NITRATE SOURCE OPTIMIZATION FOR MICROALGAE CULTIVATION FOR BIOFUELS PRODUCTION

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ABSTRACT

In response to the increasing global demand for sustainable energy, microalgae have emerged as a promising source for biofuel production due to their rapid growth rates, high lipid content, and adaptability to various environmental conditions. This study examines the effects of different nitrate sources on the growth of Tetradesmus obliquus microalgae, aiming to optimize biomass yield and biofuel synthesis, particularly biodiesel and green kerosene. The investigation involves three nitrate sources – sodium nitrate, magnesium nitrate, and urea – to understand their impact on microalgal cultivation. Daily monitoring of cell count and pH levels provides insights into growth kinetics and culture health. The dynamic relationship between nitrate sources, concentration, and these parameters is analyzed to identify trends and potential nutrient limitations. After a 10-day cultivation period, final biomass content is determined, and samples are analyzed for lipid content using the saponification method. Results reveal the influence of nitrate sources on microalgal growth and lipid accumulation. Despite urea biomass having a higher lipid fraction (5.63%), it has the lowest total biomass concentration (1.690 g.L^{-1}). In contrast, magnesium nitrate resulted in a higher final biomass concentration (2.406 g.L^{-1}) and lipid productivity (0.129 g.L^{-1}). This research contributes to optimizing microalgal cultivation, enhancing the adaptability of cultivation methods, and informing future sustainable bioenergy initiatives.

1. INTRODUCTION

The global demand for sustainable and renewable energy sources has driven significant interest in alternative biofuels. Microalgae have emerged as a promising candidate for biofuel production due to their rapid growth rates, high lipid content, and adaptability to various environmental conditions. Unlike terrestrial crops used for biofuels, microalgae do not compete with food production and can be cultivated on non-arable land, utilizing wastewater or saline water, which enhances their sustainability profile (Chisti, 2007; Alvarez et al., 2021; Zhang; Cai, 2018).

Microalgae are photosynthetic organisms that can convert sunlight, carbon dioxide, and nutrients into biomass and lipids, the latter of which can be converted into biodiesel and other biofuels. The lipid content of microalgae is a critical factor in determining their viability as a biofuel source. Lipids are primarily stored in the form of triglycerides, which can be transesterified to produce biodiesel. Optimizing the growth conditions and nutrient supply for microalgae can significantly enhance lipid accumulation, thus improving the efficiency and economic feasibility of biofuel production (Chisti, 2007; Rampi et al., 2023).

Microalgae can grow under different nutritional modes: autotrophic, heterotrophic, and mixotrophic. Autotrophic growth relies solely on photosynthesis, where microalgae convert sunlight, carbon dioxide, and inorganic nutrients into biomass. This mode is energy-efficient and sustainable but may be limited by light availability. Heterotrophic growth, on the other hand, uses organic carbon sources such as glucose or acetate, allowing microalgae to grow in the dark.

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While this can lead to higher biomass yields, it requires external organic carbon, increasing production costs. Mixotrophic growth combines both autotrophic and heterotrophic modes, allowing microalgae to utilize both light and organic carbon sources. This flexible approach can enhance biomass and lipid production, making it an attractive option for large-scale cultivation (Vo et al., 2019; Costa et al., 2022).

Nutrient availability, particularly nitrogen, plays a crucial role in microalgal growth and lipid accumulation. Nitrogen is a key component of cellular proteins and nucleic acids, and its availability can influence the metabolic pathways of microalgae. Different nitrogen sources, such as sodium nitrate, magnesium nitrate, and urea, can have varying effects on the growth kinetics and lipid production of microalgae. Understanding these effects is essential for optimizing cultivation conditions and maximizing biofuel yields (Costa et al., 2022).

Previous studies have indicated that nitrate availability can regulate lipid biosynthesis in microalgae. For instance, nitrogen limitation often triggers lipid accumulation as a survival strategy, although it may reduce overall biomass production. Therefore, balancing nitrogen supply to optimize both biomass and lipid yield is a critical aspect of microalgal biofuel research (Nagappan et al., 2019).

The aim of this study is to investigate the effects of different nitrate sources on the growth and lipid accumulation of *Tetradesmus obliquus*, a microalgal species known for its high lipid content. The main objective and specific objectives are:

Main Objective:

- To optimize biomass and total lipid yield in *Tetradesmus obliquus* using different nitrate sources.

Specific Objectives:

- To compare the effects of sodium nitrate, magnesium nitrate, and urea on the biomass yield of *Tetradesmus obliquus*.
- To determine the lipid content of *Tetradesmus obliquus* when cultivated with each nitrate source.
- To evaluate the overall lipid productivity of *Tetradesmus obliquus* under different nitrate conditions.
- To provide insights for optimizing microalgal cultivation conditions for biodiesel and green kerosene production.

The experiments were carried out at the Sustainable Energy Research and Development Center (NPDEAS) at the Federal University of Paraná (UFPR).

2. MATERIALS AND METHODS

In this section, materials and methods to achieve the proposed objectives are presented and explained. The studies were conducted at the Sustainable Energy Research and Development Center (NPDEAS) at the Federal University of Paraná (UFPR). This facility provided the necessary infrastructure and expertise for carrying out the experiments on microalgal cultivation, ensuring precise control over environmental conditions and accurate measurement of growth and lipid production parameters.

2.1 Microalgal Strain and Culture Conditions

The microalgal strain used in this study, *Tetradesmus obliquus*, was obtained from the algal culture collection of NPDEAS. The microalgae were grown in mixotrophic state, were inorganic and organic carbon source is available in the presence of a light source. The cultures were maintained in BBM medium without any nitrate source, under controlled laboratory conditions with a temperature of 22 ± 1°C, with atmospheric air feeding for agitation and to provide a CO_2 source, the experiment was also under artificial light exposure during all the time of the experiment.

2.2 Experimental Design

The experimental setup consisted of three groups, each corresponding to one of the nitrate sources. Triplicate cultures for each nitrate source were established in 10 L bottles containing 7.2 L of BBM supplemented with the respective nitrate source and 5 $g.L^{-1}$ of glucose. The initial nitrate source concentration in each bottle was standardized to 1 $g.L^{-1}$ of sodium nitrate ($NaNO_3$) for group A, 1 $g.L^{-1}$ of hexahydrate magnesium nitrate ($Mg(NO_3)_2.6H_2O$) for group B and 1 $g.L^{-1}$ of urea ($(NH_2)_2CO$) for group C. Furthermore, 0.8 L of previously grown *Tetradesmus Obliquus* were inoculated in each bottle.

The bottles were maintained in the same light and temperature conditions for a 10-day period.

2.3 Biomass Determination

At the end of the 10-day cultivation period, the cultures were harvested by centrifugation at 5000 rpm for 10 minutes. The supernatant was discarded, and the algal pellets were washed with distilled water to remove residual medium. The washed pellets were then dried at 60°C to a constant weight, and the dry biomass was measured using an analytical balance. The biomass yield was expressed in grams per liter ($g.L^{-1}$).

2.4 Lipid Extraction and Analysis

Lipid extraction was performed using the saponification method. In this process, 5 g of dried algal biomass was mixed with 25 g of sodium hydroxide (NaOH) and 250 mL of ethanol. The mixture was subjected to agitation and heat at 55°C for 1.5 hours to facilitate the breakdown of complex lipids

into fatty acids and glycerol. After the saponification reaction, the mixture was neutralized with acetic acid.

Following neutralization, hexane was added to the mixture to separate the phases. The hexane layer, containing the lipid content, was collected, and the process was repeated until all lipids were extracted.

A simple representation of this process is shown in Fig. 1

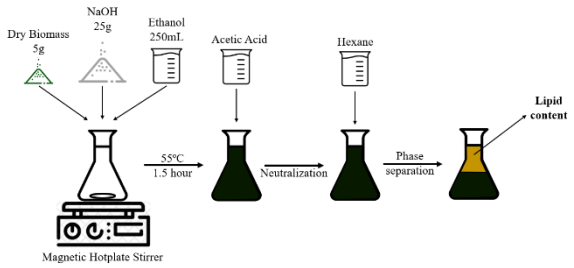

Figure 1. Lipid extraction via saponification.

Once the lipid content was separated from the mixture, the hexane was completely evaporated using a rotary evaporator. The lipid content was then determined gravimetrically and expressed as a percentage of dry biomass and as lipid productivity in grams per liter ($g.L^{-1}$). All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

The impact of different nitrate sources on the growth and lipid accumulation of *Tetradesmus obliquus* was investigated by cultivating the microalgae with sodium nitrate ($NaNO_3$), hexahydrate magnesium nitrate ($Mg(NO_3)_2.6H_2O$), and urea ($(NH_2)_2CO$). The initial nitrate concentration was standardized to 1 $g.L^{-1}$ for each nitrate source, with corresponding nitrogen concentrations of 0.165 $g.L^{-1}$ for sodium nitrate, 0.109 $g.L^{-1}$ for magnesium nitrate, and 0.466 $g.L^{-1}$ for urea.

Table 1. Initial and Nitrogen Concentrations of Different Nitrate Sources Used in the Study.

Nitrate Source	Chemical Formula	Concentration ($g.L^{-1}$)	Nitrogen %
Sodium Nitrate	$NaNO_3$	1	16.5
Magnesium Nitrate	$Mg(NO_3)_2.6H_2O$	1	10.9
Urea	$(NH_2)_2CO$	1	46.6

Table 1 demonstrates the chemical formula of each nitrate source used and also the nitrogen content of each component.

3.1 Biomass Yield

After a 10-day cultivation period, the final biomass concentrations were measured. The results indicated that magnesium nitrate led to the highest biomass yield, reaching 2.406 ± 0.120 $g.L^{-1}$, followed by sodium nitrate at 2.098 ± 0.105 $g.L^{-1}$, and urea at 1.690 ± 0.085 $g.L^{-1}$. This suggests that magnesium nitrate was the most effective nitrate source for promoting microalgal growth under the experimental conditions.

Figure 2 shows the final biomass production for each nitrate source.

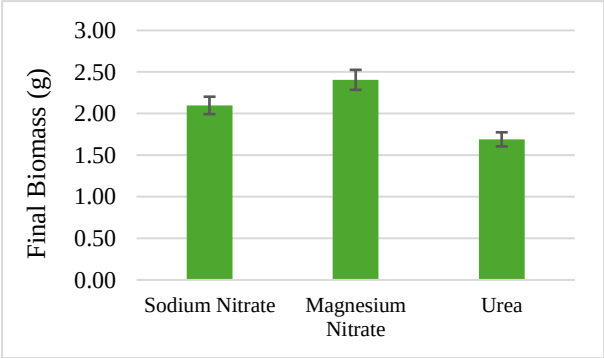

Figure 2. Final biomass production for each nitrate source.

3.2 Lipid Content and Productivity

The lipid content of the harvested biomass was analyzed using the saponification method. The lipid content percentages were 4.731 ± 0.730% for sodium nitrate, 5.353 ± 0.853% for magnesium nitrate, and 5.632 ± 0.511% for urea. Despite urea resulting in the highest lipid percentage, it had the lowest final biomass concentration, which affected the overall lipid productivity.

Figure 3 shows a comparison between the lipid content of the harvested biomass and lipid productivity for each nitrate source.

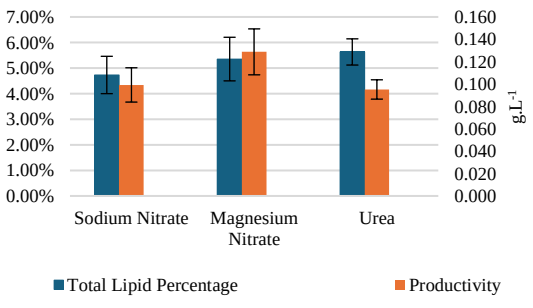

Figure 3. Lipid content in % of total biomass and total lipid productivity per liter of cultive in $g.L^{-1}$ for each nitrate source.

The lipid content of the harvested biomass was analyzed using the saponification method. The lipid content percentages were 4.731 ± 0.730% for sodium

nitrate, 5.353 ± 0.853% for magnesium nitrate, and 5.632 ± 0.511% for urea. Despite urea resulting in the highest lipid percentage, it had the lowest final biomass concentration, which affected the overall lipid productivity.

Lipid productivity, which combines both biomass yield and lipid content, was calculated as 0.099 ± 0.015 $g.L^{-1}$ for sodium nitrate, 0.129 ± 0.021 $g.L^{-1}$ for magnesium nitrate, and 0.095 ± 0.009 $g.L^{-1}$ for urea. Magnesium nitrate demonstrated the highest lipid productivity, indicating that it not only supported robust biomass growth but also promoted significant lipid accumulation.

3.3 Comparison of Nitrate Sources

The differences in growth and lipid accumulation among the nitrate sources can be attributed to the varying nitrogen availability and the microalgae's ability to assimilate and utilize these nitrogen forms. Sodium nitrate and magnesium nitrate, being inorganic sources, are readily assimilated by microalgae, supporting both growth and lipid synthesis. Urea, an organic nitrogen source, although resulting in higher lipid content, did not support biomass growth as effectively as the inorganic sources.

4 CONCLUSIONS

This study represented a significant effort to evaluate and understand the effects of different nitrate sources on the growth and lipid accumulation of *Tetradesmus obliquus* for biofuel production. The following conclusions highlight the main results and contributions of this research:

- The study successfully identified the optimal nitrate source for maximizing biomass yield and lipid productivity in *Tetradesmus obliquus*. Magnesium nitrate was found to be the most effective in achieving high biomass yield and lipid accumulation, contributing to enhanced biofuel synthesis.
- Comparison of nitrate sources on biomass yield: Magnesium nitrate resulted in the highest biomass yield (2.406 ± 0.120 $g.L^{-1}$), followed by sodium nitrate (2.098 ± 0.105 $g.L^{-1}$), with urea producing the lowest biomass concentration (1.690 ± 0.085 $g.L^{-1}$).
- Determination of lipid content: Urea led to the highest lipid percentage (5.632 ± 0.511%), although it did not correspond to the highest overall biomass yield. It is recommended to study ways to utilize the high lipid percentage obtained with urea and to investigate the use of urea in combination with other inorganic nitrate sources to further maximize oil productivity.
- Evaluation of lipid productivity: Magnesium nitrate demonstrated the highest lipid productivity (0.129 ± 0.021 $g.L^{-1}$), indicating a balance between substantial biomass growth and significant lipid accumulation.
- Insights for optimizing microalgal cultivation conditions: The study provided valuable insights into the influence of different nitrate sources on *Tetradesmus obliquus* cultivation. The superior performance of magnesium nitrate suggests its potential for large-scale microalgal cultivation for biodiesel and green kerosene production. Future research could explore the mechanisms underlying nitrogen assimilation and the impact of other nutrient sources to further enhance biofuel yields.

5. REFERENCES

Alvarez, A.L. et al. (2021). "Microalgae, soil and plants: A critical review of microalgae as renewable resources for agriculture." *Algal Research*, 54, 102200. ISSN 2211-9264. DOI:10.1016/j.algal.2021.102200.

Chisti, Y. (2007). "Biodiesel from microalgae." *Biotechnology Advances*, 25(3), 294–306. ISSN 0734-9750. DOI:https://doi.org/10.1016/j.biotechadv.2007.02.001.

Costa, I., Vargas, J., Balmant, W., Pereira Ramos, L., Zandona Filho, A., Taher, D., Mariano, A., Yamamoto, C., Conceicao, D., & Kava, V. (2022). "Microalgae-Derived Green Diesel." *Chemical Engineering & Technology*, 45. DOI:10.1002/ceat.202100647.

Nagappan, S., Kumar, R.R., Balaji, J.R., Singh, S., Verma, S.K. (2019). "Direct saponification of wet microalgae by methanolic potassium hydroxide using acetone as co-solvent." *Bioresource Technology Reports*, 5, 351-354. ISSN 2589-014X. DOI:10.1016/j.biteb.2018.05.010.

Rampi, M.G., Balmant, W., Corrêa, D.O., Santos, B., Selesu, N.F.H., Severo, I.A., Mariano, A.B., Vargas, J.V.C., & Ordonez, J.C. (2023). "Airlift Photobioreactor for Microalgae Growth from Emissions: Mathematical Modeling and Simulation." *Chemical Engineering & Technology*, 46, 1717-1725. DOI:10.1002/ceat.202200457.

Vo, H.N.P. et al. (2019). "A Critical Review on Designs and Applications of Microalgae-Based Photobioreactors for Pollutants Treatment." *Science of The Total Environment*, 651, 1549–1568.

Zhang, Z., Cai, J. “Energy Issues in Carbon Capture”. *International Journal of Energy Clean Environment*. [S. l.: s. n.], 2018. v. 19

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