



RESEARCH ARTICLE

# Ability of *Chlorella vulgaris* algae for nutrients removal in domestic wastewater and its collection by ferrate

*Đánh giá hiệu quả xử lý chất dinh dưỡng bằng tảo Chlorella vulgaris và khả năng thu hồi tảo bằng ferrate*

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In this study, we aim to employ *Chlorella vulgaris* algae for removal of nutrients in wastewater and collect the produced algae by ferrate after treatment. The growth of algae was conducted in F/2 synthetic medium and in actual domestic wastewater. The removals of nitrogen and phosphorous by algae were then investigated for low and high nutrient concentrations using wastewater after biological treatment in both batch and continuous experiments. Results showed that specific growth rates in the exponential phase were 0.23 and 0.35 day<sup>-1</sup> for F/2 medium and domestic wastewater, respectively, proving the suitability of wastewater for algae growth. The removal efficiency of ammonia, nitrate, and phosphate were 89 - 93, 64 - 76, and 69 - 88%, respectively. In the algae collection test, pH 8 is the optimal pH to remove algae and ferrate had higher algae removal ability than alum under each optimal condition with removal efficiency of 84 - 97% at dosage of 12 mg Fe/L. These results suggest that microalgae is a potential alternative for removing of nutrients in wastewater treatment due to the high uptake capacity of nitrogen and phosphorous and the effective collection of algae after treatment by ferrate.

*Mục tiêu của nghiên cứu này là đánh giá hiệu quả xử lý chất dinh dưỡng bằng tảo Chlorella vulgaris trong môi trường nước thải sinh hoạt, thông qua khả năng xử lý N và P từ nguồn nước khi tảo tăng trưởng và khả năng keo tụ để thu hồi tảo bằng ferrate. Tốc độ tăng trưởng đặc thù  $\mu$  trong môi trường F/2 và nước thải sinh hoạt lần lượt là 0,23 ngày<sup>-1</sup> và 0,35 ngày<sup>-1</sup>. Hiệu suất xử lý ammoni, nitrat và photphat lần lượt đạt 89 - 93%, 64 - 76% và 69 - 88%. Kết quả thí nghiệm keo tụ thu hồi tảo cho thấy pH = 8 là thích hợp nhất để loại bỏ tảo bằng ferrate và việc sử dụng ferrate cho hiệu quả tách tảo tốt hơn phen nhôm với lượng sử dụng ít hơn. Ở hàm lượng 12 mgFe/L, hiệu quả tách tảo đạt cao nhất từ 84 - 97%. Nghiên cứu cho thấy tiềm năng thay thế công nghệ sinh học truyền thống bằng công nghệ vi tảo trong loại bỏ các chất dinh dưỡng và khả năng thu hồi tảo hiệu quả bằng cách sử dụng ferrate.*

**Keywords:** wastewater, algae, ferrate, nutrient removal, *Chlorella vulgaris*

## 1. Introduction

Microalgae are emerging as a very promising and sustainable environmental technology due to its ability of nutrient consumption in wastewater and CO<sub>2</sub> adsorption in air. High rate algae ponds are considered as more simple but high efficient and stable technology than traditional biological treatments such as aerobic, anoxic, and anaerobic methods for removal of nitrogen and phosphorous. The microalgae grown by sunlight as energy source for photosynthesis, thus reduce the energy required for the treatment system while consume and transform a considerable amount of greenhouse gas CO<sub>2</sub> into organic biomass. Moreover, biomass of microalgae is a valuable product that could be used for food additives, biofuels, and compost production (Mulbry et al., 2005). Microalgae

technology also plays an essential role in the sustainable development of agriculture since microbial fertilizers from microalgae provide high nutrients content for plants (Zepka et al., 2010). In addition to these advantages, microalgae technology has limitations such as high cost of removing algae before discharging into the environment and the cost of harvesting microalgae. Therefore, it is necessary to looking for a feasible way for microalgae collection after water treatment in terms of technology and cost.

*Chlorella vulgaris* is a genus of unicellular green algae with size of about 2-10  $\mu$ m and commonly used in wastewater treatment due to its high survival potential in contaminated environments (Canovas et al., 1996; Janczyk et al., 2006; Safi et al., 2014). Through photosynthesis, *C. vulgaris*

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is grows rapidly with CO<sub>2</sub>, water, and small amounts of organic matter (N, P) to form organic compounds for cell growth and development (Kuhl & Lorenzen, 1964). Therefore, the method for eliminating microalgae after wastewater treatment is very important to avoid high algal density in water environment. High nitrogen and phosphorus with organic matter in wastewater could cause algal blooms and blockages, which have negative impact on the quality and flowrate of treated water (Aslan & Kapdan, 2006). The method used to isolate algae is usually flotation (Organization, 2004), filtration with sand (Naghavi & Malone, 1986) and membrane (Hung & Liu, 2006; Lee et al., 2012), and flocculation (Gheraout et al., 2010). Flotation is expensive while filtration technology is generally applicable for large algae such as *Spirulina* sp. Flocculation is widely considered as a good way to recover algae at a reasonable cost and most studies have focused primarily on the potential of aluminum, iron, chitosan and other coagulants to eliminate microalgae (De Godos et al., 2011; Divakaran & Pillai, 2002; Eldridge et al., 2012; Gheraout et al., 2010; Liu et al., 2013; Sanyano et al., 2013). Specifically, Ma and Liu (2002) used ferrate as a pre-oxidant that enhances the removal of algae by coagulation process using alum. However, there has not been any study on the application of ferrate as both pre-oxidant and coagulant for effective collection of *C. vulgaris* after wastewater treatment.

In this study, we aim to employ *Chlorella vulgaris* algae for removal of nutrients in wastewater and collect the produced algae by ferrate after treatment. The performance of algae for ammonia, nitrite, nitrate, and phosphate removals in domestic wastewater was investigated in batch and continuous tests. The applicability of ferrate for algae collection after water treatment was also evaluated.

## 2. Materials and methods

*Chlorella vulgaris* was collected from Faculty of Biotechnology, Ho Chi Minh City University of Natural Sciences, Vietnam and grown in the key laboratory of Vietnam National University in Ho Chi Minh City University of Technology using of F/2 environment (Guillard & Ryther, 1962). *C. vulgaris* was cultured at a rate of 1/20 (v/v) in 250 mL Erlenmeyer flasks containing 100 mL of F/2 medium at room temperature of 25°C, using sunlight and shaken daily to prevent algae from sticking to the bottom. *C. vulgaris* grew rapidly in 6 to 9 days in F/2 medium and 4 to 6 days in wastewater environment. Once entered stable phase, the algae were used for culturing with a volume of 4 to 20 L and placed in a water tank with dimensions of 300 × 350 × 600 mm and volume of 60 L to obtain higher volume for further experiments.

Officially, the algae density is observed by counting the number of the algae cells in a Neubanet counter or measuring the Chlorophyll-a content. However, these techniques are time consuming and complicated. Therefore the optical density (OD) – the absorbance at the

wavelength of 680 nm was used instead because it is linearly correlated with number of cells and Chlorophyll-a content (Wang et al, 2010). The OD of 5 samples (in culture media F/2 and wastewater) were measured and correlated with the number of cells. The correlations between algae density Y (million cell/mL) and the absorbance at 680 nm X (OD) were determined in both F/2 medium and domestic wastewater as following equations:

$$Y_1 = 8.7926X_1 + 1.5882 (R^2 = 0.9972, P < 0.0001) \quad (1)$$

$$Y_2 = 8.7664X_2 + 0.0429 (R^2 = 0.9998, P < 0.0001) \quad (2)$$

The algae density (number of cells) of all samples were then determined by converting the measured OD following equations (1) and (2) respectively. The wastewater used in the study is domestic wastewater after biological treatment with water quality shown in Table 1. pH was recorded using a Mettler Toledo pH meter while Chlorophyll-a and other water quality parameter was analyzed using Standard Method (SMEWW method 10200H and 4500) with a HACH DR/2800 spectrophotometer. All the analyses were replicated three times and the average values were reported.

**Table 1. Characteristics of two wastewater (W) sources**

Parameter	Unit	W1	W2
pH	-	8.5	8.1
Ammonia (NH <sub>4</sub> <sup>+</sup> -N)	mg/L	8.2 ± 0.7	15 ± 0.9
Nitrate (NO <sub>3</sub> <sup>-</sup> -N)	mg/L	37.2 ± 0.8	49.5 ± 0.5
Nitrite (NO <sub>2</sub> <sup>-</sup> -N)	mg/L	6.1 ± 0.7	3.3 ± 0.6
Phosphate (PO <sub>4</sub> <sup>3-</sup> -P)	mg/L	4.5 ± 0.5	5.7 ± 0.4

Ferrate solution was prepared by wet oxidation method followed a process reported previously (Graham et al., 2010; Tien et al., 2008) using analytical-grade chemicals from Merck (Germany). Alum Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>.18H<sub>2</sub>O was bought from Guangdong Guanghua Sci-Tech Co., Ltd. (China) and used without any further treatment. Ferrate and alum were applied for algae removal and the effect of pH (5 – 9) and dosage of alum (5 – 25 mg Al/L) and ferrate (4 – 20 mg Fe/L) on algae removal efficiency were investigated. Optical density (OD) and chlorophyll-a content were used as indicators for evaluation of algae removal efficiency.

For batch experiments, the growth of *C. vulgaris* was investigated in both F/2 medium and wastewater using the water tank with volume of 63 L for 14 days under natural sunlight. Water samples were collected daily for both environment at the same time to evaluate the algal growth. In continuous tests, water retention time is designed at 6 days for F/2 medium and 4 days for wastewater and the tests was conducted for 14 days using water tanks of the same volume of 63 L.

### 3. Results and discussion

#### 3.1 Growth of algae

The result of cell number increase in the batch test is shown in Figure 1 in terms of chlorophyll-a content and number of cell for both F/2 medium and wastewater environment (wastewater W1 with lower nutrients content). In F/2 medium, the 4 growth phases of *C. vulgaris* were observed in 1, 5, 4, and 4 days of lag, log, stable, and death phases, respectively. In domestic wastewater, the 4 growth phases were 1, 2, 3, and 8 days. This indicates that *C. vulgaris* grew faster in the domestic wastewater than in F/2 medium with specific growth rate of 0.35 days<sup>-1</sup> (for 3 days) and 0.23 days<sup>-1</sup> (for 5 days), respectively, possibly due to the suitable environmental composition of wastewater (Wang et al., 2010). These values are competitive with those reported in the literature for F/2 medium of 0.238 day<sup>-1</sup> (Ong et al., 2010) and wastewater of 0.34 day<sup>-1</sup> (Pouliot et al., 1989; Wang et al., 2010). Moreover, *C. vulgaris* uses CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> to perform photosynthesis under sunlight for their growth, thus causes the increase of pH during the log and stable phases (Figure 1).

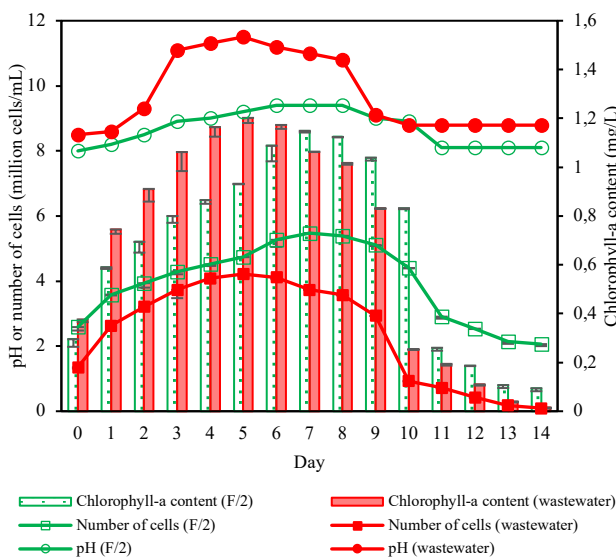


Figure 1. Growth of *Chlorella vulgaris* in batch tests

In continuous tests (Figure 2), the wastewater is continuously supplied and the treated water as well as algae in water. This help to form a steady-state operation of the system after around 4 to 6 days without death phase as in batch tests. pH is also monitored and shown a stable trend in Figure 5. With the same specific growth rates as in batch tests, the condition of domestic appears to be more favorable for *Chlorella vulgaris* growth than that of F/2 medium. pH variation was observed but only in the lag and log phase of algae growth.

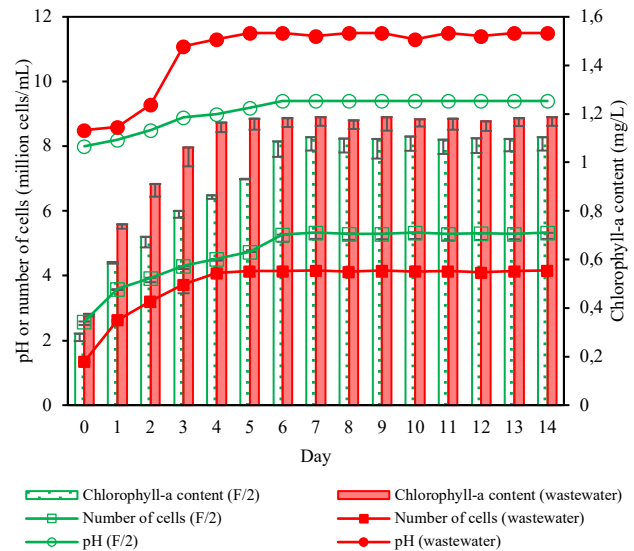


Figure 2. Growth of *Chlorella vulgaris* in continuous tests

#### 3.2 Nutrients removal ability of algae

Figure 3 and Table 2 presents the removal ability of nutrients by *C. vulgaris* during and after 7 days of experiment, respectively. These experiments were conducted using two types of wastewater (denoted as W1 and W2), in which the nutrient strength of W2 was higher than that of W1. Results showed that the growth of *C. vulgaris* lead to the rapid depletions of nitrogen and phosphorous in wastewater. The ammonia and nitrate concentrations in the effluent water decreased sharply after 5 days of operation. The removal efficiency of ammonia after 7 days reached 93% and 92% for W1 and W2, respectively, which are competitive to those reported in the literature using synthetic and domestic wastewater (Kim et al., 2010; Lau et al., 1997; Shi et al., 2007).

The nitrate removal efficiency was in range of 64 – 65% for both types of wastewater after 7 days of treatment. Algae use nitrate to produce ammonia in chloroplasts and nitrite formed during nitrate decomposition into ammonia; therefore, nitrite content increased as nitrate decreased in wastewater as *C. vulgaris* increased (Crawford, 1995).

For phosphate, the removal efficiency reached 69% and 78% after 7 days for W1 and W2. Although the transformation of phosphate by *C. vulgaris* in water is more slowly than that of nitrogen, *C. vulgaris* in this study can use phosphate at low concentrations and phosphate is not a limiting factor for growth of *C. vulgaris*. This is in agreement with research by Kim et al. (2013), in which *C. vulgaris* grows well in low phosphorus environment (1.37 – 1.85 mg/L).

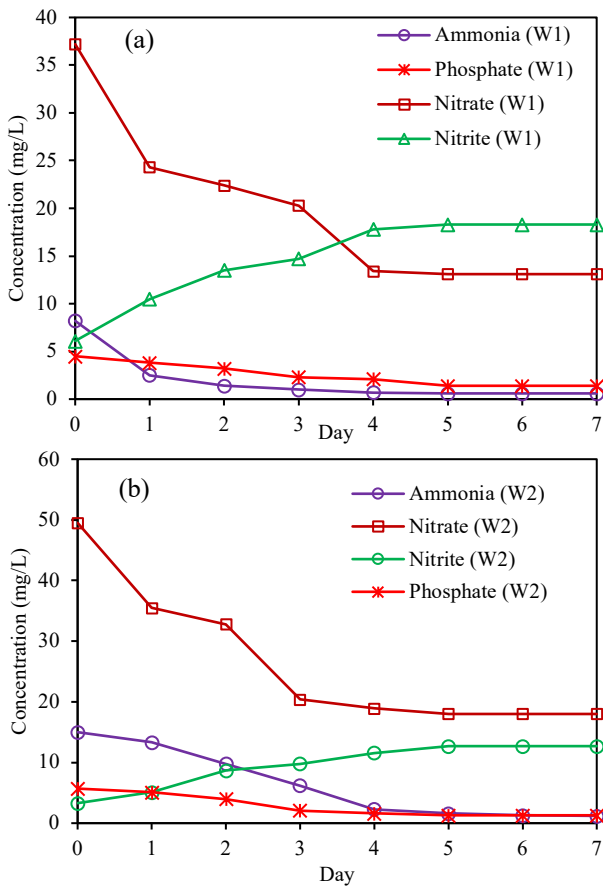


Figure 3. Concentrations of ammonium, nitrate, nitrite and phosphate during 7 days of batch experiment with two types of wastewater: (a) W1 and (b) W2

Table 2. Concentrations of ammonia, nitrate, and phosphate after 7 days of experiment

Ingredient	Concentration (mg/L)		
	W1	W2	QCVN 14:2008
Ammonia (NH <sub>4</sub> <sup>+</sup> -N)	0.6 ± 0.7	1.2 ± 0.9	5
Nitrate (NO <sub>3</sub> <sup>-</sup> -N)	13.1 ± 0.8	18.0 ± 0.5	30
Phosphate (PO <sub>4</sub> <sup>3-</sup> -P)	1.4 ± 0.4	1.3 ± 0.6	6

Similar to batch tests, the growth of *C. vulgaris* in continuous tests led to rapid changes in ammonia, nitrate, and phosphate levels in wastewater, which occurred rapidly after 4 days and then changed slowly until the end of the test period (Figure 4 and Table 13). Ammonia, nitrate, and phosphate removal efficiencies were relatively stable after 4 days of operation and reached 89 - 92%, 74 - 76%, and 57 - 88% after 14 days of operation, respectively. As results of batch and continuous tests, wastewater containing nitrogen and phosphorus seems to be suitable for growing of *C. vulgaris*.

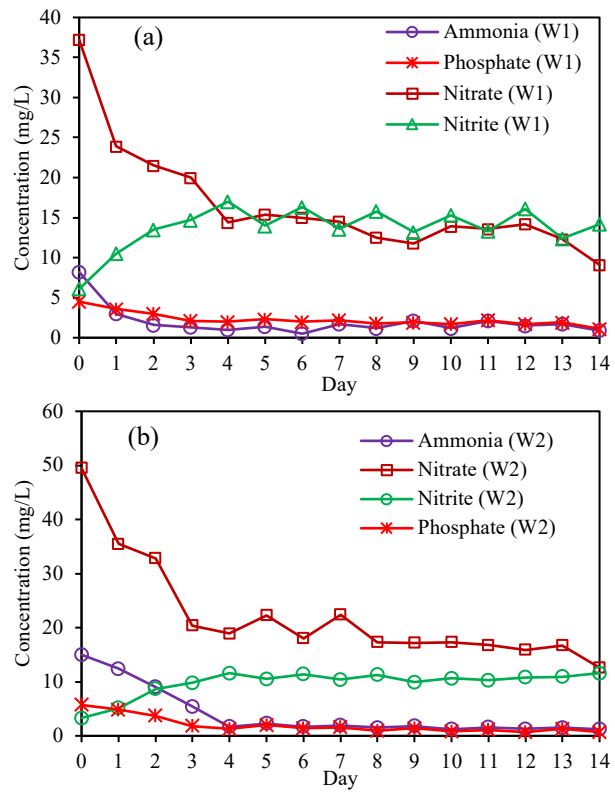


Figure 4. Concentrations of ammonium, nitrate, nitrite and phosphate during 14 days of continuous experiment with two types of wastewater: (a) W1 and (b) W2

Table 3. Concentrations of ammonia, nitrate, and phosphate after 14 days of experiment

Ingredient	Concentration (mg/L)		
	W1	W2	QCVN 14:2008
Ammonia (NH <sub>4</sub> <sup>+</sup> -N)	0.9 ± 0.2	1.2 ± 0.6	5
Nitrate (NO <sub>3</sub> <sup>-</sup> -N)	19.1 ± 1.4	12.6 ± 1.2	30
Phosphate (PO <sub>4</sub> <sup>3-</sup> -P)	1.1 ± 0.3	0.7 ± 0.1	6

### 3.3 Ability of algae collection from treated wastewater

After growing by wastewater, the produced algae must be collected not only for biomass utilization but also for achieving the quality of treated water. This study applied and compared the use of alum and ferrate for removal of algae in the effluent of wastewater treatment. The effect of solution pH (5 - 9, adjusted by adding of 0.1 M NaOH or HCl solution), alum dosage (5 - 25 mg Al/L), and ferrate dosage (4 - 20 mg Fe/L) on the collection efficiency were investigated to find suitable conditions for algae removal. Results in Figure 5 showed that both pH and coagulant dosage had a strong effect on the algae collection efficiency. The optimum conditions for ferrate and alum were obtained at pH 8 and 12 mg Fe/L with efficiency of 84 - 97% for ferrate and at pH 7 and 20 mg Al/L with efficiency of 82 - 84% for alum. This indicates that ferrate is better than alum for algae collection in terms of coagulant dosage (less ferrate used and sludge produced) and pH (less acid used due to the high pH of treated wastewater).

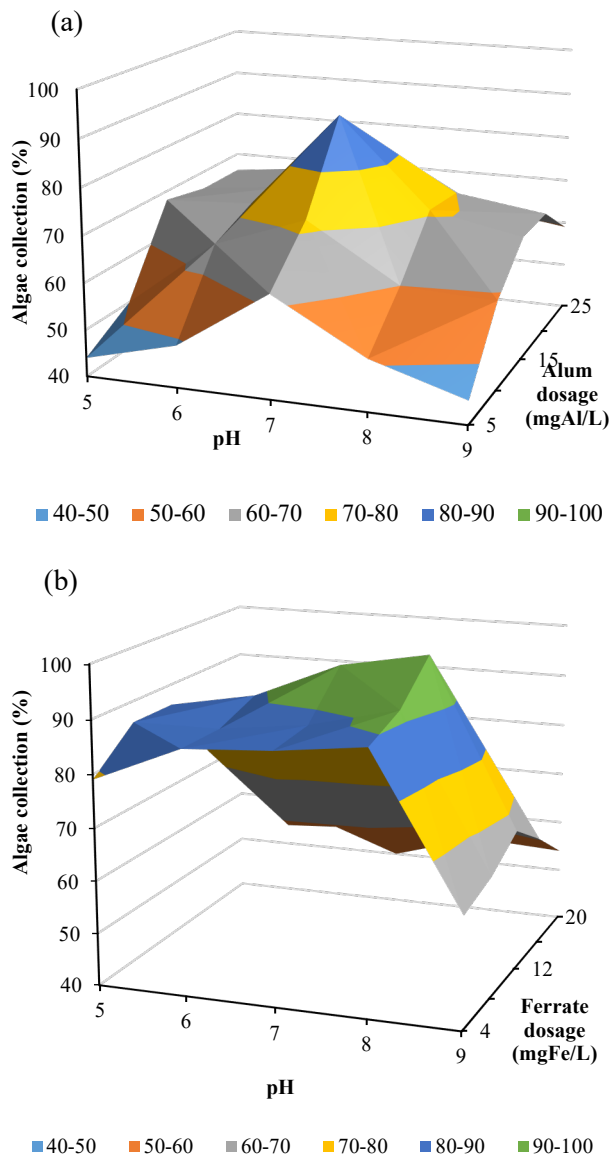


Figure 5. Effect of pH and coagulant dosage on the collection efficiency of algae using (a) alum and (b) ferrate

## 4. Conclusion

*Chlorella vulgaris* was successfully grown and applied for removal of nutrients in domestic wastewater. The treated wastewater met well Vietnamese standard of QCVN 14:2008/BTNMT column A for domestic wastewater discharge. The collection tests showed that ferrate was more efficient and suitable than alum for coagulation of algae after wastewater treatment due to its requirement of less amount use and higher pH as well as less sludge produced. These indicate that application and recovery of microalgae to remove nutrients could be a potential technology for advanced wastewater treatment in practical application.

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