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Harvesting Marine Microalgae *Nannochloropsis sp* by using Electroflocculation

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Abstract

With current changing climates, researching alternative food, value added products, and renewable energy sources is crucial. Microalgae is a promising candidate for all three categories. This study focuses on identifying the ideal photobioreactor to grow *Nannochloropsis sp*, ideal electrode material for electroflocculation and optimising parameters of electroflocculation using response surface methodology (RSM). The microalgae *Nannochloropsis sp* was grown to identify ideal indoor photobioreactor type by observing growth performance, while electrode materials C (carbon), Mg (magnesium), and Al (aluminium) were screened by measuring flocculation efficiency (FE) of *Nannochloropsis sp*. Finally, parameters (time, pH, voltage) were optimised for electroflocculation using Design Expert Version 13, analysing FE results using multiple regression statistical analysis. Ideal photobioreactor for *Nannochloropsis sp* is Erlenmeyer flasks, with dry cell weight of 1.024g/L, compared to canisters (0.914g/L) and Schott Bottles (0.771g/L). For electrode screening, Magnesium had the highest maximum as well as highest final FE of 95.4% and 94.74% respectively, while Aluminium (94.62%, 93.26%) and Carbon (88.85%, 85.73%) performed more poorly. For RSM analysis, optimum flocculation conditions were identified to be 27.05 minutes, pH 8.98, and 29.05V, for 100% FE and desirability of 1. The results and observations in this study were found to be statistically significant and reproducible.

Keywords: microalgae cultivation, *Nannochloropsis sp*, electroflocculation, response surface methodology

Introduction

Microalgae are photosynthetic microorganisms which have gained exponential interest over the past few decades, for a variety of potential applications ranging from pharmaceuticals, cosmoceuticals, and nutraceuticals, to the production of sustainable biofuels (Dominguez, 2013; Venkatesan *et al*, 2015). Microalgae are found in a variety of environments, capable of thriving in a very wide range of parameters such as temperature, salinity, pH, and intensity of light (Khan, Shin & Kim, 2018).

In a world where fossil fuels are dwindling and the need for sustainable sources of energy continues to rise, microalgae bring forth a novel avenue of pursuing biofuel research, not only helping reduce the heavy dependency we currently have on fossil fuels, but to also reduce the pollution and carbon emissions that result from the use of said fossil fuels (Milano *et al*, 2016). This makes microalgae a very strong candidate for further biofuels research.

Nannochloropsis has been extensively studied for its many value-added bioactive compounds, offering potential in nutrition, phytomedicine, medicinal drug development, biofuels, pigment sources, and more (Ma *et al*, 2021). There have been many studies and evaluation of a variety of methods for harvesting microalgae such as flocculation, filtration, flotation, sonication, centrifugation, precipitation 2 (Branyikova *et al*, 2018; Saad *et al*, 2019). Electroflocculation is considered to be highly promising in terms of microalgal harvesting because of it is cost effective, and also because the biomass recovery is extremely efficient and does not compromise the quality of said biomass (Bleeke *et al*, 2015; Krishnamoorthy *et al*, 2021)..

This research aims to identify the best photobioreactor for the cultivation of *Nannochloropsis sp*, screen the best electrode material for electroflocculation of *Nannochloropsis sp*, and optimise the parameters of electroflocculation of *Nannochloropsis sp* using research surface methodology

Materials and methods

The *Nannochloropsis sp* sample was collected from an isolation of marine water from Tanjung Kupang. This was subsequently cultured using an enrichment culture of Walne's solution (12 nutrients per litre of sterilised seawater: $NaNO_3$, $FeCl_3.6H_2O$, $MnCl_2.4H_2O$, $Na_2.EDTA$, $NaH_2PO_4.2H_2O$, $ZnSO_4.7H_2O$, $CoCl_2.6H_2O$, $NaMoO_4.2H_2O$, $CuSO_4.5H_2O$, Vitamin B12, thiamine, and biotin) to prepare the inoculum. During medium preparation, the media's pH was adjusted to pH 8.2 and then autoclaved. Following that, the inoculum was prepared. This was conducted in a 250ml conical flask in sterile conditions over a period of 7 days at an inoculum concentration of 10%. Following that, the microalga media was transferred to a 2L indoor photobioreactor (PBR) with a 1.5L working volume, where the OD readings were measured for 14 days. The conditions of these PBRs were standardised, with three different reactor vessels used: Erlenmeyer flasks, schott bottles, and modified suction canister. The aeration rate was set to 1.0L/min, pH 8.2, light intensity of 3000 lux, and the temperature was room temperature at about 25°C ± 2°C.

The optical density of the samples was measured using spectrophotometry at 680 nm, and the cell density of the sample was counted using dry cell weight. These readings were used to determine the growth performance of the *Nannochloropsis sp*. The *Nannochloropsis sp* was cultured in an indoor 2L bioreactor with different photobioreactors at 0 h dark and 24 h light cycles. The experiments were conducted in triplicate and the mean of said triplicate was then analysed for the different bioreactor vessels. The OD readings were plotted on a graph in order to make comparisons between the photobioreactor vessels and the effect of their type of growth performance of *Nannochloropsis sp*. In order to calculate biomass concentration, the Dry Cell Weight was calculated by way of the oven dry cell weight method where filtering the sample by using pre-weighted cellulose acetate membrane of 0.45µm.

To assess the efficacy of the electrode materials, C, Mg, and Al, the electroflocculation equipment was set up as shown in the schematic diagram of the electroflocculation equipment in Fig 3.5. The electroflocculation equipment was set up along with 150ml of microalgal solution. The electrodes were of the same material (C, Al, Mg) and were tested at voltage 15V at room temperature in triplicate. The OD of the solution was measured from 1 ml samples taken every 5 minutes for 35 minutes. These samples were taken from 3 cm below the surface of the solution. Following that, the graphs of the OD were charted and the flocculation efficiencies of each electrode were mapped out on a graph.

The process of optimising these parameters for flocculation utilised the Design Expert Version 13 software in order to set up experiments using statistical concepts. In order to calculate the best possible values of time, pH, and voltage, a central composite face-centred (CCF) setup was used, thereby making the experiment more efficient. The optical density of the sample was measured using spectrophotometry at 680 nm, in order to calculate the flocculation efficiency. These experiments were conducted in triplicate and plotted using the mean result of the triplicate experiments. Following that, the calculations were utilised in statistical analysis.

Results and discussion

The cultivation of microalgae for further downstream processing requires that the sample of microalgae be harvested at the end of the log phase or during the early stationary phase. 10% inoculum of *Nannochloropsis sp* (150ml) was added to the 1.5L culture medium of filtered seawater, and Walne's solution for each photobioreactor. Figure 1 below shows the graph of the growth performance of *Nannochloropsis sp* in the three different types of photobioreactors. From this figure, it can be summarily observed that the *Nannochloropsis sp* has the highest growth performance in the Erlenmeyer flask with a maximum dry cell weight of 1.024g/L at 14 days. This was followed by the growth performance observed in the suction canister, where the maximum dry cell weight at 14 days was 0.914g/L. Finally, the photobioreactor with the lowest results for growth performance was the Schott bottle, where the maximum dry cell weight was only 0.771g/L at 14 days.

Nannochloropsis had shown the highest growth performance in the Erlenmeyer flask, because the conical shape of the Erlenmeyer flask allows for better agitation of the microalgae as compared to other shapes of photobioreactors (Masojídek & Torzillo, 2014; Bácsi *et al*, 2022). As a microalgal culture begins to grow denser, light can be absorbed almost completely within just a few millimetres of the medium, and the cells present towards the centre of the photobioreactor would not receive as much light, thus impeding their optimum growth levels (Gupta *et al*, 2015; Assunção & Malcata, 2020). Therefore, it can be seen that photobioreactors with lower surface area such as the Schott bottle and modified suction canister, do not have as much light reaching the centre of the photobioreactor, reducing their growth performance compared to the Erlenmeyer flask.

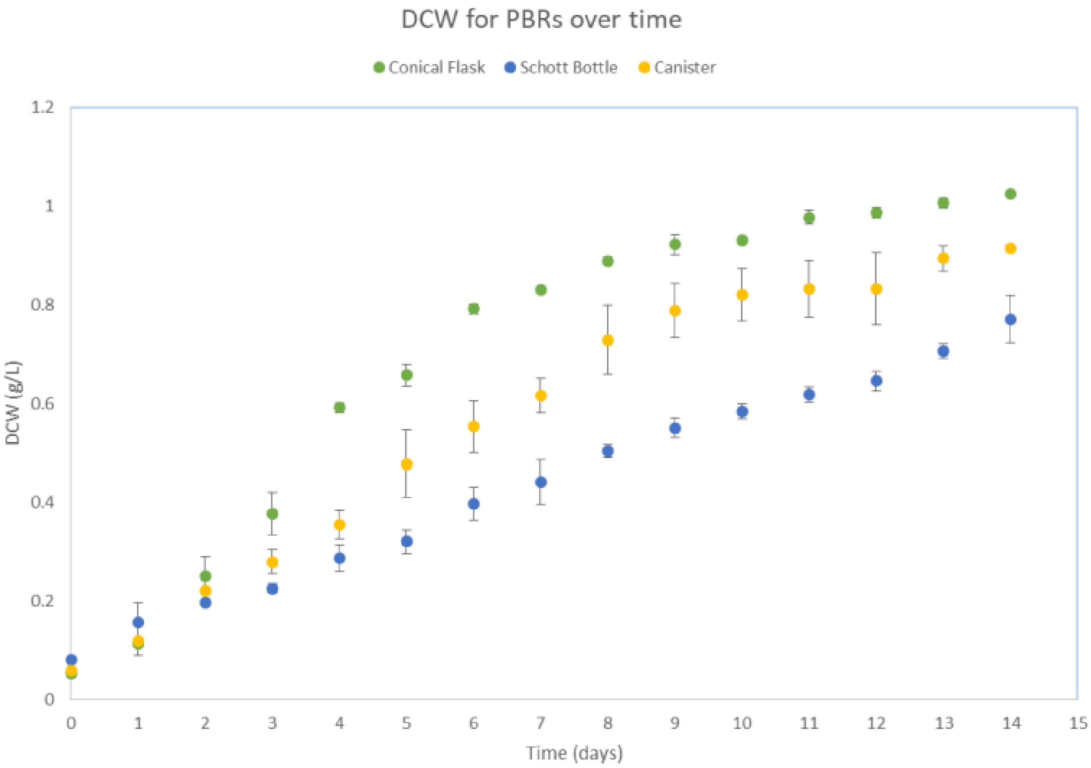


Figure 1 Growth Performance of *Nannochloropsis sp* in different types of indoor 2L photobioreactor system

The benefits of using flocculation to harvest microalgae is that it is a much quicker and simpler procedure as compared to other methods of harvesting microalgae, with much lower energy requirements as well (Gerde *et al*, 2014). Figure 2 below shows the OD readings taken every 5 minutes during electroflocculation, while Figure 3 shows the flocculation efficiency of each electrode material. From these figures, it can be summarised that Magnesium showed the maximum flocculation efficiency of 95.4%, and the highest final flocculation efficiency after 35 minutes of electroflocculation at 94.74%. Aluminium is a close second in terms of flocculation efficiency, achieving a maximum flocculation efficiency of 94.62% and a final flocculation efficiency of 93.26%. On the other hand, Carbon showed a much lower flocculation efficiency, with a maximum flocculation efficiency of 88.85% and a final flocculation efficiency of 85.73%.

A variety of existing literature thus far has studied the performance of different electrode materials; however, these were all metal electrodes, and these studies indicated that Carbon as a cheap source of materials could be explored in further studies (Vilar *et al*, 1998; Lee *et al*, 2013; Mathew *et al*, 2014; Bleeke *et al*, 2015; Cruz *et al*, 2019). These studies also did not utilise *Nannochloropsis sp* for their flocculation experiments, but rather other species (Lee *et al*, 2013; Bleeke *et al*, 2015; Cruz *et al*, 2019). In addition to the performance of each material, the economic costs and safety of each material was also assessed, with Mg being nontoxic at much higher concentrations than that of Aluminium (Bleeke *et al*, 2015). Electroflocculation with Al does have its concerns as the metal ions will inevitably end up contaminating the algal flocs that are harvested, meaning that these algal flocs would not be viable for applications that require pure algal biomass (Branyikova *et al*, 2018).

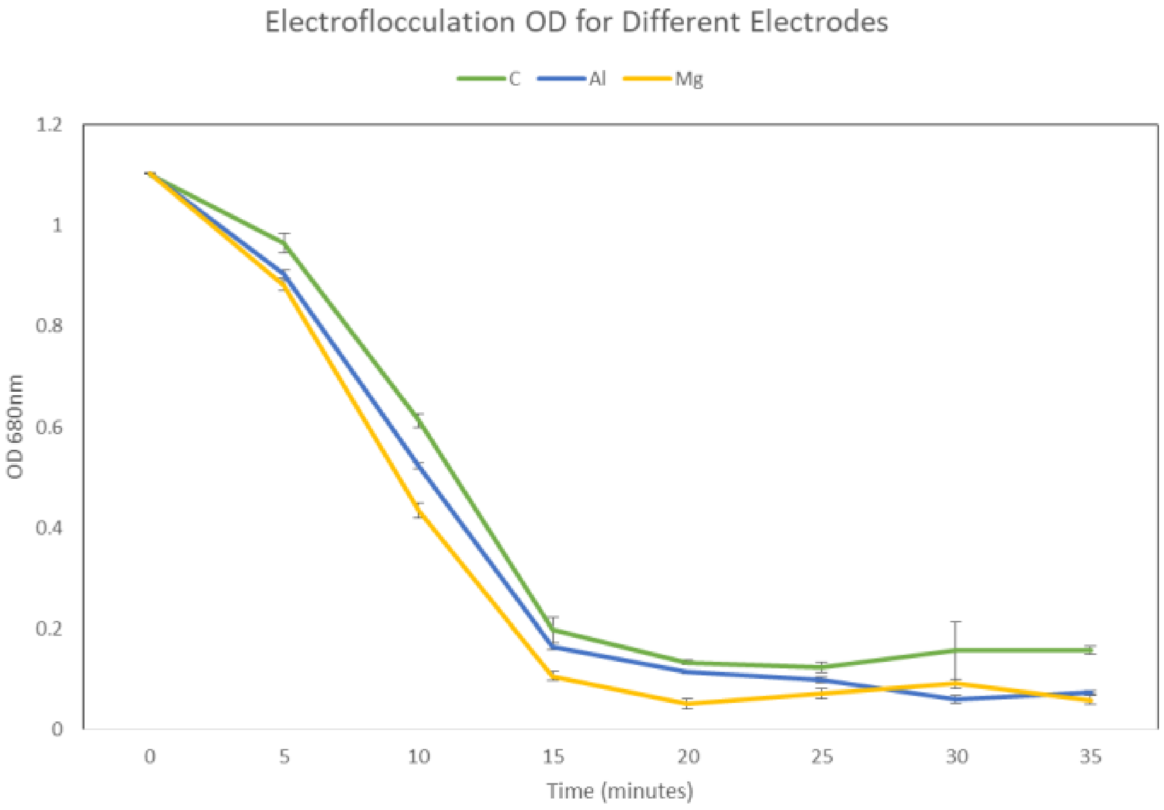


Figure 2 The Electroflocculation OD (680nm) for each electrode material

Electroflocculation is highly dependent on a large number of surface factors, which require optimisation in order to maximise the recovery of algal biomass during the harvesting process (Branyikova *et al*, 2018). For this study, the response surface methodology was utilised. The software, Design Expert Version 13 utilises multiple regression analysis, and observes the effect of multiple independent variables against a dependent variable.

The software performed analysis of variance (ANOVA) for the model, the results of which are showcased in Figure 4. The developed Model F value of the study was found to be 8.43, and the p-value of the model was 0.0013, which the software marked as significant. According to the software, there is only a 0.13% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant (Kiran *et al*, 2007). In this case A, BC, and A^2 are significant model terms. A p-value above 0.1 indicates that the terms are insignificant, which is not the case here with the statistical model of this study as the p-value is well below 0.05, indicating the level of significance for the model (Kumari & Gupta, 2019, Chicco *et al*, 2021).

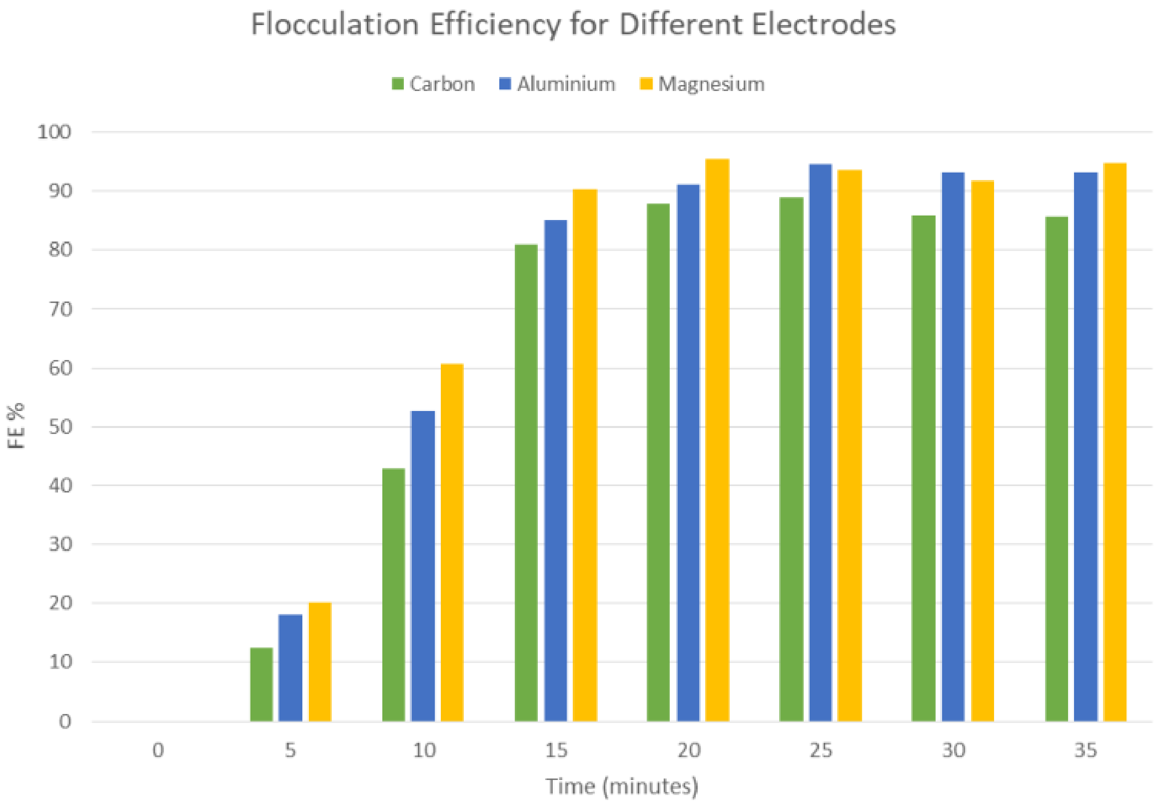
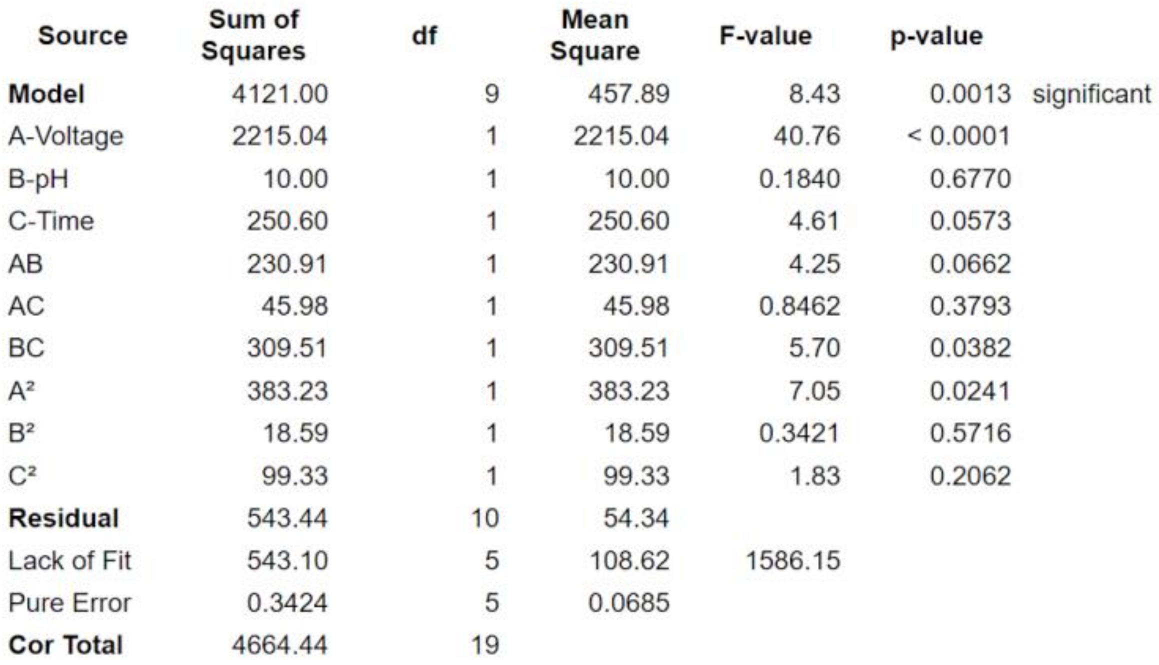


Figure 3 Flocculation Efficiency for Different Electrodes in Electroflocculation



Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4121.00	9	457.89	8.43	0.0013	significant
A-Voltage	2215.04	1	2215.04	40.76	< 0.0001	
B-pH	10.00	1	10.00	0.1840	0.6770	
C-Time	250.60	1	250.60	4.61	0.0573	
AB	230.91	1	230.91	4.25	0.0662	
AC	45.98	1	45.98	0.8462	0.3793	
BC	309.51	1	309.51	5.70	0.0382	
A^2	383.23	1	383.23	7.05	0.0241	
B^2	18.59	1	18.59	0.3421	0.5716	
C^2	99.33	1	99.33	1.83	0.2062	
Residual	543.44	10	54.34			
Lack of Fit	543.10	5	108.62	1586.15		
Pure Error	0.3424	5	0.0685			
Cor Total	4664.44	19				

Figure 4 ANOVA results of the Response Surface Methodology (RSM)

The Design Expert Version 13 calculated the optimised conditions needed for maximal flocculation efficiency of *Nannochloropsis sp* by way of electroflocculation. The optimised parameters for the optimum flocculation efficiency were found to be 29.05V, pH 8.98, and 27.05 minutes, as shown in Figure 5. The results of the optimisation had a desirability of 1.00, which indicates that the statistical model developed for the study is applicable, as D values that are closer to 1 are considered more desirable (Kumari & Gupta, 2019; Kamaroddin *et al*, 2020; Chicco *et al*, 2021).

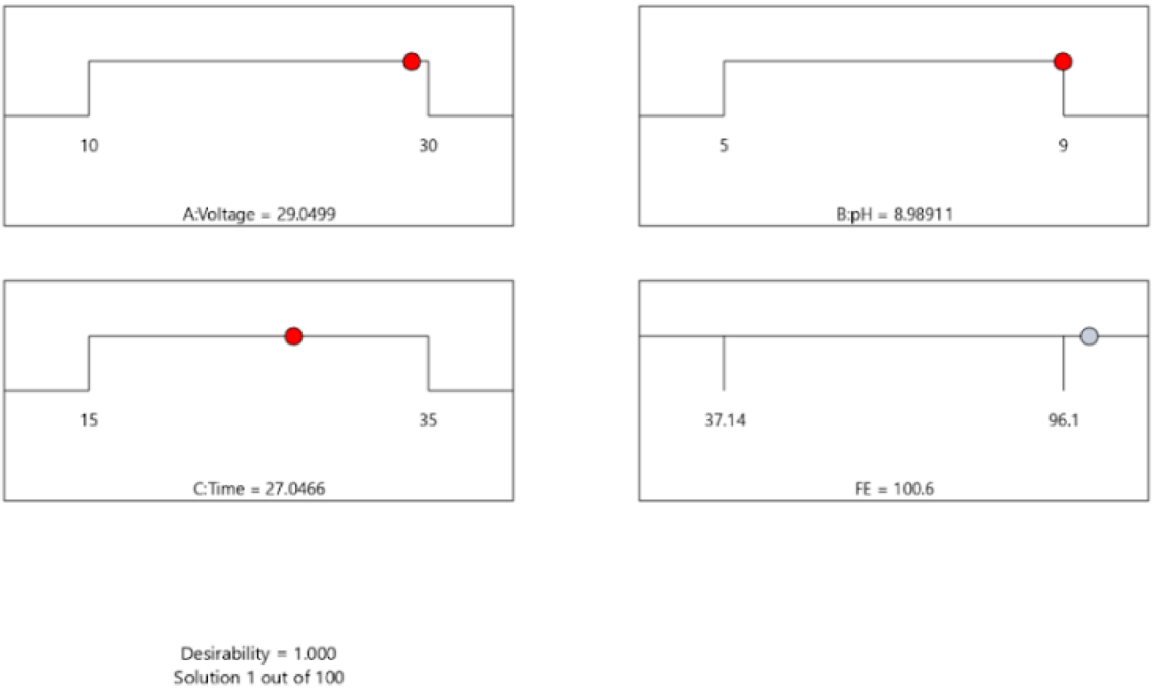
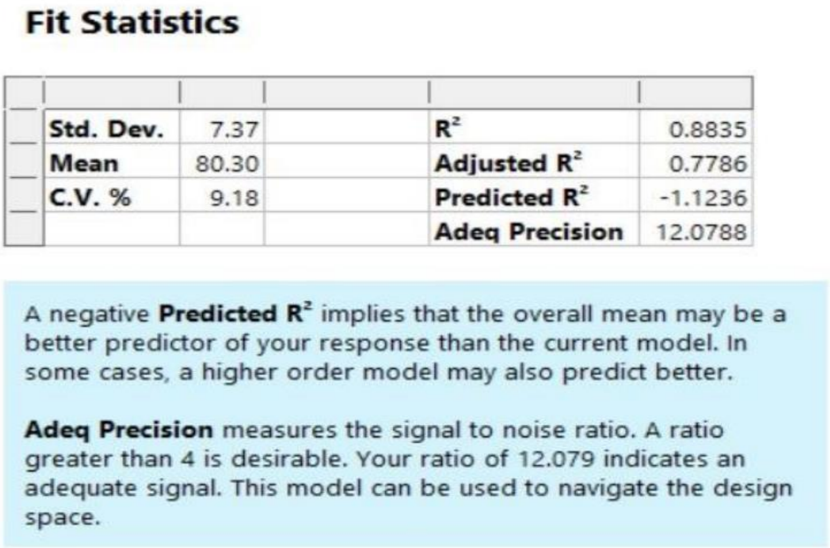


Figure 5 Desirability and numerical optimisation of the selected parameters

In addition to the ANOVA result significance stated above, the software also calculated the fit statistics of the multiple regression model, shown in Figure 6. The R^2 value of the study model was found to be 0.8835, and after adjustment of the multiple independent variables applied in this study, the adjusted R^2 was calculated to be 0.7786. Furthermore, the adequate precision of the study was found to be 12.0788. When considering the R^2 of a multiple regression model, the higher the R^2 value, the more of the results of the statistical model are considered to be affected by the independent variables of the model (Kiran *et al*, 2007; Kumari & Gupta, 2019). Therefore, with the R^2 at 0.8835 for this model, it can be stated that 88.35% of the results in the statistical model used in this experiment can be attributed to the independent variables: voltage, pH, and time. These relationships are further explored down below with 3D models of the graph. In addition to the R^2 test, the adjusted R^2 test measured and was above 0.7, and therefore the dependent variable, flocculation efficiency, generally follows and is in line with the movements of the independent variables. The adjusted R^2 test compensates for the addition of the variables into the statistical model to find out how much of the results could be explained by the independent variables (Kumari & Gupta, 2019). The Adeq precision, as stated by the software, measures the ratio of the signal to noise, where a ratio of 4 and greater is considered to be desirable. The ratio of 12.0788 indicates an adequate signal and that the statistical model is applicable to be used in the design space.



Fit Statistics

Std. Dev.	7.37		**R^2**	0.8835
Mean	80.30		**Adjusted R^2**	0.7786
C.V. %	9.18		**Predicted R^2**	-1.1236
			Adeq Precision	12.0788

A negative **Predicted R^2** implies that the overall mean may be a better predictor of your response than the current model. In some cases, a higher order model may also predict better.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 12.079 indicates an adequate signal. This model can be used to navigate the design space.

Figure 6 Fit Statistics of the study model from Design Expert Version 13

3D surface graphs were rendered to explore the relationship between two of the independent variables each. Figures 7, 8, and 9 details relationships between voltage, pH, and time in pairs. For the relationship between voltage and pH in Figure 7, it can be observed that the flocculation efficiency was better when voltage and pH were higher. Figure 8 showcases the relationship of voltage and time with regards to flocculation efficiency, and it can be observed that flocculation efficiency increases as time and voltage increase. Yet, it can be observed that after the 25-minute mark, the flocculation efficiency 8 begins to decrease once more. Finally, in Figure 9, the relationship of pH and time shows that the lower time and lower pH lead to very low flocculation efficiencies. On the other hand, after about the 25-minute mark, the flocculation efficiency is lower than before. These results are in line with the optimum desirability solution chosen by the software as shown above in Figure 5.

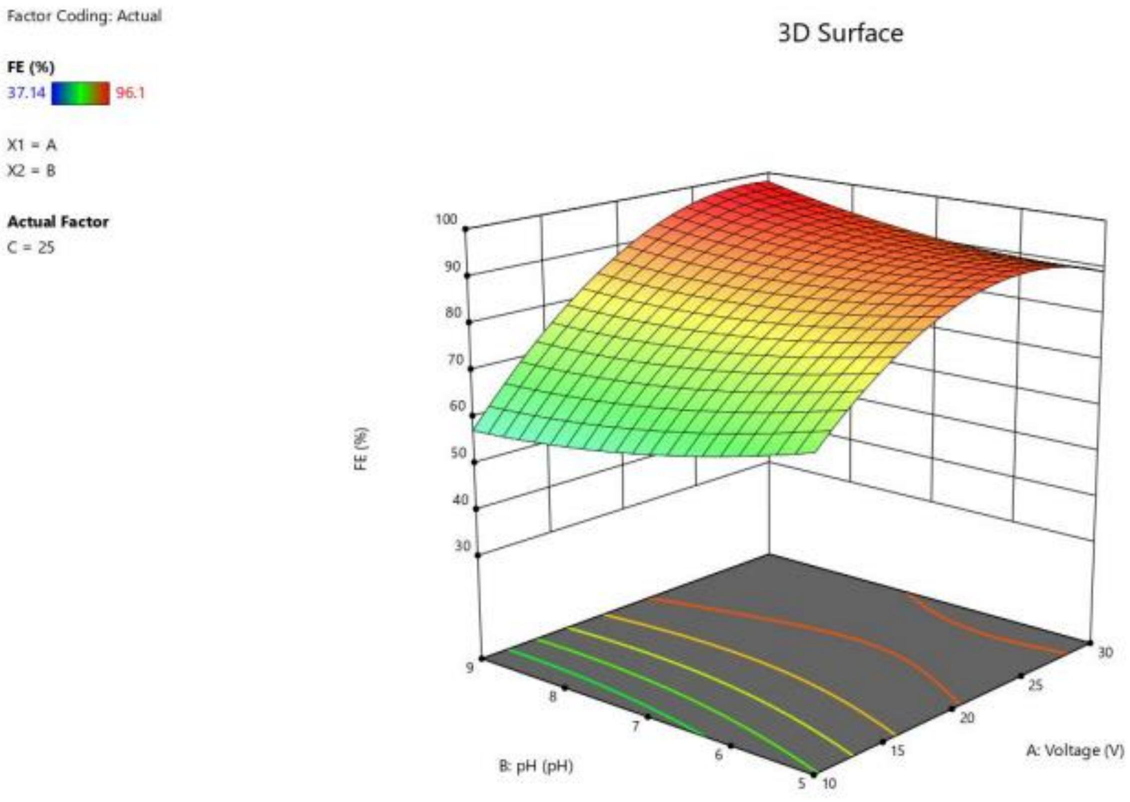


Figure 7 3D graph of the relationship between Voltage and pH

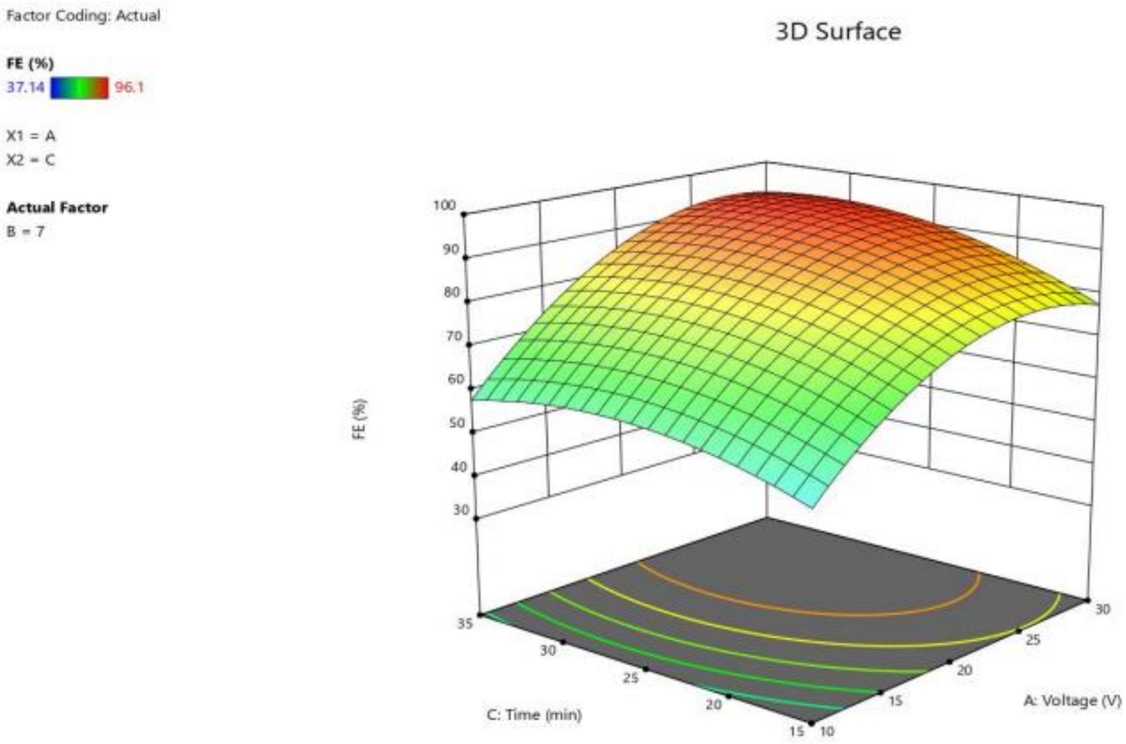


Figure 8 3D graph of the relationship between Voltage and Time

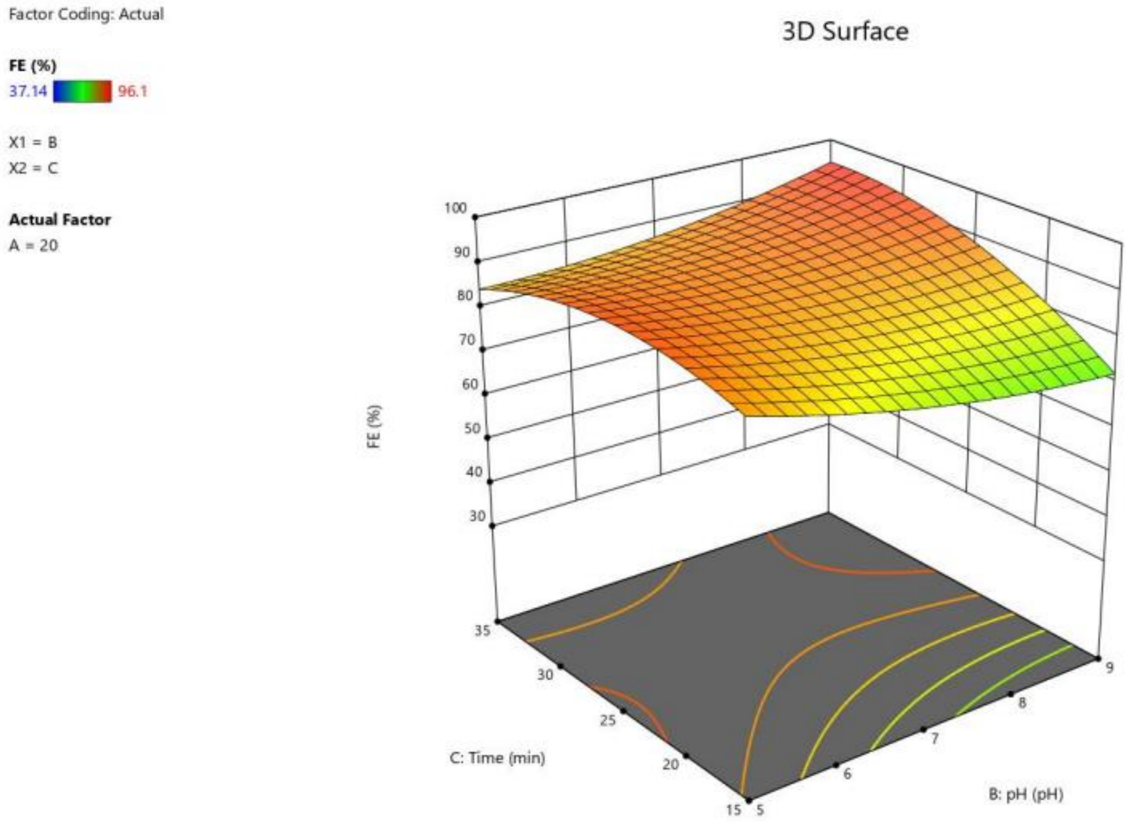


Figure 9 3D graph of the relationship between pH and Time

In addition to statistical analysis, the microscopic view of the microalgae before and after electroflocculation were taken at 40x magnification to observe any visible physiological and morphological services. Figure 10 below details *Nannochloropsis sp* before electroflocculation, harvested on day 14 of growth where it is expected to be in its early stationary phase or around the end of the log phase. The morphological features as noted in the literature review can be observed in Figure 10, where *Nannochloropsis sp* consists of small, spherical, non-motile cells (Chua & Schenk, 2017; Ma *et al*, 2021). In Figure 11, it can be observed that the microalgal cells are clumping together in floc formations, post electroflocculation and in addition to the clumping, it can be observed that some of the cells underwent lysis during electroflocculation. However, in accordance with existing literature review, the electroflocculation led to much higher amounts of biomass recovery than what occurs in a similar amount of time for other methods of harvesting microalgae (Gerde *et al*, 2014; Bleeke *et al*, 2015).

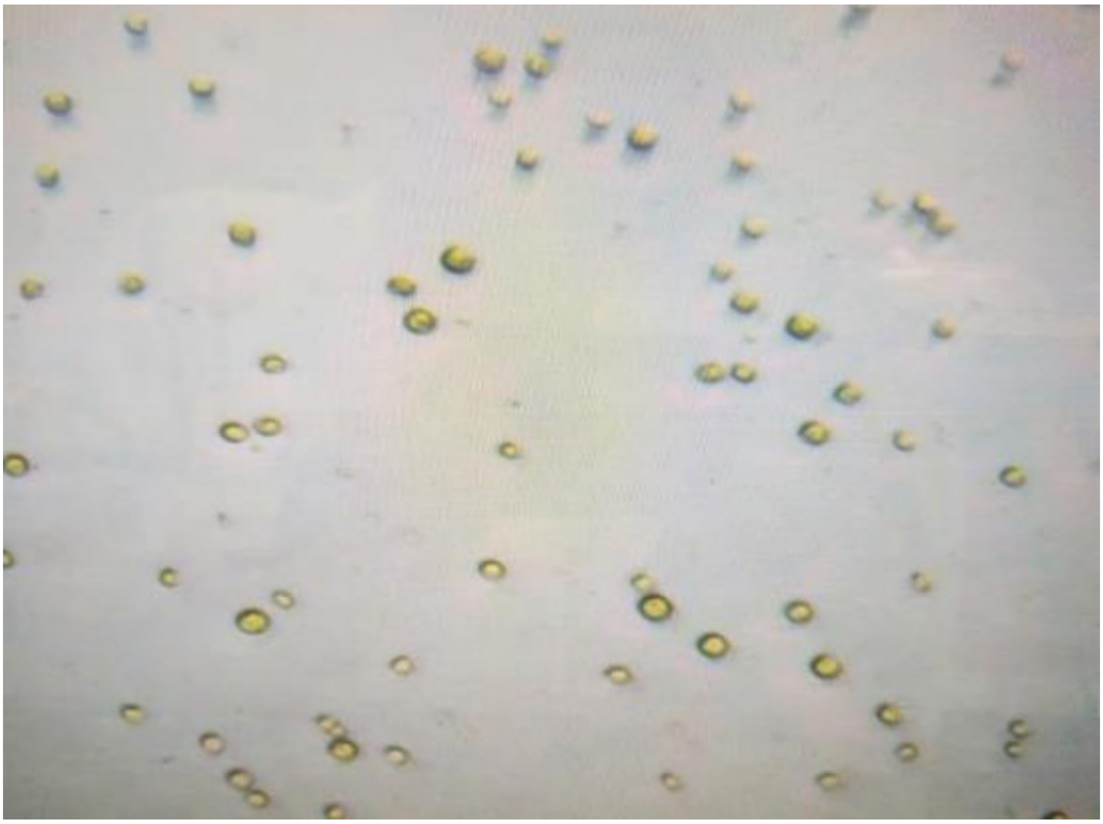


Figure 10 40x magnification of *Nannochloropsis sp* at day 14 of growth before electroflocculation

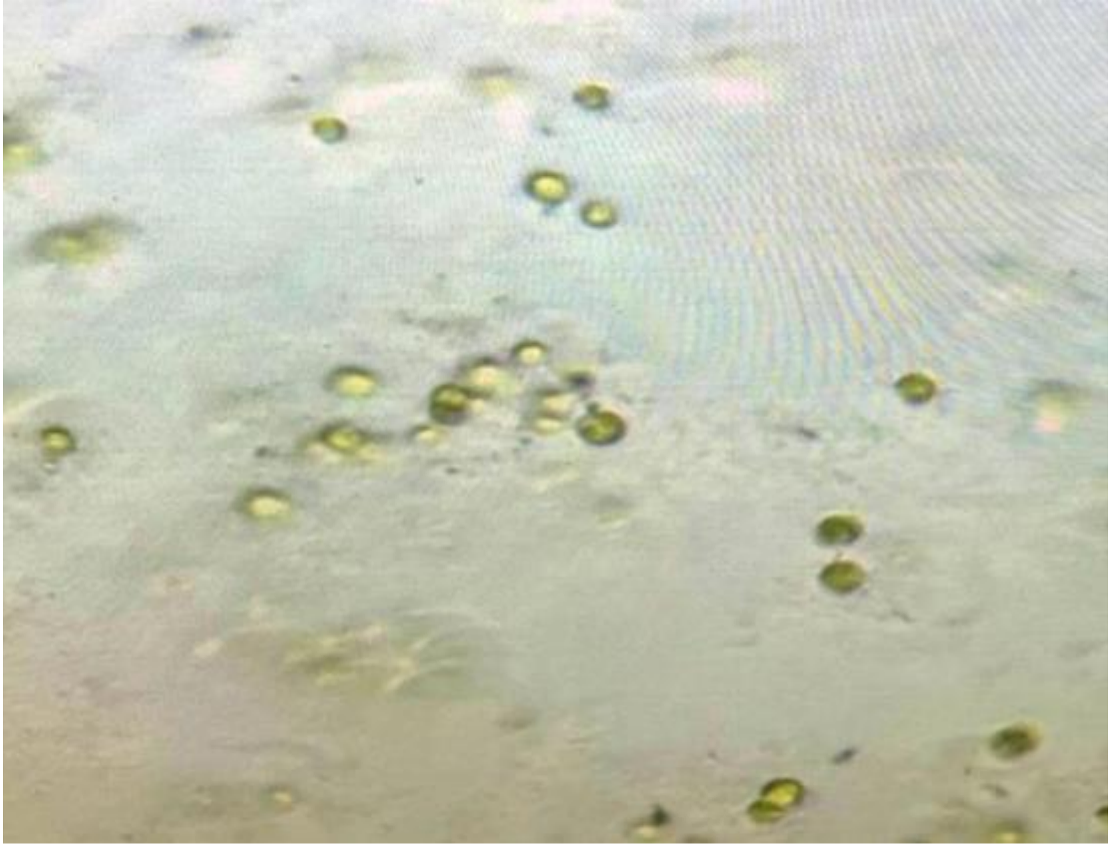


Figure 11 40x magnification of *Nannochloropsis sp* after electroflocculation

With regards to pH, *Nannochloropsis sp* is a marine microalga and its optimum pH is around pH 8 (Kagan & Matulka, 2015; Chua & Schenk, 2017). Previous studies have found that the initial starting pH of the medium in electroflocculation can affect the flocculation efficiency (Cao *et al*, 2010; Garzon-Sanabria *et al*, 2012; Hossain *et al*, 2013). In addition, when studies conducted electroflocculation on microalgae, it was found that longer times led to better flocculation efficiency, and reached the bar of at least 90% biomass recovery from the media and that time plays a very crucial role in how the microalgal biomass clumps together. (Hossain *et al*, 2013; Bleeke *et al*, 2015; Krishnamoorthy *et al*, 2021). Similar studies also found that while electroflocculation can take place at any voltage, higher voltage leads to the 90% recovery of biomass takes place much faster, so the trade off with regards to electric voltage would be the optimisation of energy costs versus the cost of time (Bleeke *et al*, 2015; Xu *et al*, 2021).

Conclusion

The observation of the growth performance of *Nannochloropsis sp* in different types of photobioreactors found Erlenmeyer flasks to be the best kind of photobioreactors for the culture of marine microalgae *Nannochloropsis sp*, with a maximum dry cell weight at 1.024g/L at 14 days. Identification of the ideal electrode material for electroflocculation of *Nannochloropsis sp* was found to be Magnesium because Magnesium showed the highest possible flocculation efficiency in comparison, and the highest final flocculation efficiency in comparison to Aluminium and Carbon. Response surface methodology utilised multiple regression analysis in order to analyse the best conditions for the flocculation of *Nannochloropsis sp* with the independent variables of pH, voltage, and time. The maximum population efficiency was found to be at 29.05 V, pH 8.98, and 27.05 minutes. The results were found to be significant statistically after conducting ANOVA tests as well as fit statistics.

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