Physico-Chemical Characteristics of Optimized Calcined Eggshell

3.7.1. SEM Analysis

The SEM images provided show significant morphological differences between the raw eggshell and the calcined eggshell. In the raw eggshell image (Figure 4a), the surface appears relatively smooth with overlapping layers, indicating the presence of the organic matrix and calcium carbonate structure typical of natural eggshells. This structure suggests a compact and less porous morphology. In contrast, the calcined eggshell image (Figure 4b) displays a markedly rougher and more fragmented surface, with irregular and granular particles. This transformation is due to the calcination treatment, which removes organic components and alters the calcium carbonate to calcium oxide. The resulting morphology is characterized by a porous and more disordered structure, with increased surface area due to particle aggregation and the breakdown of the original layered architecture. This porous structure could enhance the material's reactivity and adsorption properties, making calcined eggshells suitable for applications in catalysis or as adsorbents [34].

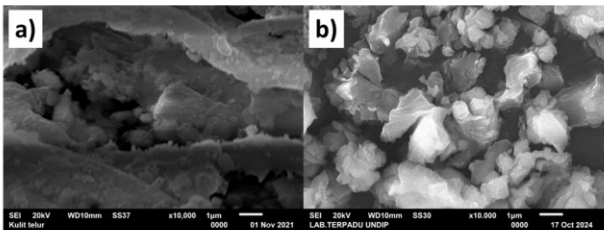

Figure 4. SEM images of (a) raw eggshell and (b) calcined eggshell

3.7.2. XRD Analysis

Figure 5 presents the XRD spectra of raw eggshells and calcined eggshells. The raw eggshell exhibits distinct diffraction peaks at 2θ values of approximately 23.1°, 29.4°, 36.0°, 39.4°, 43.2°, 47.5°, and 48.5°, which correspond to the characteristic reflections of calcium carbonate ($CaCO_3$) in the calcite phase. These peaks are indexed to crystallographic planes such as (012), (104), (110), (113), (202), (018), and (116), confirming the high crystallinity of the natural eggshell structure. The intensity of these peaks indicates well-ordered crystal packing within the $CaCO_3$ lattice. The raw eggshell is identified as having a trigonal crystal structure, indexed to the space group R-3c, matching the JCPDS card No. 083-0577 [35]. This phase represents calcium carbonate ($CaCO_3$), which is predominant in natural eggshells.

However, after treatment with NaOH and subsequent calcination at 700°C, a significant transformation occurs. The calcined sample shows a phase shift to calcium hydroxide, $Ca(OH)_2$, with a hexagonal crystal structure in the P-3m1 space group, as referenced by JCPDS card No. 04-0733 [36]. The characteristic calcite peaks at 29.4° and 39.4° diminish, while new peaks emerge at 2θ values of approximately 32.2°, 37.3°, 53.9°, 64.2°, and 67.4°, which are attributed to calcium oxide (CaO). These peaks correspond to the (111), (200), (220), (311), and (222) crystallographic planes of CaO, confirming the phase transformation.

During synthesis processes, $CaCO_3$ and NaOH are initially mixed in an aqueous solution. The addition of NaOH raises the pH of the solution, which can partially dissolve $CaCO_3$, releasing calcium ions (Ca^{2+}) and carbonate ions (CO_3^{2-}) into the solution. Upon calcination, the Ca^{2+} ions precipitate as calcium oxide (CaO), as CO_2 is released. In the final step, this CaO then interacts with residual moisture, forming $Ca(OH)_2$. This transformation highlights the role of NaOH in facilitating $CaCO_3$ dissolution and CaO formation under heat, with the resulting CaO ultimately converting to $Ca(OH)_2$ through moisture absorption.

The transformation from $CaCO_3$ to $Ca(OH)_2$ brings several benefits when using the material as an anode in MFCs. $Ca(OH)_2$ typically forms a more porous and potentially amorphous structure compared to the compact and crystalline nature of $CaCO_3$. This increased porosity provides a larger surface area for microbial adhesion, which is crucial for effective microbial colonization and electron transfer within the MFC. Additionally, the presence of hydroxide groups in $Ca(OH)_2$ creates a slightly alkaline environment, which some microorganisms prefer, thereby promoting stronger biofilm formation [37]. This biofilm acts as a conductive bridge between the electrode and microbes, enhancing MFC efficiency [38]. Furthermore, the amorphous structure and greater surface area of $Ca(OH)_2$ support more efficient ion transfer and redox reactions within the MFCs. This enhanced reactivity makes $Ca(OH)_2$ a superior material for facilitating electrochemical interactions compared to the less reactive, crystalline $CaCO_3$.

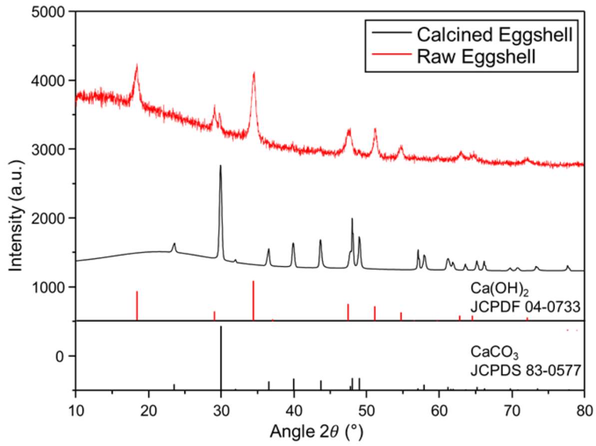

Figure 5. XRD spectra of raw eggshell and calcined eggshell

The changes in peak intensity and broadening suggest alterations in crystallite size and atomic arrangement [39]. The disappearance of calcite peaks and the formation of new CaO peaks indicate complete phase transformation. Additionally, peak broadening in the calcined eggshell pattern may suggest a reduction in crystallite size, potentially increasing the surface reactivity of the material. The transformation from $CaCO_3$ to CaO is significant for applications such as catalysis, bio-ceramics, and adsorption processes in environmental remediation. This characteristic is beneficial for MFCs due to the increased surface area and active sites, which promote enhanced electrochemical reactions and microbial adherence, improving electron transfer within the MFC environment.

3.7.3. FTIR Analysis

The FTIR spectra of raw and calcined eggshells, as shown in the Figure 6, provide key insights into the structural and chemical changes induced by thermal treatment. The raw eggshell (red spectrum) exhibits prominent peaks at 3600 cm^{-1}, 1400 cm^{-1}, and 871 cm^{-1}, characteristic of $CaCO_3$. The absorption at 3600 cm^{-1} corresponds to O-H stretching vibrations, which are attributed to hydroxyl groups from adsorbed moisture or organic materials present in the raw eggshell. The strong peak at 1400 cm^{-1} represents the asymmetric stretching mode of the CO_3^{2-}, confirming the presence of $CaCO_3$ as the primary component. Additionally, the peak at 871 cm^{-1} corresponds to the out-of-plane bending mode of the CO_3^{2-} group, further supporting the crystalline calcite phase of calcium carbonate.

Upon calcination, significant spectral changes occur, as seen in the green spectrum. The disappearance of the 3600 cm^{-1} peak indicates the removal of hydroxyl groups and organic matter, confirming that the high-temperature treatment effectively eliminates moisture and volatile components. Additionally, a shift from 1400 cm^{-1} to 1450 cm^{-1} suggests alterations in the carbonate environment, possibly due to the formation of new calcium-containing species. The persistent peak at 872 cm^{-1}, closely resembling the 871 cm^{-1} peak in raw eggshell, indicates that residual carbonate species may still be present, but in a modified phase.

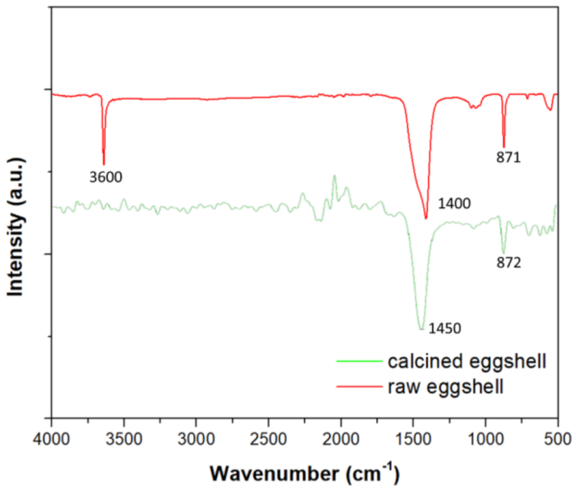

Figure 6. FTIR spectra of raw eggshell and calcined eggshell

Correlating these FTIR results with the XRD findings further clarifies the phase transformation. XRD analysis revealed that the characteristic $CaCO_3$ peaks in raw eggshells disappeared after calcination, replaced by CaO peaks. The FTIR data supports this transformation, as the loss of the 3600 cm^{-1} peak indicates dehydroxylation, while the shift in the carbonate peaks suggests a structural rearrangement. A new peak at 1450 cm^{-1} suggests the formation of $Ca(OH)_2$, likely due to CaO reacting with atmospheric moisture during cooling. This aligns with XRD findings, which identified CaO as the dominant phase post-calcination.

3.8. Comparison between Predicted and Actual Maximum Power Density

Figure 7 presents the MPD forecasted under optimal conditions using RSM. The significantly higher MPD observed for the optimized calcined eggshell@CF suggests that the calcination process enhances the electrochemical properties of eggshells, likely by increasing surface area, conductivity, or catalytic activity. The gap between the experimental and predicted MPD implies that actual improvements, such as enhanced porosity, electron transfer efficiency, or surface reactivity, contributed to superior performance. While raw eggshell@CF shows limited power generation due to constrained electrochemical reactions, the optimized calcined eggshell@CF achieves a substantially higher MPD, which peaks and then declines, likely due to mass transport limitations or internal resistances.

The observed discrepancy between the predicted MPD (76.21 mW/m^2) and the actual value (97.58 mW/m^2) can be attributed to several non-technical factors. One key factor is the variation in experimental conditions compared to the ideal parameters used in the predictive model [40]. Temperature and humidity are environmental factors that may significantly affect material performance, leading to deviations from anticipated values. Increased temperatures may enhance the mobility of charge carriers, resulting in a rise in the MPD. Additionally, little discrepancies in material properties, such as thickness or surface roughness, may influence performance.

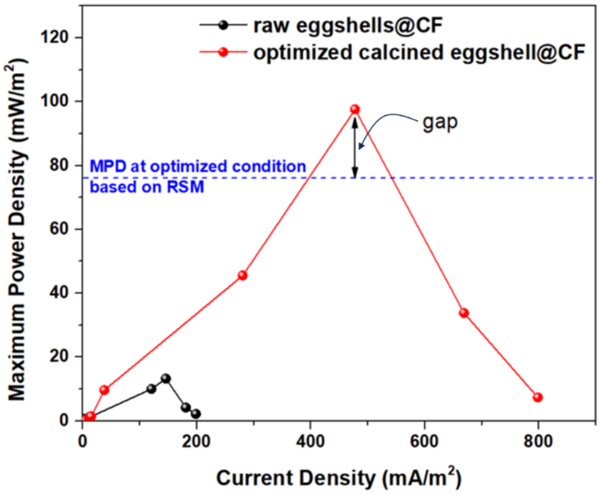

Figure 7. MPD between the projected and actual value

Unaccounted variables, like changes in the microstructure or the presence of impurities in the materials, may also contribute to the discrepancy. The model does not account for potential microstructural alterations, like the formation of grain boundaries or phase transitions. Even little impurities may alter the electrical properties of materials, leading to an elevated measured MPD. Consequently, while the model provides a theoretical prediction, real-world conditions provide complexities that may result in a greater observed MPD than first anticipated. The enhanced performance is probably attributable to a material change by heat treatment, which improves reaction kinetics, charge transfer, and electrochemical stability.

3.9. Research Implications

This study has meaningful implications for both materials science and renewable energy. By demonstrating that calcined eggshells can serve as a cost-effective and efficient anode material in MFCs, it opens up new possibilities for waste valorization and sustainable energy production. The findings emphasize the crucial role of calcination temperature and the eggshell-to-NaOH ratio in improving the electrochemical performance of MFCs. The optimal parameters identified—704.55°C and a ratio of 2.52—not only enhance the microstructural properties and crystallinity of the calcined eggshells but also improve their ability to transport electrons and support bacterial adhesion. These improvements contribute to higher MFC efficiency, leading to increased voltage and MPD. Additionally, the study employs RSM to ensure a systematic and reproducible approach, strengthening the reliability of the model for future research.

Beyond the technical advancements, this research aligns with circular economy principles by transforming discarded eggshells into valuable anode materials. By repurposing waste in this way, it helps reduce environmental impact while promoting sustainability. Overall, this study highlights the potential of waste-derived materials for cutting-edge energy applications, paving the way for more affordable and eco-friendly energy solutions.

4. Conclusion and Future Perspectives

The thorough examination of the experimental results underscores the complex interplay between temperature, the eggshell/NaOH ratio, and their collective influence on voltage and MPD in MFCs using calcined eggshell as an anode. The findings demonstrate that increasing the temperature from 550°C to 700°C typically augments both voltage and MPD, with the most pronounced enhancements seen at 700°C. Nonetheless, above 850°C, performance does not uniformly enhance, indicating a limit beyond which efficiency diminishes. The eggshell/NaOH ratio considerably influences the results, with increased ratios generally yielding improved performance until reaching an ideal threshold. At 700°C, augmenting the ratio from 0.25 to 4 results in significant improvements in voltage and MPD. The ANOVA analysis indicates that the model accounts for a substantial percentage of the variability in voltage (79.89%) and MPD (82.74%), although the adjusted and anticipated R- squared values imply possible overfitting. None of the covariates, including temperature and eggshell-NaOH ratio, exhibit statistical significance at the 0.05 level, suggesting that these variables do not significantly influence voltage or MPD. The regression analysis corroborates these data, indicating that while positive correlations exist between temperature, eggshell-NaOH ratio, and the response variables, none of these correlations are statistically significant. The absence of multicollinearity, as seen by the VIF values, indicates the independence of the model's variables. Two-dimensional and three-dimensional contour studies indicate that appropriate calcination conditions, especially an optimal temperature of 704.55°C and an optimal eggshell-NaOH ratio of 2.52, improve the microstructural characteristics of calcined eggshell, including porosity and crystallinity. These characteristics are essential for effective electron transfer and bacterial adhesion, resulting in enhanced performance of MFCs. The SEM study reveals the morphological transition from a smooth, compact structure in the raw eggshell to a rough, porous morphology in the calcined eggshell, advantageous for applications necessitating elevated surface area and reactivity. The XRD examination verifies the phase shift from calcium carbonate ($CaCO_3$) to calcium hydroxide ($Ca(OH)_2$), induced by NaOH treatment and calcination at 704.55°C, hence improving the material's appropriateness for use in MFCs owing to enhanced porosity and advantageous electrochemical characteristics. The FTIR study corroborates these results by demonstrating the elimination of organic constituents and the synthesis of novel calcium compounds during calcination. The optimized calcined eggshell has significant promise as a cost-effective and efficient material for many applications, especially in catalysis and microbial fuel cells, owing to its improved structural and electrochemical characteristics. This work offers significant insights into the transformation processes and functional enhancements of calcined eggshells, facilitating future research and development in sustainable materials science.

This discovery has a hopeful future, since it creates new opportunities for the use of waste materials in sustainable energy applications. The refined calcination method of eggshells improves the efficacy of MFCs while adhering to circular economy principles via waste valorization. Subsequent investigations may examine the scalability of this technique for industrial applications, confirming that the optimal parameters can be reliably attained on a wider scale. Furthermore, examining the long-term stability and endurance of calcined eggshell anodes under diverse environmental conditions would be essential for practical implementation. Subsequent research may investigate the feasibility of integrating calcined eggshells with other waste-derived substances to develop hybrid anodes exhibiting enhanced characteristics. This study facilitates novel methodologies in renewable energy and environmental sustainability, aiding in the development of economical and efficient MFC technologies.

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References

[1] Asim Ali Yaqoob, Asma Khatoon, Siti Hamidah Mohd Setapar, Khalid Umar, Tabassum Parveen, Mohamad Nasir Mohamad Ibrahim, Akil Ahmad, Mohd Rafatullah, Outlook on the Role of Microbial Fuel Cells in Remediation of Environmental Pollutants with Electricity Generation, *Catalysts*, 10, 8, (2020), 819 https://doi.org/10.3390/catal10080819

[2] Asim Ali Yaqoob, Mohamad Nasir Mohamad Ibrahim, Khalid Umar, Tabassum Parveen, Akil Ahmad, David Lokhat, Siti Hamidah Mohd Setapar, A glimpse into the microbial fuel cells for wastewater treatment with energy generation, *Desalination and Water Treatment*, 214, (2021), 379-389 https://doi.org/10.5004/dwt.2021.26737

[3] Saloni Kunwar, Neha Pandey, Pooja Bhatnagar, Gurasees Chadha, Neha Rawat, Naveen Chandra Joshi, Mahipal Singh Tomar, Murat Eyvaz, Prateek Gururani, A concise review on wastewater treatment through microbial fuel cell: sustainable and holistic approach, *Environmental Science and Pollution Research*, 31, 5, (2024), 6723-6737 https://doi.org/10.1007/s11356-023-31696-x

[4] Najwa Najihah Mohamad Daud, Mohamad Nasir Mohamad Ibrahim, Asim Ali Yaqoob, Amira Suriaty Yaakop, Mohd Hazwan Hussin, Chua Yong Shen, Abeer A. AlObaid, Optimizing microbial fuel cells performance: An innovative approach integrating anode materials, dual-pollutant treatment, and long-term operation, *Fuel*, 364, (2024), 131160 https://doi.org/10.1016/j.fuel.2024.131160

[5] Marcelinus Christwardana, Zahra Fauziah, Purbowatiningrum Ria Sarjono, Investigating the influence of *Saccharomyces cerevisiae* on microbial fuel cell performance through bioelectrochemical and biochemical approaches under varied operating conditions, *Biomass Conversion and Biorefinery*, 15, 6,

(2025), 8189-8202 https://doi.org/10.1007/s13399-024-05734-8

[6] F. Silva-Palacios, A. Salvador-Salinas, M. A. Quezada-Alvarez, M. Rodriguez-Yupanqui, Rojas-Flores Segundo, Nazario-Naveda Renny, Luis Cabanillas-Chirinos, Bioelectricity generation through Microbial Fuel Cells using *Serratia fonticola* bacteria and *Rhodotorula glutinis* yeast, *Energy Reports*, 9, (2023), 295-301 https://doi.org/10.1016/j.egyr.2023.05.255

[7] Roma Agrahari, Büşra Bayar, Haris Nalakath Abubackar, Balendu Shekher Giri, Eldon R. Rene, Radha Rani, Advances in the development of electrode materials for improving the reactor kinetics in microbial fuel cells, *Chemosphere*, 290, (2022), 133184 https://doi.org/10.1016/j.chemosphere.2021.133184

[8] Asim Ali Yaqoob, Mohamad Nasir Mohamad Ibrahim, Claudia Guerrero-Barajas, Modern trend of anodes in microbial fuel cells (MFCs): An overview, *Environmental Technology & Innovation*, 23, (2021), 101579 https://doi.org/10.1016/j.eti.2021.101579

[9] Aarti Tundwal, Harish Kumar, Bibin J. Binoj, Rahul Sharma, Gaman Kumar, Rajni Kumari, Ankit Dhayal, Abhiruchi Yadav, Devender Singh, Parvin Kumar, Developments in conducting polymer-, metal oxide-, and carbon nanotube-based composite electrode materials for supercapacitors: a review, *RSC Advances*, 14, 14, (2024), 9406-9439 http://dx.doi.org/10.1039/D3RA08312H

[10] Mustapha Omenesa Idris, Claudia Guerrero-Barajas, Hyun-Chul Kim, Asim Ali Yaqoob, Mohamad Nasir Mohamad Ibrahim, Scalability of biomass-derived graphene derivative materials as viable anode electrode for a commercialized microbial fuel cell: A systematic review, *Chinese Journal of Chemical Engineering*, 55, (2023), 277-292 https://doi.org/10.1016/j.cjche.2022.05.009

[11] Anusha Vempaty, Abhilasha Singh and Mathuriya, Strategic development and performance evaluation of functionalized tea waste ash-clay composite as low-cost, high-performance separator in microbial fuel cell, *Environmental Technology*, 44, 18, (2023), 2713-2724 https://doi.org/10.1080/09593330.2022.2041103

[12] Mustapha Omenesa Idris, Mohamad Nasir Mohamad Ibrahim, Nur Asshifa Md Noh, Asim Ali Yaqoob, Najwa Najihah Mohamad Daud, M. Hazwan Hussin, Abeer A. AlObaid, Assessment of biomass material as valuable electrode for high energy performance in microbial fuel cell with biodegradation of organic pollutant, *Fuel*, 371, (2024), 132059 https://doi.org/10.1016/j.fuel.2024.132059

[13] Bolanle M. Babalola, Lee D. Wilson, Valorization of Eggshell as Renewable Materials for Sustainable Biocomposite Adsorbents—An Overview, *Journal of Composites Science*, 8, 10, (2024), 414 https://doi.org/10.3390/jcs8100414

[14] Nattasak Suwannasingha, Attawut Kantavong, Suriyan Tunkijjanukij, Chakkapat Aenglong, Hong-Bing Liu, Wanwimol Klaypradit, Effect of calcination temperature on structure and characteristics of calcium oxide powder derived from marine shell waste, *Journal of Saudi Chemical Society*, 26, 2, (2022), 101441 https://doi.org/10.1016/j.jscs.2022.101441

[15] Raymond H. Myers, Douglas C. Montgomery, Christine M. Anderson-Cook, *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*, John Wiley & Sons, 2016,

[16] M. J. Salar-García, A. de Ramón-Fernández, V. M. Ortiz-Martínez, D. Ruiz-Fernández, I. Ieropoulos, Towards the optimisation of ceramic-based microbial fuel cells: A three-factor three-level response surface analysis design, *Biochemical Engineering Journal*, 144, (2019), 119-124 https://doi.org/10.1016/j.bej.2019.01.015

[17] Mohammadreza Hosseinpour, Manouchehr Vossoughi, Iran Alemzadeh, An efficient approach to cathode operational parameters optimization for microbial fuel cell using response surface methodology, *Journal of Environmental Health Science and Engineering*, 12, 1, (2014), 33 https://doi.org/10.1186/2052-336X-12-33

[18] Jagdeep Kumar Nayak, Saurabh Mishra, Amit, Uttam Kumar Ghosh, Simultaneous Production of Bio-energy and Bio-treatment of Wastewater Using Photosynthetic Microbial Fuel Cell: Optimization and Kinetic Modeling Approach, *Waste and Biomass Valorization*, 14, 1, (2023), 69-84 https://doi.org/10.1007/s12649-022-01836-4

[19] Mehdi Sedighi, Saad A. Aljlil, Mohammed Druis Alsubei, Mostafa Ghasemi, Majid Mohammadi, Performance optimisation of microbial fuel cell for wastewater treatment and sustainable clean energy generation using response surface methodology, *Alexandria Engineering Journal*, 57, 4, (2018), 4243-4253 https://doi.org/10.1016/j.aej.2018.02.012

[20] Muhammad Nihal Naseer, Asad A. Zaidi, Hamdullah Khan, Sagar Kumar, Muhammad Taha bin Owais, Yasmin Abdul Wahab, Kingshuk Dutta, Juhana Jaafar, Nor Aliya Hamizi, Mohammad Aminul Islam, Hanim Hussin, Irfan Anjum Badruddin, Hussein Alrobei, Statistical Modeling and Performance Optimization of a Two-Chamber Microbial Fuel Cell by Response Surface Methodology, *Catalysts*, 11, 10, (2021), 1202 https://doi.org/10.3390/catal11101202

[21] Shu-Hui Liu, Ting-Jun Zhu, Chi-Wen Lin, Cathodes for microbial fuel cells that improve the removal of copper ions from wastewater concomitant with power generation using the response surface methodology, *International Journal of Energy Research*, 46, 14, (2022), 20415-20424 https://doi.org/10.1002/er.7980

[22] H. Hadiyanto, Marcelinus Christwardana, Wahyu Zuli Pratiwi, P. Purwanto, S. Sudarno, Kristinah Haryani, Anh Tuan Hoang, Response surface optimization of microalgae microbial fuel cell (MMFC) enhanced by yeast immobilization for bioelectricity production, *Chemosphere*, 287, (2022), 132275 https://doi.org/10.1016/j.chemosphere.2021.132275

[23] Awais Ahmad, D. Jini, M. Aravind, C. Parvathiraja, Rabia Ali, Maryam Zaheer Kiyani, Asma Alothman, A novel study on synthesis of egg shell based activated carbon for degradation of methylene blue via photocatalysis, *Arabian Journal of Chemistry*, 13, 12, (2020), 8717-8722 https://doi.org/10.1016/j.arabjc.2020.10.002

[24] Marcelinus Christwardana, J. Joelianingsih, Linda Aliffia Yoshi, H. Hadiyanto, Binderless carbon nanotube/carbon felt anode to improve yeast

microbial fuel cell performance, *Current Research in Green and Sustainable Chemistry*, 5, (2022), 100323 https://doi.org/10.1016/j.crgsc.2022.100323

[25] Marcelinus Christwardana, Domenico Frattini, Grazia Accardo, Sung Pil Yoon, Yongchai Kwon, Optimization of glucose concentration and glucose/yeast ratio in yeast microbial fuel cell using response surface methodology approach, *Journal of Power Sources*, 402, (2018), 402-412 https://doi.org/10.1016/j.jpowsour.2018.09.068

[26] Marcelinus Christwardana, J. Joelianingsih, Satrio Kuntolaksono, Achmad Yanuar Maulana, Effect of NaOH concentration as activator on calcined eggshell and its application for yeast microbial fuel cell, *Bioresource Technology Reports*, 21, (2023), 101347 https://doi.org/10.1016/j.biteb.2023.101347

[27] Manickam Minakshi, Stephen Higley, Christian Baur, David R. G. Mitchell, Robert T. Jones, Maximilian Fichtner, Calcined chicken eggshell electrode for battery and supercapacitor applications, *RSC Advances*, 9, 46, (2019), 26981-26995 https://doi.org/10.1039/C9RA04289J

[28] Widad El Hayaoui, Naoual Tajat, Chaimae Radaa, Nadia Bougdour, Jallal Zoubir, Abderahim Idelahcen, Malika Tamimi, Samir Qourzal, Ali Assabbane, Idriss Bakas, In situ preparation of eggshell@Ag nanocomposite electrode for highly sensitive detection of antibiotic drug ornidazole in water sample, *Nanotechnology for Environmental Engineering*, 7, 3, (2022), 635-646 https://doi.org/10.1007/s41204-022-00272-y

[29] John S Tse, A chemical perspective on high pressure crystal structures and properties, *National Science Review*, 7, 1, (2019), 149-169 https://doi.org/10.1093/nsr/nwz144

[30] Alexiane Godain, Timothy M. Vogel, Pascal Fongarland, Naoufel Haddour, Influence of Hydrodynamic Forces on Electroactive Bacterial Adhesion in Microbial Fuel Cell Anodes, *Bioengineering*, 10, 12, (2023), 1380 https://doi.org/10.3390/bioengineering10121380

[31] Kaiqi Yang, Yifan Cao, Youtian Zhang, Shaoxun Fan, Ming Tang, Daniel Aberg, Babak Sadigh, Fei Zhou, Self-supervised learning and prediction of microstructure evolution with convolutional recurrent neural networks, *Patterns*, 2, 5, (2021), 100243 https://doi.org/10.1016/j.patter.2021.100243

[32] Cancan Peng, Hongli Bai, Mingwu Xiang, Changwei Su, Guiyang Liu, Junming Guo, Effect of Calcination Temperature on the Electrochemical Properties of Spinel LiMn2O4 Prepared by Solid-State Combustion Synthesis, *International Journal of Electrochemical Science*, 9, 4, (2014), 1791-1798 https://doi.org/10.1016/S1452-3981(23)07892-6

[33] M. Mahinzad Ghaziani, J. Mazloom, F. E. Ghodsi, Electrospun $MgCo_2O_4$ nanofibers as an efficient electrode material for pseudocapacitor applications: Effect of calcination temperature on electrochemical performance, *Journal of Physics and Chemistry of Solids*, 152, (2021), 109981 https://doi.org/10.1016/j.jpcs.2021.109981

[34] Ehsan kianfar, Hamidreza Sayadi, Recent advances in properties and applications of nanoporous materials and porous carbons, *Carbon Letters*, 32, 7, (2022), 1645-1669 https://doi.org/10.1007/s42823-022-00395-x

[35] Sikander Hayat, M. Tariq Siddique, Khalil Ahmad, M. Wazir-ud-Din, M. Masood Mahmood, Shakeel Ur Rehman, M. Basim Kakakhel, Sikander M. Mirza, Material characterization and kinetic parameters based thermoluminescence study of Pakistani Onyx pellets, *Journal of Luminescence*, 236, (2021), 118098 https://doi.org/10.1016/j.jlumin.2021.118098

[36] Hamid Reza Momenian, Sousan Gholamrezaei, Masoud Salavati-Niasari, Behnam Pedram, Farhang Mozaffar, Davood Ghanbari, Sonochemical Synthesis and Photocatalytic Properties of Metal Hydroxide and Carbonate (M:Mg, Ca, Sr or Ba) Nanoparticles, *Journal of Cluster Science*, 24, 4, (2013), 1031-1042 https://doi.org/10.1007/s10876-013-0595-y

[37] B. Athanassiadis, P. V. Abbott, L. J. Walsh, The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics, *Australian Dental Journal*, 52, s1, (2007), S64-S82 https://doi.org/10.1111/j.1834-7819.2007.tb00527.x

[38] Lina J. Bird, Elizabeth L. Onderko, Daniel A. Phillips, Rebecca L. Mickol, Anthony P. Malanoski, Matthew D. Yates, Brian J. Eddie, Sarah M. Glaven, Engineered living conductive biofilms as functional materials, *MRS Communications*, 9, 2, (2019), 505-517 https://doi.org/10.1557/mrc.2019.27

[39] D. S. Suresh, Abdullah Ba Shbil, Sapna Sharanappa, S. P. Vijaykumar, H. Ganesha, S. Veeresh, Y. S. Nagaraju, H. Devendrappa, Enhancement of crystallinity with porosity material through solvent and thermal treated eggshell waste for high-performance supercapacitor applications, *Journal of Materials Science: Materials in Electronics*, 35, 5, (2024), 330 https://doi.org/10.1007/s10854-024-12021-9

[40] Douglas C. Montgomery, *Design and Analysis of Experiments*, Ninth ed., John wiley & sons, 2017,