Hindawi Publishing Corporation
Journal of Renewable Energy
Volume 2013, Article ID 671545, 6 pages
http://dx.doi.org/10.1155/2013/671545

Research Article

The Effects of Trace Elements on the Lipid Productivity and Fatty Acid Composition of *Nannochloropis oculata*

Xiao Dou, Xiang-Hong Lu, Mei-Zhen Lu, Li-Sheng Yu, Rong Xue, and Jian-Bing Ji

College of Chemical Engineering and Materials Science, Zhejiang University of Technology, Zhejiang Province Key Laboratory of Biofuel, Hangzhou 310014, China

Correspondence should be addressed to Xiang-Hong Lu; luxh@zjut.edu.cn

Received 7 December 2012; Revised 2 March 2013; Accepted 8 March 2013

Academic Editor: Yongsheng Chen

The effects of trace elements on the lipid productivity and fatty acid composition of *Nannochloropis oculata (N. oculata)* were studied. The results showed that trace elements had a strong influence on not only the lipid productivity but also the fatty acid composition. The addition of Fe^{3+}, Zn^{2+}, Mn^{2+}, Mo^{6+}, and EDTA and the deletion of Cu^{2+} and Co^{2+} can increase the lipid productivity. The optimum concentrations of the trace elements in the culture medium are 6 times of Fe^{3+} and EDTA, the same concentration of Zn^{2+}, Mn^{2+}, and Mo^{6+} as the control group, but the optimum medium has no Cu^{2+} or Co^{2+}. Fe^{3+}, Zn^{2+}, Mn^{2+}, Mo^{6+}, and EDTA are indispensable during the EPA formation of *N. oculata*. The addition of Fe^{3+}, Zn^{2+}, Mn^{2+}, Mo^{6+}, and EDTA can strongly increase the content of EPA in the lipid of *N. oculata*, but the concentration of the trace elements had little influence on the level of EPA.

1. Introduction

In order to reduce the cost of biodiesel from microalgae, we can not only screen out the algal species that both grow well and have high lipid content but also explore the optimal culture condition to improve the lipid productivity of microalgae. *N. oculata* is a promising source of biodiesel because of its high growth rate and high lipid content, ranging from 31% to 68% [1].

Apart from the macroelements such as N, P, and C, the growth rate and lipid content of *N. oculata* can also be affected by trace elements. This is because trace elements are indispensable during the growing process of marine algae, and trace elements are indispensable parts of the algal cell itself and the enzyme active center of algal cell [2, 3]. There have been some reports about the effects of trace elements on the growth of microalgae. Liu and Wang [4] reported that high content of Fe^{3+} was beneficial to increase the lipid content of marine chlorella. Jeffrey and Millton [5] and Alexander and Markus [6] found that Mn^{2+} was indispensable during the growing process of microalgae and was the catalyst of photosynthesis and the activator of some kind of enzymes in microalgae. Yamochi [7] found that the addition of EDTA which could form a complex with many kinds of metal ions would promote the absorption of metal ions of microalgae and the growth of microalgae, and the complex iron was the inducement of red tide in the nutrition water. Boyer and Brand [8] found that trace elements could cause the abnormal propagation of microalgae, and suitable concentration of Fe^{3+}, Mn^{2+}, and Zn^{2+} could cause the mass rearing of microalgae. Yamasaki et al. [9] found that Mo^{6+} could control the growth and material accumulation of *Dunaliella salina*. Furthermore, too high or too low concentrations of Mo^{6+} were both harmful to the growth of *Dunaliella salina*. Their researches showed that trace elements had some effects on the growth density and lipid content of microalgae, but the effects of trace elements on the lipid productivity and fatty acid composition of *N. oculata* have not been reported yet.

In this paper, the effects of trace elements (Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, and Mo^{6+}) and EDTA on the lipid productivity and fatty acid composition of *N. oculata* were studied. The optimum dosage of trace elements that will increase the lipid productivity of *N. oculata* was determined.

Table 1: The concentration of trace elements (TEs) in different media.

TE ($mg \cdot L^{-1}$) group number	Fe^{3+}	Cu^{2+}	Zn^{2+}	Co^{2+}	Mn^{2+}	Mo^{6+}	EDTA
0	3.16	0.01	0.023	0.012	0.18	0.07	4.36
1	—	0.01	0.023	0.012	0.18	0.07	4.36
2	9.48	0.01	0.023	0.012	0.18	0.07	4.36
3	18.96	0.01	0.023	0.012	0.18	0.07	4.36
4	3.16	0.01	—	0.012	0.18	0.07	4.36
5	3.16	0.01	0.069	0.012	0.18	0.07	4.36
6	3.16	0.01	0.138	0.012	0.18	0.07	4.36
7	3.16	0.01	0.023	0.012	—	0.07	4.36
8	3.16	0.01	0.023	0.012	0.54	0.07	4.36
9	3.16	0.01	0.023	0.012	1.08	0.07	4.36
10	3.16	0.01	0.069	0.012	0.18	—	4.36
11	3.16	0.01	0.138	0.012	0.18	0.21	4.36
12	3.16	0.01	0.023	0.012	0.54	0.42	4.36
13	3.16	0.01	0.023	0.012	1.08	0.07	—
14	3.16	0.01	0.023	0.012	0.18	0.07	8.72
15	3.16	0.01	0.023	0.012	0.18	0.07	17.44
16	3.16	—	0.023	0.012	0.18	0.07	4.36
17	3.16	0.01	0.023	—	0.18	0.07	4.36

2. Materials and Methods

2.1. Microalgae. *N. oculata* was originally obtained from Marine Biological Culture Collection Centre (China) and screened for its potential ability of growth at Zhejiang University of Technology (China). *N. oculata* cells were maintained in the modified *F*/2 medium in artificial sea water, which was made by putting 26.726 g NaCl, 2.26 g $MgCl_2$, 3.248 g $MgSO_4$, 1.153 g $CaCl_2$, 0.198 g $NaHCO_3$, 0.721 g KCl, 0.058 g NaBr, 0.058 g H_3BO_3, 0.0024 g Na_2SiO_3, 0.0015 g $Na_2Si_4O_9$, 0.002 g H_3PO_4, 0.013 g Al_2Cl_6, 0.002 g NH_3, and 0.0013 g $LiNO_3$ in distilled water (per liter), at temperature 20°C, and pH = 8.0 with a light intensity of 3000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in illumination box. The composition of modified *F*/2 medium (per liter) was shown as follows: 0.225 g $NaNO_3$, 0.045 g $NaH_2PO_4 \cdot H_2O$, 0.02 g $Na_2SiO_3 \cdot 9H_2O$, 3.16 mg $FeCl_3 \cdot 6H_2O$, 0.18 mg $MnCl_2 \cdot 4H_2O$, 0.023 mg $ZnSO_4 \cdot 7H_2O$, 4.36 mg Na_2 EDTA, 0.01 mg $CuSO_4 \cdot 5H_2O$, 0.012 mg $CoCl_2 \cdot 6H_2O$, 0.07 mg $Na_2MoO_4 \cdot 2H_2O$, 1 μg vitamin B1, 1 μg vitamin B12, and 0.5 μg biotin.

The mud of algae for inoculation was collected by centrifuging the cells of *N. oculata* at the logarithmic phase at 3000 $r \cdot min^{-1}$ and then washed with distilled water for 3 times to remove the salt in the medium.

2.2. Culture System. Growth experiment was done at a different content of trace elements in 1.0 L-Erlenmeyer flasks. The medium and flasks were sterilized at UV irradiation for 30 min in a clean bench in order to prevent any contamination during the early stages of growth.

N. oculata cells with the same inoculation density ($OD_{440\,nm}$ = 0.70) grew at room temperature (25 ± 1°C), natural lighting, and pH = 8.0 with bubbling of aseptic air continuously. The concentration of trace elements in different media was listed in Table 1.

2.3. Determination of Microalgae Growth Density. The microalgae growth density was determined by measuring the optical density at 440 nm, the highest absorbance of *N. oculata* when scanned the wave-length ranging from 220 nm to 700 nm, using a UV-*V* is spectrophotometer every 24 hours. All measures were carried out in triplicate.

2.4. Preparation of Dry Algal Powder. When the *N. oculata* cells had been at the stationary phase for three days, the pH of the algal culture was adjusted to 10.5 with NaOH which was used for sedimentation, and then the culture stood for 24 hours. The algal mud was obtained by removing the supernatant and washed with distilled water 3 times to remove the salt in the algal mud and then dried for 24 hours at 70°C to get the dry algae powder, and the dry weight was obtained by weighting the algal biomass after being dried for 24 hours at 70°C.

2.5. Lipid Extraction. Lipid was extracted using the modified acid method [10]. Firstly, dry algae powder (1 g) was put in a 50 mL centrifuge tube, and then 4 mL of distilled water and 5 mL of HCl were put into the tube. The mixture was heated for 20 min in the water bath at 70°C. Then, 5 mL of ethanol was added into the mixture, and the mixture was cooled naturally. Secondly, 10 mL of diethyl ether was added into the mixture, and the mixture was shaken for 1 min. Next, the mixture was centrifuged for 2 min at 4000 r/min. The ether layer was taken into the round flask. Thereafter, 5 mL of diethyl ether was added into the residue, the above

process was repeated, and the ether layer was combined and taken into the round flask. Finally, the lipid was obtained by evaporating the ether under vacuum in a rotary evaporator.

2.6. Esterification of Algal Lipid. The *N. oculata* lipid was dissolved with chloroform and transferred into the 1.5 mL glass vial. Then, 1 mL of 1 M sulphuric acid-methanol was added into the lipid. The mixture was kept at 100°C for 1 hour under nitrogen protection and was then cooled naturally. 200 μL of distilled water was added into the mixture. The mixture was well mixed by shaking for 1 min and extracted with n-hexane 3 times. The organic phases were combined, stripped with 200 μL of distilled water 3 times, transferred into the 1.5 mL glass vial, and dried by nitrogen blowing. The obtained methyl ester was weighed.

2.7. Analysis of Methyl Ester from N. oculata Lipid. The fatty acid composition of *N. oculata* lipid was determined by a gas chromatography-mass (Agilent 7890 N), and the content of fatty acid was qualitatively characterized by a gas chromatograph with FID detector (Agilent 7890) using methyl undecanoate as internal standards.

Analysis Condition of GC. DB-WAX (30 m × 0.32 mm × 0.50 μm), the temperature program, comprised three phases: the first phase: initially the temperature was ramped from 50°C to 150°C at a rate of 10°C·min^{-1} and held for 2 min. The second phase: it was then ramped to 200°C from 150°C at a rate of 10°C·min^{-1} and held for 6 min. The third phase: it was ramped to 230°C from 200°C at a rate of 10°C·min^{-1} and held for 5 min. Carrier gas (N_2) velocity was 3 mL·min^{-1}. The detector was a hydrogen flame detector; the velocity of H_2 was 30 mL·min^{-1}, and the velocity of air was 300 mL·min^{-1}. The detector temperature was 300°C. The injector temperature was 280°C.

Analysis condition of GC-Mass. DB-5MS (30 m × 250 um × 0.25 um), the temperature program, comprised two phases: the first phase: initially the temperature was ramped from 60°C to 270°C at a rate of 10°C·min^{-1} and held for 5 min. The second phase: it was then ramped to 300°C from 270°C at a rate of 8°C·min^{-1} and held for 8 min. The injector temperature was 270°C. The temperature of the connector between GC and mass was 260°C. Carrier gas (He) velocity was 1.0 mL·min^{-1}. The ionization source of mass was EI, 70 eV, and mass range: m/z was 200~550 amu.

3. Result and Discussion

3.1. The Effects of Trace Elements on the Dry Weight of N. oculata . The effects of trace elements on the dry weight of *N. oculata* were presented in Figure 1. As can be seen, there were certain extent effects of trace elements on the dry weight of *N. oculata*.

It was shown that the dry weight of *N. oculata* decreased firstly and then increased gradually with the increase of concentration of Fe^{3+}, Zn^{2+}, and EDTA. The dry weight of *N. oculata* reached the maximum when the concentrations

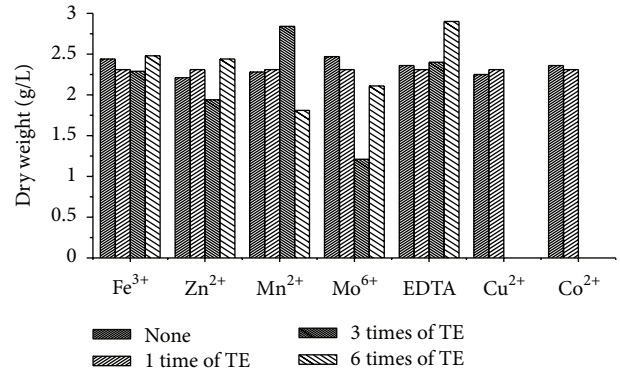

Figure 1: The effects of trace elements (TEs) on the dry weight of *N. oculata*.

of the trace elements were 6 times of the control group (modified $F/2$). It was probably because Fe^{3+} was the assistant factor of many kinds of enzymes, and it was essential for the formation of cytochrome, ferredoxin, and Mo-Fe protein. The photosynthesis of algae depended on Fe^{3+}, and at the same time Fe^{3+} was an important part of nitrate and nitrite reductase. It could remarkably increase the reduction and transfer efficiency of Nitrate and Nitrite reductase in algae [8]. The carbon source heavily utilized in the algae cell was CO_2, but CO_2 mainly existed in the form of HCO_3^- in the cell [8], CO_2 should be transferred from HCO_3^- by the catalysis of carbonic anhydrase before photosynthesis. Zn^{2+} was a part of carbonic anhydrase [11]. The increase of the concentration of Zn^{2+} would increase the efficiency of photosynthesis in algae. EDTA was the organic ligand in the medium of algae and could be used as the buffer of metal ions [12], because it could form the stable complex with most of the kinds of metal ions. In our work, the dry weight of *N. oculata* increased with the increase of the concentration of EDTA, and this might be because EDTA formed a complex with Fe^{3+}. Yang [13] found that the growth rate of algae and the uptake of Fe^{3+} were higher in complexed iron than that in colloidal iron at the same concentration of Fe^{3+}. The consistent conclusion was drawn in our work.

With the increase of concentration of Mn^{2+}, the dry weight of *N. oculata* decreased firstly, then increased, and then decreased again. When the concentration of Mn^{2+} was 3 times of the control group, the dry weight of *N. oculata* reached the maximum. The possible reason was that Mn^{2+} was the activator of some enzyme in the reaction of glycolysis and tricarboxylic acid cycle and the activator of nitrate reductase. The deletion of Mn^{2+} would affect the utilization of nitrate [14]. Qin and Zhou [15] and Guo and Yang [16] found that a certain concentration of Mn^{2+} in water would promote the growth of algae, and the different optimum concentrations of Mn^{2+} were obtained for the different algae strains. In this experiment, when the concentration of Mn^{2+} was 3 times of the control group, the dry weight of *N. oculata* reached the maximum. The addition of Mo^{6+} restrained the growth of *N. oculata*. Compared with the control group,

Table 2: The effects of trace elements on the fatty acid composition of *N. oculata*.

Trace elements	Control	Fe^{3+} (mg/L)			Zn^{2+} (mg/L)			Mn^{2+} (mg/L)		
		0	9.48	18.96	0	0.069	0.138	0	0.54	1.08
C14:0	5.82	4.85	6.02	6.10	4.90	6.45	6.74	0.00	6.01	6.00
C16:0	32.50	34.43	30.42	33.88	33.99	31.30	28.88	25.12	38.70	37.45
C16:1	21.06	39.46	25.75	24.70	38.93	24.15	23.32	40.37	24.40	23.69
C18:0	2.42	0.75	2.43	2.33	0.85	2.43	2.58	0.00	1.89	2.03
C18:1	8.15	12.53	7.62	8.12	9.69	8.04	6.35	22.60	9.51	9.67
C18:2	0.94	0.92	0.64	0.76	1.46	0.59	0.00	11.90	0.88	0.87
C20:4	6.05	1.15	6.71	5.98	1.78	6.50	7.23	0.00	5.10	5.30
C20:5	19.66	5.92	20.42	18.31	8.40	20.56	24.66	0.00	13.52	14.99
Saturated	40.74	40.03	38.87	42.31	39.74	40.18	38.2	25.12	46.6	45.48
Monounsaturated	29.21	51.99	33.37	32.82	48.62	32.19	29.67	62.97	33.91	33.36
Polyunsaturated	26.65	7.99	27.77	25.05	11.64	27.65	31.89	11.9	19.5	21.16
Trace elements		Mo^{6+} (mg/L)			EDTA (mg/L)			Cu^{2+}	Co^{2+}	
	0	0.21	0.42	0	8.72	17.44	0	0		
C14:0	3.66	6.07	6.01	4.93	5.88	6.40	6.25	5.82		
C16:0	35.62	36.41	34.34	34.98	33.35	34.43	35.60	32.34		
C16:1	43.94	25.14	24.59	39.22	23.66	24.02	22.75	22.89		
C18:0	0.00	2.07	2.12	0.72	2.30	2.29	2.26	2.40		
C18:1	12.14	8.75	8.55	11.26	8.42	8.39	9.03	7.90		
C18:2	0.00	0.78	0.82	1.30	0.92	0.00	0.82	0.92		
C20:4	0.00	5.45	5.82	1.45	6.11	5.91	5.98	6.58		
C20:5	4.64	15.34	17.76	6.14	19.36	18.57	17.32	21.09		
Saturated	39.28	44.55	42.47	40.63	41.53	43.12	44.11	40.56		
Monounsaturated	56.08	33.89	33.14	50.48	32.08	32.41	31.78	30.79		
Polyunsaturated	4.64	21.57	24.4	8.89	26.39	24.48	24.12	28.59		

the growth density of *N. oculata* with the deletion of Mo^{6+} was higher. The dry weight of *N. oculata* decreased gradually with the increase of the concentration of Mo^{2+}, but when the concentration of Mo^{2+} became 3 times of the control group, the increase of the concentration of Mo^{2+} would increase the dry weight of *N. oculata*, and the results might be because high concentration of Mo^{2+} impelled the generation of stress protein in *N. oculata* [9] which would promote algal photosynthesis and increase the *g* dry weight.

3.2. The Effects of Trace Elements on the Lipid Content of N. oculata . The effects of trace elements on the lipid content of *N. oculata* were presented in Figure 2. As can be seen, trace elements had a significant influence on the lipid content of *N. oculata*. The deletion of Fe^{3+}, Zn^{2+}, Mn^{2+}, Mo^{6+}, and EDTA would greatly cause a decrease of lipid content of *N. oculata*. In the range of the experimental concentrations, with the increase of Fe^{3+}, the lipid content increased. But with the increase of the concentration of Zn^{2+}, Mn^{2+}, Mo^{6+}, and EDTA, the lipid content of *N. oculata* increased firstly and then decreased. The lipid content went to the maximum when the concentrations of Mn^{2+}, Mo^{6+}, and EDTA were 6 times of the control group, and the concentration of Zn^{2+} was the same as the control group. For Zn^{2+}, the lipid content would increase again when the concentration of Zn^{2+} exceeded 3 times of the control group. The results showed that Fe^{3+}, Zn^{2+}, Mn^{2+}, Mo^{6+}, and EDTA were indispensable during the lipid formation of microalgae, and that appropriate concentration of the trace elements could promote the lipid formation, but too high concentration of the trace elements would produce poisonous effects on microalgae. Furthermore, ultra high concentration of trace elements would impel the generation of stress protein in *N. oculata* which would make the metabolic pathway turn from producing starch to producing lipid and thus increases the lipid content.

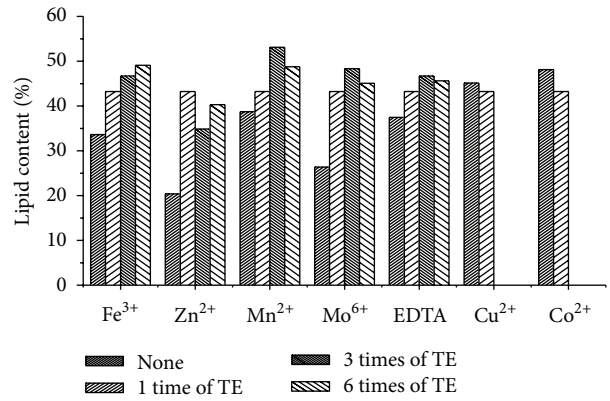

Figure 2: The effects of trace elements (TEs) on the lipid content of *N. oculata*.

Table 3: The effects of trace elements (TEs) on the lipid productivity ($mg \cdot L^{-1} \cdot day^{-1}$) of *N. oculata*.

Concentration (times of the control group)	Fe^{3+}	Zn^{2+}	Mn^{2+}	Mo^{6+}	EDTA	Cu^{2+}	Co^{2+}
None	51.28	28.15	57.6	40.69	55.25	63.48	70.8
One time	62.41	62.41	62.41	62.41	62.41	62.41	62.41
Three times	66.85	42.28	53.11	36.56	70.07		
Six times	76.11	61.46	48.77	59.44	82.69		

Table 4: The ANOVA of the effects of trace elements on the yield of lipid of *N. oculata*.

Source of variance	SS	df	*F*	*P* value	The critical value of *F*
Fe^{3+}	7604.53	7604.53	134.19	$2.49E-05$	$F_{0.05}$ (1.6) = 5.99
Zn^{2+}	4245.81	4245.81	30.50	0.0015	$F_{0.05}$ (1.6) = 5.99
Mn^{2+}	5612.17	5612.17	271.21	$3.19E-06$	$F_{0.05}$ (1.6) = 5.99
Mo^{2+}	4469.85	4469.85	50.49	0.00040	$F_{0.05}$ (1.6) = 5.99
EDTA	8477.32	8477.32	117.13	$3.68E-05$	$F_{0.05}$ (1.6) = 5.99

However, Cu^{2+} and Co^{2+} had negative effects on the lipid formation. Compared with the control group, the deletion of Cu^{2+} and Co^{2+} could increase the lipid content in *N. oculata*. The deletion of Cu^{2+} and Co^{2+} had little effects on the growth density as shown in Figure 2. Thus, the optimum culture should have no Cu^{2+} or Co^{2+}.

3.3. The Effects of Trace Elements on the Fatty Acid Composition of N. oculata . The effects of trace elements on the fatty acid composition of *N. oculata* were presented in Table 2.

As can be seen, palmitic acid, palmitoleic acid, and eicosapentaenoic acid (EPA) were the major fatty acids of *N. oculata*, comprising 65% to 85% of total fatty acids. The fatty acid composition shifted in response to the addition of trace elements. The level of saturated fatty acid shifted slightly after the addition of trace elements. Furthermore, the level of monounsaturated and polyunsaturated fatty acids increased sharply after the addition of Fe^{3+}, Zn^{2+}, Mn^{2+}, Mo^{6+}, and EDTA, but the concentration of the trace elements had little influence on the level of monounsaturated and polyunsaturated fatty acids. The deletion of Cu^{2+} and Co^{2+} had little effects on all fatty acids in *N. oculata*.

Recently, the utilization of PUFAS, such as eicosapentaenoic acid (EPA) and docosahexaenoic (DHA), for functional food and pharmacological products has received an increased attention [8]. The price of EPA with a purity of 99% in international market is 200 $US \cdot g^{-1}$. Therefore, it is a good way to increase the economic benefit of biodiesel production by a combined production of EPA from *N. oculata*.

3.4. The Effects of Trace Elements on the Lipid Productivity of N. oculata . The effects of trace elements on the lipid productivity of *N. oculata* was presented in Table 3. The ANOVA of the effects of trace elements on the yield of lipid of *N. oculata* were presented in Table 4. As can be seen, the *P* value of the trace elements on the lipid productivity of *N. oculata* was less than 0.01 which was to say that the trace elements had a large influence on the lipid productivity of *N. oculata*.

The order of factors affecting the lipid productivity was Mn^{2+}, EDTA, Fe^{3+}, Mo^{6+}, and Zn^{2+} according to the magnitude of *P* value. Based on these results, the proper control of the concentration of trace elements could increase the lipid productivity of *N. oculata*. The optimum concentrations of the trace elements in the culture medium are 6 times of Fe^{3+} and EDTA, the same concentration of Zn^{2+}, Mn^{2+}, and Mo^{6+} as the control group, but the optimum medium has no Cu^{2+} or Co^{2+}. But when using the optimum concentration of all the trace elements in one medium, we could get the highest lipid productivity; the lipid productivity was 55.53 $mg \cdot L^{-1} \cdot day^{-1}$ which was lower than the control group. The reason may be that there existed a restrain function among the different concentration of trace elements which prevented the algae from getting the highest lipid productivity.

4. Conclusions

N. oculata is a promising source of biodiesel for its high content of lipid and EPA. The trace elements significantly influence the lipid productivity and the fatty acid composition of *N. oculata*. The results contribute to improving the lipid and EPA production and providing the experimental basis that *N. oculata* can be used to produce biodiesel.

References

[1] Y. Chisti, "Biodiesel from microalgae," *Biotechnology Advances*, vol. 25, no. 3, pp. 294–306, 2007.

[2] Department of Biology and Microbiology of Wuhan and Fudan University, *Microbiology*, vol. 258, Higher Education Press, Beijing, China, 1987.

[3] D. D. Fan, *Cell Culture and Protein Engineering*, vol. 108, Chemical Industry Press, Beijing, China, 2000.

[4] Z. Y. Liu and G. C. Wang, "Dynamics of lipid accumulation vulgaris promoted by iron," *Marine Scienee*, vol. 32, no. 11, pp. 56–58, 2008.

[5] M. R. Jeffrey and R. S. Millton, "Effect of manganese and nickel on growth of selected algae in pH buffered medium," *Water Research*, vol. 33, no. 10, pp. 2448–2454, 1999.

[6] P. Alexander and H. Markus, "Effects of manganese on chlorophyll fluorescence in epiphytic cyano- and chlorolichens," *Flora-Morphology, Distribution, Functional Ecology of Plants*, vol. 201, no. 6, pp. 451–460, 2006.

[7] S. Yamochi, "Nutrient factors involved in controlling the growth of red tide flagellates Prorocentrum micans, Eutreptiella sp. And Chattonella mirina in Osaka Bay," *Bulletin of the Plankton Society of Japan*, vol. 32, no. 2, pp. 97–106, 1984.

[8] G. L. Boyer and L. E. Brand, "Trace elements and harmful algal blooms," in *Physiological Ecology of Harmful Algal Blooms*, D. M. Anderson, A. D. Cembella, and G. M. Hallegraeff, Eds., pp. 489–508, Springer, Berlin, Germany, 1998.

[9] Y. Yamasaki, T. Yokose, T. Nishikawa et al., "Effects of alginate oligosaccharide mixtures on the growth and fatty acid composition of the green algae Chlamydomonas reinhardtii," *Journal of Bioscience and Bioengineering*, vol. 113, no. 1, pp. 112–116, 2012.

[10] T. M. Zhang and S. Gui, *Effects of Zn, Fe, Mn on the Cell Multiplication of Phytoplankton in Fresh Water*, Capital Normal University, Beijing, China, 2006.

[11] Z. S. Chu, X. C. Jin, F. Yan, S. F. Zheng, Y. Pang, and Q. R. Zeng, "Effects of EDTA and iron on growth and competition of Microcystis aeruginosa and Scenedesmus quadricauda," *Huanjing Kexue/Environmental Science*, vol. 28, no. 11, pp. 2457–2461, 2007.

[12] C. M. Chen, C. Y. Zhou, and A. L. Zhen, "Limitation of environmental factors on propagation of skeletonema costatum," *Marte Science Bulletin*, vol. 15, no. 2, pp. 37–42, 1996.

[13] D. F. Yang, "The studies of phytoplankton reproduction capacity," *Marine Science*, vol. 27, no. 5, p. 19, 2003.

[14] Q. T. Zhang, Z. Q. Zhang, and S. L. Dong, "Effects of environmental factors on the growth and associated aspects of red tide alga," *Transactions of Oceanology and Limnology*, vol. 7, pp. 79–84, 2002.

[15] X. M. Qin and J. Z. Zhou, "Study on the effects of N, P, Fe-EDTA, Mn on the growth of a red tide dinoflagellate scrippsiella trochoidea," *Oceanologia et Limnologia Sinica*, vol. 28, no. 6, pp. 594–598, 1997.

[16] J. Y. Guo and X. L. Yang, "Regulation of Mo to growth and material accumulation of dunaliella salina," *Lake Salt and Chemical Industry*, vol. 36, no. 1, pp. 27–30, 2007.