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Effects of agricultural grade nutrient modulation on the biomass production and carbon fixation rate of *Isochrysis* sp. microalgae

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ABSTRACT
This study looked at what happens when the nutrients in agricultural grade (AG) medium are changed and how that changes the biomass production and CO2 fixation ability of Isochrysis sp. It aims to address the challenges in establishing biofuel stocks due to the microalgae issue. A medium optimization system (AMOS) was first used to determine the optimum level of nitrogen, phosphorus, and micronutrients in AG medium using Factorial and Box Behnken Experimental Design, which resulted in improvements to N, K, Ca, Mg, Fe, and Z with 15 mM, 10 mM, 0.5 mM, 0.8 mM, 0.3 mM, and 0.15 mM, respectively. Subsequently, the improved medium was tested in a 1L culture volume, resulting in a 2.37 gL^{-1} biomass extracted from cultivation in the improved AG medium compared to cultivation in the traditional F/2 medium (1.63 gL^{-1}). Cultures with higher Ca and Fe tested in an interim study yielded 9% and 7% enhanced biomass production compared to AG medium. The new optimized medium, which is known as TNBR-optimized medium (OM), was tested at the live coal-fired power plant in a 250 L air-lift bubbling column-type photobioreactor supplied with simulated and actual flue gas. The TNBR-optimized medium has demonstrated better algae growth, especially on actual flue gas, which has increased the concentration of CO_2. The improved CO_2 fixation rate was 0.72 $gCO_2.L^{-1}$ day^{-1}, respectively, against those obtained from the previous report – 0.52 gCO_2 L^{-1} day^{-1}. An improved medium has been formulated to cultivate *Isochrysis* sp., and the current work can be further utilized for larger-scale cultivations.

Contribution/Originality: A novel TNBR-optimized medium was formulated by modulating the nutrients of agricultural-grade (AG) medium through a Medium Optimization System (AMOS). TNBR-optimized medium has allowed better growth of *Isochrysis* sp. microalgae, enabling larger-scale cultivations and realizing the dream of making microalgae a feedstock for biofuel or biomass.

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1. BACKGROUND

The world is currently facing the challenge of increasing global fuel production to meet the demand of the ever-growing population. This sparks the idea of transitioning to renewable energy, for extensive exploitation of fossil fuels leads to their depletion and possible extinction due to their exhaustive and non-renewable characteristics (Khan, Shin, & Kim, 2018). The oil spike price during the oil crisis in the 1970s has shifted the focus of biofuel production towards utilizing aquatic species such as microalgae as a source of energy, as they exhibit high economic potential through the production of biomass, biofuel and high-value products (HVP). Examples of strains of microalgae studied for biofuel production include *Scenedesmus sp.*, *Chlorella sp.*, *Dunaliella sp.*, *Nannochloropsis sp.*, *Botrycoccus sp.*, *Spirulina*, *Phaedactylum tricornutum*, *Isochrysis galbana*, *Monodus subterraneus*, *Tetraselmis*, *C. reinhardtii*, and *Neochloris sp.* (Vadivel et al., 2020). Additionally, the effect of climate change caused by releasing Green House Gases (GHG) from mostly anthropogenic sources can be reduced by employing microalgae as a carbon mitigation tool. Compared to terrestrial plants, microalgae convert light and CO_2 into biomass more efficiently (Razzak, Ali, Hossain, & deLasa, 2017).

Nonetheless, the dream of using microalgae as a feedstock for biofuel or biomass is yet to be achieved, for there is a need to resolve the issue of the expensive operating costs of algae farms, which mainly involve cultivation and down-processing equipment. Improving microalgae's photosynthetic efficiency is among the strategies to reduce algal biomass's unitary cost (Vecchi, Barera, Bassi, & Dall'Osto, 2020). Concurrently, photosynthetic efficiency depends on microalgae cultivation at an optimum level of growth conditions and nutrients; hence, optimization of these parameters is necessary.

Algae has an elemental composition of $C_{106}H_{263}O_{110}N_{16}P$ (Zhu et al., 2019). The four most prevalent elements in microalgal biomass are carbon, hydrogen, oxygen, and nitrogen (Heinberg & Fridley, 2010). Additionally, elements such as phosphorus, sulphur, potassium, calcium, sodium, magnesium, and chlorine are required in smaller amounts. On the other hand, micronutrients play an essential role in cellular metabolism and are also needed in trace amounts. These include iron, manganese, copper, cobalt, zinc, molybdate, boron, silicon, selenium, and vanadium (Reynolds, 2006). In developing the most effective microalgae system, precise nutrient requirements for optimal growth are necessary in the sense that any nutrients present in limited or excess will be eliminated. Designing an optimized culture medium is critical for microalgal biomass production, as the precise composition can significantly affect biomass yield and quality. The availability of a defined optimal medium will enhance the carbon fixation ability and facilitate the reproducible production of microalgal biomass and premium products such as biofuels and high-value products (Stephens, Wagner, Ross Liam, & Hankamer, 2012). The medium compositions also determine the cost and complexity of the downstream purification of products. Other critical factors affecting microalgal biomass productivity include light intensity, cycling time, aeration speed, pH, temperature, dissolved oxygen and CO_2, and tolerance to toxic substances that accumulate in the culture medium. Of these, light and carbon supply (CO_2 for photoautotrophic growth and organic carbon sources for photoheterotrophic growth) are usually the most critical.

In this research, medium optimization of *Isochryis* sp. was carried out by employing a simple Full–Factorial and Box–Behnken Experimental Design to improve nutrient composition in the medium via an Automated Medium Optimization System (AMOS) as developed by Radzun et al. (2015). The design is equipped with two screening systems: a) Screen 1 (application of simple Full–Factorial to determine different types of Nitrogen and Phosphate sources); and b) Screen 2 (application of Box–Behnken Design to determine the main effects of macro- and microelements). Consequently, the newly formulated medium obtained through this system was subjected to a reproducibility test in larger-scale cultivation, and the carbon fixation efficiency of the microalgae grown in the newly improved medium was analyzed.

2. DATA AND METHODS

2.1. Organism and Medium Composition

The microalga *Isochrysis* sp. obtained from the Tenaga National Berhad Research Sdn. Bhd. (TNBR), Malaysia was pre-adapted and maintained in Tris - Acetate - Phosphate - NaCl medium (TAP - NaCl) using research-grade nutrients (15 mM NH_4Cl, 0.35 mM $CaCl_2.2H_2O$ (*Univar, USA*), 0.4 mM $MgSO_4.7H_2O$ (*Univar, USA*), 0.82 mM Na_2HPO_4 (*Sigma, USA*), 0.45 mM KH_2PO_4 (*Scharlau, Spain*), 184 uM H_3BO_3 (*AnalaR, United Kingdom*), 25.8 uM $MnCl_2.H_2O$ (*Univar, USA*), 18 uM $Fe_2(SO_4)_3.7H_2O$ (*Univar, USA*), 6.7 uM $CoCl_2.6H_2O$ (*Government Stores Department, Australia*), 6.4 uM $CuSO_4.5H_2O$ (*Chem-Supply, Australia*), 0.89 uM $(NH_4)_6MoO_4.4H_2O$ (*Univar, USA*), 20 mM Tris (*Amresco, Canada*) and 14 mM CH_3COOH (*Lab Scan, Analytical Science, Poland*) and 500 mM NaCl (Sigma, USA). The inoculum for optimization study was transferred to MRL (Microalgae Research Lab) Agricultural Grade (AG) medium, which has similar nutrient compositions to the TAP - NaCl medium. The culture was grown in 1 L conical flasks at constant 24-hour illumination (120 µmolphotons.$m^{-2}s^{-1}$ white light) with an agitation speed of 150 to 160 rpm (using *FINEPCR 2D400 Shaker, FINEPCR Co., Ltd, Korea*) at a temperature of ~25 °C. Cell density was measured and monitored daily

in 96 microwell plates (*Nunclon Delta Surface, Nunc, Denmark*) and read using a microwell plate reader (*Infinite M200 PRO series plate reader, Tecan Group Ltd.)* based on optical density at 560 nm (OD_{560}).

2.2. Nutrient Optimization via Automated Media Optimization System (AMOS)

The growth performance of *Isochrysis* sp. was optimized via Screen 1 and Screen 2 of AMOS. Screen 1 is based on incomplete factorial-designed experiments to optimize nitrogen and phosphate concentrations. Nitrogen and Phosphate concentrations that yielded the highest growth and biomass were used as constants for Screen 2. Various Nitrogen and Phosphate sources tested in this study could not be revealed due to agreements with TNBR Sdn. Bhd. Box–Behnken Experimental Design was employed in Screen 2 with varied concentrations of Ca, Mg, Cu, Fe, Zn, Mn, Mo, Se, V, and Si. Each element was set at three levels (-1, 0, +1) for low, medium, and high. The experiment was conducted in 96-well plates cultivated in the AMOS for 72 hours.

2.3. Growth Rate Determination

Screens 1 and 2 aimed to define the growth conditions that yield biomass production's highest growth rate (μ, h-1) (measured in optical density). To achieve this, the highest growth rate of each condition in each well is calculated using the formula below. The rate was then determined from the slope of the log plot:

$$u = \frac{\ln (OD560\,(2) - OD560\,(1))}{t(2) - t(1)} \quad (1)$$

Where;

μ= growth rates, OD560(2) = OD560 at time 2, OD560(1) = OD560 at time 1, t(2) = time 2 (hour), and t(1)= time 1 (hour).

2.4. Biomass

A new, improved media formulated based on screens 1 and 2 was tested in a 1L culture volume for reproducibility assessment. The culture was grown for 15 days. Algal biomass obtained after 15 days was centrifuged and heat-dried (in a hot-air oven at 65℃) for 1 week until the dry weight reached constant. The biochemical content of *Isochrysis* sp. was also conducted at UNIPEQ, Universiti Kebangsaan Malaysia (UKM).

2.5. Statistical Analysis

The main effect of individual elements tested in Screen 2 was analyzed using the main plot effect from Response Surface Analysis. The analysis was conducted on each media formulation, involving three levels of concentration coded as -1, 0, and 1 for every element.

2.6. Growth Rate and Carbon Fixation Rate Calculation

The biomass weight method assessed the microalgae carbon fixation activity, in which the CO_2 fixation rate is assumed and estimated to be in the form of biomass dry weight. The calculation sequence is as follows:

1) Calculating Growth Rate Based on Biomass Dry Weight:

$$g = \frac{W(2) - W(1)}{t(2) - t(1)} \quad (2)$$

Where, W = dry weight ($g.L^{-1}$) of biomass at point 1 and 2 and t = times (day)

2) Carbon Dioxide Absorption Rate Given by Ratio of CO_2: Algae Biomass:

$$1.882g = \frac{1 \times g \times MWc}{MWb} \quad (3)$$

Where, W = dry weight ($g.L^{-1}$) of biomass at point 1 and 2 and t = times (day)

3. RESULTS AND DISCUSSIONS

3.1. Optimization of MRL Agricultural–Grade Medium to Enhance the Growth of Isochrysis sp. using AMOS

Microalgae may achieve maximum growth rates or anything approaching them with appropriate optimization of the macroelement nutrients using generally accepted, adequate micronutrients. Agri-Grade (AG) medium MRL is an economical growth medium proposed in this study to replace the standard cultivation medium (F/2) that TNBR Sdn. Bhd. previously used to cultivate *Isochrysis* sp. based on more expensive research-grade nutrients. Screen 1 was designed as a simple full factorial in this research to test the effects of varying nitrogen and phosphate types and concentrations on the biomass yield and carbon dioxide fixation rate. The experimental design used here was significantly more extensive and involved four different types of nitrogen and one phosphate source. The first nutrient optimization process was conducted using Screen 1 on AMOS based on an incomplete Factorial Experimental Design, where various concentrations of nitrogen and phosphate were tested to determine their optimal concentrations as observed by the growth performance of *Isochrysis* sp. microalgae. Nitrogen (N) and Phosphate (P) are critical macroelements that must be supplied adequately to support algal growth. In this study, the optimized concentration of N and P were determined first so that these elements would be present in sufficient amounts for subsequent optimization of the rest of the micronutrients. Figure 1 shows the surface chart representing the data for each medium formulation with varying concentrations of N and P sources in the Screen 1 experiment.

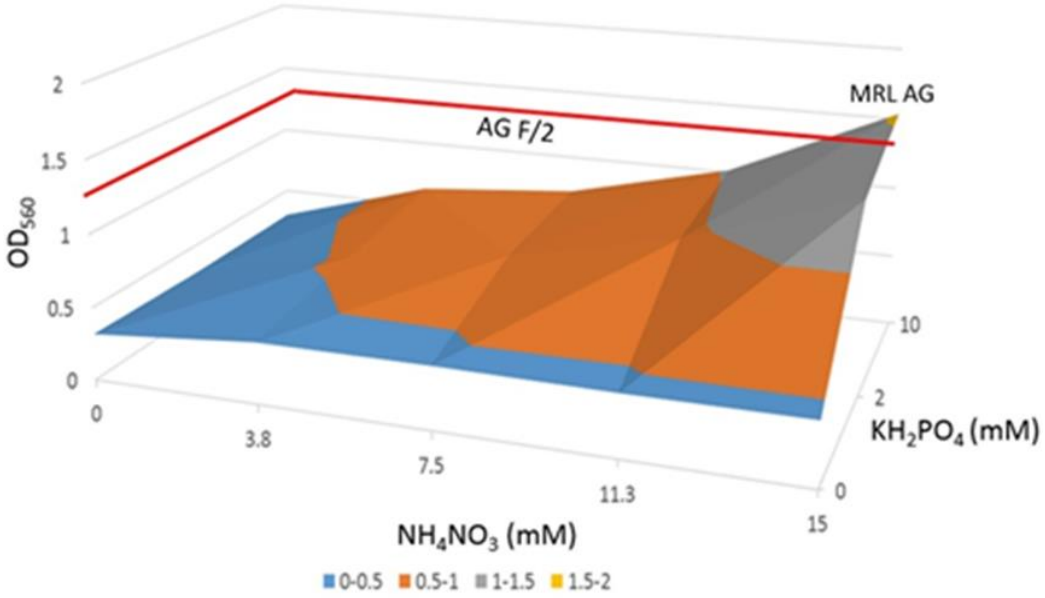

Figure 1. Surface chart representing the data for each medium formulation with varying concentration of N and P sources in screen 1 experiment.

The result presented in Figure 1 registered the highest end-point OD_{560} (1.5367) when the culture was supplied with 15 mM NH_4NO and 10 mM KH_2PO_4 as opposed to the control AG F/2 (red line) medium, which registered an end-point OD_{560} of 1.3830. Meanwhile, the growth rates for cultivation in the MRL AG medium and the control were calculated to be 0.042 h^{-1} and 0.04 h^{-1}, respectively. It is also important to note that the microalgae were pre–grown in research grade TAP – NaCl medium, by which the nitrogen supplied was NH_4Cl. The result indicates that the algae was able to adapt to the new N source and even yielded a higher end point OD_{560} of approximately 10% higher than the AG F/2 control medium. Generally, NH_4 is the preferred N source for most algae as it can be directly incorporated into amino acids, while other N sources, such as nitrite and nitrate, must first be reduced to ammonium before they can be utilized. While NH_4 is found to be a suitable N source for *Isochryis* sp. growth, NH_4 may be toxic at a certain level.

Screen 2 was subsequently conducted based on Screen 1 to determine the optimal concentration of other macro- and microelements. Screen 2 highlighted the application of Box–Behnken experimental design to determine the most or nearly optimal concentration, yielding a higher growth productivity of Isochrysis sp. microalgae. Each factor or independent variable is placed at one of three equally spaced values to achieve this. At least 3 levels are needed to fit a quadratic model that contains squared terms and products of two factors. The three levels are coded as -1, 0, and 1, representing the variable or treatment at low, centre/middle (average), and high values, respectively. In this study, Screen 2 was devised to optimize combinations of 10 nutrients, including calcium, magnesium, boron, iron, copper, manganese, zinc, selenium, vanadium, and silicon concentrations at 3 different concentrations (-1, 0, and 1 corresponding to low, middle, and high concentration, respectively), which were provided across a 4-fold concentration range – i.e., 0.5x, 1x and 2x the average literature value) at the best nitrogen and phosphate levels identified in Screen 1. The Box-Behnken design was chosen for the development of Screen 2 because the increased number of variables (10 nutrients with 3 concentration levels) means that the full-factorial design becomes infeasible (it would require 3^{10} experiments as generated by the MiniTab 15 software (MiniTab Inc, USA).

In Screen 2, the cultures were supplied with a constant amount of NH_4NO_3 (15 mM) and KH_2PO_4 (10 mM), while other elements were varied. Figure 2 illustrates the growth of *Isochrysis* sp. in the Screen 2 media formulation. In the Screen 2 system, the variables are Ca, Mg, Cu Fe, Zn, Mn, Mo, Se, V, and Si concentration, supplied at 3 levels, i.e., +1 (high level), 0 (medium level), and -1 (low level).

The main effect of each element from the response surface analysis portrayed in Figure 2 showed a significant increment (double in amount) in Ca, Mg, Fe, and Zn concentration compared to cultivation in the initial AG F/2 medium, with a reported growth rate of 0.06 h^{-1} and 0.04 h^{-1} respectively. 0.5 mM $CaCl.2H_2O$, 0.8 mM $MgSO_4.7H_2O$, 0.3 mM $Fe_2(SO_4)_3.7H_2O$, and 0.15 mM $ZnSO_4.7H_2O$ were observed to be the optimum concentrations for better growth of *Isochrysis* sp. The concentration of other microelements essential for *Isochrysis* sp. growth did not show significant increments, suggesting that the amount subject to growth is sufficient for optimum growth. Interestingly, the OD reading dropped at a higher silicon concentration, suggesting that Si impedes growth. Through *Screen 2*, improvements were made to Ca, Mg, Fe, and Zn, as these elements were shown to be in limitation. Ca and Fe are of interest, as previous studies conducted using an Automated Media Optimization System showed that high calcium and iron concentrations have significantly improved the growth performance of various microalgae species (Radzun et al., 2015).

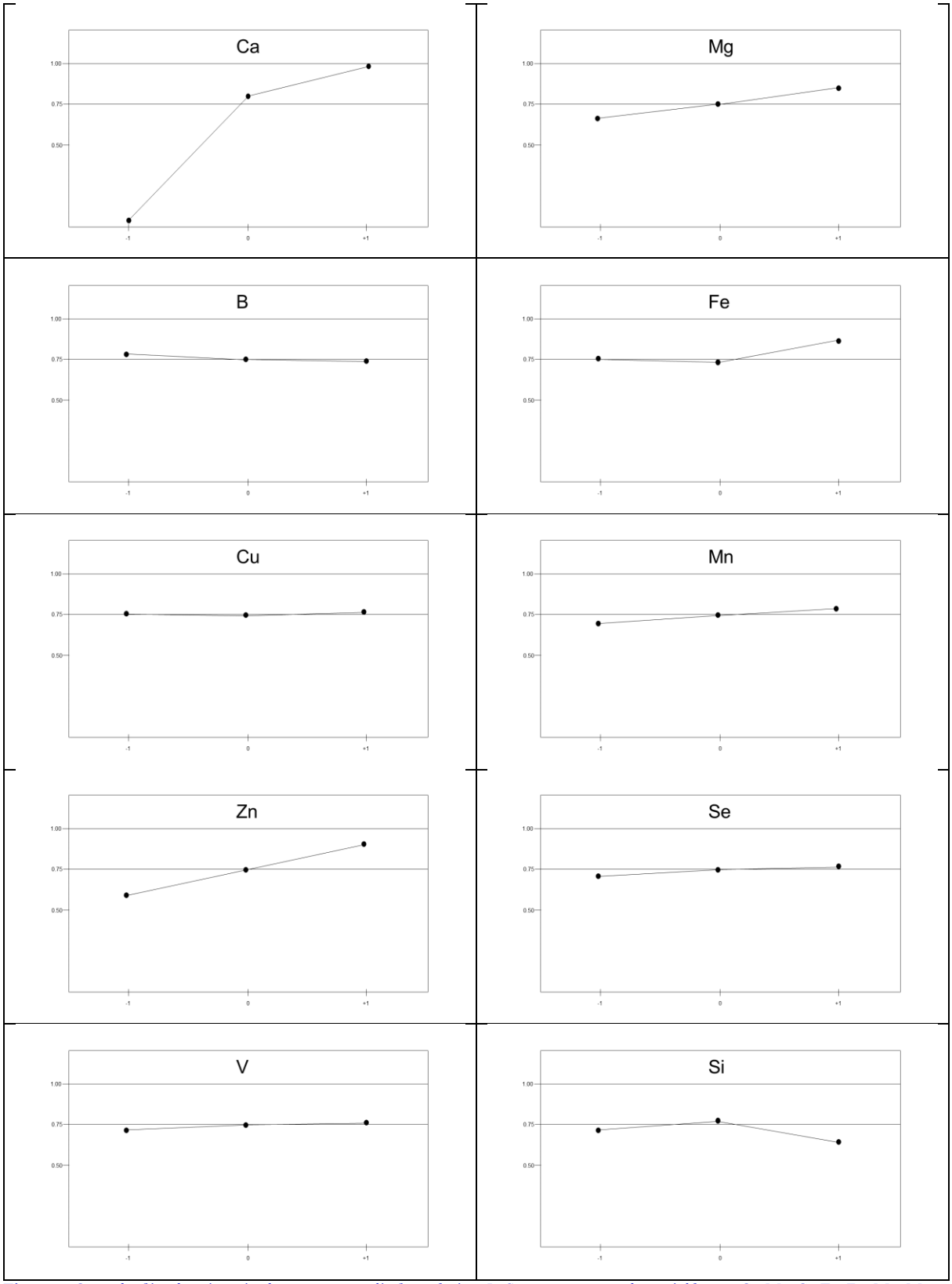

Figure 2. Growth of isochrysis sp. in the screen 2 media formulation. In Screen 2 system, the variables are Ca, Mg, Cu Fe, Zn, Mn, Mo, Se, V and Si concentration, supplied at 3 levels i.e., +1 (high level), 0 (medium level) and -1 (low level).

In another experiment set, culture supplied with high Ca yielded 4.32 g/L biomass, a 9% increment in biomass production. Calcium has been reported to play a role in the flexibility of cell walls in plants, and the same is assumed for microalgae. In general, calcium performs cross–linking with negatively charged pectin polymers of the cell wall. Excess calcium in the system is thought to limit cell expansion, increase cell rigidity, and inhibit growth. Low calcium concentrations improves cell wall flexibility, allowing room for cell expansion and division (Hanifzadeh, Garcia, & Viamajala, 2018). While this study suggests a higher Ca concentration for enhanced productivity, it is always good to define the most optimal concentration to avoid growth inhibition due to excessive or deficiency of such.

Meanwhile, iron was supplied at a higher concentration in this experiment and yielded an increment of 7% from the culture in the MRL AG medium, as postulated in Figure 3. Figure 3 highlights the growth performance analysis of *Isochrysis* sp. in the interim media. The medium formulation of Flask A represents a medium containing high Ca, while Flask B represents a medium with high Fe.

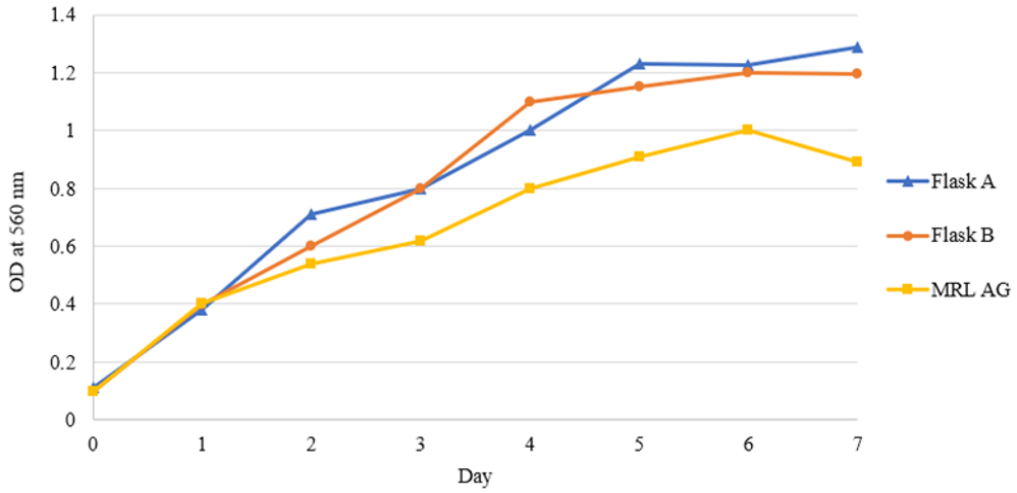

Figure 3. Growth performance analysis of Isochrysis sp. in interim media. medium formulation of flask a represents medium containing high amount of ca while flask b represents medium with high Fe.

Being a cofactor for about 140 enzymes, iron is an essential nutrient for the general growth of all living organisms. In photosynthetic organisms, iron participates in the biosynthesis of chlorophyll and thylakoids and the development of chloroplasts. Iron depletion in the system leads to simultaneous chlorophyll loss and chlorophyll structure degradation (Ermis, Guven-Gulhan, Cakir, & Altinbas, 2020). Iron is needed for catalytic activities, the electron transport system, taking in nitrate and nitrogen, and making hydrogen through photosynthesis at the cellular level (Sabzi, Shamsaie Mehrgan, Rajabi Islami, & Hosseini Shekarabi, 2021). Additionally, iron protects Reactive Oxygen Species (ROS) in higher plants, algae, and prokaryotes by binding to superoxide dismutase (SODs) (Abdoli, Ghassemi-Golezani, & Alizadeh-Salteh, 2020). Though it is essential to note that the bioavailability of iron in phytoplankton and algal environments is often debatable, its solubility, especially in a mildly alkaline condition, is extremely low (Yarimizu, Cruz-Lopez, & Carrano, 2018). The findings of this study suggest that a higher concentration of Fe is needed to enhance the growth of *Isochyrsis* sp. This may explain the solubility problem of iron when it is supplied at a lower concentration; hence, more iron is required. These findings suggest that *Isochrysis sp.* used in this study can tolerate high Ca and Fe, yielding more biomass. Nonetheless, it is anticipated to know the combined effect of both high Ca and Fe on the algae's growth.

3.2. Biomass Quantification

A novel cultivation medium, namely TNBR – OM medium, was formulated according to Screen 1 and Screen 2 data. The medium was tested for reproducibility analysis and biomass production in a 1 Litre culture volume, and the growth was recorded after 15 days. Cultivation of *Isochrysis* sp. was generated as in Table 1 and compared with the AG F/2 medium.

Table 1. Dried biomass obtained from TNBR – OM and AG F/2 media and respective biochemical composition. Dried biomass samples. dried biomass samples from cultivation in TNBR – OM and AG F/2 Media were also analyzed for carbohydrate, protein and lipid at UKM UNIP.

Medium	TNBR - OM	AG F/2
Dried biomass (g/L)	2.37	1.63
Percent of carbohydrate (%)	13.7	13.2
Percent of protein (%)	11.5	3.1
Percent of fat (%)	0.2	0.0

Isochrysis sp. in TNBR – OM exhibited a 45% biomass increment compared to the cultivation in AG F/2. Meanwhile, biochemical analysis of the dried biomass obtained from cultivation in the new medium and AG F/2 medium yielded high carbohydrate and protein contents of 23% and 13.7%, respectively. *Isochrysis sp.* is one of the promising candidates for biofuel production because it can store excess carbon as a lipid (Anto, Karpagam, Renukadevi, Jawaharraj, & Varalakshmi, 2019). Contrastingly, *Isochrysis sp.*, in this study, did not yield as much lipid as anticipated. Nonetheless, high-dry biomass obtained via cultivation in the new, improved medium can be used as feedstock for biogas and animal feed production. It is also important to note that the TNBR - OM produced higher biomass with a significant amount of protein composition compared to the AG F/2 medium.

3.3. Effects of Microalgae Growth and CO_2 Fixation Rate Supplemented with Flue Gas

The availability of nutrients in the culture affects how much carbon microalgae consume and fix (Huang, Luo, Xu, & Wang, 2019). Additionally, an optimal amount of CO_2–enriched air must be supplied to the system in order to achieve maximum growth potential (Thomas, Mechery, & Paulose, 2016). Though CO_2 obtained from the environment would not be sufficient to support maximum yield due to the low concentration of CO2 in the air (360 ppm). The main reason is that O_2 and CO_2 compete for the active site of Rubisco in Calvin–Benson Cycle (Kang et al., 2018). In most cases, the probability of O_2 binding to Rubisco is higher, hindering carbon fixation from occurring (Way, Dusenge, & Duarte, 2019); hence, increasing the concentration of CO_2 can increase the rate of CO_2 fixation. Flue gas was chosen as a source of CO_2 in this study due to a higher CO_2 concentration of up to 20% (Cuellar-bermudez, Garcia-Perez, Rittmann, & Parra-Saldivar, 2014), while waste flue gases from combustion processes typically contain more than 15% CO_2, which, in principle, will provide sufficiently high CO_2 concentrations for macroscale production of microalgae. Many flue gas mitigation strategies have been discussed using microalgae, but as the cost of preliminary separation of CO_2 gas is high, direct utilization of power plant flue gas has been considered in microalgal biofuel production systems due to being economical (Thomas et al., 2016).

To find out how well Isochyris sp. grown in the new TNBR medium took up carbon, the algae cultures were given either simulated flue gas (4% CO2 + other gases) or real TNBR flue gas. Figure 4 indicates the effect of simulated and actual flue gas on the growth of *Isochrysis* sp. cultivated using AG F/2 and TNBR-Optimized Medium (OM).

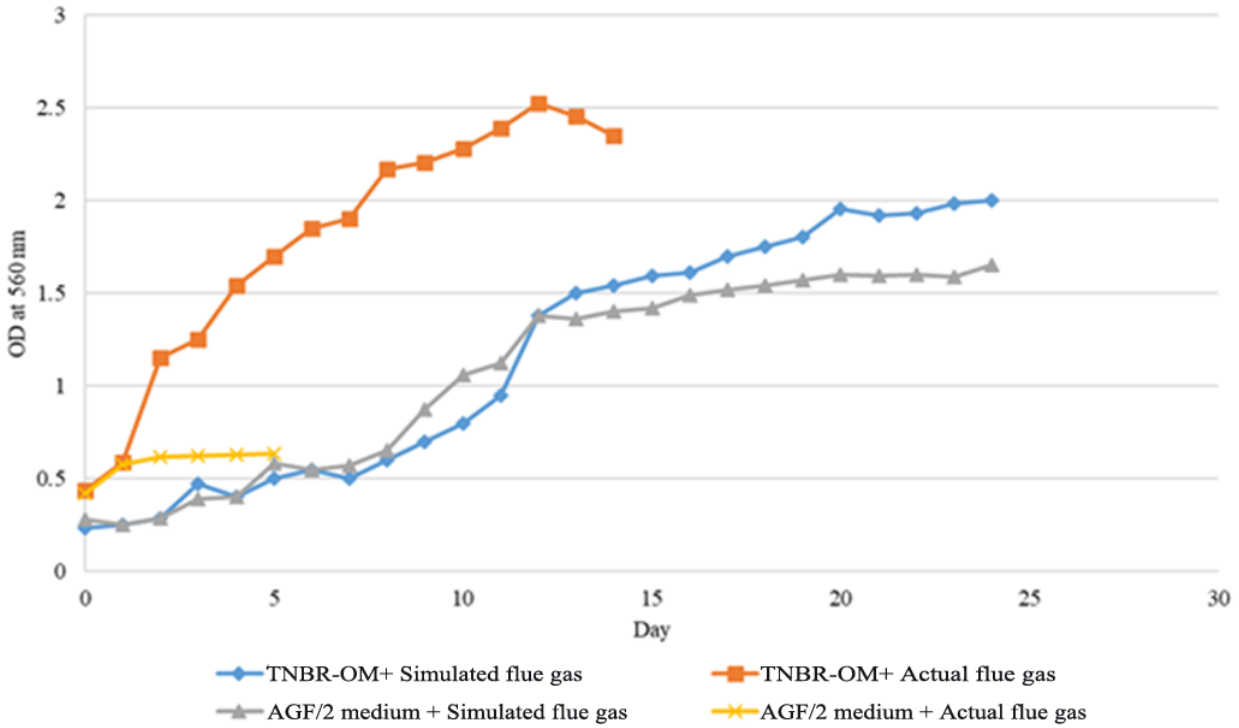

Figure 4. Effect of simulated and actual flue gas on the growth of isochrysis sp. Cultivated using AG F/2 and TNBR - Optimized medium (OM).

Under simulated flue gas at TNBR's Algae Park, TNBR – OM registered a specific growth rate of 0.1703 compared to that of AG F/2 of 0.1418 – a 20% improvement. While carbon is among the essential nutrients in microalgal growth, a certain level of carbon dioxide may inhibit their growth. Conversely, the growth performance was 90% better when the algae were exposed to flue gas at the power station in TNB Janamanjung. Most microalgae can fix a concentration of 1 -10% carbon dioxide. Flue or exhaust gas typically exhibits a 10–30% carbon dioxide concentration, which is somewhat higher and may be toxic for most microalgae. Interestingly, *Isochrysis* sp. grown in the TNBR – OM grew in actual flue gas, suggesting that this species may have a high tolerance to high CO_2 concentrations. It was also observed that the maximum optical density (OD) was achieved within 2 weeks compared to other cultures in different cultivation conditions, which took almost a month to complete the growth cycle. A review of the carbon fixation rate is presented in Table 2.

Table 2. Chemical use and cost of nutrients.

Medium	Old AG F/2 medium	TNBR – OHM medium
Total media (Chemicals) costs for the facility per year	RM 6,6630 /USD 1527	RM2,324/ USD 78
Total amount of CO2 fixed per year	234 kg	324 kg
Cost of media to fix	RM27(USD 6.14) / kg	3.1

Cultivation of *Isochrysis* sp. in TNBR – OM medium recorded the highest carbon fixation rate of – 0.72 g/L.day^{-1}. Interestingly, the rate obtained from this study was higher than the previous work reported using the same species

with a carbon fixation rate of 0.32 g/L.day^{-1} (Nagajyoti, Lee, & Sreekanth, 2010). This observation suggests two important consequences. Firstly, more CO_2 can be fixed biologically. With better growth and CO_2 fixation rates using the new nutrient medium, the anticipated amount of CO_2 that can be fixed annually is 324 kg, compared to 234 kg with AG F/2 medium. These are based merely on the current culture volume pilot capacity existing at TNBJ, i.e., 6 x 250 litres = 1,500 litres. Secondly, the new media offers reduced chemicals and fixed costs per kg of CO_2, which are close to four times cheaper.

4. CONCLUSION

In this study, we provide a new method of improving cultivation medium using a high-throughput screening-based - Automated Microalgae Nutrient Screening System. A new, improved cultivation medium for *Isochrysis* sp. was successfully formulated and tested in a TNBR power plant using a 250 L photobioreactor to determine the growth and carbon fixation rate of *Isochrysis* sp. microalgae. To conclude, cultivation media and other environmental factors are imperative to achieve higher microalga growth and biomass production. Furthermore, using an efficient and lower-cost culture medium may reduce the economic cost of microalgae cultivation. Determining optimal cultivation parameters (such as pH, agitation, and flue gas concentrations) and system (photobioreactor) are essential areas of future research to ensure the maximum productivity of microalgae.

ABBREVIATIONS

AG: Agricultural Grade; AMOS: Automated Medium Optimization System; GHG: Greenhouse Gases; HVP: High-value Products; MRL: Microalgae Research Lab; OD: Optical Density; OM: Optimized Medium; ROS: Reactive Oxygen Species; SODs: Superoxide Dismutase; TAP - NaCl: Tris - Acetate - Phosphate - NaCl medium and TNBR: Tenaga National Berhad Research Sdn. Bhd.

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Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.
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REFERENCES

Abdoli, S., Ghassemi-Golezani, K., & Alizadeh-Salteh, S. (2020). Responses of ajowan (Trachyspermum ammi L.) to exogenous salicylic acid and iron oxide nanoparticles under salt stress. *Environmental Science and Pollution Research*, *27*(29), 36939-36953. https://doi.org/10.1007/s11356-020-09453-1

Anto, S., Karpagam, R., Renukadevi, P., Jawaharraj, K., & Varalakshmi, P. (2019). Biomass enhancement and bioconversion of brown marine microalgal lipid using heterogeneous catalysts mediated transesterification from biowaste derived biochar and bionanoparticle. *Fuel*, *255*, 115789. https://doi.org/10.1016/j.fuel.2019.115789

Cuellar-bermudez, S. P., Garcia-Perez, J. S., Rittmann, B. E., & Parra-Saldivar, R. (2014). Photosynthetic bioenergy utilizing CO : An approach on fl ue gases utilization for third generation biofuels. *Journal of Cleaner Production*, *98*, 53–65.

Ermis, H., Guven-Gulhan, U., Cakir, T., & Altinbas, M. (2020). Effect of iron and magnesium addition on population dynamics and high value product of microalgae grown in anaerobic liquid digestate. *Scientific Reports*, *10*(1), 1-12. https://doi.org/10.1038/s41598-020-60622-1

Hanifzadeh, M., Garcia, E. C., & Viamajala, S. (2018). Production of lipid and carbohydrate from microalgae without compromising biomass productivities: Role of Ca and Mg. *Renewable Energy*, *127*, 989–997. https://doi.org/10.1016/j.renene.2018.05.012

Heinberg, R., & Fridley, D. (2010). The end of cheap coal. *Nature*, *468*(7322), 367-369. https://doi.org/10.1038/468367a

Huang, Y., Luo, L., Xu, K., & Wang, X. C. (2019). Characteristics of external carbon uptake by microalgae growth and associated effects on algal biomass composition. *Bioresource Technology*, *292*, 121887. https://doi.org/10.1016/j.biortech.2019.121887

Kang, T., Wu, H., Lu, B., Luo, X., Gong, C., & Bai, J. (2018). Low concentrations of glycine inhibit photorespiration and enhance the net rate of photosynthesis in Caragana Korshinskii. *Photosynthetica*, *56*(2), 512-519. https://doi.org/10.1007/s11099-017-0688-1

Khan, M. I., Shin, J. H., & Kim, J. D. (2018). The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products *Microbial Cell Factories*, *17*(1), 1–21.

Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. (2010). Heavy metals, occurrence and toxicity for plants: Areview. *Environmental Chemistry Letters*, *8*(3), 199-216. https://doi.org/10.1007/s10311-010-0297-8

Radzun, K. A., Wolf, J., Jakob, G., Zhang, E., Stephens, E., Ross, I., & Hankamer, B. (2015). Automated nutrient screening system enables high-throughput optimisation of microalgae production conditions. *Biotechnology for Biofuels*, *8*(1), 1-17. https://doi.org/10.1186/s13068-015-0238-7

Razzak, S. A., Ali, S. A. M., Hossain, M. M., & deLasa, H. (2017). Biological CO2 fixation with production of microalgae in wastewater–a review. *Renewable and Sustainable Energy Reviews, 76*, 379-390. https://doi.org/10.1016/j.rser.2017.02.038

Reynolds, C. S. (2006). The ecology of phytoplankton. In (pp. 535-590). Cambridge: Cambridge University Press.

Sabzi, S., Shamsaie Mehrgan, M., Rajabi Islami, H., & Hosseini Shekarabi, S. P. (2021). Changes in biochemical composition and fatty acid accumulation of Nannochloropsis oculata in response to different iron concentrations. *Biofuels, 12*(1), 1-7. https://doi.org/10.1080/17597269.2018.1489672

Stephens, B., Wagner, E., Ross Liam, L., & Hankamer, L. (2012). 1 introduction – integration in microalgal biotechnology, in Microalgal Biotechnology: Integration and Economy, no. December, C. Posten, Clemens; Walter, Ed. In (pp. 267–306). Berlin: Walter De Gruyter.

Thomas, D. M., Mechery, J., & Paulose, S. V. (2016). Carbon dioxide capture strategies from flue gas using microalgae: A review. *Environmental Science and Pollution Research, 23*(17), 16926-16940. https://doi.org/10.1007/s11356-016-7158-3

Vadivel, M., Arvindnarayan, S., Kumar, G., Shobana, S., Dharmaraja, J., Nguyen, D. D., . . . Prabhu, K. K. S. (2020). Biodiesel potentiality of microalgae species: Evaluation using various nitrogen sources. *Waste and Biomass Valorization, 11*, 1671-1679. https://doi.org/10.1007/s12649-018-00552-2

Vecchi, V., Barera, S., Bassi, R., & Dall'Osto, L. (2020). Potential and challenges of improving photosynthesis in algae. *Plants, 9*(1), 1-25. https://doi.org/10.3390/plants9010067

Way, D. A., Dusenge, M. E., & Duarte, A. G. (2019). Plant carbon metabolism and climate change: Elevated CO 2 and temperature impacts on photosynthesis, photorespiration and respiration. *New Phytologist, 221*(1), 32-49. https://doi.org/10.1111/nph.15283

Yarimizu, K., Cruz-Lopez, R., & Carrano, C. J. (2018). Iron and harmful algae blooms: Potential algal-bacterial mutualism between Lingulodinium polyedrum and Marinobacter algicola. *Frontiers in Marine Science, 5*, 1-12. https://doi.org/10.3389/fmars.2018.00180

Zhu, L., Li, S., Hu, T., Nugroho, Y. K., Yin, Z., Hu, D., . . . Hiltunen, E. (2019). Effects of nitrogen source heterogeneity on nutrient removal and biodiesel production of mono-and mix-cultured microalgae. *Energy Conversion and Management, 201*, 112144. https://doi.org/10.1016/j.enconman.2019.112144