



NUTRIENT RECOVERY AND POLLUTANT REMOVAL FROM PIGGERY WASTEWATER BY *SPIRULINA* CULTIVATION

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Abstract. *Spirulina* is noticed for great applications on food supplements, animal feeds, pharmaceutical, cosmetics, biofuel, fertilizer, etc. *Spirulina* cultures in wastewaters could enhance the feasibility of commodities due to its ability to reduce the cost of biomass production and remove pollutants in wastewaters. This study investigates the effects of wastewater pretreatment using various aeration periods (i.e. from 3 to 7 days), the supplement of bicarbonate and N:P ratios on the growth of *Spirulina* sp. to produce protein-rich biomass. The work showed that *Spirulina* sp. had the ability to effectively remove ammonium, with the highest efficiencies up to 99.8 %. However, the high concentration of ammonium in wastewater, from 125 mgNH₄⁺-N/L upward, caused the decline in the growth rate of *Spirulina*. The growth and remediation potential of *Spirulina* sp. were in the best condition with the N:P ratio of the medium in the range of 19-22. This study suggested a procedure to cultivate *Spirulina* sp. in piggery wastewater and remove pollutants efficiently.

Keywords: *Spirulina*, wastewater treatment, nitrogen recovery, N:P ratio.

Classification numbers: 3.1.

1. INTRODUCTION

Spirulina has always obtained an interest in functional foods, pharmaceuticals, animal feeds, bioplastic, biofuels, etc. [1]. However, besides products intended for human consumption which need to be cultivated under strict hygiene and health safety, the remaining products may be cultured in suitable types of wastewater. Therefore, research on the cultivation of *Spirulina* in wastewater to utilize nitrogen (N), phosphorus (P) and beneficial minerals while reducing the cost of cultivation has been carried out for a long time and is still being developed [2]. In Viet Nam, the use of nutrients in wastewater for algae cultivation, concomitantly collecting biomass for useful products has been interested recently. Wastewater from pig farms is one of very common types of waste stream in Viet Nam. They were usually treated in biogas or anaerobic digester, which significantly reduced SS and BOD₅ contents. However, the effluent still contained high nitrogen and phosphorus concentration, which did not meet the regulatory

discharged standard. To eco-friendly remove N and P, microalgae cultivation could be the best solution since N and P are supplements for microalgae development. N and P play a major role in cell metabolism. More specifically, N is required for the synthesis of proteins and nucleic acids, as well as photosynthetic pigments such as chlorophyll-a and phycobiliprotein [3]. Phosphorus is an important constituent of nucleic acids (RNA and DNA), membrane phospholipids, ATP and P-rich ribosomes [3]. The Redfield C:N:P ratio of 106:16:1 is often observed in natural and exponentially growing phytoplankton populations [4]. The growing in wastewater of many microalgae species, mostly green ones, e.g. *Chlorella*, *Scenedesmus*, were evaluated based on four following criteria: (i) fast growth rate; (ii) high nutrient removal rate; (iii) strong adaptability to different type of wastewater and local climate; and (iv) high biomass productivity [2, 5]. However, there were less investigations in the effect of N:P ratios to the *Spirulina* performance and its nutrient recovery. The objective of the current study was to determine nutritional conditions to propose a procedure for optimal *Spirulina* sp. cultivation and effective removal of pollutants in wastewater.

2. MATERIAL AND METHODS

2.1. Species and culture maintenance

Spirulina sp. HH, a native species which was isolated from Ba Mau lake, was cultured in Zarrouk's medium in 250 ml Erlenmeyer flask, at room temperature, continuous shaking, 90 rpm and illumination with the fluorescent intensity of 2000 lux.

2.2. Wastewater

The piggery wastewater from Thanh Hung pig farm in Thanh Oai district, Ha Noi, was taken from the effluent of the anaerobic pond (with HDPE cover) and then filtered through several layers of cloths to separate sludge. Then, it was stored in 20-liter containers and kept in the refrigerator for following experiments.

2.3. Experiments

2.3.1. Determination of optimal doses of bicarbonate for culture supplement

Adding 3, 5 and 7 g/L sodium bicarbonate to 3-fold diluted of piggery wastewater and then culturing *Spirulina*. The control medium was the same diluted wastewater, but no bicarbonate supplement. Cell densities of *Spirulina* were measured daily, and then the specific growth rates were calculated (Eq.1). The dry biomass at day 7 of each supplement level was examined daily to know the productivity (Eq.2).

2.3.2. Effect of aeration duration to pretreat piggery wastewater prior *Spirulina* cultivation

Piggery wastewater was aerated in three durations, i.e. for 3 (CN-3), 5 (CN-5) and 7 days (CN-7). After aeration, all trials were diluted with distilled water to reduce the NH_4^+ concentration to $100 \pm 10 \text{ mgNH}_4^+\text{-N/L}$. Then it was added with an optimal dose of NaHCO_3 , which was mentioned in section 2.3.1. The initial inoculum of *Spirulina* was 0.4 - 0.5 in optical density. Cell density, pH of culture media and concentrations of COD, NH_4^+ , PO_4^{3-} were monitored daily, until the stationary phase of *Spirulina*.

2.3.3. Effect of NH_4^+ concentration in the input medium

The optimal pretreated wastewater, received after section 2.3.2 was diluted to get various NH_4^+ concentrations, i.e. 30, 50, 75, 100, 125 and 160 $\text{mgNH}_4^+\text{-N/L}$. Then, the diluted piggery wastewater samples were used as media for *Spirulina* cultivation. Cell density and NH_4^+ concentration were examined daily.

2.3.4. Effect of N:P ratios of wastewater on the *Spirulina* cultivation

The best concentration of ammonium found above was added with various volumes of K_2HPO_4 500 gP/L in order to prepare different medium of *Spirulina*, which the ratios of N:P were 15:1, 19:1, 22:1 and 25:1. Then four types of media were supplied with 5 g/l bicarbonate. All trials were conducted in 1500-mL transparent plastic bottles with 800 ml of working solutions. They were aerated continuously with air bubbles and irradiated with fluorescence light intensity ~ 2000 lux.

2.4. Measurement and analytical methods

2.4.1. Algae biomass

The cell density of *Spirulina* was determined via the optical density of algae solution at the wavelength of 680 nm. The biomass concentration (X, g/L) was the dry biomass in filter papers, which was determined by filtering a volume of algae solution and then dried at $70\text{-}80$ °C until unchanged weight. The growth rate (μ) of *Spirulina* and biomass productivity were determined as follows:

$$\mu(\text{day}^{-1}) = \frac{\ln X_t - \ln X_0}{t - t_0} \quad (1)$$

$$P(\text{g/l.d}) = \frac{X_t - X_0}{t - t_0} \quad (2)$$

where X_0 and X_t are the biomass concentrations at the beginning and the end of the log phase. t_0 and t are the time from beginning and the end of the log phase; P is overall biomass productivity.

2.4.2. COD, ammonium, phosphate

The filtrate of 20 - 35 ml sample was analyzed following parameters COD, NH_4^+ , PO_4^{3-} , according to standard methods for water and wastewater analysis [6]. pH was determined by pH meter.

3. RESULTS AND DISCUSSION

3.1. Determination of optimal dose of bicarbonate for *Spirulina* culture supplement

In order to reduce the strength of pollutants in piggery wastewater to adapt *Spirulina* growth, the original wastewater CN was diluted to gain ammonium concentration below 100 $\text{mgNH}_4^+\text{-N/L}$. The diluted CN had pH around 7.6 - 8 and poor amount of inorganic carbon which were unsuitable for the growth of *Spirulina*. Therefore, different doses of bicarbonate (i.e. 3, 5, 7 g/L) were added into the diluted wastewater. The supplement of bicarbonate resulted to change growth rate and biomass productivity of *Spirulina* noticeably (see Table 1). Observing

the culture in no-bicarbonate medium found that the color of *Spirulina* culture was yellowish-green for 4-5 days and then turned to collapse quickly, earlier than other media. The growth rate of *Spirulina* sp. HH in diluted, no-bicarbonate medium was in well range, compared with various literature reports, for example the one in the Depraetere's study (i.e. 0.1 vs. 0.15 d⁻¹) [7]. In our previous study, same kind of piggery wastewater was successfully cultivated *Chlorella* sp., but unneeded bicarbonate [5]. In contrast, *Spirulina* sp. HH needs more carbon supplement from bicarbonate or carbon dioxide into wastewater. In this experiment, the best growths were in both 5 and 7 g/L bicarbonate adding, as shown in Table 1. For saving chemical, the supplement of 5 g/L bicarbonate was the best dose and would be applied to the next trials.

Table 1. Growth rate and biomass productivity of *Spirulina* after 7 days culturing in the diluted wastewater with different supplements of NaHCO₃.

Types of medium	NaHCO ₃ supplement (g/L)	Biomass productivity (g.L ⁻¹ .day ⁻¹)	Specific growth rate (day ⁻¹)
Diluted wastewater to [NH ₄ ⁺] ≤ 100 mgNH ₄ ⁺ -N/L (~ 4 folds), no aeration before culturing <i>Spirulina</i> sp. HH	0	0.04	0.1
	3	0.06	0.18
	5	0.18	0.24
	7	0.17	0.23

3.2. Effect of various aeration periods on wastewater as media for *Spirulina* growth

The effluent of the piggery wastewater remained a high concentration of pollutants, especially stinky smell, high content of ammonium and phosphorus despite the digest in an anaerobic treatment system. These pollutants might cause harm to *Spirulina* development, especially at a high level of ammonium [8, 9]. In this study, we propose that further aerobic treatment of the effluent of anaerobic pond would remove more volatile compounds, and therefore it might reduce the need for dilution water prior to culturing *Spirulina*. For that reason, the samples of piggery wastewater were aerated for 3, 5 and 7 days (namely CN-3, CN-5, CN-7), then diluted two folds to CN-5 and CN-7 samples, three folds to CN-3 to reduce ammonium concentration below 100 mgNH₄⁺-N/L prior to culturing *Spirulina*. The study aimed to find a pretreatment procedure of piggery wastewater as a medium for *Spirulina* cultivation.

According to Tables 1 and 2, aerated wastewater provided better growth for *Spirulina* sp.HH than the no-aeration one. The growth rates and the biomass productivity increased significantly, i.e. 0.24 d⁻¹ vs. 0.33 d⁻¹ and 0.18 vs 0.51 g.L⁻¹.day⁻¹ respectively. When the wastewater was bubbled for 3 days, harmful substances such as stinky odor and ammonium remained in higher content than the longer duration of aeration (smelling test at experimental bottles, data not shown). The results in Table 2 showed that *Spirulina* CN-3 grew slower than the one in CN-5 but slightly faster than CN-7. Among them, the medium CN-5 (i.e. 5 days aeration) supported the fastest cell growth and the best biomass productivity of *Spirulina*. Therefore, the aeration of biogas wastewater for 5 day was a necessary step to take out unwanted volatile constituents. Briefly, the pre-treatment of 5-day aeration was the best solution of pretreatment prior to cultivating *Spirulina* in piggery wastewater.

Table 2. Growth rate and biomass productivity of *Spirulina* HH after 7 days in different-aeration medium.

Types of medium	Name	Aeration (day)	Biomass productivity (g.L ⁻¹ .d ⁻¹)	Specific growth rate (d ⁻¹)
Piggery wastewater wo. aeration	CN-0	0	0.04	0.1
Piggery wastewater with aeration	CN-3	3	0.4	0.27
	CN-5	5	0.51	0.33
	CN-7	7	0.35	0.25

Concomitantly with the process of biomass synthesis, COD, NH₄⁺ and PO₄³⁻ in the medium were removed in different manners. The best yield was found for NH₄⁺ removal when the efficiencies reached 99.8 % after 7 days cultivation (Fig. 1). pH of medium raised up and maintained above 9 since day 3 of cultivation, that transformed NH₄⁺ to NH₃ and precipitated phosphate partially [1]. COD in all solutions were almost similar, that means it was removed mainly by aerobic digestion. Fig 1 showed that the efficiencies of PO₄³⁻ removal in solutions with *Spirulina* were lower than the one without. The results agree with the fact that bacterial consumed more phosphate for the same amount of biomass synthesis than algae, because the optimal N:P ratios of bacteria and microalgae are 5:1 vs. 20:1, respectively [2].

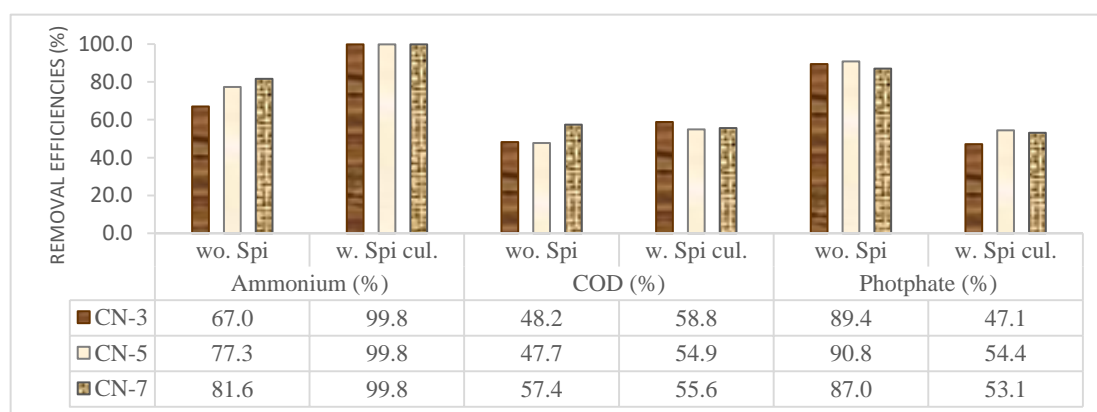


Figure 1. Removal efficiencies of COD, NH₄⁺, PO₄³⁻ after 7 days, with and without culturing *Spirulina* sp. HH.

3.3. Effect of initial NH₄⁺ concentration in the input mediums

The wastewater after 5-day aeration (CN-5) was adjusted with distilled water to reach the concentration of NH₄⁺ from 30 - 160 mgNH₄⁺-N/L. *Spirulina* inoculum was added in the same amount of cell density of 0.5 g/L and maintained all cultures at the same room temperature, continuous air blowing. Figures 2a and 2b show that the input ammonium concentration of 75-100 mgNH₄⁺-N/L was the optimal ammonium levels for microalgae to develop robustly and obtained the highest biomass concentration. NH₄⁺ concentration from 125 mgNH₄⁺-N/L upward shows the long lag phase and low cell optical density at stationary phase (Fig. 2a). Along with the algal growth, NH₄⁺ concentration was reduced almost completely in the first 2-4 days with input NH₄⁺ from 100 mgNH₄⁺-N/L downward. When the input concentration of NH₄⁺ was increased (i.e. from 125 mgNH₄⁺-N/L upward), it inhibited *Spirulina* HH growth and caused to decreased of biomass productivity as well removal efficiencies of pollutants. The performances

in this study agreed with Nimptsch and Pflugmacher's report, that high ammonium concentration caused to stress the cell membrane, increase intracellular oxidative stress, causing the degradation of pigments [9].

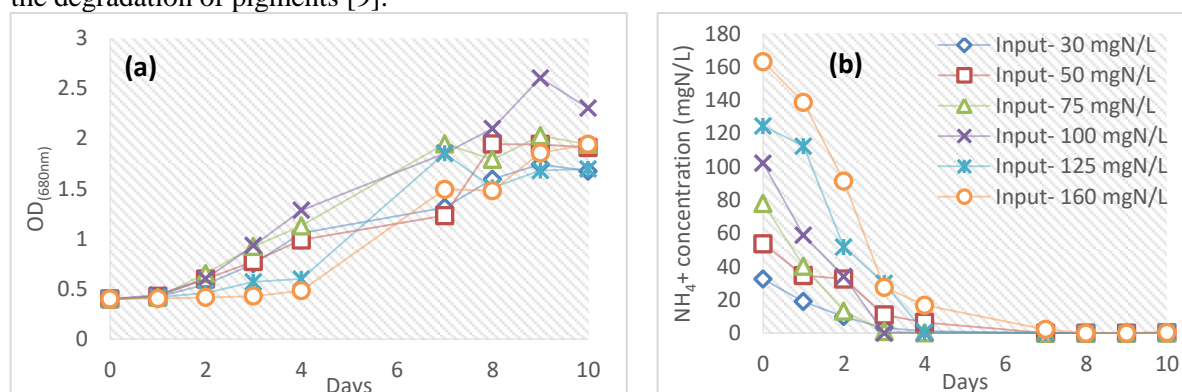


Figure 2. Growth curve of *Spirulina* (a) and variability of NH_4^+ concentration daily (b) in mediums with various input NH_4^+ levels.

3.4. Effect of N:P ratios of wastewater on the cultivation of *Spirulina*

In this experiment, the N:P ratio of the pretreated CN-5 was 39:1. After diluting 3 folds, the CN-5 was added with various volumes of stock solution of KH_2PO_4 500 mgP/L to make several media, that varied ratios of N:P, e.g. 15:1, 19:1, 22:1, 25:1. As shown in Table 3, the *Spirulina* adapted well in the medium with N:P ratio from 19:1 to 22:1. Especially, the biomass harvested from culture with N:P = 22 possessed the highest protein. The results are same as Xin's findings, which conducted on aeration systems with artificial sewage [10]. This shows that the N: P ratio has a strong impact on the growth of algae. Additionally, the threshold of initial NH_4^+ concentration in wastewater also has an important impact on the culture process.

Concomitantly the algae growth, the consumption of nutrients took place to remove pollutants in wastewater. Their efficiencies were displayed in Table 3. The efficiencies of COD removal were rather low, just about 17.6 - 28.6 % because *Spirulina* could not uptake organic carbon in wastewater. Ammonia was removed almost completely due to *Spirulina* consumption and volatilization off high-pH cultures (pH~10-11).

Table 3. Characteristics of *Spirulina* cultures in various N:P ratios and removal efficiencies of pollutants in medium (CN-5 diluted wastewater).

N/P ratio	Biomass productivity ($\text{g}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$)	Specific growth rate (d^{-1})	Total Protein content (% of DW)	Removal efficiencies of pollutants after algal cultivation (%)		
				COD	NH_4^+	PO_4^{3-}
15:1	0,24	0.2	64.7	18.2	99.7	55.5
19:1	0.45	0.27	68.9	17.6	99.9	48.3
22:1	0.5	0.28	70.4	23.5	99.8	46.5
25:1	0.35	0.24	61.8	28.6	99.7	40.4

Based on the outcomes, a procedure of culturing *Spirulina* sp. HH in piggery wastewater is suggested as follows:

- Step 1: pretreat wastewater, collected piggery wastewater after anaerobic pond was filtered and then aerated for 5 days.
- Step 2: dilute wastewater, adjust the concentration of NH_4^+ from 100 $\text{mgNH}_4^+\text{-N/L}$ downward.
- Step 3: based on the nitrogen concentration of piggery wastewater, calculated a volume of phosphate solution needed to have the ratio of N:P in the range of 19:1 to 22:1. The phosphate sources could be chemicals or from other kinds of rich-phosphate wastewater.
- Step 4: add 5 g/L of sodium bicarbonate into the above medium. Then, continuously provide air-bubbles to *Spirulina* cultures and illuminated with natural sunlight or artificial light.

4. CONCLUSION

Spirulina sp. HH could be cultured in 5-day aerated piggery wastewater and grown better in diluted piggery added 5 g/L bicarbonate and the N:P ratios from 19 to 22. The input level of ammonium in wastewater critically affected to *Spirulina* sp. HH growth and must not be greater than 100 $\text{mgNH}_4^+\text{-N/L}$ for algal survival. The best biomass yield was 0.51 $\text{g. L}^{-1} \cdot \text{d}^{-1}$ and the removal efficiency of ammonium and phosphate up to 99.9 % and 48.3 %, respectively. The study suggests a procedure to successfully cultivate *Spirulina* in digested piggery wastewater. Studying *Spirulina* biomass properties and its application in fertilizer and bioplastic will be conducted in next stage of the project for sustainable production of *Spirulina* biomass from piggery wastewater.

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