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**Article**

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Energy Reports

**Provided in Cooperation with:**

Elsevier

*Suggested Citation:* Onay, Melih (2020) : Enhancing carbohydrate productivity from Nannochloropsis gaditana for bio-butanol production, Energy Reports, ISSN 2352-4847, Elsevier, Amsterdam, Vol. 6, Iss. 1, pp. 63-67, <https://doi.org/10.1016/j.egy.2019.08.019>

This Version is available at:

<https://hdl.handle.net/10419/243711>

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6th International Conference on Energy and Environment Research, ICEER 2019, 22–25 July,  
University of Aveiro, Portugal

# Enhancing carbohydrate productivity from *Nannochloropsis gaditana* for bio-butanol production

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Received 16 July 2019; accepted 16 August 2019

## Abstract

It presents an effective bio-butanol production with various acid hydrolysis treatment strategies  $\text{H}_2\text{SO}_4$ , HCl and  $\text{H}_3\text{PO}_4$  (1, 2, 3, 4 and 5%) from *Nannochloropsis gaditana* grown under different light intensities (40, 80, 120 and  $160 \mu\text{molm}^{-2} \text{s}^{-1}$ ). Microalgae were cultivated at  $24 \pm 2$  with f/2 medium for control. According to our results, HCl 3% yielded the highest carbohydrate productivity with ( $21.3 \pm 0.9$  %) and bio-butanol production ( $2.9 \pm 0.24$  g/L). In addition, we obtained the highest carbohydrate content at  $160 \mu\text{molm}^{-2} \text{s}^{-1}$  of light intensity. These findings suggest that *Nannochloropsis gaditana* can be used for effective carbohydrate content and large scale bio-butanol production in the future.

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Peer-review under responsibility of the scientific committee of the 6th International Conference on Energy and Environment Research, ICEER 2019.

**Keywords:** Acid hydrolysis treatment; Bio-butanol production; Biofuel; Microalgae; *Nannochloropsis gaditana*

## 1. Introduction

Algae can be classified to 2 classes. They are macro and micro. These microorganisms are photoautotrophic microorganisms. They need light,  $\text{CO}_2$  and macro and micro elements such as N, P, S and K for proliferation of cells [1]. Microalgae can be used for pharmaceutical, cosmetic, food and biofuel industry. They are 3rd generation renewable energy and they have distinguishable facilities compared to former renewable energy resources. In addition, they have no conflict with plant derivative samples for grounds used for cultivation. They can cultivate quickly and consist of immense masses [2,3]. They multiply their cell nuclei in low than 24 h and can cultivate a hundred times quickly than plants with real nuclei [4]. Algae show a significant property in the use of carbon dioxide due to their skill to capture carbon dioxide ten–fifty times more effectively than the green plants and they convert the sun to chemical energy [5]. In addition, there are various sources of biofuel such as bio-diesel, bio-ethanol and bio-butanol. Bio-butanol is revealed by the acetone–butanol–ethanol (ABE) method. At bio-butanol production, ABE method maintains fermentation of biomass. Traditionally, *C. acetobutylicum* can be used for

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<https://doi.org/10.1016/j.egy.2019.08.019>

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Peer-review under responsibility of the scientific committee of the 6th International Conference on Energy and Environment Research, ICEER 2019.

anaerobic procedure. It generates butyric and acetic acids with acidogenesis. In the next process, acetone, butanol and ethanol occur. Biomass of microorganism needs high sugar concentration to maintain high bio-butanol production [6,7]. Photosynthesis procedure is the crucial step that turns sun into chemical energy. This energy leads to accumulation of biomass and metabolic compounds such as lipid and carbohydrate for photosynthetic organisms [8]. Light has main property for photosynthesis. It is driving force and abiotic factor for determination of cellular metabolism. In addition to light, pH, temperature, compounds and amount of CO<sub>2</sub> affect FPBR systems and they should be controlled strictly [9]. Light quantity and quality cause to changes for microalgae. Microalgal biomass can decrease under much more light and content of biofuel can change. Actually, this is a defense mechanism for microalgae. They can increase carbohydrate and lipid content when these harsh environments appear [10]. Spectral composition of light affects metabolic compounds of microalgae. Red light increases hydrocarbon production and carbohydrate production [11,12]. Sun energy maintains main pigments such as chlorophyll a, b and carotenoids. They maintain bands of absorption. Blue and red wavelengths show the basic spectrum bands of these pigments [13]. Blue and red wavelengths have high amounts of biomass but green one has slope value for biomass production [14]. In this paper, we used the green microalgae. *Nannochloropsis gaditana* is a unicellular microalga, a member of the class Eustigmatophyceae, phylum Heterokontophyta. *Nannochloropsis sp.* has taken attention due importance of structural properties. Indeed, researchers generally studies metabolic parameters such as lipid for bio-diesel [3]. On the contrary, we researched its different role for biofuel. In current paper, we carried out role of bio-butanol from *Nannochloropsis gaditana* in various acid hydrolysis treatment strategies H<sub>2</sub>SO<sub>4</sub>, HCl and H<sub>3</sub>PO<sub>4</sub> (1, 2, 3, 4 and 5%) and various light intensities (40, 80, 120 and 160 μmol m<sup>-2</sup> s<sup>-1</sup>).

## 2. Materials and methods

### 2.1. Cultivation of *Nannochloropsis gaditana*

It was gotten from Van Yuzuncu Yil University collection. *Nannochloropsis gaditana DEE003* was grown at 24 ± 2 and f/2 was prepared to f/2 procedure [15]. Microalgae was carried out at 680 nm using spectrophotometer (Merck-Prove-300) under different light intensities (40, 80, 120 and 160 μmol m<sup>-2</sup> s<sup>-1</sup>).

### 2.2. Monitoring of microalgae

Microalgae were studied at 680 nm using spectrophotometer under different light intensities (40, 80, 120 and 160 μmol m<sup>-2</sup> s<sup>-1</sup>). Ash free dry weights (AFDW) were maintained for connection between OD and dry weight. For harvesting, microalgae samples were centrifuged at 3000 g in 6 min and pellet was collected.

### 2.3. Acid treatment procedure

Dry mass was mixed with various acid derivative disruption strategies H<sub>2</sub>SO<sub>4</sub>, HCl and H<sub>3</sub>PO<sub>4</sub> (1, 2, 3, 4 and 5%) in 1/10 ratio.

### 2.4. Calculation of total carbohydrate

The Anthrone method with minor modifications was used for this study [16]. Glucose was selected as a standard at various volumes.

### 2.5. Biobutanol production

Acid hydrolyzed microalgae slurries were used for determination of bio-butanol content. Bio-butanol content was determined to Maiti and modified acetone–butanol–ethanol (ABE) method spectrophotometrically [17].

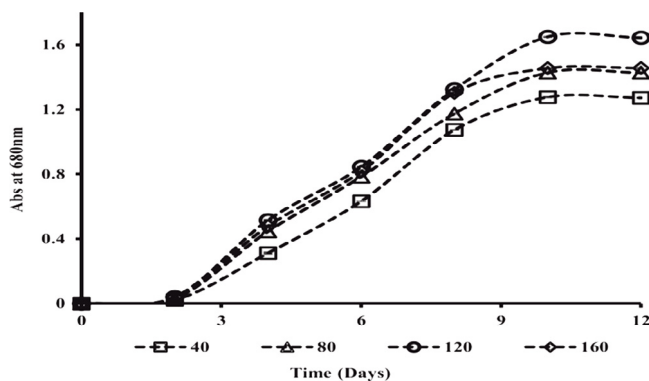


Fig. 1. Growth curves of *Nannochloropsis gaditana* at 40, 80, 120 and 160  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to OD at 680 nm versus time.

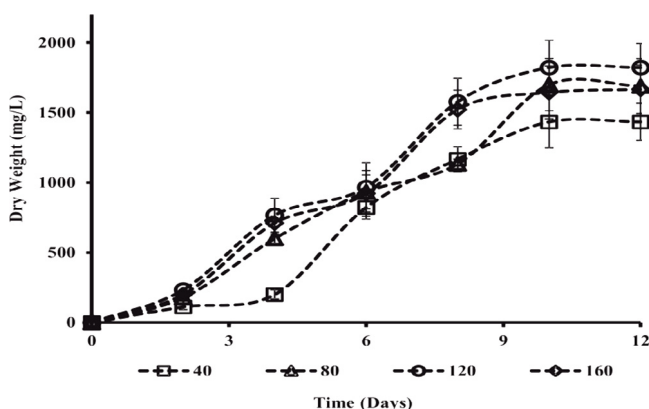


Fig. 2. Growth curves of *Nannochloropsis gaditana* at 40, 80, 120 and 160  $\mu\text{mol m}^{-2} \text{s}^{-1}$  with dry weight values (mg/L) vs. time (days).

### 3. Results and discussion

Curve values of *N. gaditana* are most likely changeable under various light intensities (40, 80, 120 and 160  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Lines of *Nannochloropsis gaditana* were determined at 40, 80, 120 and 160  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at 24 °C at 16:8 (light: dark) with OD. Line values of *N. gaditana* were displayed in Fig. 1.

120  $\mu\text{mol m}^{-2} \text{s}^{-1}$  had the maximum absorbance value with 1.64 and 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$  displayed the lowest value with 1.27. 80 and 160  $\mu\text{mol m}^{-2} \text{s}^{-1}$  had values with 1.43 and 1.46, respectively.

Also, we examined growth rates of *Nannochloropsis gaditana* with dry weight (mg/L). Results were found in parallel with OD and their line values were shown in Fig. 2. The highest biomass concentration carried out 120. 40 showed the lowest mass concentration. Also, biomass of *Nannochloropsis gaditana* was 540.63  $\text{mg L}^{-1}$  Ma et al. [18]. In other words, various macro and micro components caused to lower values for biomass content in its paper [18].

In addition to this, we determined some parameters of *Nannochloropsis gaditana* and these parameters were shown in Table 1.

The highest biomass productivity and specific growth rate were  $0.182 \pm 0.01 \text{ g L}^{-1} \text{ d}^{-1}$  and  $5.29 \pm 0.13 \text{ d}^{-1}$  in 120  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of light intensity, respectively. The lowest ones were  $0.143 \pm 0.01 \text{ g L}^{-1} \text{ d}^{-1}$  and  $5.10 \pm 0.16 \text{ d}^{-1}$  in 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of light intensity. On the contrary, 120  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of light intensity had the lowest doubling time with 0.131  $\text{d}^{-1}$ . 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of light intensity had highest doubling time with 0.136  $\text{d}^{-1}$ .

Carbohydrate concentrations and productivities of *Nannochloropsis gaditana* were carried out under various light intensities (40, 80, 120 and 160) at different acid concentrations  $\text{H}_2\text{SO}_4$ , HCl and  $\text{H}_3\text{PO}_4$  (% 1, 2, 3, 4 and 5). The highest carbohydrate concentration was found in HCl 3%. All experimental set up was prepared according to HCl 3%. Carbohydrate values were calculated as % dwt and carbohydrate productivities were found as  $\text{g L}^{-1} \text{ d}^{-1}$ . The results were given in Table 2.

**Table 1.** Growth parameters of time of *Nannochloropsis gaditana* under light intensities (40, 80, 120 and 160).

Light intensities [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	Biomass concentrations [ $\text{g L}^{-1}$ ]	Biomass productivities [ $\text{g L}^{-1} \text{d}^{-1}$ ]	Specific growth rates [ $\text{d}^{-1}$ ]	Doubling time [d]
40	$1.43 \pm 0.11$	$0.143 \pm 0.01$	$5.10 \pm 0.16$	0.136
80	$1.70 \pm 0.11$	$0.170 \pm 0.01$	$5.24 \pm 0.12$	0.132
120	$1.82 \pm 0.11$	$0.182 \pm 0.01$	$5.29 \pm 0.13$	0.131
160	$1.64 \pm 0.11$	$0.164 \pm 0.01$	$5.19 \pm 0.14$	0.134

**Table 2.** Carbohydrate content and productivity of *Nannochloropsis gaditana* under various light intensities (40, 80, 120 and 160) with HCl 3%.

Light intensities ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Carbohydrate content (dwt %)	Carbohydrate productivity ( $\text{g L}^{-1} \text{d}^{-1}$ )
40	$17.3 \pm 0.9$	$0.025 \pm 0.001$
80	$15.0 \pm 0.6$	$0.026 \pm 0.002$
120	$16.0 \pm 0.6$	$0.029 \pm 0.003$
160	$21.3 \pm 0.9$	$0.035 \pm 0.002$

Carbohydrate content with HCl 3% was  $21.3 \pm 0.9\%$  dwt for  $160 \mu\text{mol m}^{-2} \text{s}^{-1}$  of light intensity and this value was the highest one among them.  $120 \mu\text{mol m}^{-2} \text{s}^{-1}$  had  $16.0 \pm 0.6\%$  dwt. The lowest value was  $15.0 \pm 0.6\%$  dwt for  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Similarly, the maximum carbohydrate productivity was  $0.035 \pm 0.002 \text{ g L}^{-1} \text{d}^{-1}$  for  $160 \mu\text{mol m}^{-2} \text{s}^{-1}$  of light intensity. The minimum carbohydrate productivity was  $0.025 \pm 0.001$  for  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ . As a result, while  $120 \mu\text{mol m}^{-2} \text{s}^{-1}$  of light intensity had the highest productivity with 0.182.  $160 \mu\text{mol m}^{-2} \text{s}^{-1}$  of light intensity showed the highest carbohydrate productivity with  $0.035 \pm 0.002$ . In literature, *Nannochloropsis gaditana* was studied in detail for biodiesel production. Lipid productivity of *N. gaditana* was studied by some extended researcher. For example, Song carried out *N. gaditana 1049* for metabolic parameters such as lipid. He evaluated that it is acceptable for biodiesel production [19]. Hu demonstrated that TG of *N. gaditana* rised with N starvation [20]. Also, we produced bio-butanol from *Nannochloropsis gaditana* at  $160 \mu\text{mol m}^{-2} \text{s}^{-1}$  of light intensity. Biomass slurry including sugar were studied for measurement of bio-butanol and carried out spectrophotometrically. The results were given in Table 3.

**Table 3.** Bio-butanol content and yield from *Nannochloropsis gaditana* in  $160 \mu\text{mol m}^{-2} \text{s}^{-1}$  of light intensity.

Light intensities ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Initial sugar content (g/L)	Bio-butanol content (g/L)	Bio-butanol yield (g/g sugar)
160	20	$2.9 \pm 0.24$	$0.145 \pm 0.01$

In this study, 20 g of sugar was collected by means of concentration for bio-butanol production. Sugar was fermented and bio-butanol concentration was calculated. Bio-butanol and yield were 2.9 and 0.145 g/g sugar at  $160 \mu\text{mol m}^{-2} \text{s}^{-1}$  of light intensity, respectively.

#### 4. Conclusion

The bio-butanol production efficiency of *Nannochloropsis gaditana* under various light intensities (40, 80, 120 and  $160 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and different acid hydrolysis treatment strategies  $\text{H}_2\text{SO}_4$ , HCl and  $\text{H}_3\text{PO}_4$  (1, 2, 3, 4 and 5%) in FPBR was evaluated in the current paper. These findings suggest that *Nannochloropsis gaditana* can be used for effective carbohydrate content and large scale bio-butanol production in the future.

#### Acknowledgment

We would like to thanks DEE of YYU for their supports.

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