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# Acceleration of Lipid Accumulation in Oleaginous Diatom *Navicula* sp. Under Nitrogen Limitation

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## ABSTRACT

Diatom biomass gain attention globally as a source of lipid due to their high growth rate and biomass composition which can compete with fossil fuel for biodiesel production. Accumulations of natural lipid can be enhanced by various stress factors in diatoms. In this study, the effect of different nitrogen concentration on biomass and lipid production was investigated by cultivation of oleaginous diatom *Navicula* sp. for biodiesel production. The cultivation performed in phases where initially the culture was cultured in standard media for seven day and followed in different concentration of nitrogen such as limitation (0.35 mM), standard as media (1.76 mM), Repletion (3.5 mM) and free of nitrogen (0 mM) as second phase for eight days. The cells were grown under light intensity of 150 µmol photon $m^{-2}s^{-1}$(12:12 h day: light period) temperature (21±1 °C). Diatom *Navicula* sp. showed highest growth and biomass production (734±15 mg/L) in nutrient f/2 standard media and extracted 19% of lipid. However, 24% of lipid yield was extracted which is significantly high from the biomass (528±10 mg/L) cultured in limited nitrogen media. Nitrogen limitation enhanced the fatty acid as saturated fatty acid C16:0, unsaturated fatty acid C16:1 and polyunsaturated fatty acid C18:3. *Navicula* sp., have potential to accumulate lipid in nitrogen limitation condition biodiesel production.

**Keywords:** Biodiesel; biomass; diatom, *Navicula* sp.; nitrogen limitation

## INTRODUCTION

Diatoms (Bacillariophyta) are unicellular photosynthetic creatures with silica cell walls. It can also trap $CO_2$, grow in a wide range of aquatic settings, and store oil

at a far higher density than other plants (around 50 wt % of biomass). Diatoms have a brief life cycle and do not require arable land [1,2,3].

Lipid production from diatom must be extremely efficient and cost-effective in order to be commercially viable, because biodiesel is such a low-value product [4-7]. Therefore, the lipid-producing diatom strain chosen should have a high lipid content as well as a high cell growth rate which include *Thalassiosira pseudonana, Chaetoceros affinis, Navicula saprophila* and *Skeletonema marinoi* have high oil content, ranging from 20% to 50% by weight, making them suitable for biofuel generation [3-8].

The key elements that govern lipid accumulation in microalgae include light irradiation, temperature, and other chemical stimuli such as pH, salinity, mineral salts, and nitrogen deficiency [1,4,9]. Among that, macronutrient deprivation is one of the most popular strategies due to its great efficiency [7,10]. As a result, diatom lipid content and fatty acid production are critical criteria for producing high-quality biodiesel fuel with the appropriate qualities [11-12]. Continuous nitrogen deprivation enhances the lipid and carbohydrate content of microalgae, but also slows their development rate, lowering their overall productivity [6].

Continuous nitrogen deprivation enhances the lipid and carbohydrate content of microalgae, but also slows their development rate, lowering their overall productivity [6]. To address this issue, a two-stage culture approach has recently gained popularity, in which microalgae are grown in a nutrient-rich medium to obtain large cell biomass and then transferred to a nutrient-deficient medium to promote lipid and carbohydrate accumulation [12]. Therefore, this study is to investigate the biomass and lipid productivity, *Navicula* sp. cells grown in f/2 medium with different concentration of nitrogen where it is limited, excess and free from the standard f/2 conventional medium. The fatty acid content profile of the diatom also was analyzed to see if the produced diatom lipid could be used to make biodiesel.

## MATERIALS AND METHODS

### Strain and Culture Medium

*Navicula* sp. culture was obtained from Algae Culture Collection Centre and Laboratory, Universiti Malaysia Pahang. The culture was maintained in natural seawater supplemented with f/2 nutrients. The *Navicula* sp. inoculum for the experiment was preculture in 1000 ml sterilised (20 min at 120 °C) Erlenmeyer flasks with 500 mL f/2 media under light intensity of 150 µmol photon $m^{-2}s^{-1}$, temperature (21±1 °C) and with shaking (80 r.p.m.). The growth of culture was monitored by taking absorbance reading using Genesys UV-VIS Spectrophotometer.

## Experimental Conditions

The experiment was conducted in artificial seawater with f/2 nutrients with 120 µM $Na_2SiO_3.5H_2O$ to grow the culture. 10% of the actively growing culture of *Navicula* sp. was inoculated in 500 ml of standard f/2 culture medium in 1000 ml Erlenmeyer flask. All the cells were grown at 21±1°C, 150 µmol photon $m^{-2}s^{-1}$, of light intensity with 12:12 h dark: light cycle. The growth of culture was monitored for the first 7 days by taking optical density of culture in 1 mL of cuvette using a spectrophotometer at 680 nm. On day eight media was added in culture where in early stationary phase, with different concentration as limited (0.20 mM), standard concentration as control (1.76 mM), repleted (3.5 mM) and free of nitrogen (0 mM) in f/2 medium. The growth of culture was monitored again for the next 8 days. On day 15, grown cells were harvested by centrifugation at 7826 ×*g* for 10 min and dried at 70 °C in a heat chamber for 18 h.

## Lipid extraction and Fatty Acid Methyl Ester Analysis

Lipids were extracted by Bligh and Dyer assisted with Ultrasound technique. Dry biomass was weighed and mixed in 50 mL of hexane (1:8, w/v) solution in falcon tube. The mixture was vortexed for 30 s. The tube was placed in a water bath at 70±2°C, 1000-watt power for 60 min and disrupted the cells. The mixture was centrifuged at 3293 ×*g*, 10 °C for 5 min and collected the supernatant. Repeated the step by adding 50 mL of hexane solvent with same biomass, until it turns to colourless. The extracted lipid was trans esterified by using hexane/methanolic-KOH (2:1, 1%) [13]. The gas chromatography mass spectrometry (GC-MS) uses for the detection of fatty acid methyl esters content.

## RESULTS AND DISCUSSION

### Effect of Nitrogen Concentration in Different Concentrations of *Navicula* sp.

The effect of nitrogen limitation and repletion in diatom *Navicula* sp. with growth, biomass and lipid production were investigated. Before harvesting the cells, the morphological structure of cells was examined under light microscope and found changes on the boat shaped diatom based on their environment of media.

The growth begins to deviate after the media is added in with different concentrations of nitrogen in the following days of experiment as shown in Figure 1a. Continuous nitrogen limitation enhances the lipid and carbohydrate content of microalgae, but it drastically reduces biomass production. Highest biomass yield obtained from standard nitrogen media (734±12 mg/L) However, Nitrogen limitation reduced the biomass production and averagely obtained (528±15 mg/L) after 15 days cultivation. The least growth and biomass production found in repleted media due to excess amount nutrients cause toxic effects to the cells. As a result, it

inhibited the growth and biomass production of *Navicula* sp. Previous studies reported that, *Navicula* sp. obtained 369 mg/l biomass after six days of batch culture [13-15]. In response to nitrogen deficiency, the diatom was able to boost light intake and divert carbon metabolism toward lipid synthesis [15].

The Bligh and Dyer method assisted with ultrasound technique highly suited for lipid extraction from the dry biomass of *Navicula* sp. Highest lipid (24%) was obtained from the biomass harvested from the limited nitrogen media and followed by control media (21%). The lowest lipid (4%) obtained from the repleted nitrogen media due to limited growth of cells. In nitrogen free media found lower lipid yield (18%) even though obtained highest biomass production. Many studies have found that when nitrate levels are low, marine diatoms produce more lipids [11-15].

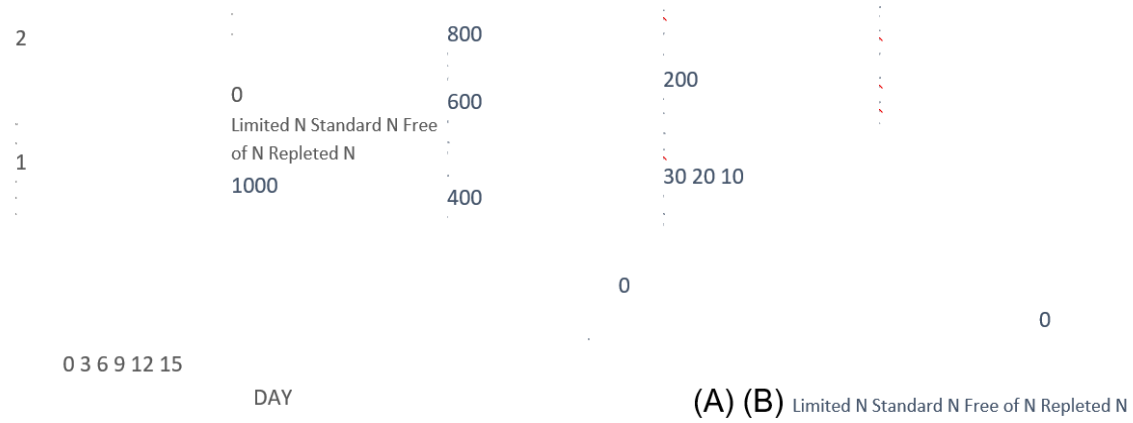


**Figure 1** Growth curve (A) and biomass and lipid production (B) of *Navicula* sp. cultivated in different concentrations of nitrogen.

## Fatty Acid Composition Analyses

Gas chromatography mass spectrometry analysis revealed the fatty acid methyl ester which is present in *Navicula* sp. extracted and trans esterified lipids. Significant criteria for improved biodiesel quality are composition of fatty acids of marine diatoms is carbon number between C14:0, C16:0, C16:1, C18:0, C18:1, and C20:5 (n-3) (14-15). Palmitic acid (C16:0) was the most abundant fatty acid in the repleted nitrogen medium (45.91%), followed by mono-saturated palmitoleic (C16:1) at 33.78% in free of nitrogen media. Highest polyunsaturated fatty acid (C20:4) found from free of nitrogen media at 12.67%.

## CONCLUSIONS

This study highlighted the potential of *Navicula* sp. has the source for biofuel production at optimum conditions. The effect of nitrogen was compared between the first and second phase of cultivation. Nitrogen limitation influences the accumulation of lipid in cells (obtained 24% of cell dry weight). Nitrogen limitation also enhances the biomass and lipid productivity in the cells.

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