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# Enhancing microalgal biohydrogen production: Unlocking higher yields with hydrothermal pretreatment with niobium phosphate

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## ABSTRACT

Microalgae cultivated in wastewater hold promise as a substrate for biohydrogen (bioH<sub>2</sub>) production. However, their rigid cell walls pose a challenge to fermentability. In this context, this study evaluated hydrothermal pretreatment with niobium phosphate (NbP) at 100–180 °C for 0–70 min, using up to 75 % NbP (relative to the dry weight of microalgal biomass). The hydrothermal pretreatment at 180 °C for 10 min with 75 % NbP released 7431 mg total carbohydrates (CHT) L<sup>-1</sup>, increasing the availability of fermentable substrates in subsequent dark fermentation (DF). When this pretreated biomass was subsequently fermented at pH 5.0 (sample PB5), bioH<sub>2</sub> production reached 1.03 mmol H<sub>2</sub> mol<sup>-1</sup> CHT, with a maximum cumulative output of 0.17 mmol H<sub>2</sub> and a CHT conversion efficiency of 83.6 %. In contrast, pH 5.5 and 6.0 reduced bioH<sub>2</sub> yields and promoted methanogenic activity, while no pH control resulted in negligible bioH<sub>2</sub> evolution. In conclusion, hydrothermal pretreatment with niobium phosphate and pH improvement synergize to enhance hydrogenogenesis, integrating wastewater treatment and renewable biohydrogen production.

## 1. Introduction

The global energy transition toward sustainable and low-carbon systems is intensifying, with renewable energy sources such as solar, wind, hydropower, and biofuels expected to play a central role in decarbonizing power, heat, and transportation sectors. Renewable electricity generation is projected to reach over 17,000 TWh by 2030, an increase of almost 90 % from 2023 levels, meeting the combined power demand of China and the United States [1]. Despite this progress, achieving net-zero carbon emissions necessitates integrating renewable biofuels into the energy mix, particularly in sectors where electrification remains challenging, such as industrial processes and heavy transport [2]. Among renewable energy sources, hydrogen (H<sub>2</sub>) stands out as a versatile energy carrier with high energy density, minimal carbon footprint, and diverse production pathways [3].

Dark fermentation (DF) of organic biomass has emerged as a promising technology for bioH<sub>2</sub> production, leveraging microbial consortia to convert primarily carbohydrates (CHT) into H<sub>2</sub> and volatile fatty acids (VFAs) under anaerobic conditions [3]. This process is particularly advantageous due to its ability to valorize diverse feedstocks, including agricultural residues and wastewater-grown biomass, without requiring complex external inputs like high-voltage electricity or pure oxygen [4]. However, achieving high H<sub>2</sub> yields is still a challenge.

Microalgae have garnered attention as a feedstock for bioH<sub>2</sub> production owing to their rapid growth rates and ability to be cultivated in wastewater, avoiding competition with arable land and food production [5]. Furthermore, wastewater-grown microalgae contribute to resource recovery through nutrient removal, reducing the environmental impact of wastewater discharge [6]. The main species commonly found in wastewater, *Chlorella vulgaris* and *Tetrademus obliquus*, possess robust

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