STUDY ON THE ABILITY TO TREAT BILGE WASTEWATER FROM FISHING BOAT BY CULTIVATING AND HARVESTING THE MICROALGAE *CHLORELLA VULGARIS*

NGHIÊN CỨU KHẢ NĂNG XỬ LÝ NƯỚC THẢI LA CANH TỪ TÀU CÁ BẰNG CÔNG NGHỆ NUÔI TRỒNG VÀ THU HOẠCH VI TẢO *CHLORELLA VULGARIS*

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Abstract - The current situation of pollution in fishing ports of many coastal localities in Vietnam is caused by the discharge of bilge water from fishing vessels directly into the sea. This wastewater has the characteristics of containing large amounts of organic matter and nutrients, with high value of COD, total nitrogen (TN) and total phosphorus (TP), high salinity, and contaminated with mineral oil. This paper studies on the cultivation of microalgae *Chlorella vulgaris* in this wastewater medium for the purpose of wastewater treatment and biomass recovery. The results show that this microalgae strain can adapt in this wastewater medium, with salinity <7‰. After 15 days of microalgae culture and harvesting by ECF method, the wastewater quality was improved prominently with pollution indicators reduced by over 90%. From the 10^{th} day, the density of microalgal biomass reached the maximum threshold, corresponding to 0.93g/L of dry biomass, and the concentration of Chlorophyll a was 31.65 μg/ml.

Key words - *Chlorella vulgaris*; bilge wastewater; wastewater treatment; growth of microalgae

Tóm tắt - Thực trạng ô nhiễm tại các cảng cá của nhiều địa phương ven biển Việt Nam hiện nay có nguyên nhân từ việc xả thải nước la canh từ tàu cá trực tiếp xuống biển. Nước thải này có đặc điểm chứa lượng lớn hữu cơ và dinh dưỡng, với các chỉ số COD, tổng Nitơ, photpho cao, độ mặn cao, bị nhiễm dầu khoáng. Bài báo nghiên cứu thử nghiệm nuôi trồng vi tảo *Chlorella vulgaris* trong môi trường nước thải này nhằm mục đích xử lý nước thải và thu hồi sinh khối. Kết quả cho thấy, chủng vi tảo này có thể thích nghi được trong môi trường nước thải này, với độ muối <7‰. Sau 15 ngày nuôi trồng vi tảo và thu hoạch bằng phương pháp ECF, chất lượng nước thải được cải thiện rõ rệt với các chỉ số ô nhiễm giảm trên 90%. Từ ngày thứ 10 mật độ sinh khối tảo đạt ngưỡng tối đa tương ứng 0,93g/L sinh khối khô, và nồng độ *Chlorophyll a* là 31,65 μg/ml.

Từ khóa - *Chlorella vulgaris*; nước thải la canh; xử lý nước thải; sinh trưởng vi tảo

1. Introduction

With 3.260 km of coastline, Vietnam has a fisheries reserve of 4.2 million tons and maximum sustainable yield (MSY) of 1.7 million tons per year [1], with five major fishing centers in 5 provinces: Hai Phong, Da Nang, Khanh Hoa, Ba Ria - Vung Tau and Kien Giang. The exploitation, processing and export of seafood products in Vietnam have grown stronger in recent years, significantly contributing to economic growth with an increase in value from 2.2 billion in 2015 to 3.2 billion USD in 2019 and tends to grow continuously over the years [2].

However, the current situation in fishing ports of many coastal localities in Vietnam is a rather serious environmental pollution problem. One of the reasons for this pollution is the lack of awareness and freedom to discharge all kinds of solid waste as well as bilge wastewater from fishing vessels directly into the sea near the fishing port area. The bilge water generated from the seafood storage cellar, due to the washing of products and the storage tank, flows down to the engine tunnel and is pumped out. This wastewater has the characteristics of containing a large amount of organic matter and nutrients, with high value of COD and total nitrogen (TN) and total phosphorus (TP), high salinity (due to the use of seawater to wash the tank), contaminated with mineral oil and has a low temperature (due to melting ice). This is an urgent requirement of the Management Board of fishing ports because there is no appropriate collection and treatment orientation [3].

Treatment of different types of wastewater by physicochemical or biological (non-microalgae) methods can often be inefficient or energy-intensive [4].

Microalgae have long been recognized as an important microorganism in wastewater, playing an essential role in wastewater treatment: Directly through the absorption of organic and inorganic nutrients from the waste; and indirectly through the oxidation of wastewater to help aerobic bacteria continue to decompose waste. Microalgae can use the abundant sunlight shining on the wastewater surface as their energy requirement to grow and at the same time remove pollutants. Microalgae can also grow in arid environments and in water with high salinity. Producing 1 kg of microalgae biomass consumes 1.88 kg of CO_2 during the wastewater treatment process [5] and generates a large amount of sludge mainly composed of microalgae. From microalgal biomass, others energy recovery options can be gasification and pyrolysis to produce syngas and biochar, fermentation and hydrothermal liquefaction of microalgae biomass for production bio-ethanol, bio-butanol and bio-oil. Microalgae are not limited to removing nutrients from wastewater; There are recent studies reporting the use of microalgae to remove pharmaceutical compounds and pesticides from agro-industrial wastewater [6], handle heavy metals and hydrocarbons [7]. Thus, with all its ability to grow, adapt and resist toxic conditions in wastewater environments, microalgae make it a versatile organism to treat a wide variety of wastewater originating from various industrial as well as agricultural activities. Furthermore, with the integration of the biorefinery concept, the harvested microalgae biomass can be used to produce carbon-neutral biofuels, high-value bioactives, fish feed and animals, bio-fertilizers, bio-plastics; while helping to reduce CO_2 emissions according to the circular principle, thereby promoting the concept of a zero-emission circular economy [4]. Since the late 2000s, instability in the oil market caused an energy crisis on a global scale, which further spurred the international community to invest in biofuels derived from microalgae in the combined with wastewater treatment, aiming to both generate energy and reduce environmental pollution. Wastewater treatments by microalgae are currently being studied as a way to cut greenhouse gas emissions to limit global warming and produce sustainable biofuels [8].

Many studies have shown the microalgae strain *C. vulgaris* to be a potential candidate for wastewater treatment applications [9], combined with biomass production for energy or food purposes [10]. Research on culturing microalgae *C.vulgaris* in wastewater issue from biogas digester shows that this microalgae strain has the potential to remove 80.9% of nitrogen N and 58.7% of phosphorus P content in wastewater after only 5-7 days of cultivation [11]. For wastewater from seafood processing, the mean nutrient removal rates over the entire culture period were 4.98 and 1.91 mg/L.day for N and P [12] respectively. In sewage water, the ability to remove nitrates, COD and BOD is up to 93%, 95% and 92% respectively [13]. Not only well adapted to organic-rich wastewater, *C. vulgaris* also has the ability to remove hydrocarbons and iron in wastewater from the steel industry with values of 75 and 97.9%, respectively [7]. This microalgae strain is also used to treat color, heavy metals and COD in textile dyeing wastewater with an efficiency of up to 74% [14]. The latest study by M. Barahoei et al. [15] showed that this microalgae strain is well adapted to wastewater with high salinity, ranging from 1000-5000 ppm (1-5‰) and reduce salinity by 80 and 40%, respectively.

With the potential to treat various types of wastewater of the microalgae *C. vulgaris* strain, this study investigated the cultivation of *C. vulgaris* in the medium of bilge wastewater taken directly from the fishing vessel boats at Tho Quang fishing port, Da Nang city and evaluating its treatment capacity for this type of wastewater in the circular economy orientation.

2. Materials and methods

2.1. Microalgae Source

In this study, experiments were performed with microalgae *C. vulgaris* with a cell size of 5-10 µm, purchased from the Algae Technology Department of the Vietnam Institute of Science and Technology, in the solution form. Use the preservation method by spreading on agar plates to preserve the strain source. *Chlorella vulgaris* was then activated and propagated under light conditions of 4000 lux for 7-10 days in Antoine medium [16] before inoculation at 10% concentration in wastewater culture medium. The biomass concentration when inoculated into the medium reached 1g/L.

2.2. Culture medium

Using bilge wastewater collected directly from the cellar of fishing vessels after loading and unloading all products, at Tho Quang fishing port, Da Nang city. Samples were collected in 20 L inert plastic cans. These samples were stored in a cool and dark room, without any filtration, to preserve sample properties. The organic composition, COD and total mineral oil of the wastewater varied by fishing vessel. The parameters of wastewater are analyzed before and after each cycle of cultivating and harvesting microalgae biomass to evaluate the adaptation process and treatment efficiency of this technology.

2.3. Cultivation method

Microalgae culture was carried out in transparent PET bottles of 1,5 liter and 5 liter containing respectively 1.25 liter and 4 liter of culture fluid (culture medium + 10% in volume of activated microalgal at level 2). Small flasks used in the stage of adaptation of microalgae in wastewater medium at various compositions. The number of flasks need to ensure the repeatability of the study was at least 2 times. Using aeration into each bottle with the same aeration rate of 650ml/min, in which CO_2 was added aiming to evaluate the role of CO_2 in the process of adaptation and growth of microalgae in wastewater. The experimental samples were illuminated by the 20W led lights with cover, lighting cycle 12h light: 12h dark. The luminous intensity is measured by the luminous flux sensor, corresponding to the value of 13.000 lux. This is a light intensity value that is quite similar to outdoor natural light in the orientation of using natural light to grow microalgae [11]. The cultivation model is shown in Figure 1.

Figure 1. *Experimental cultivation model in the laboratory*

The growth rate was assessed by measuring the concentration of *Chlorophyll a* at the same time in each culture day. Harvesting of biomass was carried out when the growth entered the decline phase (corresponding to 2 days of decrease on the growth curve).

2.4. Evaluation of growth rate

There are a number of methods to qualitatively assess the density of microalgal biomass in the culture medium to determine the growth capacity of microalgae such as: measure the optical density (OD); determine the concentration of *Chlorophyll a*; measure viscosity of the medium; or count the number of algal cells [17]. In this study, because of the high turbidity and TSS content of wastewater medium, the method of determining the concentration of *Chlorophyll a* is used to accurately assess the growth of microalgae in the wastewater medium, as well as determine the efficiency of microalgae biomass recovery by Electrolysis - Coagulation - Flotation method (ECF).

According to this method, the sample is placed in an eppendorf tube, then centrifuged at 6000 rpm at 4ºC for 5 minutes to collect sample biomass after discard the supernatant. Add 1ml of cooled 90% acetone to the eppendorf tube containing the precipitate. Then centrifuge at 13000 rpm 4ºC for 5 minutes. Decant the supernatant and measure the OD at 664 nm, 647 nm. The concentration of *Chlorophyll a* was determined according to the Jeffrey and Humphrey's equation

$$\text{Chlorophyll a } (\mu g/ml) = 11.93 * E_{664} - 1.93 * E_{647} \quad (1)$$

2.5. Harvest method

Using Electrolysis Coagulation Flocculation (ECF) method to harvest microalgae biomass. All electrolysis experiments were performed at room temperature for 30 min in a 3 liter plastic tank (21 x 15.6 x 8.1cm) containing 2 liters of algae solution per harvest. The anode is aluminum tubes with a diameter of 1.1 cm and a length of 20cm, the cathode is a stainless-steel plate (20 x 15cm) placed parallel and close to the bottom of the tank. The distance between the electrodes is 3 cm. Use the DC supply with a current of 0.35A and a voltage of 9V.

2.6. Calculation of dry biomass

The dry biomass was determined through the method of calibrating the relationship between the concentration of *Chlorophyll a* and the biomass according to the linear equation:

$$y = 0.0289x + 0.0107 \qquad R^2 = 0.9958 \qquad (2)$$

Where, y - the amount of dry biomass (g/l) and x - the concentration of *Chlorophyll a* (µg/ml).

3. Results and discussion

3.1. Effect of wastewater composition on the adaptability of microalgae

The analysis results of the composition of bilge wastewater (Table 1) show that this type of wastewater has a very large fluctuation in wastewater parameters, especially related to COD, BOD, total N and total P depending on the samples taken from different fishing vessels.

Table 1. *Wastewater parameters and analytical methods*

Parameters	Unit	Method	Value		
			Sample 1	Sample 2	Sample 3
pH		TCVN 6492:2011	7.6	7.2	7.4
Salinity	‰	SMEWW 2520B:2012	3.7	6.8	11.4
TSS	mg/L	TCVN 6625:2000	790	1430	1060
COD	mg/L	SMEWW 5220D:2012	1480	4450	920
BOD_5	mg/L	SMEWW 5210D:2012	970	3340	532.7
N-NH4	mg/L	TCVN 5988-1995	137.05	119.37	29.14
N-NO3	mg/L	TCVN 6180:1996	Non detected	1.05	2.36
T - N	mg/L	TCVN 6638:2000	317.49	326.08	57.25
T - P	mg/L	TCVN 6202:2008	26.42	36.17	18.74
Mineral oil	mg/L	SMEWW 5520B&F:2012	12.34	10.57	9.74

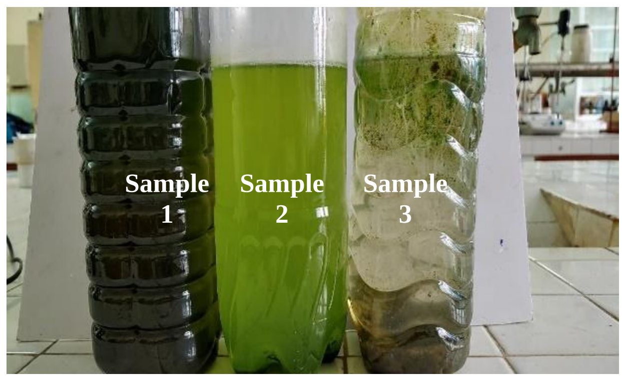

Figure 2. *Investigation of the adaptation of microalgae in wastewater medium with variable salinity*

Conducting the cultivation of microalgae in these wastewater samples after 10 days, the results obtained in Figure 2 reveal that the COD parameter, organic composition and mineral oil composition in wastewater do not have a great influence on the adaptation of *C. vulgaris* compared with the salt concentrations. Specifically, these microalgae adapted best in medium of the sample 1, followed by sample 2. For sample 3, although the COD, T-N, T-P and mineral oil were all lower, but the salt concentration was too high, the microalgae were difficult to adapt, so they died and settled to the bottom of the bottle. This result is also consistent with the study of M. Barahoei et al. [15] when investigating the effect of salt concentration on the adaptability of microalgae *C. vulgaris*.

3.2. Effects of culture conditions on the growth of microalgae C. vulgaris in the bilge wastewater

Some culture factors strongly affect the growth of microalgae, beyond the medium nutrient, there is also the light intensity and the CO_2 aeration [16]. For the purpose of cultivating microalgae under conditions of natural light aiming to handle the environmental pollution (wastewater and CO_2 emissions), in this study, we fixed the light source at a luminous flux of 13.000 lux, which is the equivalent value to outdoor light intensity [11] in all performed experimentations. Four experiments were performed with a fixed aeration rate of 650 ml/min, without and with CO_2 addition: 5; 10; and 15 ml/min, respectively. The culture medium corresponds to the wastewater of sample 2. The results of monitoring the concentration of Chlorophyll over time every 2 days show that the life cycle of the algae is 20 days. The maximum biomass density is around 10-14 days, corresponding to the maximum value of the equilibrium phase. After the cultivation period, the biomass was recovered by the ECF method, and the biomass was determined.

The results of *Chlorophyll a* concentration through the growth chart of the microalgae *C. vulgaris* (Figure 3) showed that this microalgae grew and developed better in the conditions with aeration of both air and CO_2 than in the case of only aeration and no CO_2 supply. The concentration of CO_2 also strongly affects the growth of microalgae, increasing the amount of CO_2 helps to increase the adaptation and growth of microalgae. The highest biomass density was achieved on the 10th day of culture in case supplemented with 15ml/min of CO_2, reaching 0.93g/L of dry biomass, corresponding to *Chlorophyll a* concentration of 31.65 µg/ml. D. Pavlik et al. has shown in his research that it is possible to add up to 7% CO_2 in the gas composition to help increase the growth rate of microalgae to 0.03g/L.h [18]. The additional CO_2 content depends on the composition of the wastewater, the higher the nutrient content of the wastewater, the more nutrient absorption capacity of microalgae is required for wastewater treatment, and the more intense photosynthesis is required, which is mainly related to light intensity and CO_2 addition. With the main goal of cultivating microalgae to treat bilge wastewater, the addition of CO_2 contained in the exhaust gas of the engine when ships operate in the fishing port area is also a potential orientation in combined exhaust treatment [18].

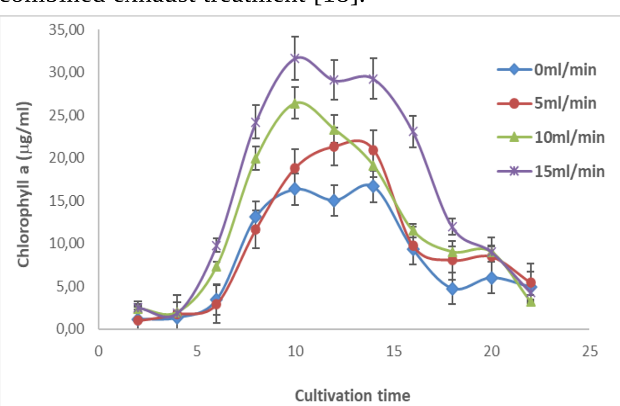

***Figure 3**. Effect of CO_2 concentration on the growth of microalgae C. Vulgaris*

3.3. Evaluation of the wastewater treatment ability of microalgae C. vulgaris

The analysis results of the wastewater medium before and after the cultivation period and harvesting of microalgae biomass by ECF method are shown in Table 2. It can be seen that microalgae have the ability to absorb a large amount of Nitrogen, Phosphorus, and significantly reduce the COD, and BOD_5 parameters. In addition, the harvest method of electrolysis coagulation and flocculation also contributes to the removal of TSS, and mineral oil in this wastewater.

***Table 2.** Analysis results of bilge wastewater samples before and after microalgae cultivation and harvesting*

Parameters	Unit	Value		
		Beginning	After cultivation and harvesting	Effectiveness (%)
pH		7.2	7.5	
Sal.	‰	6.8	6.2	8.82
TSS	mg/L	1430	16.2	98.87
COD	mg/L	4450	144.6	96.75
BOD_5	mg/L	3340	26.7	99.20
T - N	mg/L	326.08	20.9	93.60
T - P	mg/L	36.17	6.3	82.70
Mineral oil	mg/L	10.57	3.4	67.92

4. Conclusions

The experimental results obtained from this study show that it is possible to apply *C. vulgaris* microalgae culture technology and harvest by ECF flocculation electrolysis, to treat bilge wastewater from fishing vessels. The recovered microalgae biomass will be utilized for many different purposes: as animal feed, or for energy production such as direct burning for heat or biofuel production (biogas, bioethanol, biodiesel). This is a solution that both solves the environmental problem and solves the energy problem.

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