

PAPER • OPEN ACCESS

## Effect of $\text{NaNO}_3$ and $\text{NaCl}$ concentration on *Nannochloropsis oculata* cell biomass and FAME composition for biodiesel production

To cite this article: A F Zulkifli *et al* 2018 *J. Phys.: Conf. Ser.* **1123** 012071

View the [article online](#) for updates and enhancements.

You may also like

- [Onboard Science Instrument Autonomy for the Detection of Microscopy Biosignatures on the Ocean Worlds Life Surveyor](#)  
Mark Wronkiewicz, Jake Lee, Lukas Mandrake *et al.*
- [Corrosion of a Carbon Steel Cylindrical Band Exposed to a Concentrated NaCl Solution Flowing through an Annular Flow Cell](#)  
Alvaro Soliz and Luis Cáceres
- [The Effects of NaCl on Hydrogen Permeation and Sulfide Stress Cracking Resistance of C110 High-strength Steel](#)  
Lianlian Liu, Lin Chen and Raymundo Case



**ECS**  
The  
Electrochemical  
Society  
Advancing solid state &  
electrochemical science & technology

**DISCOVER**  
how sustainability  
intersects with  
electrochemistry & solid  
state science research

# Effect of $\text{NaNO}_3$ and $\text{NaCl}$ concentration on *Nannochloropsis oculata* cell biomass and FAME composition for biodiesel production

A F Zulkifli<sup>1</sup>, A Ramli<sup>1</sup>, J W Lim<sup>1</sup> and M K Lam<sup>2</sup>

<sup>1</sup>Department of Fundamental and Applied Science, Universiti Teknologi PETRONAS, 32610 Bandar Seri Iskandar, Perak, Malaysia

<sup>2</sup>Department of Chemical Engineering, Universiti Teknologi PETRONAS, 32610 Bandar Seri Iskandar, Perak, Malaysia

[anita\\_ramli@utp.edu.my](mailto:anita_ramli@utp.edu.my)

**Abstract.** In the present study, the growth of microalgal strain *Nannochloropsis oculata* was studied in different concentration of  $\text{NaNO}_3$  and  $\text{NaCl}$  as nutrient factors in culture medium to investigate its effect on the cell biomass and fatty acid methyl esters (FAME) composition. The microalgae were exposed to nitrogen and salt stress in two different experiment set-ups. The nitrogen source,  $\text{NaNO}_3$  was supplied at different concentration which are 25, 50, 75, and 100 mg/L while  $\text{NaCl}$  concentration was fixed at 25 g/L whereas  $\text{NaCl}$  concentration were tested at 0, 10, 25 and 35 g/L while  $\text{NaNO}_3$  was fixed at 75 mg/L. The results observed indicated that the growth of microalgae sample cultivated in  $\text{NaNO}_3$  concentration of 100 mg/L and  $\text{NaCl}$  concentration of 25 g/L is higher compared to that cultivated in other concentration of  $\text{NaNO}_3$  and  $\text{NaCl}$ , respectively after being analysed by the cell optical density. The specific growth rate shows similar results as treatment of 100 mg/L  $\text{NaNO}_3$  and 25 g/L  $\text{NaCl}$  have highest growth rate. The FAMEs content of different cultivation condition produces different composition of saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFAs) and poly-unsaturated fatty acids (PUFAs).

## 1. Introduction

The depletion rate of fossil fuels is rising to a concerning level due to the fact that it is a non-renewable energy while the daily energy demand had upsurge tremendously. The third-generation biofuel, microalgae had been into limelight for its high potential as a candidate for biodiesel production due to high photosynthetic efficiency compared to the conventional crops which are classified as first and second-generation biofuels [1]. The main advantages of microalgae as feedstock especially for biodiesel production are the ability to produce huge amount of triacylglycerols (TAGs), high growth rates, able to fix  $\text{CO}_2$  as it is converted to  $\text{O}_2$ , able to adapt to various environment condition and utilize nutrients from wastewater [2].

Microalgae has the ability to accumulate significant amount of TAGs which are reported to be the best substrate for biodiesel. In transesterification process the TAGs react with methanol to produce fatty acid methyl esters (FAME) and glycerol [3]. Marine microalgae *Nannochloropsis sp.* has been reported to produce a promising quantity of oils due to its fast growth and high lipid production (28.7%), mainly palmitic acid (C16) and significant amount of unsaturated fatty acids [4].



Studies have shown that the quantity of microalgae biomass production and lipid composition are affected by the growth conditions such as (temperature, light intensities, pH, duration of cultivation, CO<sub>2</sub> concentration and salinity) as well as nutrient composition (nitrogen, phosphate, carbon, iron concentration) [5,6]. To achieve both high biomass and lipid production at the same time are often a challenging task in microalgae cultivation as they are inversely proportional with each other where only one of the elements would produce a significant amount to satisfy the needs of biodiesel oil production. Therefore, it is crucial to apply an optimal cultivation method to produce high biomass and lipid production concurrently.

A stress condition applied to the microalgae may increase the lipid production as the unfavourable growth conditions are causing the microalgae to modify the lipid biosynthetic pathways towards the formation and accumulation of neutral lipids in the form of TAGs [2]. However, the concern on the applied stress conditions for the microalgae culture would result in lower productivity of the whole culture because of the decrease in growth rates [7]. This research aims to study the effect of NaNO<sub>3</sub> and NaCl concentrations as nitrogen and salinity factors, on *N. oculata* growth and lipid composition. The optical density, specific growth rate and FAME contents were used to determine the impact of these factors on microalgae for biodiesel production.

## 2. Materials and methods

### 2.1. Strain and starter culture

The marine microalgae (*N. oculata*) was obtained from sources of Centre for Biofuel and Biochemical Research (CBBR), Universiti Teknologi PETRONAS. The stock culture of microalgae was inoculated into a 1 L Erlenmeyer flask containing artificial seawater enriched with f/2 medium. The compositions of the media were kept within its standards for nutrient sufficient cultivation. The flasks were all kept under 24 hours illumination using fluorescence white light (Philip TL-D 36W/865, light intensity of 60-70 μmol/m<sup>2</sup>s) with temperature around 27± 2 °C, initial pH 8 and supplemented with 0.04% CO<sub>2</sub>.

### 2.2. Experimental setup

The microalgae culture cultivations were exposed to different concentration of NaNO<sub>3</sub> (25, 50, 75, and 100 mg/L) while another bioreactor was set-up for different NaCl concentration (0, 10, 25 and 30 g/L) NaCl. Compositions of other components were kept the same within normal artificial seawater and f/2 medium concentrations.

### 2.3. Determination of optical density

The cell absorbance is measured daily using Visible Spectrophotometer (Thermo Scientific 30 GENESYS Spectrophotometer) at wavelength 680 nm (OD<sub>680</sub>) in triplicates until day 14. It resembles biomass concentration from the microalgae sample culture colour saturation which could be used to measure microalgae growth.

### 2.4. Determination of microalgae specific growth rate

The growth rate of each sample was characterized based on the daily biomass concentration. The specific growth rate, referring to μ of each cultivation sample was calculated from the slope of the linear regression of cultivation days and natural log of biomass concentration during the exponential growth phase [8].

$$\mu = (\text{Ln } M_0 - \text{Ln } M_1) / (t_0 - t_1) \quad (1)$$

μ = specific growth rate

M<sub>0</sub> = biomass concentration at the beginning of exponential phase (t<sub>0</sub>)

M<sub>1</sub> = biomass concentration at the end of exponential phase (t<sub>1</sub>)

### 2.5. Harvesting for synthesis of biodiesel

150.0 mL of culture medium were recovered by centrifuge (Thermo Scientific Medifuge Centrifuge) at 3600 rpm for 10 minutes. The mixture was washed with distilled water and recovered by centrifuge twice. The supernatant was discarded and the remaining pellet was dried at 105 °C for 24 hours until a constant weight was achieved. The dried mass was measured using an analytical balance. Lipid extraction step were performed using modified Bligh and Dyer [9]. The dried microalgae were immersed into water and mixture of ethanol: ethyl acetate (1:2 v/v) with ratio of (2:3 v/v). Each sample was then centrifuged at 3000 rpm for 10 minutes. The upper layer (lipid and ethyl acetate mixture) was removed via suction using micropipette and transferred into another bottle. The mixture was dried to constant weight under compressed air blow.

### 2.6. Biodiesel analysis

The lipid extracted from the microalgae then undergoes transesterification to produce biodiesel. The process comprises of homogenizing every 10 mg of extracted lipid with 2 mL of KOH in methanol (1.5 mg/mL) via agitation. The samples were then put into a water bath shaker at 60 °C for 3 hours. The biodiesel layer was dried under compressed air blow until it reached constant weight. The biodiesel was then analysed using a Shimadzu GC-2010 gas chromatography with flame ionization detector (GC-FID) fitted with BPX-20 column to determine the fatty acid methyl ester (FAME) content. Helium was used as a carrier gas at a flow rate of 1.73 mL/min and pressure of 83.9 kPa. The initial column temperature was set at 150 °C and later increased to 240 °C at the rate of 5 °C/min while both the injector and FID temperatures were set at 250 °C. The injection volume was 1 µL with a split ratio of 10:1. Methyl heptadecanoate (C17:0) was used as an internal standard to quantify the individual FAME content.

## 3. Results and Discussions

### 3.1 Effect of different $\text{NaNO}_3$ and $\text{NaCl}$ concentration on microalgae growth

The growth of microalgae is divided into four phases, which are lag, exponential, stationary phase, and finally death phase [10]. Figure 1 shows the effect of  $\text{NaNO}_3$  and  $\text{NaCl}$  contents of the *N. oculata* cell growth measured using visible spectrophotometer at 680 nm.

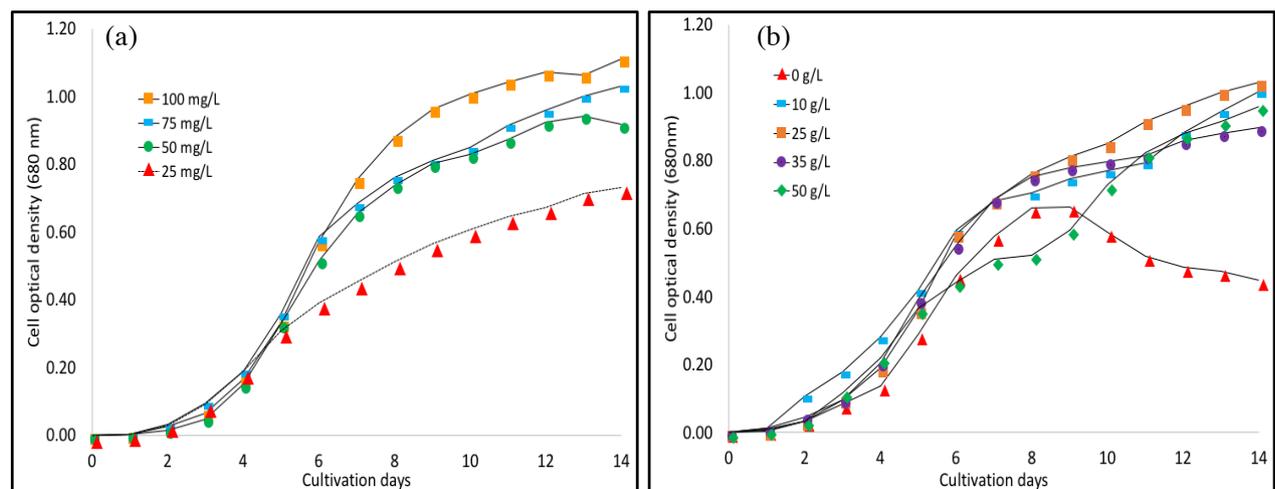


Figure 1: Cells optical density measured at 680 nm for microalgae cultivated in different  $\text{NaNO}_3$  concentration (25, 50, 75 and 100 mg/L)-(a) and  $\text{NaCl}$  concentration (0, 10, 25, 35, 50 g/L)-(b).

The optical density is used to measure the growth of microalgae where it resembles the intensity of chlorophyll pigments in the microalgae cells. The wavelength was set at 680 nm to measure the chlorophyll  $\alpha$  content in *N.oculata*. Figure 1(a) exhibits the absorbance of microalgae against cultivation days until day 14 for different concentration of  $\text{NaNO}_3$ . During the microalgae growth at different concentration of  $\text{NaNO}_3$ , 100 mg/L microalgae cultivation showed the highest absorbance reading after exponential phase. This is due to the abundance of nutrient availability in the cultivation medium. The result is in accordance with results reported by other researcher where the depleted nitrogen concentration (25 mg/L) shows the lowest cell optical density at 680 nm [11]. As nitrogen is proven to be the main nutrient composition, high amount of nitrogen would give microalgae sufficient energy to reproduce even after exponential phase.

Salinity is one of the important growth conditions vital for microalgae growth, lipid content and biochemical composition especially the marine species [12]. Figure 1(b) shows 25 g/L NaCl shows the highest cell density as compared to other salinity concentration, suggesting that only the most similar concentration of sea water had the most optimal condition for microalgae growth. In addition, low salinity level at 10 g/L NaCl may also assist in growth of the microalgae where there are no major differences of microalgae growth compared to the normal sea water concentration. At 0 g/L NaCl, the growth decreases after day 8 proving that saline species microalgae could not grow well in non-saline environment.

### 3.2. Effect of different $\text{NaNO}_3$ and NaCl concentration on microalgae specific growth rate

**Table 1:** Microalgae specific growth rate on different treatment of  $\text{NaNO}_3$

$\text{NaNO}_3$ concentration (mg/L)	Specific growth rate, $\mu$ ( $\text{d}^{-1}$ )
25	0.193
50	0.295
75	0.297
100	0.307

**Table 2:** Microalgae specific growth rate on different treatment of NaCl

NaCl concentration (g/L)	Specific growth rate, $\mu$ ( $\text{d}^{-1}$ )
0	0.243
10	0.277
25	0.297
35	0.254
50	0.194

The specific growth rate of microalgae depends on few vital factors including the nutrient compositions and the ability of microalgae to reproduce in certain conditions [13]. Tables 1 and 2 shows that the highest growth rate is observed when the culture medium contains 100 mg/L  $\text{NaNO}_3$  and 25 g/L NaCl. The results are in agreement with the results reported in Figure 1. This could be due to microalgae receives sufficient nutrition and saline condition almost similar to sea water. Other treatments are having quite similar growth rate even when stress was applied except for the lowest  $\text{NaNO}_3$  concentration, at 25 mg/L with specific growth rate of 0.193 due to low nutrient supply affects the growth of microalgae. However, the specific growth rate only provides information on production rate of microalgae at exponential phase but not the whole biomass content and lipid composition.

### 3.3. Composition of FAME produced as an effect of $\text{NaNO}_3$ and $\text{NaCl}$ concentration

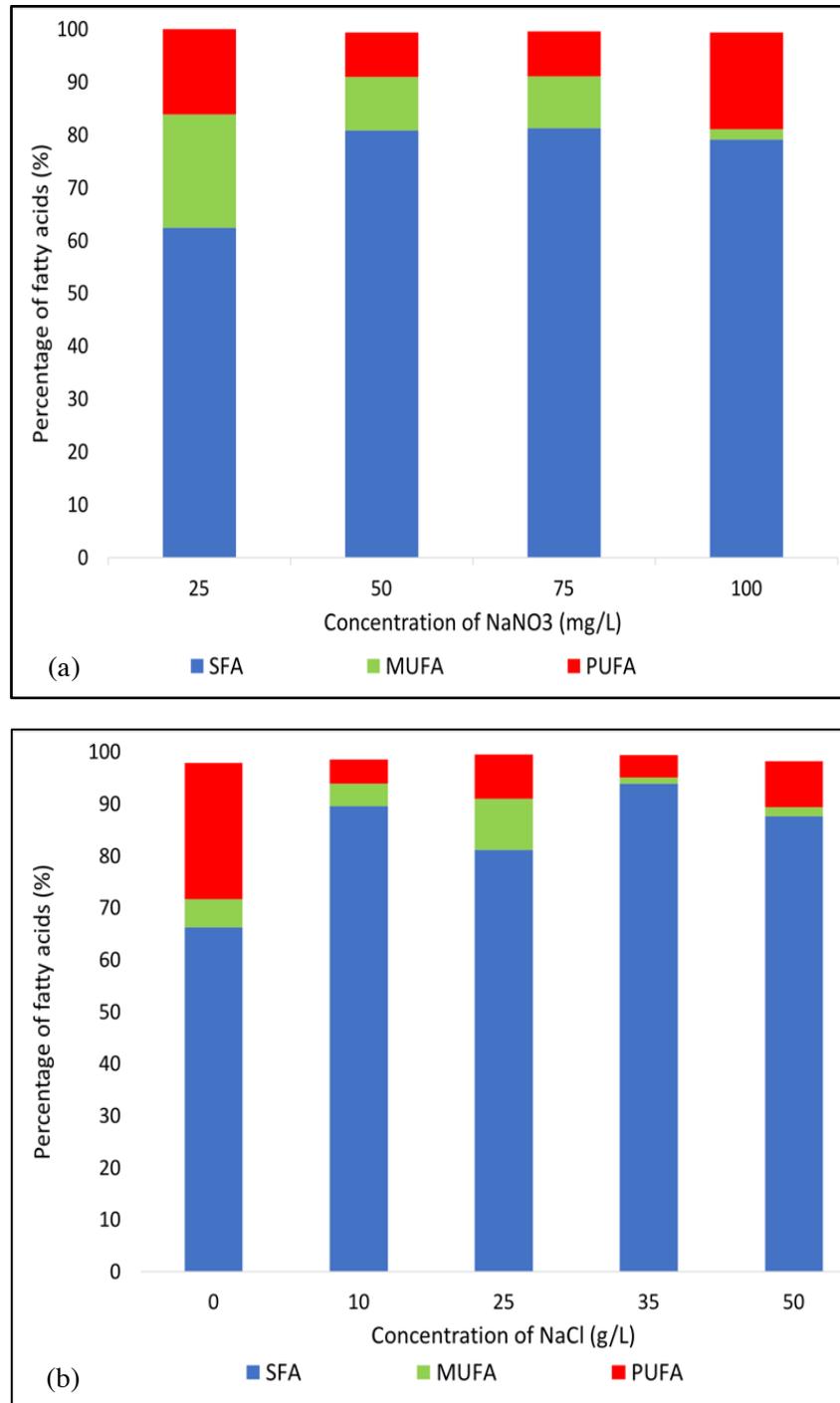


Figure 2: Composition of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) at different concentration of  $\text{NaNO}_3$  (a) and different concentration of  $\text{NaCl}$  (b).

Figure 2 shows the FAME composition for two different concentrations of nitrogen,  $\text{NaNO}_3$  and salinity,  $\text{NaCl}$ . The results of FAME analysis are similar to that reported by Ra *et al.* [13] as they found that *N. oculata* is mainly consists of palmitic acid (C16:0) and oleic acid (C18:0) which are classified under SFA group. The biodiesel standard requirements, namely, ASTM D6751 standard in US and EN 14214 standard in Europe had provide a guideline for the accepted biodiesel properties. High saturated fatty acids composition would give a better cetane number, kinematic viscosity and oxidative stability [14]. These factors are the main specification for a standard requirement for biodiesel. Elevated SFA content will result in high cetane number, kinematic viscosity and oxidative stability but poorer performance at low-temperature [15]. On the other hand, MUFA has the ability to improve biodiesel's cold flow as well as good oxidative stability [16] while high PUFA resulted in good cold flow but the biodiesel is easily oxidized thus not reaching a satisfactory level for biodiesel [2].

These results are in agreement with that reported by Wang *et al.* [15], on which the nutrient composition especially nitrogen would affect both cell growth and the biochemical composition of microalgae. Different salinity treatment also gave different composition of fatty acids despite *N. oculata* was originated from saline environment [17]. The SFAs content in all treatment shows high percentage where it is more than 60% and considered to be good amount according to the biodiesel standard. In the study of nitrogen concentration, 25 mg/L and 100 mg/L  $\text{NaNO}_3$  show higher PUFA as compared to less stress nitrogen condition. The results for salinity study also show the same pattern where 0 g/L and 50 g/L  $\text{NaCl}$  exhibits higher PUFAs than that of other concentrations. The presence of adequate amount of PUFA is necessary for the cold flow property of biodiesel. Overall, the combination of high number of SFAs (>60%) and the low number but adequate PUFAs (<20%) indicates that the *N. oculata* species has great potential to produce high quality biodiesel even when being cultivated in stress condition especially 50 and 75 mg/L  $\text{NaNO}_3$  and 10 and 35 g/L  $\text{NaCl}$  concentrations.

#### 4. Conclusion

The present work was carried out to observe the microalgae growth and FAME composition when being treated with different  $\text{NaNO}_3$  and  $\text{NaCl}$  concentrations. The microalgae cell optical density and specific growth rate shows similar results on which treatment has better growth performance. The FAME analysis provides sufficient data to choose between 50 and 75 mg/L  $\text{NaNO}_3$  and 10 and 35 g/L  $\text{NaCl}$  concentrations should be used in microalgae cultivation for biodiesel production. A further investigation should be conducted to determine the effect of combination of stress to the biomass growth and lipid production on *N. oculata*.

#### Acknowledgements

The authors are grateful to the Yayasan Universiti Teknologi PETRONAS research grant via YUTP for funding this research and UTP for financial assistance to Ms Amelia Farahani Zulkifli.

#### 5. References

- [1] Ahmad A L, Yasin N H, Mat, C J, Derek and Lim J K 2011 *Renew Sust Energ Rev* **15** 584-93
- [2] Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M and Darzins A 2008 *Plant J* **54** 621-39
- [3] Chisti Y 2007 *Biotechnol Adv* **25** 294-306
- [4] Gouveia L and Oliveira 2012 *J Ind Microbiol Biotechnol* **36** 269-74
- [5] Malakootian M, Hatami B, Dowlatshahi S and Rajabizadeh A 2015 *Env Health Eng and Man J* **2** 141-47
- [6] Thompson G A 1996 *Biochim Biophys Acta* **130** 313-26
- [7] Adams C, Godfrey V, Wahlen B, Seefeldt L and Bugbee B 2013 *Bioresour Technol* **131** 188-94
- [8] Song M, Haiyan P, Wenrong H and Guixia M 2013 *Bioresour Technol* **134** 353

- [9] Bligh E G and Dyer W J 1959 *Can J Biochem Phys* **37** 911-17
- [10] Moazami N, Alireza A, Reza R, Mehrnoush T, Roghieh E and Ali S N 2012 *Biomass Bioenergy* **39** 449
- [11] Rizwan M, Mujtaba G, Rashid N and Lee K 2017 *Chem Biochem Eng* **31** 199-207
- [12] Gu N, Lin Q and Li G 2012 *Eng Life Sci* **12** 631-37
- [13] Ra C H, Kang C H, Jung J H, Jeong G T and Kim S K 2016 *Bioresour Technol* **212** 254-61
- [14] Knothe G 2013 *Algae for Biofuels and Energy*, ed Borowitzka M A and Moheimani N R (New York: Springer) p 210
- [15] Wang X W, Liang J R, Luo C S, Chen C P and Gao Y H 2014 *Bioresour Technol* **161**124-30
- [16] Hoekman S K, Broch A, Robbins C, Cenicerros E and Natarajan M 2012 *Renew Sust Energy Rev* **16** 143-69
- [17] Khatoon H, Rahman N A, Banerjee S, Harun, N, Suleiman S S, Zakaria N H, Lananan F, Hamid S H A and Endut A 2014 *Int Biodeter Biodegr* 95 11