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TẠP CHÍ KHOA HỌC

MỤC LỤC

trang

BIOMASS PRODUCTION OF ALGAE *Nannochloropsis oculata* AT LARGE SCALE IN QUYNH LUU DISTRIC, NGHE AN PROVINCE

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ABSTRACT

The research was conducted to complete technological protocol of biomass production of algae *Nannochloropsis oculata* with both culture methods in plastic bags and photo-bioreactor systems. The results show that in the nylon bag system, algae reached a peak of biomass density at day-8 of culture with 80 million cells per ml. In the photo-bioreactor systems, algae were higher density (180 million cells per ml), and longer period of culture (15 days of culture) than in plastic bags. The total products of 404.4 kg of fresh concetrated algae and 57.6 kg of dried algae powder which were suitable produced under climate conditions in Nghe An. All the algae products met the quality and requirements for use as food for aquatic animals as well as raw materials for the production of functional foods for humans.

Keywords: *Nannochloropsis oculata;* algae; photo-bioreactor; plastic bags culture.

1. Introduction

Marine microalgae in general and algae *Nannochloropsis sp* are the basic food, the first in the natural live-food chain. Algae *Nannochloropsis oculata* is used as direct and indirect food (supplementary food) in these hatcheries of marine fish, crustaceans, and molluscs [2]. The products of algae *N. oculata* are stocked in different ways such as: liquid, fresh concentrated and dried powder.

Some studies show that algae *Nannochloropsis oculata* has a content of 41.6% protein, 19.4% lipid, 13.1% carbohydrate, 5% EPA and 1% carotenoid. In addition, algae also contain vitamin E, phytosterols, polysaccharides, and amino acid profile such as: aspartic, threonine, serine, glutamic, glycine, alanine cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, proline. Algae *N. ocultata* also has many excellent functions/effects on humans such as: blood sugar regulation, health recovery, immunity enhancement, anti-bacterial, fungi, viruses, anti-oxidation and anti-inflammation, and enhancement of circulation and heart function, anti-allergy, reducetion of omega-3 unsaturated fatty acids, enhancement of brain functions and increasement of memory, vitality, detoxify, prevention and treatment assistence of cancer disease [1].

The extraction of omega-3 fatty acids (eicosatetraenoic acid-EPA) from the alga *N. oculata* can substitute EPA derived from liver and oil of salmon, shark... which was high cost, and depends on nature resource. Thus, the positive development of algae *N. oculata* biomass, food safety and hygiene are necessary, and contributing to stable of aquatic seed production, as well as providing the clean raw materials for production of functional food in Nghe An condition.

2. Methods

2.1. Research object and materials

- Research object: algae *Nannochloropsis oculata*

- Materials: medium (F/2) for algae culture, plasticbags (60 L each), photo-bioreactor system with a volume of 1000 L, aeration buble supplied in the middle of plasticbags and in photo-bioreactor systems, pump (capacity 20 W), centrifuge and electricity dry machine.

2.2. Research period and site

- Research period: 2022 August to 2023 August

- Research site: Joint Stock Company of VN Algae Science Technology in hamlet-6, Quynh Luong commune, Quynh Luu district, Nghe An province.

2.3. Research contents

Application of advanced technology on development of biomass production models of algae and processing process of fresh concentrated and dried powder algae *N. oculata* at large scale.

2.4. Research Method

2.4.1. Biomass algae production

The protocol of maintain culture and biomass culture of algae *Nannochloropsis oculata* were transferred by Aquaculture Research Sub-Institute for North Central [6].

Seawater supplied to the culture system had salinity of 25-30‰ were filtered, sterilized with chlorine, and F/2 nutritional medium were supplied at the ratio of 3 ml/l of water [6].

Algae biomass production in plastic bags: 250 fifty litter bags, were hanged on the 150 cm high shelf. The aeration and CO_2 were continuously supplied during culture period.

Production of algae biomass in the photo-bioreactor system: one system has a length of 30 m, connected by 22 glass tubes with a diameter of 34 mm into 2 parallel rows. The volume of the system was 1000 L. Algae were circulated in the system by a pump with a capacity of 20 W/h (Fig. 2). The model used 8 photo-bioreactor systems with a total volume of 8000 L. The initial algae density for culture in this system was 1 million cells per ml.

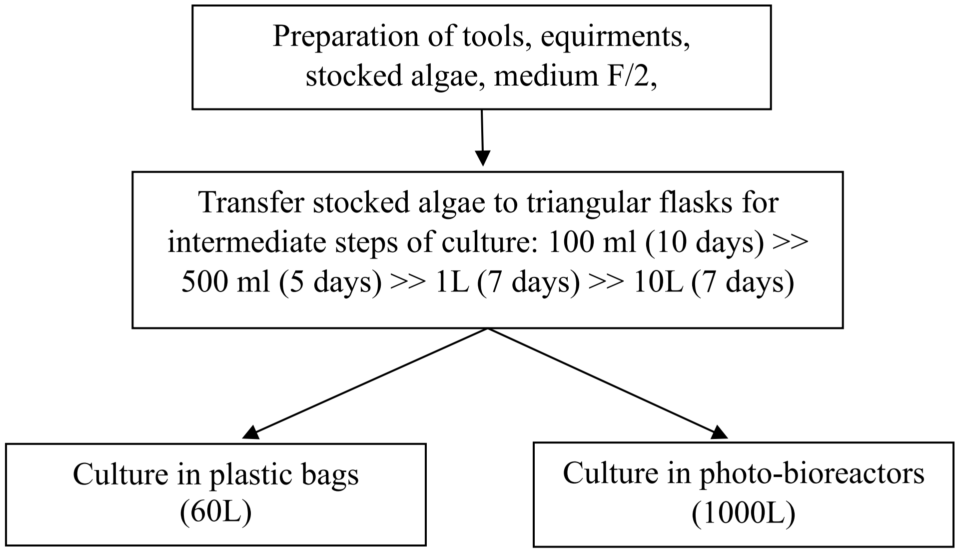

Figure 1: *Flow chart of protocol of algae N. oculata biomass production* [6]

Figure 2: *The photo bioreactor system (left hand) and plastic bags (right hand)*

2.4.2. Measurement of environmental parameters

- Illumination intensity measurement: measured by Lux/Fc Light Meter TM 204 machine (Taiwan), to measure the illumination intensity above the plastic bag or photobioreactor systems.

- Temperature measurement: measured temperature of algae liquid in plastic bags or photobioreactor systems by the mercury thermometer with 0.2 graduations at 7:00 a.m and 2:00 p.m every day.

- pH: use of pH meter (HI 8314 - 04 /Hanna, Italy) to measure pH in algae liquid in plastic bags or photo-bioreactor systems twice a day.

- Salinity: measured by refractometer (1528M/Atago, Japan) before pumping the water into the culture systems.

2.4.3. Determintion of algae growth

- The growth of algae N. oculata was determined by measurement of optical density at wavelength 680 nm (OD680) or counted the number of algae cells by the Burker-Turk counting chamber (Germany).

- Counting method and formula for calculation of algae density according to Coutteau (1996): number of algae (cells/ml) = [(n1 + n2)/160]×106×d. In which: n1: number of algae cells in the first counting chamber; n2: number of algae cells in the second counting chamber; d: dilution ratio.

2.4.4. Harvest and processing of biomass algae

The protocol of processing algae *Nannochloropsis oculata* (fresh concentrated and dried powder) was transferred by Aquaculture Research Sub-Institute for North Central [6].

Harvesting method of fresh algae biomass: harvested by KAl $(SO_4)_2.12H_2O$) with a concentration of 4% for the precipitation to collect the sedimented algae.

- Processing method for fresh concentrated algae: harvest concentrated algae by directly used of centrifugation at a speed of 3,500 rpm for 10 minutes.

- Processing method for dried powder algae: used of electric dryer to dry concentrated algae at 35^0C for 360 minutes

2.4.5. Quality analysis of the post-harvest algae

Three algae samples (triplicated) were analysised at the Laboratory of the Center for Drug - Food Testing and Applied Research, Hanoi Department of Science and Technology.

- Salmonella was determined by Vietnam Standard 4829:2005 [7], e. coli was determined according to Vietnam Standard 7924-2:2008 [8], and coliform was determined according to Vietnam standard 6848:2007 [9].

- Total aerobic microorganisms were determined by Vietnam Standard 4884:2015 [10].

- Heavy metal parameters: Pb, Cd, Hg were determined by GFA-AAS method [11].

- Nutritional parameters: protein level according to Vietnam Standard 4328 - 2007 [12]; lipid level was determined by Vietnam Standard 4331 - 2007 [13]; carbohydrate was determined by Vietnam Standard 4327-2007 [14].

3. Results and Discusion

3.1. Biomass production of algae Nannochloropsis oculata

During culture period algae biomass, some environmental factors were monitored, such as: illumination intensity in ranged of 19,452 to 44,135 lux; water temperature in ranged of 27.8 to 33ºC; pH fluctuated in between 7.2 to 9.4. Thus, illumination intensity, temperature and pH did not affected on photosynthesis and growth of algae *N. oculata* [2], [4].

The growth rate of algae in biomass production culture is shown in Fig 3. The growth rate of algae was quite similar during 9 days of culture in both systems (plastic bags and photo-bioreactions). In the plastic bags, algae density reached a peack with 80 million cells per ml date-8 of culture.

In the photo-bioreaction system, algae density was reached higher (180 million cells per ml), and longer culture period than its in the bags. This can be explained that the diameter tubes in photo-bioreaction system have diameter (3.4 cm) smaller than the plastic bags (50 cm), there algae were exposed to the light therefore photosynthesize better compared to those in plastic bags. Referring to the results of Cam D. T. V. et al., the algae density reached from 30 to 50 million cells per ml when they produced biomass of algae for milkfish larvae rearing [3]. The biomass of algae in this research was also higher density compared to the photo-bioreaction system of Trung B. B. et al., that cultured in glass tube with diameter of 34 mm, reached a density of 61 million cells per ml [5].

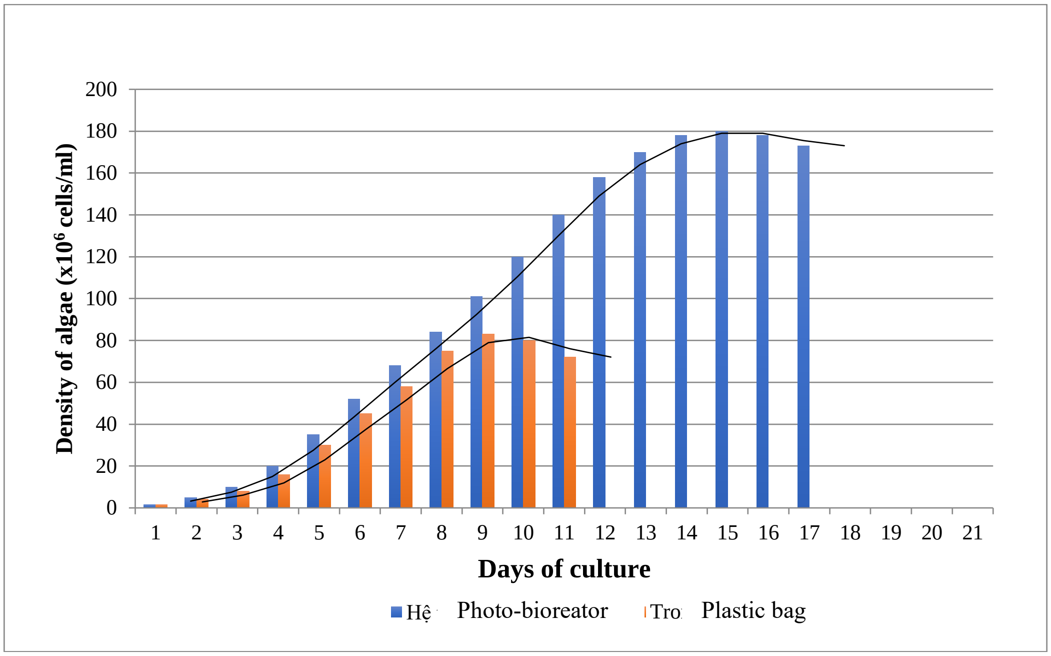

Figure 3: *Density of algae Nannochloropsis oculata (milion cells per ml) by days of culture*

The above results indicate that, both culture systems (plastic bags and photo-bioreactor system) can be applied for growing algae *Nannochloropsis oculata* in Nghe An. However, plastic bags culture is more easier to operate than the photo-bioreactor system.

3.2. Production of fresh concentrated algae Nannochloropsis oculata

3.2.1. Production of fresh concentrated algae in plastic bags

The results of harvest algal biomass grown in plastic bags are presented in Table 1. The results show that the total yield of algal in plastic bags from 27 harvest times from 2022 October to 2023 June reached 298.800 L. Algae density at harvesting time was in ranged of 70 to 92 milion cells per ml. Algae biomass harvested by flocculation method, transferred to a centrifuge to remove the water obtained 420.8 kg of concentrated algae. The obtained density and biomass of algea each culture batchs have stable, which demonstrated the highly stable of culture techniques.

Table 1: *Harvested fresh concentrated algae in plastic bags*

Time (month/ year)	**Weight of harvesting algae per batch (Litre)**			**Algae density achieved (cells/ml)**	**The amount of algae put into the centrifuge (Litre)**	**Concentrated algae (kg)**
	Batch 1	Batch 2	Batch 3			
10/2022	11.900	12.250	12.100	$70 \div 90 \times 10^6$	36.250	52.4

Time (month/ year)	**Weight of harvesting algae per batch (Litre)**			**Algae density achieved (cells/ml)**	**The amount of algae put into the centrifuge (Litre)**	**Concentrated algae (kg)**
	Batch 1	Batch 2	Batch 3			
11/2022	12.000	12.000	12.100	$72 \div 90 \times 10^6$	36.100	52.2
12/2022	12.000	12.150	11.850	$78 \div 88 \times 10^6$	36.000	51.0
01/2023	12.000	12.250	11.900	$78 \div 92 \times 10^6$	36.150	51.8
02/2023	11.900	12.050	12.000	$74 \div 82 \times 10^6$	35.950	50.2
03/2023	12.000	12.050	12.050	$76 \div 86 \times 10^6$	36.100	51.2
04/2023	11.800	11.750	11.550	$74 \div 80 \times 10^6$	35.100	49.0
05/2023	8.500	8.850	8.650	$74 \div 83 \times 10^6$	26.000	34.6
06/2023	7.250	7.000	6.900	$70 \div 80 \times 10^6$	21.150	28.4
Total					**298.800**	**420.8**

3.2.2. Production of fresh concentrated algae in photobioreactor

Table 2: *Harvested fresh concentrated algae in photo-bioreactions system*

Time (Month/ year)	**Harvested times**	**Algae density (cells per ml)**	**Amount of harvesting algae per batch (L)**	**The amount of algae put into the centrifuge (L)**	**Concentrated algae (kg)**
10/2022	2	160 ÷ 180 milion	6.000	12.000	32.8
11/2022	2	168 ÷ 182 milion	8.000	16.000	44.6
12/2022	2	170 ÷ 180 milion	8.000	16.000	43.8
01/2023	2	160 ÷ 178 milion	8.000	16.000	43.4
Total	**8**			**60.000**	**164.6**

The yield of algae biomass harvested in the photo-bioreactor system (Table 2) was 60,000 L, with densities in range of 160 to 182 milion cells per ml, higher than those in plastic bags. The algae liquid solution was then centrifuged to remove the water, obtained 164.6 kg of fresh concentrated algae. That result shows that to obtain 1 kg of fresh concentrated algae required 364.9 L liquid of algae. The result was similar to Pham My Dung et al. (2021), when she got 1kg of fresh concentrated algae *N. oculata* needed to centrifugate 350 L of those liquid of algae [4]. Thus, at the end of the project the total fresh concentrated algae products where 585.4 kg came from both plastic bags culture and photo-bioreactor culture system. Of which, 181 kg of fresh concentrated algae were dried, and 404.4 kg of fresh concentrated algae was the remaining.

3.3. Production of dried powder algae Nannochloropsis oculata

10 g sample (triplicates) of fresh concentrated algae were dried at 35°C for 350 minutes obtained 3.15 ± 0.04 g dried algae. The results in Table 3 show that a total of 181

kg of concentrated algae were dried to get a yield of 57.6 kg of dried algae with the ratio of dried algae/concentrated algae ranged from 0.31 to 0.32. Dried algae were stored in plastic bags by the vacuum method to keep them in good quality situation.

Table 3: *Weight of algae N. oculata powder after drying*

Time (Month/ year)	**Culture system**	**Concentrated algae use for drying (kg)**	**Weight of dried algae collection (kg)**	**Dried algae/fresh algae rate**
October 2022	Plastic bags	20.0	6.3	0.32
	Photo-bioreactor	12.0	3.7	0.31
November 2022	Plastic bags	15.0	4.8	0.32
	Photo-bioreactor	16.0	5.1	0.32
December 2022	Plastic bags	15.0	4.8	0.32
	Photo-bioreactor	12.0	3.8	0.32
January 2023	Plastic bags	16.0	5.2	0.33
	Photo-bioreactor	12.0	3.8	0.32
February 2023	Plastic bags	15.0	4.8	0.32
March 2023	Photo-bioreactor	16.0	5.1	0.32
April 2023	Plastic bags	15.0	4.8	0.32
May 2023	hoto-bioreactor	9.0	2.9	0.32
Total		**181**	**57.6**	

Thus, the model made a total products of 57.6 kg of dried algae *N.oculata* powder.

Figure 4: *Fresh concentrated algae (left) and dried algae (right)*

3.4. The quality of algae N. oculata products

The quality analysis of 3 concentrated algae samples (samples 1, 2, 3) and 3 dried algae powder samples (samples 4, 5, 6) shows in Table 4.

Quality analysed of algae samples show that microbiological paremeters (*E. coli*, coliform, total aerobic microorganisms, salmonella), heavy metal (lead, cadmium, mercury) were consistented with the standards. National Technical Regulations on microbial contamination in food (QCVN 8-3:2012/BYT) and National Technical Regulations on limitation of heavy metal contamination in food (QCVN 8-2 :2011/BYT). The nutrition value was different between fresh concentrated algae and dried powder algae. For the fresh concentrated algae, protein content in ranged of 6.13÷6.66%; lipid 0.18-0.24%, carbohydrates 2.08 ÷ 2.13%. For the dried powder algae, protein content in ranged of 40.2 ÷ 41.2%, lipid 0.18 ÷ 0.21%, carbohydrates 8.54 ÷ 8.88%.

Table 4: *Qualities of algae N.oculata samples*

No	Name of Indicators	Unit	Require (*)	Fresh concentrated algae samples		
				Sample 1	Sample 2	Sample 3
Microbiological indicators						
1	*E. coli*	Cfu/g	≤ 3	KPH(LOD:10)	KPH(LOD:10)	KPH(LOD:10)
2	*Coliforms*	Cfu/g	≤10	KPH(LOD:10)	KPH(LOD:10)	KPH(LOD:10)
3	Total aerobic microorganisms	Cfu/g	≤ 10.000	$1.5x10^1$	$3.5x10^1$	$3.5x10^1$
4	*Salmonella*	25g	KPH	KPH	KPH	KPH
Nutritional indicators						
1	Protein	%	≥ 6%	6.66	6.13	6.13
2	Lipid	%	≥ 0.18%	0.24	0.18	0,18
3	Carbonhidrate	%	≥ 2%	2.13	2.08	2.08
Heavy metal						
1	Lead	ppm	1.0	KPH (<0.05)	KPH (<0.05)	KPH (<0.05)
2	Cadmium	ppm	0.05	KPH (<0.03)	KPH (<0.03)	KPH (<0.03)
3	Mercury	ppm	0.50	KPH (<0.01)	KPH (<0.01)	KPH (<0.01)
				Result of dried algae powder samples		
				Sample 4	Sample 5	Sample 6
Microbiological indicators						
1	*E. coli*	Cfu/g	≤ 3	KPH(LOD:10)	KPH(LOD:10)	KPH(LOD:10)
2	*Coliforms*	Cfu/g	≤10	KPH(LOD:10)	KPH(LOD:10)	KPH(LOD:10)
3	Total aerobic microorganisms	Cfu/g	≤ 10.000	$1.5x10^2$	$1.5x10^2$	$1.2x10^2$
4	*Salmonella*	25g	KPH	KPH	KPH	KPH
Nutritional indicators						
1	Protein	%	≥ 6%	41.2	41.2	40.2
2	Lipid	%	≥ 0.18%	0.21	0.21	0.18
3	Carbonhidrate	%	≥ 2%	8.88	8.88	8.54
Heavy metal						
1	Lead	ppm	1.0	KPH (<0.05)	KPH (<0.05)	KPH (<0.05)
2	Cadmium	ppm	0.05	KPH (<0.03)	KPH (<0.03)	KPH (<0.03)
3	Mercury	ppm	0.50	KPH (<0.01)	KPH (<0.01)	KPH (<0.01)

Note: *refer to Vietnamese Standards: 1. QCVN 8-3:2012/BYT: National Technical Regulations on microbial contamination in food; 2. QCVN 8-2:2011/BYT: National technical regulation on limitation of heavy metal contamination in food; KPH is not detected; ppm (parts per million), converted 1 ppm = 1mg/l; Cfu/g is a unit commonly used to calculate the number of bacteria in 1 g of product

Thus, the fresh concentrated and dried powder algae *N.oculata* produced at VN Algae Science and Technology Joint Stock Company met the requirements for making aquatic food and raw materials for human functional food production.

4. Conclusion

The process of biomass production and processing of algae *N.oculata* which was suitable for Nghe An climate conditions has been completed in this study. Therefore, a total of 404.4 kg of fresh concentrated algae, and 57.6 kg of dried powder algae have been produced meeting quality criteria, and the requirements of food for aquatic animals and raw materials for functional foods for humans.

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TÓM TẮT

SẢN XUẤT SINH KHỐI TẢO *Nannochloropsis oculata* QUY MÔ HÀNG HÓA TẠI HUYỆN QUỲNH LƯU, NGHỆ AN

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Ngày nhận bài 14/8/2023, ngày nhận đăng 04/9/2023

Nghiên cứu đã hoàn thiện quy trình công nghệ sản xuất sinh khối tảo *Nannochloropsis oculata* với hai hình thức nuôi trong túi nilon và nuôi trong hệ thống ống kín. Với hệ thống túi nilon, tảo đạt mật độ sinh khối cực đại vào ngày thứ 8 với mật độ 80 x10^6 tế bào/ml. Ở hệ thống ống kín, tảo đạt mật độ cao hơn, thời gian nuôi dài hơn so với nuôi trong túi nilon, mật độ tảo cực đại ở ngày thứ 15 (180x10^6 tế bào/ml). Kết quả nghiên cứu đã sản xuất được 404,4 kg tảo tươi cô đặc và 57,6 kg tảo bột khô phù hợp với điều kiện khí hậu Nghệ An. Các sản phẩm tảo đạt các chỉ tiêu chất lượng, đáp ứng yêu cầu làm thức ăn cho thủy sản và làm nguyên liệu sản xuất thực phẩm chức năng cho con người.
Từ khoá: Tảo *Nannochloropsis oculata*; nuôi sinh khối tảo; thức ăn thuỷ sản.