CULTIVATING MICROALGAE, CHLORELLA SP. IN NITRATE REPLETE CONCENTRATION FOR BIODIESEL PRODUCTION

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Abstract—**The impact of various nitrate replete concentration is investigated in terms of biomass yield, cell concentration and nitrate concentration. The media used in this study is Bold's basal Medium as the medium contain nitrate that act as nutrient and varied for the purpose of finding best concentration to produce high biomass yield. The chemical used for the medium is fairly easy to obtained thus used as the media. The nitrate concentration is varied as 30 g nitrate/L, 35 g nitrate/L, 40 g nitrate/L,45 g nitrate/L and 50 g nitrate/L concentration. The sample is culture in Schott bottle with light presents and aerated with air compressor. The results show that the best concentration to obtain highest biomass yield is 45 g nitrate/L concentration. 45 g nitrate/L concentration gives highest optical density reading at day 9 with 2.100 ± 0.070 and also shows the highest cell concentration with 703 ± 29 x 10^6 cells/mL. The dry algae produced by 45 g nitrate/L concentration after the end of the cycle is 291 ± 9 mg which is the highest compare to other concentration. As the biomass yield is already increased using 45 g nitrate/L concentration, other methods to increase lipid content can be pair with nitrate replete method and can be further studied in future.**

Keywords— Biodiesel, Microalgae, Nitrate replete, Biomass, Lipid yield.

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I. INTRODUCTION

Biodiesel have been an alternative for gasoline and fuels as biodiesel is more environmental friendly. There are many types of biodiesel which is grouped into generations. The first-generation biodiesel is made from feedstock while the second generation is from non-human consumption such as agricultural waste. Microalgae-based biodiesel is the third generation of biodiesel which is favorable compare to the others.

Biodiesel component is mainly form by fatty acid methyl esters (FAME) which can be found in abundance in lipid. As biodiesel is extracted from lipid, the amount of biodiesel is dependent on lipid yield. Lipid content can be increase by introducing stress to the microalgae in the nitrate deplete stage. Unfortunately, during nitrate deplete stage, the biomass decreases while lipid content increase, which will significantly reduce lipid yield when biomass is reduced. Biomass yield have to be increased so that lipid yield is not significantly reduced.

In this study, the microalgae are cultivated in various nitrate replete concentration to explored the impact of cultivating in various concentration in terms of biomass yield, cell concentration and optical density. The nitrate replete concentration is varied as 30 g nitrate/L, 35 g nitrate/L,40 g nitrate/L, 45 g nitrate/L and 50 g nitrate/L concentration. This study can help as a precursor for two stage cultivation.

II. METHODOLOGY

A. Cultivating microalgae Chlorella sp. in various nitrate concentration

The microalgae are cultured in Bold's Basal Medium (BBM) which contained 10 ml of the following chemical: $NaNO_3$ (25 g/L), $CaCl.2H_2O$ (2.5 g/L), $MgSO_4.7H_2O$ (7.5g/L), K_2HPO_4 (7.5 g/L), KH_2PO_4 (17.5 g/L), NaCl (2.5 g/L), and 1 ml for the following chemical: EDTA(50g/L),KOH(31 g/L), $FeSO_4.7H_2O$ (4.98 g/L), H_2SO_4 (1 mL), H_3BO_3(11.42 g/L), $ZnSO.7H_2O$ (8.82g/L), $MnCl_2.4H_2O$(1.44g/L), MoO_3(0.71g/L), $CuSO_4.5H_2O$ (1.57 g/L) and $Co(NO_3)$. 6H2O (0.49g/L) (Bischoff & Bold, 1963). The microalgae Chlorella sp, was extracted and the volume of microalgae is 10% from the total volume of media. The cycle last for 10 days and each sample is recorded. The modified media for 35 g nitrate/L,40 g nitrate/L,45 g nitrate/L and 50 g nitrate/L concentration is modified by adding more $NaNO_3$ based on the percentage concentration. The sample is cultured in Schott 500mL bottle and exposed to light. The sample also aerated using air compressor (Y. Shen, 2014)

B. Growth rate analysis

The growth rate of the sample can be determined by measuring optical density and measuring biomass yield. The optical density reading is taken using spectrophotometer. Biomass yield is determined by filtering 15 mL of the sample and filter using Glassman Micro Filter Paper. The filter paper is washed with 20mL of distilled water, dried in oven for 2 hours at 105°C. The weight of filter paper, filter paper with microalgae is taken and recorded. The biomass is calculated using this formula:

Growth rate (d^{-1}) = ($\ln DW_t - \ln DW_o$) / t , where DW_t is for biomass weight on t day and DW_o is for initial day. [3]

Cell concentration data was taken from optical density reading. Standard curve is plotted based on optical density and cell concentration (Luveshan Ramanna, 2013). The cell concentrations are calculated based on the standard curve.

C. Nitrate Concentration Analysis

The nitrate concentration is taken every day after filtering 15 mL sample during biomass yield analysis. 10 mL of filtrate is taken and analyze. The sample is analyze using cadmium reduction method provided by Hach Spectrometer. The 10mL filtrate is mixed with NitraVer 5 Nitrate Reagent Powder Pillow. The sample is then shaken vigorously for 1 minute. The sample is then put to rest for 5 minutes before reading taken. Program used for the spectrophotometer is 355 N Nitrate HR PP.

D. Cell harvesting

The microalgae are centrifuged at 6000 rpm in 10°C. The microalgae are washed twice with distilled water to get rid of contaminants and then dry in oven for 24 hours in 60°C (Sangeeta Negi, 2015). The dried algae are used in lipid analysis.

III. RESULTS AND DISCUSSION

This study was done to find optimum concentration that can produce highest biomass yield. High biomass yield is needed as during nitrogen deplete stage, lipid content is increased but biomass decrease. It is profitable to produce higher biomass yield so the lipid yield is not significantly reduced. The best concentration can be determined by optical density, cell concentration, biomass yield and nitrate concentration.

A. Optical Yield

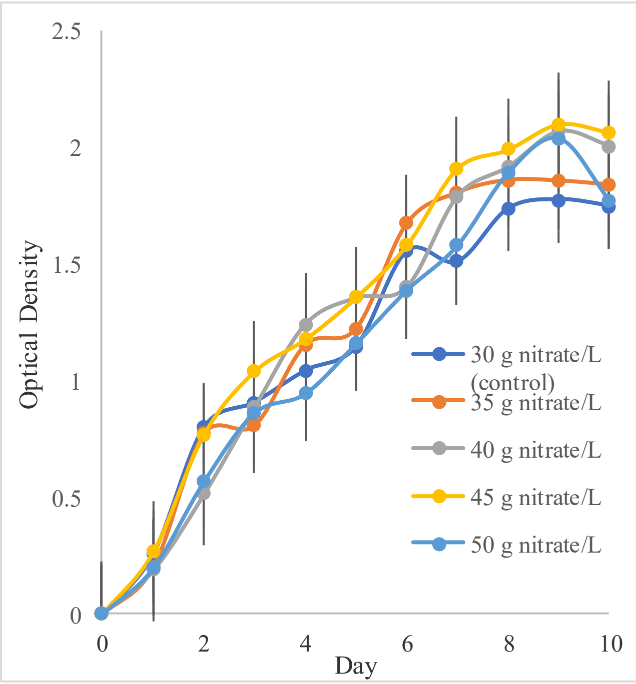

Figure 1 Optical Density of Various Nitrate Concentration

From **Figure 1,** At 50 g nitrate/L concentration, the growth slow down because the amount of nitrogen is in excess causing inhibition to occurs. The optical density from day one to nine of 30 g nitrate/L and 50 g nitrate/L shows almost similar growth. During Day 6, the highest optical density is 35 g nitrate/L, but during other days, 35 g nitrate/L shows low optical density reading.

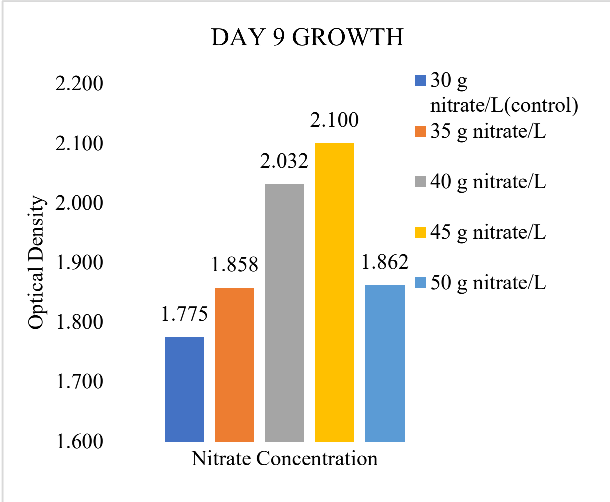

Figure 2 Optical Density in Day 9

From **Figure 1**, it shows that most nitrate concentration reached peak at day 9. As shown in **Figure 2**, the highest concentration on day 9 is 45 g nitrate/L. 40 g nitrate/L concentration is the second highest. Concentration 35 g nitrate/L and 50 g nitrate/L shows almost similar growth in terms of optical density.

Most concentration shows peak in optical density in day 9 except for 35 g nitrate/L concentration which peak in day 8. 30 g nitrate/L that cultured as control peak with reading of 1.775 ± 0.035. 35 g nitrate/L concentration peak reading on day 8 is 1.861 ± 0.070, 40 g nitrate/L and 50 g nitrate/L concentration with reading of 2.068 ± 0.018 and 2.036 ± 0.022 respectively. The highest reading is 45 g nitrate/L concentration in day 9 with 2.100 ± 0.070

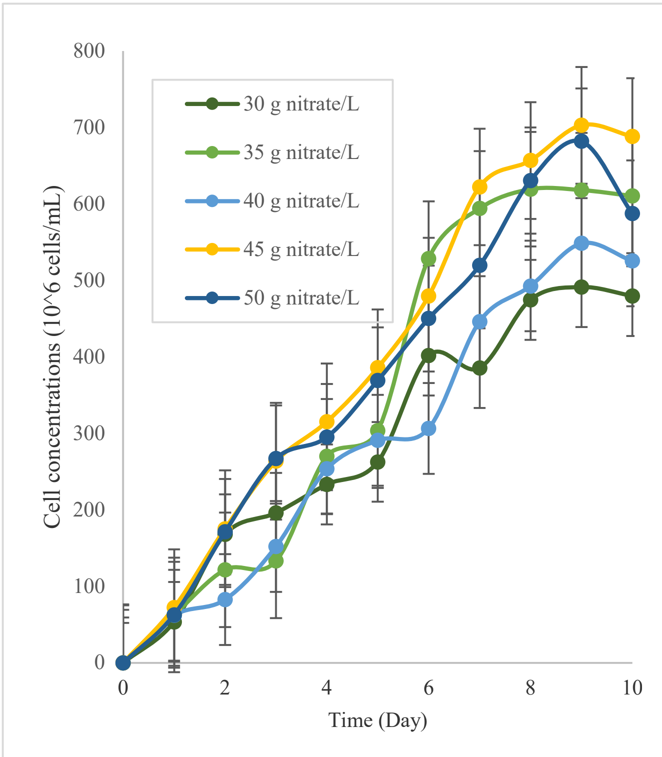

Figure 3 Cell Concentration of Various Nitrate Concentration.

Cell concentration as shown in **figure 3** is calculated based on optical density reading obtained. Similarly, with previous graph, the trend shows that the highest nitrate concentration that produce highest cell concentration is 45 g nitrate/L. The highest cell concentration is of 45 g nitrate/L concentration on day 9 with 703 ± 29 x 10^6 cells/mL.

B. Biomass Yield

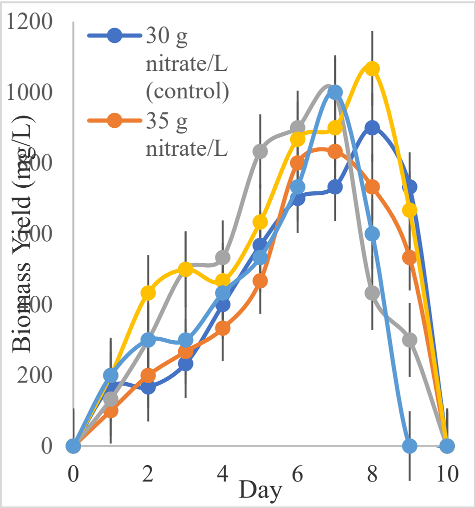

Figure 4 Biomass Yield for Various Nitrate Concentration

Biomass yield is taken daily by weighing the filtered algae. Similarly, with optical density graph, the biomass yield peaked at day 9. The lowest nitrate concentration shown is 50 g nitrate/L while the highest is 45 g nitrate/L. 35 g nitrate/L reached peaked at day 7 and decrease in day 8 and 9 this is probably because of nitrate concentration reduced by day 7. The highest biomass yield is on day 9 by 45 g nitrate/L at 1066.67 mg/L. 40 g nitrate/L follows with 1000 mg/L on day 9. In some days, 35 g nitrate/L and 50 g nitrate/L shows lower biomass yield than 30 g nitrate/L. While, 40 g nitrate/L and 45 g nitrate/L is almost always slightly higher than 30 g nitrate/L. During last day of cultivation, 30 g nitrate/L shows the highest reading with 733.33 mg/L while 40 g nitrate/L is the lowest at 433.33 mg/L and 45 g nitrate/L is right behind 30 g nitrate/L with 666.67 mg/L. Even though, 30 g nitrate/L shows high biomass yield at the end of cycle, 30 g nitrate/L did not exceed 40 g nitrate/L and 45 g nitrate/L biomass yield in most of the days.

C. Comparison of various nitrate concentration.

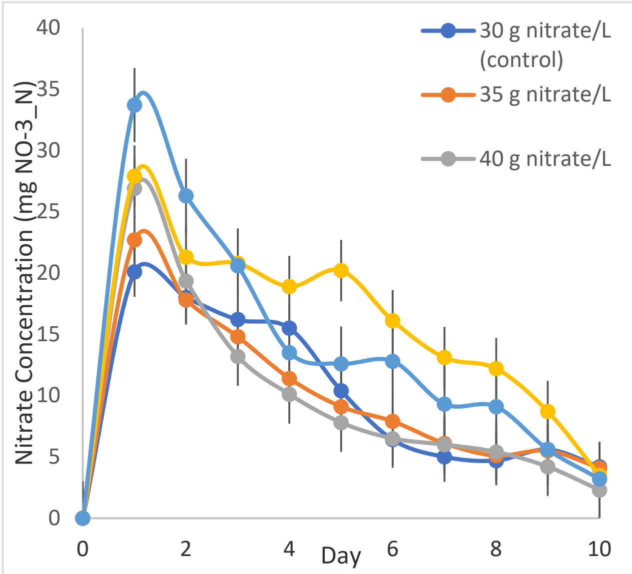

Figure 5 Nitrate Concentration Every Day of Various Nitrate Concentration

Microalgae consume nitrate to increase in biomass, for that it is necessary to record the trend of nitrate concentration. The data was taken after diluted to 2 dilution factor(DF). Dilution factor 2 is needed because the concentration of 50 g nitrate/L at the beginning exceed 30 mg/L which is the limit for reading of the spectrometer. All concentration shows significant drop from Day 1 to Day 4 except for concentration 45 g nitrate/L which shows almost stagnant reading between Day 2 and Day 3. From Day 3 onward the trend is slowing compare to others concentration. It can be related to optical density of 45 g nitrate/L on day 2 and day 3 which rises slowly compare to others concentrations.

At the end of the cycle, 30 g nitrate/L concentration shows the highest nitrate left with 4.20 ± 0.10 mg NO_3-N/L, followed by 35 g nitrate/L concentration with 4.10 ± 1.7 mg NO_3-N/L. Although 45 g nitrate/L shows high biomass yield compare to others, the nitrate concentration at last day of cycle is not the lowest at 3.5 ± 0.1 mg NO_3-N/L followed by 50 g nitrate/L concentration with 3.2 ± 0.6 mg NO_3-N/L. The lowest nitrate concentration at the end of the cycle is 40 g nitrate/L concentration with 2.3 ± 0.7 mg NO_3-N/L

D. Cell harvested

The weight of each dry algae is taken record and send to lipid analysis. The heaviest dry algae are 45 g nitrate/L with 291 ± 9 mg. 40 g nitrate/L weight almost similar with 45 g nitrate/L, 252 ± 2 mg with exception of 50 g nitrate/L where the weight is lower than 40 g nitrate/L although more concentrated than 40 g nitrate/L with 208 ± 7 mg. The volume of the media before centrifuge is noted. 45 g nitrate/L concentration with media volume of 388 mL produce about 291 ± 9 mg. While, 40 g nitrate/L concentration with 393 mL media produce only 252 ± 2 mg. Even though the volume of 40 g nitrate/L is larger than 45 g nitrate/L, the dried algae produce is lesser.

E. Growth analysis based on nitrate concentration and days

The growth of microalgae shows decline after certain days regardless the nitrate concentration. This is because the amount of nitrate decreases as time while the microalgae keep growing. This cause the nitrate as nutrients unable to meet demands of microalgae thus the microalgae growth decline. This can be seen starting on day 9 in figure 1,3 and figure 4.

Higher amount of nitrate also did not guarantee the biomass yield, as nitrate at 50 g nitrate/L concentration shows lesser biomass yield compare to 40 g nitrate/L concentration. Although using different media, Y. Shen et. al. and Ting Ting et. al. also proves there is decline in growth after certain concentration [1][2]. Y. Shen et. Al. indicates that the decline is cause by inhibition of nitrate because the concentration is too high [1]. Ting Ting et. al. states that after certain glucose concentration, the graph turns plateau [2].

IV. Conclusion

In this study, the best concentration to culture microalgae to produce highest yield was determined by biomass yield, cell concentration and nitrate concentration value. Five different nitrate concentrations with exception of one nitrate concentration as a control was experimented. Out of all concentration of 30 g nitrate/L, 35 g nitrate/L, 40 g nitrate/L, 45 g nitrate/L and 50 g nitrate/L, the best concentration proves to be 45 g nitrate/L concentration. Although 40 g nitrate/L shows similar growth, at the end of the cycle, 45 g nitrate/L give better biomass yield than 40 g nitrate/L. The other 2 concentrations, 35 g nitrate/L and 50 g nitrate/L produce less biomass yield than 40 g nitrate/L. Thus 45 g nitrate/L is better concentration to increase biomass yield for biodiesel production in terms of biomass yield, cell concentration and nitrate concentration.

Another finding during this experiment is, the best day to culture microalgae is 10 days, with day 9 shows biomass yield and cell concentration at peak reading.

Acknowledgment

Thank you to my supervisor, Prof Dr Norazah Abd Rahman and Universiti Teknologi Mara. Special thanks to Mrs Norazalina and colleagues Mr. Ahmad Naufal Ahmad Nizar for assistance.

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