# REMOVAL OF INORGANIC NUTRIENT AND ORGANIC CARBON FROM WASTEWATER OF BINH DIEN MARKET USING GREEN ALGA *CHLORELLA* SP

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**Abstract**

Traditional markets play a major role in socio-economics and constitutes a significant aspect of Vietnamese culture. However, wastewater streams discharged from the markets are generally characterized by a lot of inorganic nutrients and organic substances originated from fresh food processing units. They could lead to serious water contamination if discharged without proper treatment. This study applied microalgae *Chlorella* sp. for eliminating inorganic nutrients ($NO_3^-$-N, $NH_4^+$-N and $PO_4^{3-}$-P) and organic carbon (Chemical oxygen demand-COD) from wastewater of the Binh Dien market. The removal efficiencies reached for $NH_4^+$-N > 86%, for $NO_3^-$-N > 72%, and for $PO_4^{3-}$-P > 69%, respectively, at algal density of 49 x $10^4$ cell $mL^{-1}$, and for COD > 96% at algal density of 35 x $10^4$ cell $mL^{-1}$ after five cultivating days. The effluence satisfied the Vietnamese standard, column B, of the National technical regulation on industrial wastewater (QCVN 40:2011/BTNMT). The results demonstrated that the culture system composed of green algal *Chlorella* sp. could be a potential candidate for the removal of nutrients and organic carbon by a wastewater treatment process from the Binh Dien market.

**Key words:** *Chlorella* sp.; chemical oxygen demand; inorganic nutrient; Binh Dien market

## 1 INTRODUCTION

A Wastewater discharged from traditional markets has been considered as one of the major contributors to domestic pollution causing wide spread concerns [1, 2]. Pollutants in the wastewater streams, mostly containing nutrients and organic substances, usually come from the wet processing section (where meat, fish, poultry, fruits, and vegetable are handled and sold), food preparation, and public toilets [1]. If not properly treated, the pollutants can seriously harm the environment and human health. To solve this problem, a lot of techniques such as coagulation, electroflotation, filtration, etc. [3, 4] has been carried out prior to discharge or disposal. However, the processes are not mostly cost-effective, environmentally friendly, and produce large quantities of toxic sludge.

Recently, microalgae based biological methods have received great attention for the effective treatment of nutrients and organic pollutants. Microalgae has been successfully used to treat the slaughterhouse wastewater [5], dairy wastewater [6], municipal wastewater [7], textile wastewater [8], and even wastewater containing heavy metal [9]. However, there has been very few studies on the treatment of pollutants from traditional market wastewater by a microalgae method reported so far.

The objective of this study is to test the ability of *Chlorella* sp. to remove inorganic nutrients ($NO_3^-$-N, $NH_4^+$-N and $PO_4^{3-}$-P) and organic pollutants (chemical oxygen demand (COD)) from wastewater of the Binh Dien market.

## 2 MATERIALS AND METHODS

### 2.1 Culture medium

The green algal *Chlorella* sp. was supplied by the Institute of Microbiology & Biotechnology of the Vietnam National University, and cultivated at the Department of Environmental Science of the Sai Gon University. The algae were cultivated in 800 mL conical glass tubes in the lab. The wastewater effluent (10 L each) was collected from an equalization tank of the wastewater plant of the Binh Dien market (Ho Chi Minh City, Vietnam) at 10.00 a.m. and was used as a culture medium. The cultures were maintained at 28±2 ◦C for a 16 h/8 h light/dark period, and were bubbled with compressed air at flowrate of 400 mL/min (with 2% $CO_2$) within 10 days. The characterization of wastewater was monitored in triplicate with ±5% error and compared with the Vietnamese standard, column B, of the National technical regulation on industrial wastewater (QCVN 40) in average values as summarized in Table 1.

**Table 1: Characterization of traditional market wastewater**

| Parameters | Mean values | *QCVN 40 (Column B) |
|---|---|---|
| pH | 6.2 | 5.5 -9.0 |
| COD (mg $L^{-1}$) | 3200 | 150 |
| $NO_3^-$-N (mg $L^{-1}$) | 82.69 | 40 |
| $NH_4^+$-N (mg $L^{-1}$) | 22.74 | 10 |
| $PO_4^{3-}$-P (mg $L^{-1}$) | 17.8 | 6 |
| Salinity (‰) | 3.0 | - |

* Vietnam national standard requirement

A volume sample of *Chlorella* sp. suspended in BG-11 medium [10] was added to the wastewater effluent. The cell density of *Chlorella* sp. in the wastewater was controlled.

### 2.2 Experimental setup

The experiments were conducted in batch by using 5000 mL flasks containing 4000 mL of wastewater. At the beginning of each series of experiments, a volume of culture medium was inoculated to flasks with a suspension of pre-cultured cells. The initial *Chlorella* sp. concentration varied between 0 – 70 x$10^4$ cell $mL^{-1}$ [11, 12]. The flasks were aerated to provide $CO_2$ and for mixing via an air pump (flowrate of 400 mL/min with 2% $CO_2$) [13]. The experiments were conducted in triplicate at room temperature (28±2 ◦C) for 5 days.

### 2.3 Analytical methods

The liquid samples for the water analysis were collected every day at 11 a.m. during the experimental period. The collected samples were centrifuged at 5,000 rpm for 15 min and the supernatants were collected for the analyses of $NO_3^-$-N, $NH_4^+$-N and $PO_4^{3-}$-P following the Standard Methods for the Examination of Water and Wastewater [14]. The pH, DO, and Salinity values were measured with a portable multimeter (2100P Portable Turbidimeter, Hach, USA). The cell density of *Chlorella* sp. was determined by a direct microscopic count method using the Sedgewick Rafter chamber [15, 16]. All these measurements were done in triplicate and the mean value of the data is reported in this study.

## 3 RESULTS AND DISCUSSION

### 3.1 Growth study

The growth curve of *Chlorella* sp. in the growth stage is shown in Figure 1. The best cell density reached was 10.0 x$10^6$ cell $mL^{-1}$ during six cultivated days with the normal physical water parameters (pH: 7.5 – 10, DO: 6.5 – 8, and temperature: 28 – 310C). Further cultivated day, the cell yields dropped. This result was even higher than the previous findings where the maximum cell density of *Chlorella* sp. was around 6.5 x$10^6$ cell $mL^{-1}$ [11]. This high cell density of algae reveals that *Chlorella* sp. seems to be dominant in pure culture through the cultivated process.

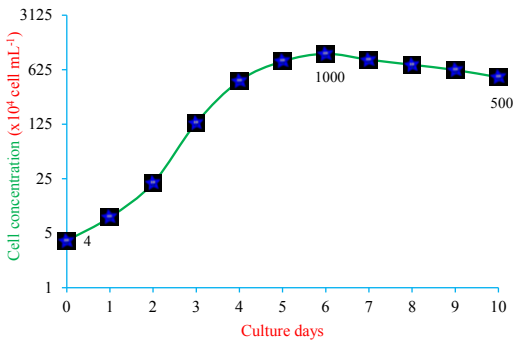


**Fig. 1 The growth curve of Chlorella sp. under aerobic culture condition for ten days**

### 3.2 Nutrient and chemical oxygen demand removal

The variation of pollutants from wastewater of the Binh Dien public market with respect to culture time at the different initial concentrations for 5 day operation is depicted in Figure 2. All effluent parameters achieved the Vietnam national standard requirements, column B (QCVN 40:2011/BTNMT).

As shown in Figure 2a, $NH_4^+$-N was completely removed (around 3 ±0.5 mg $L^{-1}$) from the media after 120 hours of cultivating. However, the $NH_4^+$-N removal efficiency was around 86 ±1.5% for the algal concentration of 49 x $10^4$ cell $mL^{-1}$, and further decreased to 83 ±1.1% when the $NH_4^+$-N algal concentration increased (70 x $10^4$ cell $mL^{-1}$). The figure also shows that no significant ammonia removal was observed in control experiments. These results could be explained so that the *Chlorella* sp. concentration was saturated in the wastewater and could not react efficiently when further increased. The observation is in agreement with the results reported by Wang et al. [7]. The trend is clearer for the removal of nitrate (Figure 2b) – the nitrate concentration significantly reduced when the wastewater had supplied *Chlorella* sp. (72±0.9%) as compared with the control experiments (28±1.1%) after 5 day cultivation. The higher the algal concentration is, the higher nitrate is removed and this is confirmed by the study of Jeanfils et al. [17] who stated that algae could utilize nitrate as a main growth nutrient.

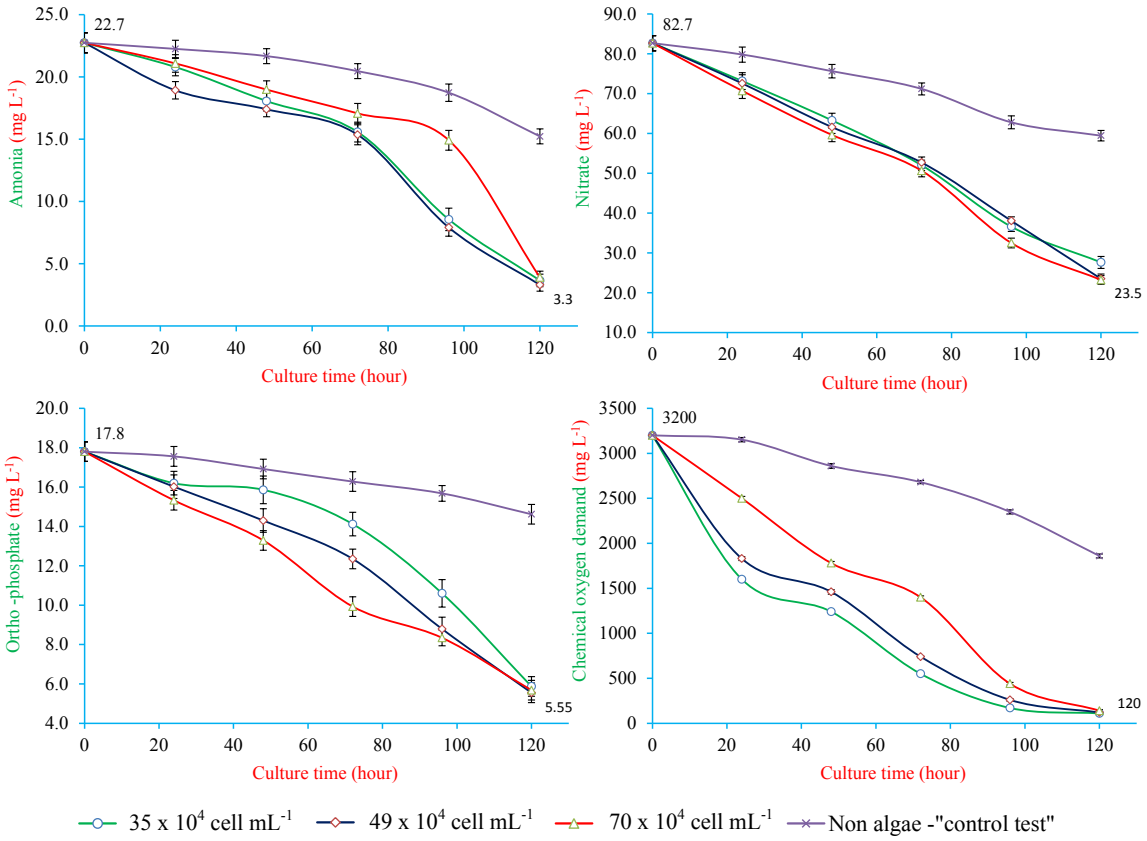


**Fig. 2 Variations of the a) ammonia/ammonium, b) nitrate, c) chemical oxygen demand, and d) ortho -** phosphate **concentrations throughout the growth of *Chlorella* sp. in the experimental treatments**

For the phosphorus removal process (Figure 2c), the final ortho-phosphorus concentration was around 5.6 ± 0.6 mg $L^{-1}$ with the removal efficiency of 69 ± 1.1% for the *Chlorella* sp. concentration of 49 x $10^4$ cell $mL^{-1}$. The other C*hlorella* sp. concentrations resulted in mostly less efficiencies than the mentioned concentration. The excess amount of *Chlorella* sp. could be one of the reasons for low removal efficiencies as increasing the algal concentration. The removal efficiency was higher than the findings by González et al. [18], where only 55 ± 1.0% phosphorus removal from agroindustrial wastewater by 216 h batch cultivation of C. *vulgaris* and *Scenedesmus dimorphus* is stated. These results indicate that *Chlorella* sp. is very effective in removing ortho-phosphorus.

The removal efficiency for organic pollutants (COD) is shown in Figure 2d. It can be ascertained that the percentage COD removal decreases with the increase in the *Chlorella* sp. concentration from 35 to 70 x $10^4$ cell $mL^{-1}$ for the initial COD concentration of 3200 ± 2.9 mg $L^{-1}$. The best removal efficiency of 97 ± 1.1% is reached at algal concentration of 35 x $10^4$ cell $mL^{-1}$. The results could be due to the fact that the organic chemical in the wastewater is a favorite medium for the growth of *Chlorella* sp. Besides, it has been found that the

*Chlorella* metabolic pathway can be altered supplying organic substances (COD) which allow it to adapt to heterotrophic growth rather autotrophic [19].

## 4 CONCLUSIONS

This study was conducted to assess the effect of *Chlorella* sp. on removing inorganic nutrients ($NO_3^-$-N, $NH_4^+$-N and $PO_4^{3-}$P) and organic carbon (Chemical oxygen demand-COD) from the wastewater collected from the Binh Dien market, Ho Chi Minh city, Vietnam. The experimental results indicated that *Chlorella* sp. successfully removed the pollutants after 5 days cultivation, the best removal efficiencies of inorganic nutrient were 86± 1.5%, 72 ± 0.9%, and 69 ± 1.1% at algal density of 49 x $10^4$ cell $mL^{-1}$ for $NH_4^+$-N, $NO_3^-$N, $PO_4^{3-}$P, respectively. Accordingly, the best removal efficiency of organic pollutants reached 97± 1.1% at algal density of 35 x $10^4$ cell $mL^{-1}$. These results indicated the potential of using *Chlorella* sp. as a "green" method to remediate wastewater containing nutrient or organic pollutants.

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