

TẠP CHÍ

KHOA HỌC

TRƯỜNG ĐẠI HỌC VINH

VINH UNIVERSITY JOURNAL OF SCIENCE

SRRIK

KHOA HỌC TỰ NHIÊN
KỸ THUẬT VÀ CÔNG NGHỆ

A

NATURAL SCIENCES, ENGINEERING
AND TECHNOLOGY

TẬP 52
SỐ 4/2023

VUJS

ISSN1809 - 2229



TRƯỜNG ĐẠI HỌC VINH
VINH UNIVERSITY

CONTENTS

	pp
1. Le Van Thanh, Pham Nhu Y , <i>A mean convergence theorem for triangular arrays of rowwise and pairwise m_n-dependent random variables.</i>	5
2. Pham Thanh Loan, Hoang Thi Le Thu , <i>Evaluation of the growth, yield and quality of <i>Rehmannia glutinosa</i> (DH19) in the midland and mountainous region of Northern Vietnam.</i>	12
3. Nguyen Xuan Tien, Le Tien Huu, Ho Thi Phuong, Nguyen Van Lam , <i>Assessment of water resource variation in the Lam river basin from 1970 to 2022.</i>	21
4. Thi Minh Nguyen, Huu Lam Phan, Hong Quang Nguyen, Thanh Nghia Cao, Ngoc Minh Luong, Thi Kim Thu Nguyen, Thi Minh Tam Nguyen, Thi Huyen Thuong Ho, Dinh Lam Vu, Thi Quynh Hoa Nguyen , <i>A simple design of broadband cross-polarization converter for the THz frequency range.</i>	28
5. Hoang Thi Phuong , <i>Designing an automatic beverage mixing system based on the internet of things.</i>	39
6. Dien Thi Hong Ha , <i>Developing a supportive device for wrist injury rehabilitation training.</i>	49
7. Le Thi My Chau, Nguyen Tan Thanh, Dinh Thi Phuong An, Pham Thi Lan Anh, Ngo Thi Men, Nguyen Thi Ha Vy, Bui Minh Tuong Linh, Tran Quang Nhat, Tran Nghia Hung, Nguyen Thi Bich Ngoc, Cao Thu An , <i>Application of natural-based anti-fungal formulations for bamboo and wooden household products.</i>	59
8. Ong Vinh An, Dang Huy Huynh, Hoang Xuan Quang, Tran Kien , <i>Regular activities of adult <i>Ptyas mucosa</i> (Linnaeus, 1758) in a farming conditions of Nghe An Province, Viet Nam.</i>	71
9. Tran Hau Khanh , <i>Antimicrobial activity of essential oils from leaf of <i>Syzygium tsoongii</i> (Merr.) Merr. & Perry, <i>Syzygium bullockii</i> (Hance) Merr. & Perry and <i>Syzygium zeylanicum</i> (L.) dc. in Ha Tinh Province.</i>	85
10. Tran Thi Hien, Dao Thi Hang, Pham Van Phi , <i>Researching and developing a smart lock system applying artificial intelligence.</i>	93
11. Chu Chi Thiet, Nguyen Thi Le Thuy, Tran Thi Ngoc Anh, Ho Viet Cuong, Pham My Dung, Nguyen Thi Thanh , <i>Biomass production of algae <i>Nannochloropsis oculata</i> at large scale in Quynh Luu District, Nghe An Province</i>	104

12. **Luong Thi Yen Nga, Nguyen Huy Bang, Le Thi Dung, Hoang Minh Dong, Trang Huynh Dang Khoa, Nguyen Thi Thu Hien, *Influence of spontaneously generated coherence and related phase on an all-optical switching in a three-level ladder-type system with incoherent pumping.* 114**
13. **Dang Thi Soa, Nguyen Thu Hang, Vu Thi Thuy, *Studying the drug use characteristics and commenting on the results of cerebral infarction treatment at Nghe An General Friendship Hospital Stroke Center in 2023.* 123**
14. **Nguyen Thanh Phuong, Luong Huu Bac, Quan Thi Minh Nguyet, Do Thi Kim Thoa, Nguyen Ngoc Trung, Tong Quang Cong, Tran Quoc Tien, *Realization of 650 nm fiber-coupled diode lasers module with output beam redirection for application in phototherapy and photodynamic inactivation of bacterials.* 135**

MỤC LỤC

	trang
1. Lê Văn Thành, Phạm Như Ý , <i>Một số định lý về sự hội tụ theo trung bình của mảng tam giác các biến ngẫu nhiên m_n-phụ thuộc đôi một theo hàng</i>	5
2. Phạm Thanh Loan, Hoàng Thị Lệ Thu , <i>Đánh giá khả năng sinh trưởng, năng suất và chất lượng của giống Địa hoàng 19 (DH19) tại vùng trung du miền núi phía Bắc Việt Nam</i>	12
3. Nguyễn Xuân Tiên, Lê Tiến Hữu, Hồ Thị Phương, Nguyễn Văn Lam , <i>Đánh giá sự thay đổi tài nguyên nước trên lưu vực sông Lam giai đoạn từ năm 1970 đến 2022</i>	21
4. Nguyễn Thị Minh, Phan Hữu Lâm, Nguyễn Hồng Quảng, Cao Thành Nghĩa, Lương Ngọc Minh, Nguyễn Thị Kim Thu, Nguyễn Thị Minh Tâm, Hồ Thị Huyền Thương, Vũ Đình Lâm, Nguyễn Thị Quỳnh Hoa , <i>Thiết kế vật liệu biến hoá phân cực chéo băng thông rộng làm việc trong vùng tần số THz</i> . .	28
5. Hoàng Thị Phượng , <i>Nghiên cứu xây dựng hệ thống pha chế đồ uống tự động ứng dụng công nghệ IoT</i>	39
6. Điền Thị Hồng Hà , <i>Nghiên cứu xây dựng thiết bị hỗ trợ quá trình luyện tập phục hồi chân thương cổ tay</i>	49
7. Lê Thị Mỹ Châu, Nguyễn Tân Thành, Đinh Thị Phương An, Phạm Thị Lan Anh, Ngô Thị Mến, Nguyễn Thị Hà Vy, Bùi Minh Tường Linh, Trần Quang Nhật, Trần Nghĩa Hưng, Nguyễn Thị Bích Ngọc, Cao Thu An , <i>Ứng dụng công thức kháng nấm từ thiên nhiên cho sản phẩm tre, gỗ gia dụng</i>	59
8. Ông Vĩnh An, Đặng Huy Huỳnh, Hoàng Xuân Quang, Trần Kiên , <i>Quy luật hoạt động của rận ráo trâu <i>Ptyas mucosa</i> (Linnaeus, 1758) trưởng thành trong điều kiện nuôi tại tỉnh Nghệ An, Việt Nam</i>	71
9. Trần Hậu Khanh , <i>Hoạt tính kháng vi sinh vật của tinh dầu từ lá loài Trâm trái trắng (<i>Syzygium tsoongii</i> (Merr.) Merr. & Perry), Trâm bullock (<i>Syzygium bullockii</i> (Hance) Merr. & Perry) và Trâm tích lan (<i>Syzygium zeylanicum</i> (L.) DC.) ở Hà Tĩnh</i>	85
10. Trần Thị Hiền, Đào Thị Hằng, Phạm Văn Phi , <i>Nghiên cứu cải tiến hệ thống khóa thông minh ứng dụng trí tuệ nhân tạo</i>	93
11. Chu Chí Thiết, Nguyễn Thị Lệ Thủy, Trần Thị Ngọc Ánh, Hồ Việt Cường, Phạm Mỹ Dung, Nguyễn Thị Thanh , <i>Sản xuất sinh khối tảo <i>nannochloropsis oculata</i> quy mô hàng hóa tại huyện Quỳnh Lưu, Nghệ An</i>	104

12. **Lương Thị Yên Nga, Nguyễn Huy Bằng, Lê Thị Dung, Hoàng Minh Đồng, Trang Huỳnh Đăng Khoa, Nguyễn Thị Thu Hiền,** *Ảnh hưởng của độ kết hợp được tạo bởi phát xạ tự phát và bơm không kết hợp lên chuyển mạch toàn quang trong môi trường nguyên tử ba mức bậc thang khi có mặt của pha tương đối.* **114**
13. **Đặng Thị Soa, Nguyễn Thu Hằng, Vũ Thị Thủy,** *Nghiên cứu đặc điểm sử dụng thuốc và nhận xét kết quả điều trị nhồi máu não tại Trung tâm Đột quỵ Bệnh viện Hữu nghị Đa khoa Nghệ An năm 2023.* **123**
14. **Nguyễn Thanh Phương, Lương Hữu Bắc, Quãn Thị Minh Nguyệt, Đỗ Thị Kim Thoa, Nguyễn Ngọc Trung, Tống Quang Công, Trần Quốc Tiến,** *Nghiên cứu chế tạo mô đun chuyển hướng bức xạ laser bán dẫn ghép sợi quang 650 nm ứng dụng trong quang trị liệu và quang sinh diệt khuẩn.* **135**

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

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ARTICLE INFORMATION ABSTRACT

Journal: Vinh University
Journal of Science
ISSN: 1859-2228

Volume: 52
Issue: 4A

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Received: 14 August 2023
Accepted: 08 September 2023
Published: 20 December 2023

Citation:
Chu Chi Thiet, Nguyen Thi Le
Thuy, Tran Thi Ngoc Anh,
Ho Viet Cuong, Pham My
Dung, Nguyen Thi Thanh
(2023). Biomass production of
algae *Nannochloropsis oculata*
at large scale in Quynh Luu
district, Nghe An province.
Vinh Uni. J. Sci.
Vol. 52 (4A), pp. 104-113
doi: 10.56824/vujs.2023a109

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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 concentrated 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. oculata* 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, reduction of omega-3 unsaturated fatty acids, enhancement of brain functions and increasement of memory, vitality, detoxify, prevention and treatment assistance 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 bubble 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 CO₂ 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.

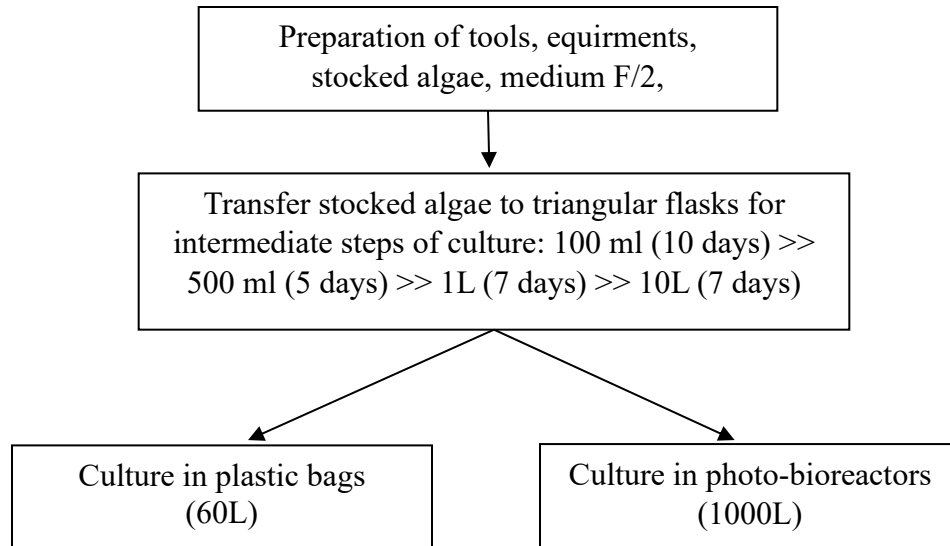


Figure 1: Flow chart of protocol of algae *N. oculata* biomass production [6]



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. Determination 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 Burkert-Turk counting chamber (Germany).

- Counting method and formula for calculation of algae density according to Coutteau (1996): number of algae (cells/ml) = $[(n1 + n2)/160] \times 106 \times d$. 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 (SO₄)₂.12H₂O 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 35⁰C for 360 minutes

2.4.5. Quality analysis of the post-harvest algae

Three algae samples (triplicated) were analysed 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 Discussion

3.1. Biomass production of algae *Nannochloropsis oculata*

During culture period algae biomass, some environmental factors were monitored, such as: inllumination intensity in ranged of 19,452 to 44,135 lux; water temperature in ranged of 27.8 to 33⁰C; pH fluctuated in between 7.2 to 9.4. Thus, inllumination 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].

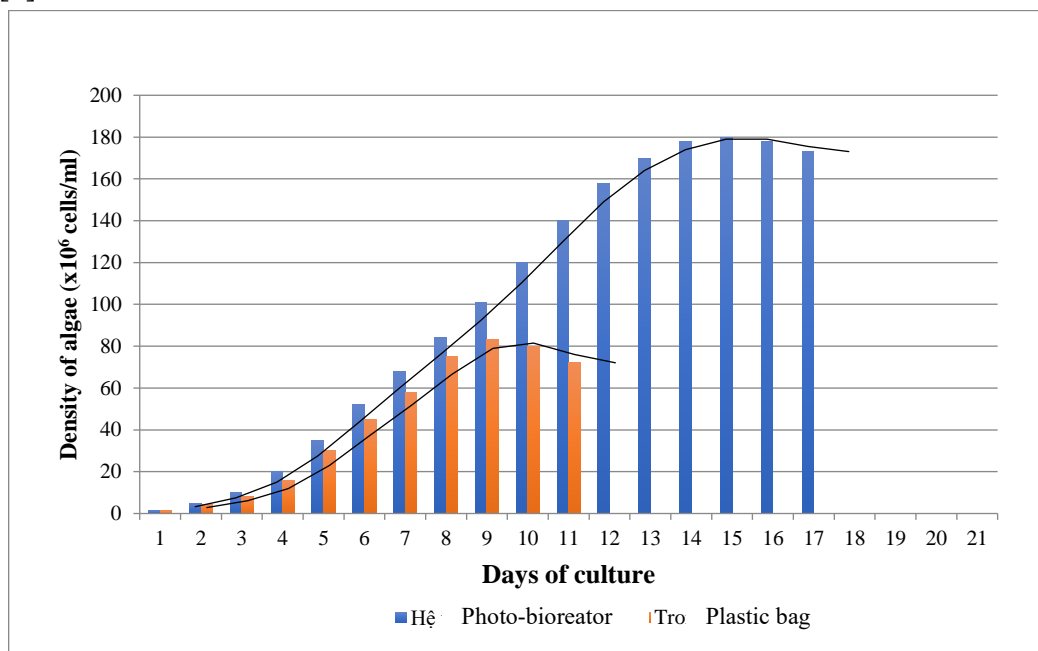


Figure 3: Density of algae *Nannochloropsis oculata* (million cells per ml) by days of culture

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 algae 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 ÷ 90 x 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)	Harveted 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 parameters (*E. coli*, coliform, total aerobic microorganisms, salmonella), heavy metal (lead, cadmium, mercury) were consistent 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	<i>E. coli</i>	Cfu/g	≤ 3	KPH(LOD:10)	KPH(LOD:10)	KPH(LOD:10)
2	<i>Coliforms</i>	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	<i>Salmonella</i>	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	<i>E. coli</i>	Cfu/g	≤ 3	KPH(LOD:10)	KPH(LOD:10)	KPH(LOD:10)
2	<i>Coliforms</i>	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	<i>Salmonella</i>	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 80×10^6 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 (180×10^6 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 thủy sản.