EXPLORING MICROALGAE FOR SUSTAINABLE BIOPRODUCT SYNTHESIS AND VINASSE TREATMENT

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ABSTRACT

Microalgae play an important ecological role by participating in vital life-sustaining reactions. These microorganisms are considered cellular factories capable of synthesizing biomass containing added-value molecules. Depending on the environmental conditions, microalgae can redirect their metabolic pathway to accumulate specific molecules of economic interest. Moreover, they contribute significantly to environmental remediation, especially in wastewater treatment. This study aimed to investigate the metabolic response of microalgae mangrove isolated *Selenastrum* sp. Strain B9 under different cultivation conditions, utilizing sugarcane vinasse as effluent and assessing its potential for effluent treatment. Results indicated that under nitrate limitation, microalgae produced higher carbohydrate concentration, constituting up to 32% of the biomass, while in vinasse-supplemented complete medium, protein production predominated. In both conditions, total phenol remotion was performed and 88% of the ammoniacal nitrogen was reduced. These findings contribute to understanding the biotechnological potential of microalgae *Selenastrum* sp. strain B9.

Keywords: Biotechnological potential. Circular Bioeconomy. Phytoremediation. Wastewater.

1 INTRODUCTION

Microalgae are photosynthetic microorganisms that use sunlight, water, inorganic compounds, and carbon dioxide (CO_2) to synthesize biomass comprising proteins, carbohydrates, and lipids[1]. This biomass holds significant commercial and environmental value, notably in biorefinery applications. These facilities represent an effective means of maximizing product diversity from a single biomass source. Its derived products encompass fatty acids for nutritional supplements such as omega-3s and biodiesel, pigments for pharmaceuticals and cosmetics, proteins for biopolymers and animal feed, and carbohydrates for third-generation biofuels[1–4].

Microalgae play a crucial role in various sectors of the circular bioeconomy, integral to the Sustainable Development Goals (SDGs) outlined in 2030 Agenda[5,6]. Thriving in diverse environments characterized by temperature variations, light intensity, and nutrient availability[7], microalgae demonstrate viability for growth in wastewater[8]. This capacity contributes significantly to pollution remediation, as these microorganisms effectively remove phosphorylate compounds and assimilate nitrogen during industrial wastewater treatment processes[9]. Furthermore, utilizing effluent as growth substrates for microalgae cultivation can substantially reduce production costs[8]. This strategic approach aims to maximize the utilization of commercially valuable molecules while minimizing waste generation[6,10].

Among the various wastes, vinasse represents a challenge for the sugar-alcohol industry due to its large-scale production, where 10 to 15 liters of vinasse are produced per liter of ethanol, as well as its toxic composition, characterized by high organic matter and nutrients content and an acidic pH [2,11–13]. Cultivating microalgae in vinasse can induce biochemical stress in cells, stimulating the accumulation of biomolecules. Furthermore, these microorganisms can convert proteins and peptides into lipids or carbohydrates under nitrogen-limited conditions, enhancing compound accumulation[14]. Therefore, this study aims to explore microalgae cultivation in vinasse under different conditions to evaluate biomolecule production and agro-industrial waste treatment efficacy.

2 MATERIAL & METHODS

The microalgae *Selenastrum* sp. strain B9, isolated from the mangrove forest of Baixada Santista (23°53'46.4"S 46°25'13.2"W), was used for tests involving vinasse as a growth medium. Two experimental conditions were tested with 20% vinasse: (A) diluted with complete BG-11 medium and (B) diluted with a nitrate-limited BG-11 medium (25% nitrate). A control medium without vinasse addition was included (C). Vinasse underwent prior treatment with sodium hydroxide (NaOH), centrifugation, and autoclaving[3].

The *Selenastrum* sp. strain B9 was maintained under continuous agitation with 0.04% CO_2 bubbling and a constant light intensity of 185 µmol m^{-2} s^{-1} for 24 hours per day over 8 days. Daily absorbance ($DO_{750\ nm}$) reading was conducted to monitor

cell growth. Pigments were extracted using methanol and quantified[15]. Microalgal biomass was analyzed for biochemical composition. Carbohydrate composition was determined daily using the acid hydrolysis method[16], while lipid production was monitored daily using a spectrofluorometer with Nile Red[17]. Lipids from biomass on the final cultivation were extracted and quantified gravimetrically[18,19], and proteins were extracted[20] and analyzed by Bradford. Effluent treatment was analyzed for phenol[21] and ammonia nitrogen removal using an ammonia selective ion electrode (HI4101, Hanna). The carbohydrate/nitrogen ratio was calculated based on the concentration of total organic carbon and total nitrogen present in the vinasse and the concentration of nitrogen in the culture medium.

3 RESULTS & DISCUSSION

Growth of *Selenastrum* sp. strain B9 was conducted by measuring OD_{750nm} and monitoring pigment production (Figure 1). The μmax was faster in the condition A (1.18 d^{-1}) compared to the condition B (1.07 d^{-1}) and the condition C (0.59 d^{-1}). A similar trend was observed for pigment production, where chlorophyll *a* and *b* levels were lower under nitrate-limitation (condition B) due to destabilization of chlorophyll structure and subsequent generation of reactive oxygen species (ROS), negatively impacting photosynthesis[22,23].

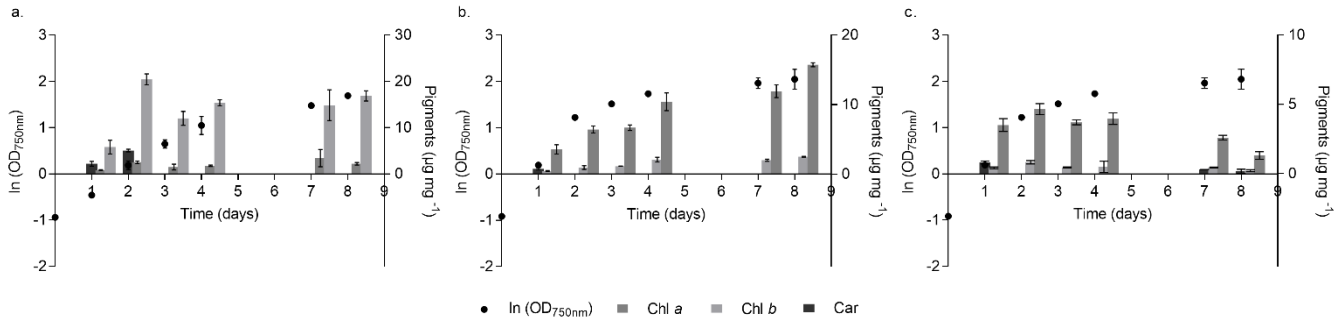

Figure 1 Cell growth curve in ln (OD) and pigment production over 8 days, under different conditions, where a. control, b. vinasse and, c. vinasse with nitrate restriction.

The carbon/nitrogen (C/N) ratio influences the metabolic responses of microalgae. An increased C/N ratio allows microalgae to assimilate more carbon, favoring compound storage within cells[24]. Thus, modifying substrate concentration and nutrients represents a strategy to enhance desired bioproduct accumulation[25]. Nitrogen availability is one of the most critical factors for microalgal growth, affecting essential pathways. Under nutrient limitation, microalgae redirect their protein metabolism towards synthesizing lipids or carbohydrates[22,26]. In this study, it was observed that condition B (C/N 11) induced a substantial increase in carbohydrate accumulation (32.33 ± 2.04%) compared to condition A (C/N 3) (15.77 ± 0.59% carbohydrate/biomass) (Figure 2).

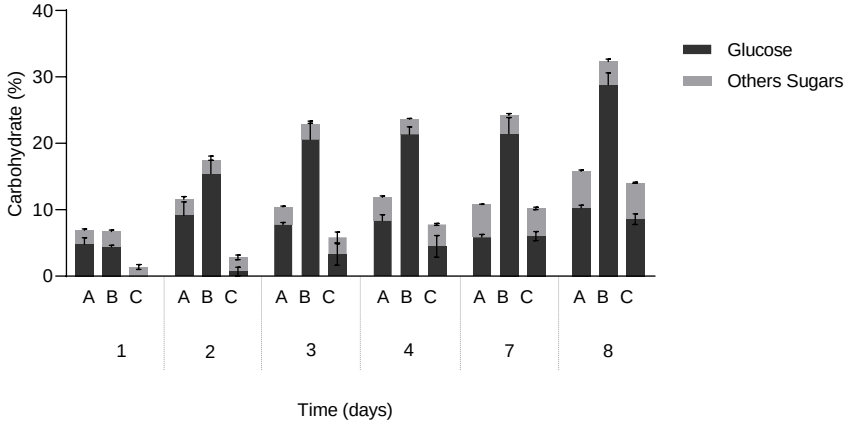

Figure 2 Carbohydrate production over 8 days under different conditions. A. vinasse diluted in BG-11 complete medium; B. vinasse diluted with a nitrate-limited BG-11 medium (25% nitrate). and C. control medium without vinasse addition

Maximum lipid accumulation in vinasse conditions was observed on the second day of cultivation, indicating lipid consumption in the final biomass. The biochemical composition of *Selenastrum* sp. strain B9 showed a different profile in each condition. In the condition A (lower C/N), protein production was higher, whereas condition B (higher C/N) favored carbohydrate production (Figure 3). Protein synthesis is related to nitrogen availability, as nitrogen is converted to amino acids during nitrate-ammonia reduction reactions[22]. Under stress conditions, carbon conversion to carbohydrates leads to decreased lipids and protein concentrations, whereas lower C/N ratios promote increased protein production[26,27]. Vinasse treatment was efficient in both conditions, completely removing phenol and 88% of ammoniacal nitrogen (87.8% ± 4.07 in condition A, and 88.12% ± 2.04 in condition B).

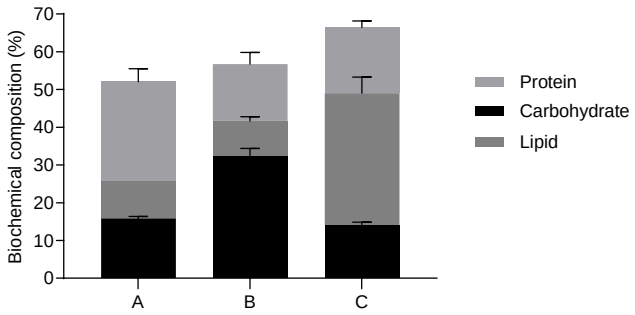

Figure 3 Biochemical profile (proteins, lipids and carbohydrates) of the final biomass. A. vinasse diluted in BG-11 complete medium; B. vinasse diluted with a nitrate-limited BG-11 medium (25% nitrate). and C. control medium without vinasse addition

4 CONCLUSION

The biotechnological potential of the microalgae *Selenastrum* sp. strain B9 has been investigated concerning its growth capacity, vinasse treatment efficacy, and synthesis of commercially relevant products. Strategies are required to enhance its accumulation depending on the bioproduct of interest. In this study, strain *Selenastrum* sp. B9 exhibited a carbohydrate accumulation of up to 32% biomass under vinasse and nitrogen-limitation conditions, and protein accumulation of up to 26% under vinasse diluted in complete medium. These carbohydrates are highly valuable as they can be utilized for various purposes, such as biohydrogen and bioethanol production. This approach contributes to the circular bioeconomy by valorizing agro-industrial wastes.

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