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MANGROVE-DERIVED *COELASTRELLA* MICROALGA: A SUSTAINABLE BIOMASS FOR ETHANOL PRODUCTION

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ABSTRACT

Third-generation (3G) ethanol holds significant potential as an alternative to fossil fuels to meet the growing global energy demands and mitigate the adverse effects of climate change. This study investigates the biotechnological potential of *Coelastrella* sp. B2 strain, a microalgae isolated from mangroves, for 3G ethanol production. Underdetermined cultivation conditions, this strain demonstrated rapid growth and high carbohydrate accumulation, essential for efficient ethanol production. The subsequent hydrolysis, under optimized conditions, and fermentation process achieved a considerable efficiency of 83%, highlighting the strain's significant potential to overcome current production challenges associated with 3G ethanol and contribute to developing sustainable energy solutions.

Keywords: CO_2 mitigation; Microalgae biomass; Polysaccharide characterization; Hydrolysis; Third-generation bioethanol.

1 INTRODUCTION

Population growth and modern lifestyles have significantly increased global energy demand[1,2]. Fossil fuels account for around 80% of this demand, but their use has negative environmental impacts and is linked to climate change[1,3–5]. It is estimated that the continued use of these fuels could increase 3 to 5ºC in global temperature by 2100[2,6,7].

The transport sector is fundamental in generating greenhouse gas emissions[8]. Therefore, the replacement of fossil fuels with low-carbon emission fuels is urgent. In this context, ethanol, obtained from biomass such as sugarcane, corn and lignocellulosic wastes, is an advantageous option[9,10]. However, these generations face environmental challenges, such as the food versus fuel dilemma and potable water use[4,11–13]. Consequently, third-generation (3G) ethanol has emerged as a promising alternative to overcome these challenges.

3G ethanol is produced from microalgae, microorganisms capable of converting light, CO_2, water, and nutrients into biomass, glucose, and oxygen[14]. Microalgae can accumulate large quantities of carbohydrates, which can be hydrolyzed and fermented to produce ethanol[15,16]. However, obtaining 3G ethanol faces economic and industrial challenges, such as strain selection, cultivation conditions, and nutritional requirements[17–19]. It is necessary to choose microalgae strains that are both productive and adaptable to various environmental conditions[16,19,20]. Furthermore, aligning 3G ethanol production with the biorefinery concept by associating it with other high-value bioproducts is strategic[17,21].

Mangroves are ecosystems characterized by variable conditions that pressure biota to adapt[22]. In the Baixada Santista region (São Paulo, Brazil), they face significant anthropogenic impacts[23,24], requiring adaptation and offering potential for biotechnological applications such as biofuel production. Microalgae isolated from these mangroves are particularly promising due to their adaptability and resilience; however, their biotechnological remains underestimated [22].

Given this context, this study aimed to explore the biotechnological potential of the *Coelastrella* sp. B2 strain, isolated from mangrove in Baixada Santista (São Paulo, Brazil), by assessing its carbohydrate accumulation and the subsequent utilization of its biomass to produce 3G ethanol.

2 MATERIAL & METHODS

The microalga *Coelastrella* sp. B2 strain (GenBank PP467629, isolated from 23°56'40.4"S and 46°23'07.6"W), was cultivated under autotrophic conditions using BG-11 medium with magnetic stirring, light (approx. 175 µmol m^{-2} s^{-1}) and continuous 5% (v/v) CO_2 bubbling. The experiments were conducted in triplicate and divided into two stages: (I) the cell growth phase, with complete medium, and (II) the carbohydrate accumulation phase, with BG-11 N-*free* medium. At the end of the experiments, the biomass was collected by centrifugation (4000 rpm, 20 min) and freeze-dried.

Cell growth (OD_{750nm}) and biomass production were periodically monitored during the first stage of cultivation. Using the final biomass, the contents of carbohydrates[25] and their composition[26], lipids[27], and proteins[28] were determined to characterize the biomass. The hydrolysis conditions were previously optimized through experimental design (2^3-centered face, 17 experiments)

and response surface methodology[29]. The formation of fermentation inhibitors was also assessed before subsequent fermentation[30] using *Saccharomyces cerevisiae*, isolated from the 1G ethanol production process. Fermentation was carried out in 96-well plates using a YPD medium as a control. Cell growth was periodically monitored (OD_{600nm}), and at the end of the assay, the fermented content was recovered and analyzed for sugar consumption and ethanol production[30].

3 RESULTS & DISCUSSION

Under the cultivation conditions studied, *Coelastrella* sp. strain B2 exhibited rapid cell growth, as evidenced by its specific growth rate (μmax) of 0.101 h^{-1} and cell generation time (gt) of 6.876 h, with the onset of the exponential growth phase occurring after 24 hours. Additionally, biomass production during the cell growth phase was 0.980 ± 0.091 g L^{-1} with a maximum productivity of 0.288 g L^{-1} d^{-1} at 48 hours of testing (Figure 1).

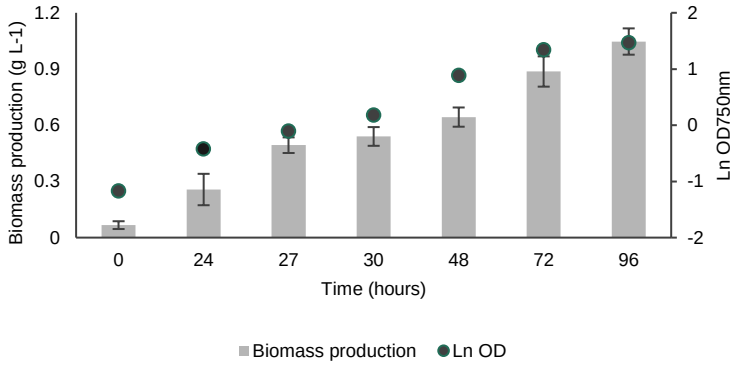

Figure 1 Cell growth of *Coelastrella* sp. B2 in terms of optical density (OD) and biomass production.

Upon reaching the stationary cell growth phase, all the biomass produced was transferred to the BG-11 N-free medium. Under these conditions, the cells cease to multiply and instead accumulate bioproducts as an energy reserve[31]. Nitrogen deprivation, in particular, is considered the most efficient stressor for inducing carbohydrate accumulation in microalgae[32,33]. This is evidenced by the biomass production measured at the end of the 7-day phase, 2.325 ± 0.164 g L^{-1}, representing more than double the amount obtained during the cell growth phase.

The macromolecules accumulated by the strain were analyzed. Total carbohydrates were assessed daily throughout the accumulation phase (Fig. 2A) and characterized in terms of the structural fraction (cell wall monosaccharides) and the non-structural fraction (soluble sugars and starch) with the biomass obtained on the last day, as well as lipids and proteins (Fig. 2B). Compared to the content of lipids and proteins, carbohydrates were the most accumulated macromolecule, demonstrating the potential for utilizing strain B2 for the production of 3G ethanol.

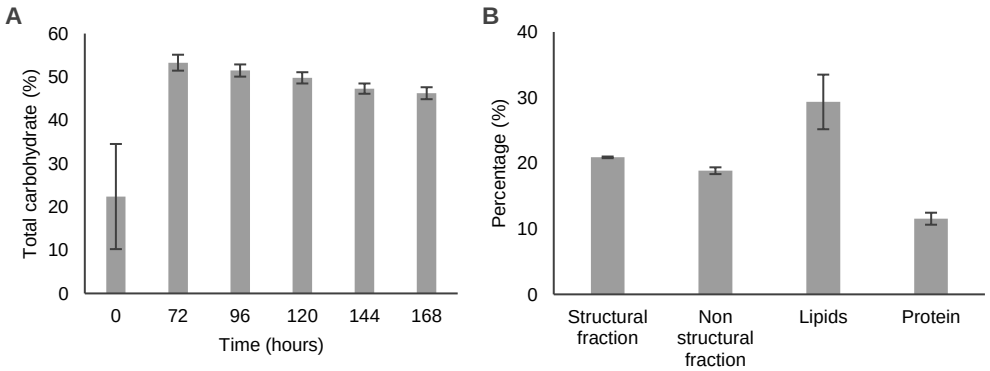

Figure 2 Evaluation of carbohydrate production over time (**A**) and characterization of the structural and non-structural polysaccharide, lipid, and protein fraction accumulated by *Coelastrella* sp. B2 (**B**).

The hydrolysis conditions were optimized. The response surface graphs (data not shown) determined the optimal hydrolysis conditions for autoclave time (60 min), sulphuric acid concentration (3% v/v), and biomass concentration (1% w/v), achieving a glucose recovery of over 32%. The mathematical model was obtained with a 90% confidence level (Table 1).

Table 1 Mathematical model for acid hydrolysis evaluating the independent variables autoclave time (X_1), sulphuric acid concentration (X_2), and biomass concentration (X_3) under the dependent variable glucose (Y) for the biomass of *Coelastrella* sp. B2.

Model equation	*p*-Value	Lack of fit	R^2
$Y = 24.314 + 1.022.X_1 + 0.744.X_2 - 0.290.X_3 + 4.328.X_1^2 + 0.353.X_2^2 + 0.062.X_3^3 - 0.418.X_1.X_2 - 0.532.X_1.X_3 - 0.842.X_2.X_3$	0.004	0.897	0.925

In the hydrolysate obtained under optimized conditions (60 minutes, 3% H_2SO_4 (v/v) and 1% biomass (w/v)), the presence of fermentation inhibitors such as organic acids and furan derivatives was verified. Specifically, only acetic acid (0.060 g L^{-1}) and levulinic acid (0.150 g L^{-1}) were formed at concentrations well below those reported to inhibit fermentation[34–36]. Therefore, fermentation was carried out using *S. cerevisiae* (Table 2). The results were promising, with a process efficiency of 83%. Studies

generally report efficiencies ranging from 41% to 87%[33,37,38], further demonstrating the potential of *Coelastrella* sp. B2 strain to overcome production obstacles in obtaining 3G ethanol.

Table 2 Sugar consumption and ethanol production during hydrolysate fermentation from strain B2 using *S. cerevisiae.*

Medium	Cellular growth			Sugars (g L^{-1})				Ethanol (g L^{-1})
	μmax (h^{-1})	gt (h)	R^2	Glucose		Others		
				Initial	Final	Initial	Final	
YPD	0.359	1.931	0.998	12.491 ± 0.000	N/D	N/D	N/D	3.999 ± 0.190
B2	0.072	9.627	0.997	11.117 ± 0.000	0.008 ± 0.001	5.208 ± 0.000	0.694 ± 0.160	6.709 ± 0.789

4 CONCLUSION

3G ethanol has the potential to overcome several obstacles associated with its earlier generations. However, achieving its viability has proven challenging. The choice of strains is a critical factor in the success of the process. In this regard, *Coelastrella* sp. strain B2 has shown high potential for 3G ethanol production, as it has accumulated more than 50% (w/w) of carbohydrates and achieved a production efficiency of over 83% in ethanol. Additionally, a significant lipid fraction (29.333 ± 4.163%) makes it attractive for exploiting other high-value bioproducts, such as fatty acids, aligning with the biorefinery concept.

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