Optimization of Microalgae Biomass Pretreatment for Biohydrogen Production by Dark Fermentation

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Highlights:

- Wastewater-grown microalgae biomass can be used as substrate to generate biohydrogen.
- Controlling temperature, reaction time and catalyst quantity are important to maximize carbohydrates release.
- 180°C for 10 minutes improves carbohydrates release.

Keywords: niobium phosphate; wastewater; pretreatment.

INTRODUCTION

The use of renewable energy sources, such as biohydrogen produced from microalgae, is important in combating climate change and overcoming the energy crisis. This process holds great promise as its main byproduct is water, contributing to carbon neutrality and bioenergy sustainability. Additionally, cultivating microalgae in wastewater promotes sustainability by utilizing waste to stimulate algal growth, purifying the water, reducing pollution, and lowering greenhouse gas emissions (Satheesh et al., 2023). Microalgae sequester carbon dioxide (CO_2) and convert it into biomass rich in lipids, proteins, and carbohydrates (Satheesh et al., 2023), which are essential for the production of biofuels such as biohydrogen, bioethanol, biodiesel, and other bioproducts. Dark fermentation, an efficient method for producing biohydrogen from microalgae, occurs under anaerobic conditions and generates a significant amount of hydrogen.

Microalgae biomass pretreatment is required to increase the efficiency of the fermentation process, preventing the degradation of carbohydrates and the formation of inhibitors. Various pretreatment methods exist, such as physical, chemical, biological, and combined, each with its advantages and disadvantages. Catalysts, such as niobium phosphate ($NbOPO_4$), can accelerate the conversion of monosaccharides during pretreatment, improving process efficiency. Moreover, niobium-based catalysts have gained attention for biomass enhancement due to their low cost, specific acidic properties, and excellent thermal stability. A key advantage of these catalysts is their high water tolerance, important given that water is often generated or present in biomass refining processes (Kang et al., 2021), and their solid nature allows them to be reused (Lima et al., 2023). However, it is important to minimize the production of inhibitory compounds, such as furans and phenolics, which can hinder microorganism growth and biohydrogen production (Monlau et al., 2014).

Considering these issues, pre-treatment of microalgae biomass grown in wastewater is a sustainable solution for biohydrogen production via dark fermentation, aligning with the circular economy.

METHODOLOGY

The experiments were conducted at the Sanitary and Environmental Engineering Laboratory (LESA) of the Federal University of Viçosa, Brazil. The experimental area includes high-rate algae ponds (HRAPs) and biofilm reactors (BRs) at a pilot scale, designed for wastewater treatment and algal biomass production. The HRAPs have dimensions of 1.28 m in width, 2.86 m in length, 0.5 m in depth, and a surface area of 3.3 m², equipped with steel paddles driven by an electric motor. The BR, with an area of 0.5 m², was installed near the HRAPs, exposed to air and solar radiation, and lined with polyester to promote biofilm growth. After cultivation, the biomass was harvested by sedimentation (HRAP) and scraping (BR), followed by freeze-drying for future analyses (Silva et al., 2024).

Biomass pretreatment was performed in a 300 mL Parr hydrothermal reactor with controlled temperature and stirring speed (500 rpm), using a biomass-to-water ratio of 3/50 (12 g of dry biomass and 200 g of distilled water). Several combinations of temperature (100, 140, 180 °C), time (0-70 min) and amounts of $NbOPO_4$ (0, 3, 9 g) were tested. Nitrogen gas was used to remove dissolved oxygen from the system. Total carbohydrate content was analyzed by the phenol-sulfuric acid method with readings at 490 nm using a glucose standard curve (DuBois et al., 1956). Concentrations of monosaccharides such as glucose, galactose, mannose, xylose, and arabinose, and furans (furfural and hydroxymethylfurfural (HMF)) were determined by high-performance liquid chromatography (HPLC).

RESULTS AND CONCLUSIONS

This study investigated the influence of temperature on the hydrothermal pretreatment of microalgae biomass for biohydrogen production via dark fermentation. The release of carbohydrates increased with higher temperatures, as evidenced by Phanduang et al. (2019), where elevated temperatures facilitated the breakdown of polymers into simple sugars. However, higher temperatures and prolonged reaction times can lead to sugar degradation, reducing the final yield, as described by Sen et al. (2016).

It was observed that a temperature of 180°C (Figure 3) resulted in the highest carbohydrate production at a reaction time of 10 minutes, which aligns with the findings of Phanduang et al. (2019), suggesting that longer times may lead to sugar degradation. On the other hand, the presence of the $NbOPO_4$ catalyst at 100°C (Figure 1) had a negative impact, with higher carbohydrate concentrations obtained without the catalyst, indicating the breakdown of sugars into compounds like furfural and HMF, as observed by Phanduang et al. (2019). These compounds are known microbial inhibitors, negatively affecting biohydrogen production, as confirmed by Monlau et al. (2014).

Figures 1, 2, and 3 show the amount of carbohydrates in solution after treatment in the hydrothermal reactor, considering different reaction times and catalyst amounts at 100, 140, and 180°C, respectively.

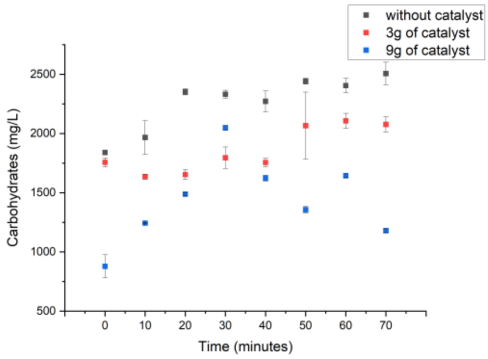

Figure 1. Reaction performed at 100°C.

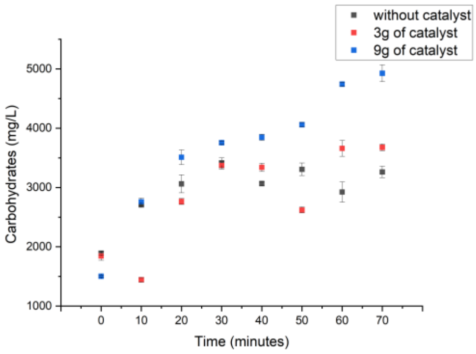

Figure 2. Reaction performed at 140°C.

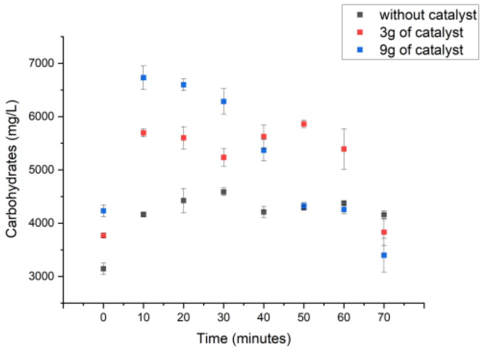

Figure 3. Reaction performed at 180°C.

The biomass studied consisted of a mixture of *Chlorella vulgaris* (47.75%), *Tetradesmus obliquus* (50%), and *Limnothrix planctonica* (2.25%), with an average carbohydrate content of 24%, which is consistent with mixed microalgae cultures (Liu et al., 2022). Different species of fermentative bacteria exhibit varying capabilities to degrade carbohydrates. For example, *Clostridium butyricum* can metabolize a wide range of sugars, making it highly versatile for hydrogen production from various biomasses (Wang and Yin, 2021). HPLC analysis revealed that glucose was the main component in the solution, which is suitable for hydrogen production by *Clostridium butyricum* (Wang and Yin, 2021).

Additionally, the production of inhibitory byproducts, such as furfural and HMF, was more pronounced with longer exposure times, higher temperatures, and greater amounts of catalyst, as observed in the HPLC analysis and described by Lima et al. (2023). The same study reports that glucose is particularly prone to dehydration, forming HMF in the presence of $NbOPO_4$. These byproducts compromise the efficiency of the fermentation process by reducing microbial activity.

Thus, the optimal temperature to maximize carbohydrate release while minimizing the formation of inhibitors was around 180°C, with reaction times of 10 minutes. Furthermore, the use of $NbOPO_4$ must be carefully controlled to avoid excessive acidification of the medium, which leads to sugar degradation and the formation of compounds detrimental to the fermentation process.

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REFERENCES

DuBois, Michel., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, Fred., 1956. Colorimetric Method for Determination of Sugars and Related Substances. Anal Chem 28, 350–356. https://doi.org/10.1021/ac60111a017

Kang, S., Miao, R., Guo, J., Fu, J., 2021. Sustainable production of fuels and chemicals from biomass over niobium based catalysts: A review. Catal Today 374, 61–76. https://doi.org/10.1016/j.cattod.2020.10.029

Lima, S., García-López, E.I., Adawy, A., Marcì, G., Scargiali, F., 2023. Valorisation of Chlorella sp. biomass in 5-HMF through a two-step conversion in the presence of Nb2O5 and NbOPO4 and optimisation through reactive extraction. Chemical Engineering Journal 471, 144583. https://doi.org/10.1016/j.cej.2023.144583

Liu, Xiaoxu, Liu, Xiaomin, Yu, M., Zhu, Z., 2022. Characteristics and driving conditions of flash drought in different grassland ecosystems. Science of The Total Environment 849, 157923. https://doi.org/10.1016/j.scitotenv.2022.157923

Monlau, F., Sambusiti, C., Barakat, A., Quéméneur, M., Trably, E., Steyer, J.-P., Carrère, H., 2014. Do furanic and phenolic compounds of lignocellulosic and algae biomass hydrolyzate inhibit anaerobic mixed cultures? A comprehensive review. Biotechnol Adv 32, 934–951. https://doi.org/10.1016/j.biotechadv.2014.04.007

Phanduang, O., Lunprom, S., Salakkam, A., Liao, Q., Reungsang, A., 2019. Improvement in energy recovery from Chlorella sp. biomass by integrated dark-photo biohydrogen production and dark fermentation-anaerobic digestion processes. Int J Hydrogen Energy 44, 23899–23911. https://doi.org/10.1016/j.ijhydene.2019.07.103

Satheesh, S., Pugazhendi, A., Al-Mur, B.A., Balasubramani, R., 2023. Biohydrogen production coupled with wastewater treatment using selected microalgae. Chemosphere 334, 138932. https://doi.org/10.1016/j.chemosphere.2023.138932

Sen, B., Chou, Y.-P., Wu, S.-Y., Liu, C.-M., 2016. Pretreatment conditions of rice straw for simultaneous hydrogen and ethanol fermentation by mixed culture. Int J Hydrogen Energy 41, 4421–4428. https://doi.org/10.1016/j.ijhydene.2015.10.147

Silva, T.A., Pereira, A.S.A. de P., Ferreira, J., Lorentz, J.F., de Assis, M.L., Assemany, P.P., dos Reis, A.J.D., Calijuri, M.L., 2024. Enhancing microalgae biomass production: Exploring improved scraping frequency in a hybrid cultivation system. J Environ Manage 355, 120505. https://doi.org/10.1016/j.jenvman.2024.120505

Wang, J., Yin, Y., 2021. Clostridium species for fermentative hydrogen production: An overview. Int J Hydrogen Energy 46, 34599–34625. https://doi.org/10.1016/j.ijhydene.2021.08.052