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EFFECTS OF NITROGEN SUPPLEMENTATION IN REPLETE CONDITION ON THE BIOMASS YIELD AND MICROALGAE PROPERTIES OF CHLORELLA SOROKINIANA

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ABSTRACT

Microalgae has received a noble attention in recent years as an alternative feedstock for biofuel production as its capability to produce lipid and has high growth rate. Biodiesel from microalgae is another route to solve fossil fuel dependence, reduce climate changes and enhance food security. High yield of biodiesel produced from microalgae is dependent on large quantities of neutral lipids accumulated in each microalgae cell. The number of microalgae cell can be increased by manipulating the culture conditions and nutrients feed in term of the concentration. Thus, this present work was aimed to encourage continuous cell division and produced high biomass yield of Chlorella sorokiniana (C. sorokiniana) under nitrogen replete condition in view of its capability to produce lipid. This work also presents the physical and chemical properties of C. sorokiniana. The American Society for Testing and Materials (ASTM) standard methods were implemented to examine the microalgae properties, including proximate and ultimate analyses. Microalgae C. sorokiniana supplemented with nitrate concentration of 43.65 mg NO_3-NL^{-1} was found to have a high biomass yield of 940 ± 10mgL^{-1} and specific growth rate 0.224 ± 0.001 d^{-1}.In comparison, the lowest biomass yield and specific growth rate was in C. sorokiniana supplemented with 48.90 mg NO_3-NL^{-1} were 760 ± 6 mgL^{-1} and 0.134 ± 0.001 d^{-1} respectively. On the other hand, TGA analysis showed C. sorokiniana supplemented with 33.95 mg NO_3-NL^{-1} contains high volatile matter (VM) 68.50 and low ash content 9.77in %wt wet basis than other nitrate concentration levels. While, C. sorokiniana supplemented with nitrate concentration of 43.65 mg NO_3-NL^{-1}contains average VM and ash content of 51.96 and 33.39 in %wt wet basis respectively. Similarly, in ultimate analysis, C. sorokiniana supplemented with 33.95mg NO_3-NL^{-1} and 43.65 mg NO_3-NL^{-1} contained carbon content of 46.16and 37.681 %wt moist basis respectively. These result demonstrated that as low as 25% of nitrate increment from control BBM media gained high VM and carbon content. In contrast, as high as 75% of nitrate incrementfrom control BBM media increase biomass yield and specific growth rate which in return lower the VM and carbon content.

Keywords: microalgae, C. sorokiniana, nitrogen replete condition and biomass yield.

INTRODUCTION

Microalgae are a single or multicellular organism with fairly complex and differentiated forms. Microalgae are known as one of the oldest life-forms and have simple development system. Unlike first and second generations of biofuels, biodiesel production from microalgae is about 15-300 times more than traditional crops on an area basis. Microalgae are chosen over first and second generations of biofuels because it synthesize and accumulate large quantities of neutral lipids (20-50 % dry weight of biomass) [1].

Chlorella sorokiniana, is under genus of Chlorella sp. which known to have a great potential to be used for biodiesel production as it has high biomass yield and lipid content. Biomass yield is one of the most important factors for biodiesel made from microalgae in order to reduce the process cost. Hence, the effect of commonly used nitrogen sources, which is nitrate with five concentration levels of C.sorokiniana was highly interested. The objective of this study was to improve the biomass yield of C.sorokiniana in nitrogen replete condition and investigate the potential of this algal species as a feedstock for biodiesel production. Firstly, the relationship between biomass yield and nitrogen consumption was studied in order to improve the growth rate and select the best nitrate concentration for C.sorokiniana. Then, proximate and ultimate analyses have been conducted on biomass of C.sorokiniana. Proximate analyses have been the commonly method to characterized biomass and other energy fuel. Ultimate analyses on the other hand, are widely used for determination of single element such as carbon, hydrogen, nitrogen, sulfur and oxygen. This helps to asses C.sorokiniana as a feedstock for biofuel production even further. Finally, evaluations of volatile products were performed by Fourier Transform Infrared Spectrometry (FTIR).

MATERIALS AND METHODS

Algae strain and subculture

C.sorokiniana microalgae strains was maintained in Bold Basal Medium (BBM), which consisted of $NaNO_3$ 2.94 mM, $CaCl_2 \cdot 2H_2O$ 0.17 mM, $MgSO_4 \cdot 7H_2O$ 0.3mM, K_2HPO_4 0.43 mM, KH_2PO_4 1.29 mM, NaCl 0.43 mM, EDTA 0.17mM, KOH 0.55mM, $FeSO_4 \cdot 7H_2O$ 0.01 mM, H_3BO_3 0.18 mM, $ZnSO_4 \cdot 7H_2O$ 0.03 mM, $MnCl_2 \cdot 4H_2O$ 0.007 mM, MoO_3 0.005 mM, $CuSO_4 \cdot 5H_2O$ 0.006 mM, $Co(NO_3)_2 \cdot 6H_2O$ 0.002 mM. A stock culture of 35 ml C.sorokiniana was cultured in 500 mL cylindrical Schott bottle with stainless steel plate Thread:GL45 (15 cm length, 8cm diameter) (TGI, Germany) with 350 mL working volume of BBM under 28±1 °C and illuminated

with 2500 lux light intensity. The bottle was air-aerated with a flow rate of 125 mL/min via bubbling from the bottom of Schott bottle using a silicone tubing 3 x 5mm (ID x OD). The air was filtered by syringe filter 25mm, pores 0.45um (PTFE) at the inlet. After 4 days of cultured, this was used as a starter culture for further experiments. The inoculation culture was used for experiments at algal cell concentration reached about 10^6 cellsmL^{-1}. Light intensity was measured using a High Intensity Dual Scale Light Meter (Extech Instrument, USA). All cultures were performed in two independent duplicate.

Influence of nitrogen concentrations

The experiments were conducted at either the control BBM media (supplementation of 29.60 mg NO_3-N/L) or one of the modified BBM media (supplementation of 34.03, 41.11, 47.47 or 53.64 mg NO_3-NL^{-1}) as shown in Table-1. The initial inoculum density was 4.9x10^8 cellsmL^{-1}. Then, the culture was incubated in 500 mL cylindrical Schott bottle with stainless steel plate Thread: GL45 (15 cm length, 8cm diameter) (TGI, Germany) under 28±1 °C and illuminated with 2500 lux light intensity. The bottle was air-aerated with a flow rate of 125 mL/min via bubbling from the bottom of Schott bottle using a silicone tubing 3 x 5mm (ID x OD). The air was filtered by syringe filter 25mm, pores 0.45um (PTFE) at the inlet.

The cultures were incubated for another ten days. Nitrate concentration was measured daily using cadmium reduction method, Method 8171 by Powder Pillow as described by [2].

The initial pH was 7 and was measured daily with a pH meter (Mettler Toledo AG8603, China). Experiments were performed thrice. Optical densities, cell concentrations and biomass yield of C.sorokiniana were then measured. For proximate and ultimate analysis, cells were harvested using centrifugation at 2500rpm for 5 min and pellets were freeze dried vacuum.

Table-1. C.sorokiniana cultured in various nitrogen concentrations.

Nitrogen source	**Increment** (%)	**Nitrate Conc. (**mg NO_3-NL^{-1}**)**	**Nitrogen Conc. (**mM**)**
Nitrate	0	29.80	2.94
	25	33.95	3.68
	50	39.90	4.41
	75	43.65	5.15
	100	48.90	5.88

Analytical procedure

Cell concentrations (cellsmL^{-1}) was determined using a Hemocytometer Set Double Cell Standard Improved Neubauer (Optik Labor, Sussex, UK) by cell counts on a Biological microscope (Meiji Techno Co., Ltd., Japan) at 40xmagnification.

$$Cell\ count = no\ of\ cells x\ 10^4\ x\ Dilution\ factor \quad (1)$$

Cell dry weight biomass was determined by filtering the microalgae cultures onto filter paper. Filter paper used was a pre-dried and pre-weighed express plus PES (pore size 0.22 um, diameter 90 mm) (Merck/Germany). Samples of 15 ml microalgae cultures were filtered using vacuum filter (Corning), rinse twice with distilled water, dry at 105°C for 2 h, cool in a desiccator and weigh to give the cell dry weight (mg/L). The specific growth rate is determined using below equation during the exponential period (1-10 days).

$$\mu = \frac{(\ln x_2 - \ln x_1)}{(t_2 - t_1)}, \quad (2)$$

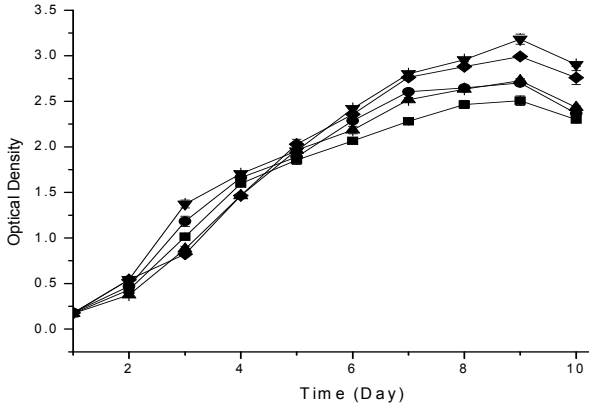
where W_1 and X_2 are the cell dry weight (mg/L) at time t_2 and t_1 (day) respectively.

Freeze dried algae sample has been analysed for proximate analysis using Thermogravimetric Analyser (TGA), Mettler Toledo TGA/SDTA 851, Switzerland where 20 mg of freeze dried microalgae sample been placed into 150µL ceramic crucible. Nitrogen gas was used as the carrier gas at flow rate of 20 mLmin^{-1} to protect the samples from oxidation process. Microalgae samples were heated from 25 °C to 1000 °C at a rate of 20 °C min^{-1}. Weight loss of the samples and its rate were continuously recorded during the process. The fixed carbon content was determined by subtracting the summation of the moisture content (MC), volatile matter (VM), and ash content from the total sample mass. The ultimate analysis for elemental content (Carbon (C), hydrogen (H), nitrogen (N), sulfur (S) and oxygen (O)) of freeze dried algae sample was tested by Elemental Analyzer, Thermo Electron, Flash EA 1112 Series. FTIR spectroscopy was used to determine all the chemical bonding including double and triple bonds in the freeze dried C.sorokiniana sample. Presence of any hydrocarbon in the freeze dried sample can be determined for further analysis.

RESULTS AND DISCUSSIONS

The effect of nitrogen supplementation on the growth of C.sorokiniana

The effect of commonly used nitrogen sources, which is nitrate with five concentration levels of C.sorokiniana was investigated. Effect on optical density and cell concentration were shown in Figure-1. The final algae cell concentrations increased when nitrogen concentration when up from 29.80 mg NO_3-NL^{-1} to 43.65 mg NO_3-NL^{-1}, but at high concentration of nitrate 48.90 mg NO_3-NL^{-1} both optical density and cell concentration showed inhibitory effects. At nitrate concentration of 43.65 mg NO_3-NL^{-1}, C.sorokiniana reached the highest optical density, cell concentrations and cells productivity of 3.174, 1090 x 10^6 cellsmL^{-1} and 121 x 10^6 cellsmL^{-1} respectively. Compared with nitrate concentration of 29.80 mg NO_3-NL^{-1}, culture of C.sorokiniana showed optical density, cell concentration and cell productivity were only 2.508, 885 x 10^6 cellsmL^{-1} and 98 x 10^6 cellsmL^{-1}, respectively. These results showed that C.sorokiniana had higher optical density, cell concentration and cell productivity with nitrate concentration of 43.65 mg NO_3-NL^{-1}but the high nitrate concentration 48.90 mg NO_3-NL^{-1} showed inhibitory effects.



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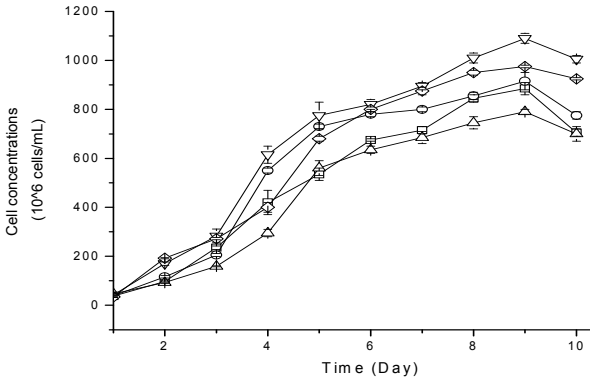


(b)

Figure-1. Effects of nitrogen supplementation of the culture medium on the optical density (a) and cell concentrations (b) (29.80 mg NO_3-NL^{-1}, square; 33.95 mg NO_3-NL^{-1}, circle; 39.90 mg NO_3-NL^{-1}, up triangle; 43.65 mg NO_3-NL^{-1}, down triangle; 48.90 mg NO_3-NL^{-1}, diamond).

In Table-2, the maximum biomass yield achieved by five concentration levels of nitrate were: 852 ± 8 mgL^{-1} (growth rate 0.214 ± 0.001 d^{-1}) with 29.80 mg NO_3-NL^{-1}, 852 ± 6 mgL^{-1} (specific growth rate 0.214 ± 0.001 d^{-1}) with 33.95 mg NO_3-NL^{-1}, 688 ± 6 mgL^{-1} (specific growth rate 0.193 ± 0.001 d^{-1}) with 39.90 mg NO_3-NL^{-1}, 940 ± 10mgL^{-1} (specific growth rate 0.224 ± 0.001 d^{-1}) with 43.65 mg NO_3-NL^{-1} and 760 ± 6 mgL^{-1} (specific growth rate 0.134 ± 0.001 d^{-1}) with 48.90 mg NO_3-NL^{-1}. The biomass yield value obtained under nitrate replete condition in this experiment was higher than that obtained from control BBM media [3], who reported a biomass yield of 95.5 ± 10 mgL^{-1} and growth rate 0.178 d^{-1}. Other example, in [4] cultivated C.sorokiniana in nitrate control Kuhl medium; they reported biomass yield was 680 mgL^{-1} and specific growth rate of 0.63 d^{-1}.

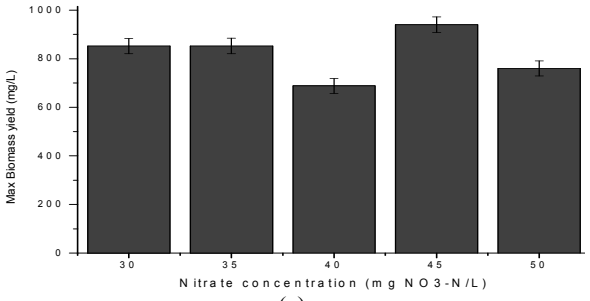
In nitrate concentration of 43.65 mg NO_3-NL^{-1}, nitrogen could be up taken vigorously to the maximum level and encourage the cell concentration to increase thus increase the frequency of the cell-cell encounters. This will lead to the enhancement of the attachment of microalgae and as a consequence increase biomass yield and growth rate. When the initial nitrate concentration of C.sorokiniana was continued to be enriched to 48.90 mg NO_3-NL^{-1}, the maximum biomass yield dropped to 760 ± 6 mgL^{-1}, which was probably due to the nitrogen inhibition. Nitrate concentration at 29.60 and 34.03 mg NO_3-NL^{-1} showed not much different as the specific growth rate and max biomass concentration were comparable to each other. Based on the maximum biomass yield of 940 ± 10mgL^{-1}, nitrate concentration of 43.65 mg NO_3-NL^{-1} was selected as the optimal concentration for biomass yield of C.sorokiniana.

Table-2. Culture of C.sorokiniana under different nitrogen supplementations in replete condition.

Nitrate Conc. (mg NO_3-NL^{-1})	Optical density	Cell Conc. (10^6 cells mL^{-1})	Cell productivity (10^6 cells $mL^{-1}d^{-1}$)
29.80	2.508 ± 0.053	885 ± 25	98 ± 3
33.95	2.704 ± 0.028	915 ± 35	102 ± 4
39.90	2.741 ± 0.024	790 ± 10	88 ± 1
43.65	3.174 ± 0.054	1090 ± 20	121 ± 2
48.90	3.054 ± 0.021	975 ± 5	108 ± 1

Table-3. Culture of C.sorokiniana under different nitrogen supplementations in replete condition (cont)

Nitrate Conc. (mg NO_3-NL^{-1})	Growth rate (day^{-1})	Biomass yield (mgL^{-1})
29.80	0.214 ± 0.001	852 ± 8
33.95	0.214 ± 0.001	852 ± 6
39.90	0.193 ± 0.001	688 ± 6
43.65	0.224 ± 0.001	940 ± 10
48.90	0.134 ± 0.001	760 ± 6



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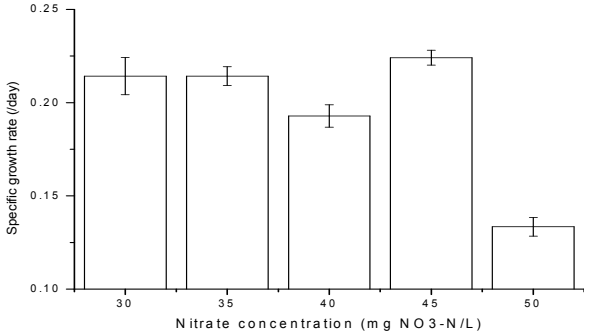


(b)

Figure-2. Max biomass yield (a) and specific growth rate (b) obtained by different supplementation of nitrate concentration.

The relationship between nitrogen consumption and biomass yield was shown in Figure-3. As expected, the biomass has increase with the increasing of nitrate supplementation. The biomass has increased from 150 mgL^{-1} to 533 mgL-1 with the consumption of nitrate from 48.90 to 40.04 mg NO_3-NL^{-1} in the first three days for nitrate supplementation of 48.90 mg NO_3-NL^{-1}. From 4 to 6 days, nitrate was slowly consumed from 34.15 to 31.45 mg NO_3-NL^{-1}, resulting in continuous biomass yield increase to 800 mgL^{-1}. After that, the consumption of nitrate continuously decreasing to 12.4 mg NO_3-NL^{-1} when the biomass yield achieved the maximum of 900 mgL^{-1} on day 8th before dropped to 733 mgL^{-1}. On the other hand, nitrate supplementation of 43.65 mg NO_3-NL^{-1} showed slightly lower biomass yield than 48.90 mg NO_3-NL^{-1} from day 1 until day 6. However, on day 7, the biomass yield of 43.65 mg NO_3-NL^{-1} achieved similar with 48.90 mg NO_3-NL^{-1}. Surprisingly, the biomass yield of 43.65 mg NO_3-NL^{-1} achieved the highest biomass yield of all at 1034 mgL^{-1} on the next day. Nitrate concentration in all cultures reach lower values on day 10 in parallel with decreasing of biomass yield. These values obtained from the experiment were comparable with those found in recent study [5]. Based on research by [5], the biomass yield of microalgae under N-replete were maximum of 810 ± 60 and minimum of 590±50 mgL^{-1}.

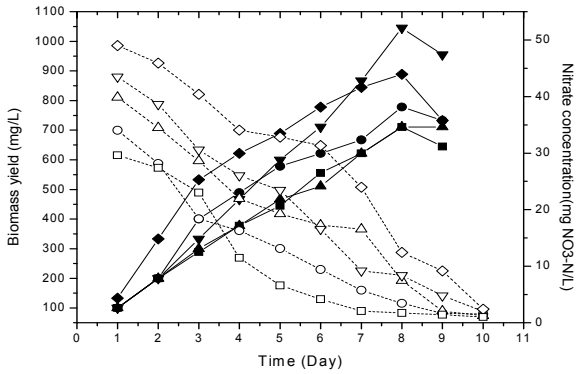
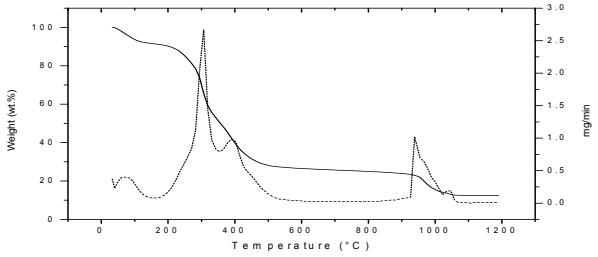


Figure-3. The change of biomass yield with the consumption of nitrate. Dash and solid lines represent nitrate concentration and biomass yield respectively (29.80 mg NO_3-NL^{-1}, square; 33.95 mg NO_3-NL^{-1},circle; 39.90 mg NO_3-NL^{-1}, up triangle; 43.65 mg NO_3-NL^{-1}, down triangle; 48.90 mg NO_3-NL^{-1}, diamond).

Proximate and ultimate analysis of C.sorokiniana

TGA was used as a tool to provide the thermal property information of C.sorokiniana. Figure-4 shows example of TGA profiles of C.sorokiniana samples cultured in five different nitrate concentrations at heating rate of 20°Cmin^{-1}. In proximate analyses by TGA, there are three main decomposition steps, first is corresponding

to dehydration (temperature < 180 °C), followed by devolatilization (temperature 180-540 °C) and last is corresponding to the slow decomposition of the solid residue (temperature >540 °C) [6]. The result obtained in this work observed that, MC loss occurs between temperatures 25 °C until 110 °C. The main decomposition step, which is devolatilization occurs in the 110 °C to 900 °C range, which involved at least two overlapped steps, observed in the corresponding DTG curve. These peaks was indicated as the loss of lipids and some decomposition of lignocellulosic polymers [7]. As shown in Figure-4, the DTG peak temperatures been observed for C.sorokiniana for all five different nitrate concentrations were first step; dehydration at 50-72 °C, second step; devolatilization for two peaks at 306-315 and 390 °C, and third step; solid residue decomposition at 936-958 °C.



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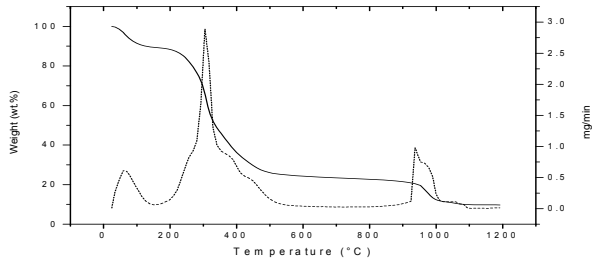

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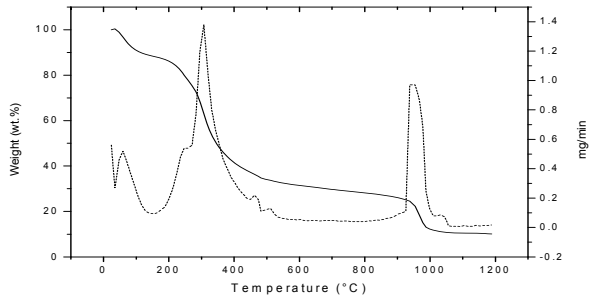

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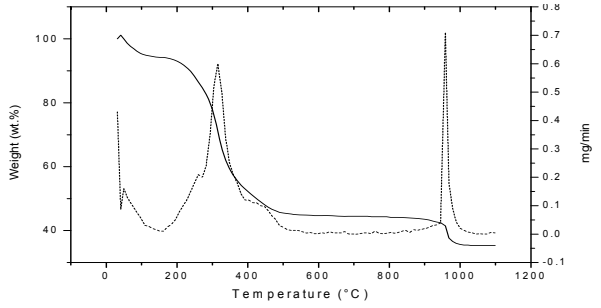

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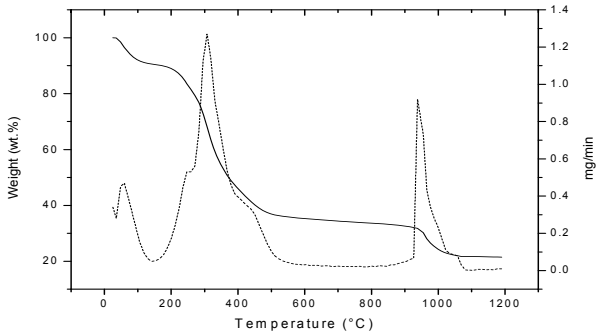

(e)

Figure-4. Themogravimetric profile of C.sorokiniana obtained by different supplementation of nitrate concentration. Dash and solid lines represent TGA and DTG respectively. (29.60 mg NO_3-NL^{-1} (a), 34.03 mg NO_3-NL^{-1} (b), 41.11 mg NO_3-NL^{-1} (c), 47.47 mg NO_3-NL^{-1} (d) and 53.64 mg NO_3-NL^{-1} (e)).

Based on Table-3, microalgae C.sorokiniana supplemented with nitrate concentration of 43.65 mg NO_3-NL^{-1} contained almost three times higher ash of 35.39% than C.sorokiniana supplemented with lower nitrate concentration. Amount of ash indicates higher biomass yield is produced as a result of high cell productivity of 121 ± 2 x 10^6 cells $mL^{-1}d^{-1}$. Meanwhile, when the initial nitrate concentration of C.sorokiniana was continued to be enriched to 48.90 mg NO_3-NL^{-1}, there was reduction in ash content. These result showed that microalgae C.sorokiniana capable to consume as high as nitrate concentration of 43.65 mg NO_3-NL^{-1} and produced high amount of biomass which represent in terms of ash and fixed carbon. Microalgae C.sorokiniana supplemented with nitrate concentration of 33.95 mg NO_3-NL^{-1} produced the highest VM of 76.39% and the lowest value of ash (10.9%) in %wt dry basis.

Table-4. The proximate analyses of C.sorokiniana on dry basis.

Condition	Content (%)	Nitrate concentration (mg NO_3-NL^{-1})		
		29.80	**33.95**	**39.90**
%wt wet basis	MC	7.87	10.32	11.60
	Ash	11.53	9.77	10.32
	VM	68.57	68.50	63.44
	FC	12.03	11.39	14.62
	S	0.00	0.00	0.00
%wt dry basis	MC	-	-	-
	Ash	12.51	10.90	11.68
	VM	74.43	76.39	71.78
	FC	13.06	12.71	16.54
	S	0.00	0.00	0.00
% wt dry-ash free	MC	-	-	-
	Ash	-	-	-
	VM	85.07	85.74	81.27
	FC	14.93	14.26	18.73
	S	0.00	0.00	0.00
Dry mineral matter free	MC	-	-	-
	Ash	-	-	-
	VM	84.90	85.60	81.07
	FC	15.10	14.40	18.93
	S	-	-	-
%wt wet basis	MC	6.543	9.64	
	Ash	35.39	21.55	
	VM	51.96	58.29	
	FC	6.09	10.50	
	S	0.00	0.00	
%wt dry basis	MC	-		
	Ash	37.87	23.86	
	VM	55.61	64.52	
	FC	6.52	11.62	
	S	0.00	0.00	
% wt dry-ash free	MC	-	-	
	Ash	-	-	
	VM	89.50	84.74	
	FC	10.50	15.26	
	S	0.00	0.00	
Dry mineral matter free	MC	-	-	
	Ash	-	-	
	VM	88.96	84.34	
	FC	11.04	15.66	
	S	-	-	

Ultimate analyses were used to determine the elemental contents such as carbon, hydrogen, oxygen, nitrogen, and sulfur. The elemental analyses are crucial in order to fully understand the condition of microalgae biomass as a biodiesel feedstock and the correlation to the gross calorific values. As shown in Table-3, in dry ash free basis, the highest carbon content was in C.sorokiniana supplemented in 33.95 mg NO_3-NL^{-1} which is 51.48%. However, the oxygen content showed quite a higher value which is 38.80% in similar nitrate concentration. To be a successful biodiesel feedstock, carbon content should be in higher value while oxygen content should be in an inverse manner. This is due to the carbon content correlates proportionally to the heating value of the biodiesel, and oxygen content is undesirable to the biodiesel. Results showed that microalgae C.sorokiniana supplemented with nitrate concentration of 43.65 mg NO_3-NL^{-1} surprisingly had the lowest value of oxygen content; 13.92% and acceptable value of carbon content of 40.32%. Most of the microalgae cultured had none of sulfur content which is very good and highly desirable for environmental friendly biodiesel. While, for nitrogen content, results showed that nitrogen content was in high level for all nitrate concentration condition. To produce high quality biodiesel made from microalgae feedstock, additional treatments should be added to the process in order to satisfy the requirement standard.

Table-5. The ultimate analyses of C.sorokiniana.

Condition	Content	Nitrate concentration (mg NO_3-NL^{-1})		
		29.80	**33.95**	**39.90**
%wt wet basis	C	43.74	46.16	44.30
	H	1.068	5.91	7.39
	N	2.75	3.35	4.63
	S	0.000	0.00	0.00
	O*	40.91	34.79	33.35
	Ash	11.52	9.77	10.32
	MC	7.87	10.32	11.60
%wt Moist Basis	C	43.74	46.16	44.30
	H	3.03	8.49	10.29
	N	2.75	3.35	4.63
	S	0.000	0.00	0.00
	O*	47.91	43.96	43.66
	Ash	11.52	9.77	10.32
	MC	-	-	-
% wt dry-ash free	C	47.48	51.48	50.12
	H	1.16	6.60	8.36
	N	2.99	3.74	5.24
	S	0.00	0.00	0.00
	O*	44.41	38.80	37.73
	Ash	-	-	-
	MC	-	-	-

Table-6. The ultimate analyses of C.sorokiniana (cont)

Condition	Content	Nitrate concentration (mg NO_3-NL^{-1})	
		29.80	**33.95**
%wt wet basis	C	43.74	46.16
	H	1.06	5.91
	N	2.75	3.35
	S	0.00	0.00
	O*	40.91	34.79
	Ash	11.52	9.77
	MC	7.87	10.32
%wt Moist Basis	C	43.74	46.16
	H	3.03	8.49
	N	2.75	3.35
	S	0.00	0.00
	O*	47.91	43.96
	Ash	11.52	9.77
	MC	-	-
% wt dry-ash free	C	47.48	51.48
	H	1.16	6.60
	N	2.99	3.74
	S	0.00	0.00
	O*	44.41	38.80
	Ash	-	-
	MC	-	-

FTIR analysis of C.sorokiniana

The biomass of C.sorokiniana was characterized by Fourier Transform Infrared (FTIR) spectroscopy to identify functional groups and macromolecular analysis. Figure-5 showed the FTIR spectrum of C.sorokiniana obtained by different supplementation of nitrate concentration which showed reflectance bands attributable in terms of carbohydrates, proteins and lipids content. FTIR spectroscopy used infrared spectrum in order to determine the biochemical composition of the algae accurately [8]. C. sorokiniana cultured in rich nitrate concentration demonstrate high carbohydrate content due to the intense bands at 1160, 1086, 1050 and 1036 cm^{-1}. The total lipid content and amide I refers to low intensity bands at 3000-2800 cm^{-1} and 1740 $cm^{-1}$and 1655 cm^{-1} respectively [9]. Bands centered at 3600-3000 cm^{-1} indicate water content in the biomass sample. If low water content was observed, it was confirmed that the raw biomass sample were dried. All condition of C,sorokiniana cultured in different supplementation of nitrate concentration show lower peaks in 3000-2800 cm^{-1} and 1740 cm-1 for lipid content, but more significant for the C.sorokiniana supplemented with 33.95 mg NO_3-NL^{-1} than 29.8 mg NO_3-NL^{-1}. These result show that C.sorokiniana supplemented with 33.95 mg NO_3-NL^{-1} was observed to have high content of lipid, carbohydrates and amide I compared to others nitrate concentration level.

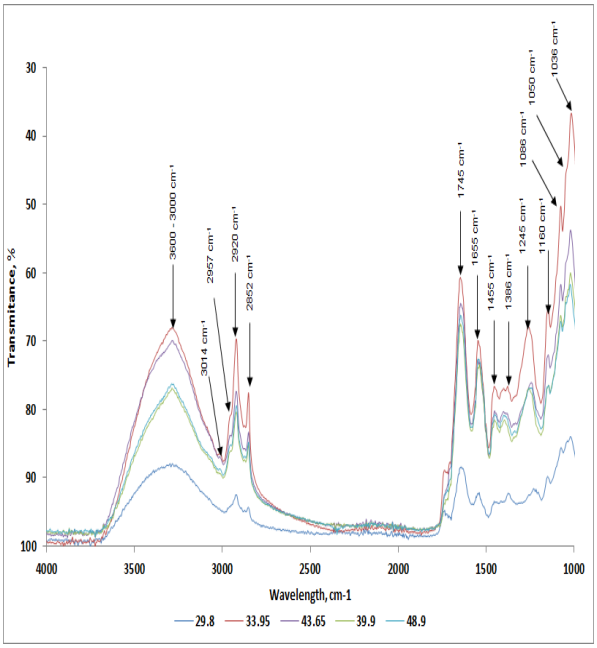

Figure-5. FTIR spectrum of C.sorokiniana obtained by different supplementation of nitrate concentration (unit in mg NO_3-NL^{-1}).

Table-7. C.sorokiniana FTIR spectrum wavelength range.

Wavelength range (cm^{-1})	Peak (cm^{-1})				
	Nitrate concentration (mg NO_3-NL^{-1})				
	29.80	**33.95**	**39.90**	**43.65**	**48.90**
3600-3000	3297	3287	3286	3287	3288
3014	3008	3014	3013	3014	3014
2957, 2920, 2852	2957, 2922, 2852	2957, 2920, 2852	2957, 2919, 2852	2957, 2921, 2852	2957, 2920, 2852
1745	1745	1745	1745	1745	1745
1655	1640	1647	1645	1646	1645
1545	1546	1546	1539	1547	1547
1455	-	1450	1454	1452	1452
	-	24	18	20	20
1386	1386	1386	1386	1386	1386
1245	1240	1261	1259	1243	1241
1200-900	1150	1148	1148	1150	1148
1160,1086, 1050, 1036	1075	1076	1075	1076	1075

Table-8. C.sorokiniana FTIR spectrum wavelength distribution.

Wavelength range (cm^{-1})	Bond	Functional group
3600-3000	vO-H stretch, vN-H stretch vC-O stretch	Water, Amide, Carbohydrates
3014	vC-H of C=CH chains of lipid	Chains of lipid
2957, 2920, 2852	v_sCH_2 and $v_{as}CH_2$, v_sCH_3 and $v_{as}CH3$	Fatty acids
1745	vC=O	Ester functional groups from lipids and fatty acids
1655	vC=O	Amides associated with protein (Amide I)
1545	δN-H	Amides associated with protein (Amide II)
1455	$\delta_{as}CH_3$ and $\delta_{as}CH_2$	Lipids and proteins
1386	$\delta_{as}CH_3$ and $\delta_{as}CH_2$, v_sC-O	Proteins, Carboxylic groups
1245	v_{as}C-O	Nucleic acids, phosphoryl groups, Phosphorylated proteins
1200-900	vC-O-C	Polysaccharides
1160,1086, 1050, 1036	vC-O	Carbohydrates

CONCLUSIONS

This research was to encourage continuous cell division and produced high biomass yield of Chlorella sorokiniana under nitrogen replete condition in view of its capability to produce lipid. Based on the results, C. sorokiniana had maximum biomass yield of 940 ± 10mgL^{-1} (specific growth rate 0.224 ± 0.001 d^{-1}) when cultured in nitrate concentration of 43.65 mg NO_3-NL^{-1}. However, the TGA and ultimate analysis showed C. sorokiniana supplemented with 43.65 mg NO_3-NL^{-1} contain average VM and carbon content. Consequently, 75% of nitrate increment from control BBM media will increase the biomass yield and growth rate but decrease the VM and carbon content of C. sorokiniana. Therefore, a two stage culture is highly interested to increase the VM and carbon content which is favourable in producing a high heating value biodiesel.

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