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Cultivation of *Spirulina platensis* using raw piggery wastewater for nutrients bioremediation and biomass production: effect of ferrous sulfate supplementation

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ABSTRACT

In the present study, an integrated process of raw piggery wastewater treatment and *Spirulina platensis* biomass production was proposed and effect of ferrous sulfate supplementation on *S. platensis* growth and biochemical composition was primarily investigated. Results showed that supplementation of 0.05 g L^{-1} ferrous sulfate in the raw piggery wastewater could increase maximum biomass and chlorophylls production to 98.67 and 0.156 mg L^{-1} d^{-1}, respectively, compared to 74.22 and 0.134 mg L^{-1} d^{-1}, respectively, of the control. Moreover, protein, total carbohydrate, total lipids and iron content of the harvested biomass ranged from 45.31%–55.15%, 8.53%–12.32%, 10.22%–11.49% and 1.31–2.03 mg g^{-1}, respectively, indicating that ferrous sulfate supplementation is an effective approach to enhance *S. platensis* biomass productivity and increase iron content in *S. platensis* biomass, which facilitates to lower *S. platensis* biomass production cost and upgrade biomass value.

Keywords: Raw piggery wastewater; *Spirulina platensis*; Nutrients removal; Ferrous sulfate supplementation

1. Introduction

Over the last decades, the livestock and poultry sectors have been undergoing a scaling-up transformation from a traditional household production mainly for self-consumption or local-market distribution to intensive industrial production in China [1]. These changes helped the livestock and poultry production industry to be more productive and economically efficient. Whereas, concentrated livestock and poultry production mode are more inclined to generate an excessive amount of organic solid wastes, wastewater containing a high concentration of nutrients and greenhouse gases which exceed the local environmental capacity. Therefore, improper management of various wastes from intensive livestock and poultry farms can easily lead to water, soil and air pollution and subsequently hurt ecological balance [2,3]. Currently, livestock and poultry farm wastes and wastewater are generally treated through anaerobic digestion followed by conventional aerobic activated sludge decomposition. The conventional livestock wastes and wastewater treatment processes can achieve high efficiency in chemical oxygen demand (COD) removal but are inefficient in nitrogen and phosphorus removal. Excessive quantities of nitrogen and phosphorus discharged from livestock and poultry farms can lead to eutrophication of natural water body [4].

On the other hand, nitrogen and phosphorus are essential elements for microalgae growth. Thus, there is great potential to cultivate microalgae using wastewater from livestock and poultry farms as a nutrient source. Moreover, the demand for meat is soaring as the world population grows rapidly, which promotes the rapid development of livestock and the poultry industry. In this context, new sources of protein are necessary to be explored to relieve the strong dependence

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on food crop derived protein for animal feed additive. Microalgae, which is free of competition with human beings in freshwater and arable land, has great potential to be an alternative source of protein to conventional feedstocks. Furthermore, microalgae have advantages of faster growth rate, higher protein productivity over field crops, e.g. soybean [5]. More importantly, many microalgae can grow in wastewater, which can save huge quantities of freshwater and nutrients [6]. Among all kinds of microalgae species, *Spirulina platensis*, multicellular and filamentous cyanobacteria, has been receiving great concern from researchers in the past decades because of its high protein content (60%~70% of dry weight) [7]. Therefore, *S. platensis* biomass has been recognized as a promising protein source for both humankind and animals [8]. The cost-effective production of *S. platensis*-based protein, however, still faces many challenges from the upstream and downstream process. To date, many studies have focused on cultivating *S. platensis* using livestock farm wastewater to simultaneously obtain value-added *S. platensis* biomass and purified wastewater [9–13].

Also, the microalgal growth and chemical compositions are highly dependent on trace elements [14]. Among which, zinc (Zn) and iron (Fe) are not only important micro-elements in the process of photosynthesis functioning and respiration of *S. platensis*, but also are necessary nutritious for animal growth. Zhou et al. [15] studied the effect of various initial Zn^{2+} concentration on *S. platensis* growth and chemical composition of the obtained biomass. Nevertheless, to the best of our knowledge, the influence of ferrous on the growth and chemical composition of *S. platensis* is still unclear.

Therefore, the objectives of this study were to: (1) determine the effect of ferrous sulfate on the *S. platensis* growth in raw piggery wastewater, (2) evaluate the nutrients removal ability of *S. platensis* cultivated in raw piggery wastewater with different loading of ferrous sulfate, and (3) identify the influence of ferrous sulfate supplementation on the chemical composition of *S. platensis* biomass.

2. Materials and methods

2.1. Materials

The *S. platensis* strain was obtained from Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences (Guangzhou, China). The raw piggery wastewater was collected from the outlet of a floor flushing effluent drainage ditch in a local pig farm near the campus of Shaoguan University (Shaoguan, China). The obtained raw piggery wastewater was filtered through gauze and gravity sedimented overnight followed by vacuum filtration to remove suspended solids before use. The physicochemical compositions of the raw piggery wastewater were analyzed and are presented in Table 1.

2.2. Cultivation of S. platensis

The *S. platensis* was firstly subjected to pre-culture in Erlenmeyer flasks in the laboratory without temperature control (room temperature ranged 26°C–35°C during the experiment). Light energy was provided by two 16 W fluorescent lights (Foshan Lighting Co. Ltd., Foshan, China) fixed 20 cm above the top of Erlenmeyer flasks. The illumination intensity and time were set to be 3,000 ± 100 lux and 24 h d^{-1}, respectively.) The preculture of *S. platensis* was carried out using standard Zarrouk's medium as nutritious source [16], which consisted of 16.8 g $NaHCO_3$, 0.5 g K_2HPO_4, 1.25 g $NaNO_3$, 1.00 g K_2SO_4, 1.00 g NaCl, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.04 g $CaCl_2{\cdot}2H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.08 g Na_2–EDTA (ethylenediaminetetraacetic acid disodium salt dihydrate) and 1 mL A_5 trace metal solution per liter. The A5 trace metal solution contained 2.86 g H_3BO_3, 1.81 g $MnCl_2{\cdot}4H_2O$, 0.222 g $ZnSO_4{\cdot}7H_2O$, 0.39 g Na_2MoO_4, 0.079 g $CuSO_4{\cdot}5H_2O$, 0.049 g $Co(NO_3)_2{\cdot}6H_2O$ per L of deionized water. Air was provided through the bottom of each Erlenmeyer flask for culture mixing. Subsequently, *S. platensis* at logarithm growth phase was harvested and inoculated to six one-L (working volume) Erlenmeyer flasks with each containing 1 L raw piggery wastewater 50% diluted by distilling water (to reduce the turbidity of wastewater based on pre-tests) to achieve an initial cell density of 0.30 g L^{-1}. For ferrous strengthening treatments, 0.05 g and 0.10 g ferrous sulfates were supplemented to the wastewaters, which was approximately 5 and 10 times of iron concentration in Zarrouk's medium [16] and was designated as $0.05FeSO_4$ and $0.10FeSO_4$, respectively. Two controls without *S. platensis* inoculation or ferrous sulfate addition were prepared for comparison (designated as 0inoculate and $0FeSO_4$, respectively). All treatments were incubated at ambient temperature (26°C–35°C), and illuminated with two 16 W white fluorescent tubes (Foshan Lighting Co. Ltd., Foshan, China) fixed at 20 cm above the flasks and keep them working all day round with light intensity of 3,000 ± 100 lux on the surface of the flasks. Meanwhile, air with 3%–5% CO_2 (v/v) was sparged through the bottom of each Erlenmeyer flask to be used as an additional carbon source and for culture mixing.

Table 1
Physico-chemical properties of raw piggery wastewater

Parameters	Value
pH	8.60 ± 0.03
Suspended solid (mg L^{-1})	2.87 ± 0.14
TN (mg L^{-1})	252.95 ± 11.90
Ammonium (mg L^{-1})	77.83 ± 1.86
TP (mg L^{-1})	74.00 ± 2.31
COD (mg L^{-1})	1421.28 ± 2.16

2.3. Determination of S. platensis growth

Biomass concentration is an important indicator to characterize microalgae growth. In this work, 5 mL culture was sampled from each flask and filtered through a dried and pre-weighed Whatman filter paper (w_1, g) every 2 d. The biomass obtained was washed twice with distilled water, dried at 105°C until constant weight (w_2, g) was achieved. The biomass concentration was calculated by Eq. (1):

$$\text{Biomass concentration}\left(\text{g L}^{-1}\right) = \left(w_1 - w_2\right) \times \frac{1{,}000}{5} \quad (1)$$

The biomass productivity and specific growth rate during the culture period were obtained by Eqs. (2) and (3), respectively:

$$\text{Biomass productivity}\left(\text{mg L}^{-1}\text{ day}^{-1}\right)=\frac{\left(X_t-X_0\right)}{\left(t-t_0\right)} \tag{2}$$

$$\text{Specific growth rate}\left(\text{day}^{-1}\right)=\ln\frac{\left(\frac{X_t}{X_0}\right)}{\left(t-t_0\right)} \tag{3}$$

where X_t and X_0 are biomass concentration at time t and t_0 (at the beginning of cultivation), respectively.

After 15 d cultivation, biomass was harvested by gravity sedimentation (lasted for 5 h) followed by centrifugation (5,000 rpm, 10 min). Biomass pellets were freeze-dried at –80°C for chemical composition analysis.

2.4. Chemical composition analysis

The pigments including chlorophyll-a (C_a), chlorophyll-b (C_b) in the biomass were extracted by 2 mL of 80% acetone aqueous solution and measured at wavelengths of 663, 646 and 470 nm, respectively. The pigments concentration (C_t) was calculated by Eqs. (4)–(6) [17].

$$C_a=12.21A_{663}-2.81A_{646} \tag{4}$$

$$C_b=20.13A_{646}-5.03A_{663} \tag{5}$$

$$C_t=\frac{\left(1{,}000A_{470}-3.27C_a-104C_b\right)}{198} \tag{6}$$

Protein and total carbohydrate content, as well as fatty acid profiles of *S. platensis* biomass, were analyzed as described in our previous work [18]. Total lipid was determined by the Bligh and Dyer method [19]. Ash content was determined gravimetrically. Briefly, dried algae biomass (m_{biomass}, g) were introduced into pre-weighed crucibles and incinerated in a muffle furnace (Shanghai Shanzhi instrument and equipment Co. Ltd., Shanghai, China) at 550°C for 5 h. The iron contents in the biomass were determined according to the method reported by Saywell and Cunningham [20] with minor modifications. Specifically, the ashed samples obtained above were solubilized by 2 M HCl followed by filtration (pore size 0.45 μm, Tianjin Jinteng Laboratory Equipment Co., Ltd., China). The obtained filtrate was diluted to 25 mL by deionized water. O-phenanthroline (analytical grade, Guangzhou Chemical Reagent Factory, Guangzhou, China) and Hexahydrate ammonium ferrous sulfate (Xilong Chemical Co. Ltd., China) solution were used as a chromogenic agent and standard solution, respectively. The iron concentration (C_{Fe}, μg mL^{-1}) was determined by spectrophotometer (722S, Shanghai Lengguang Technology Co. Ltd., Shanghai, China). The iron content in the biomass ($\text{IC}_{\text{biomass}}$) was calculated by Eq. (7):

$$\text{IC}_{\text{biomass}}\left(\text{mg g}^{-1}\right)=\frac{C_{\text{Fe}}\times25\times10^{-3}}{m_{\text{biomass}}} \tag{7}$$

2.5. Wastewater quality determination

Approximately 10 mL culture was taken out from each flask every 2 d during the cultivation process for pH, COD, ammonium, total nitrogen (TN) and total phosphorus (TP) concentration analysis according to the experimental design after deionized water was added to the culture medium to compensate for water loss via evaporation. The samples were first centrifuged at 5,000 rpm for 5 min. Then, the supernatants were filtered using 0.22 μm nylon membrane filters (Tianjin Jinteng Laboratory Equipment Co., Ltd., China). Finally, the filtrates were appropriately diluted for COD, ammonium, TN and TP concentration measurement according to the Chinese National Standard methods [21]. The removal percentage (RP) and removal rate (RR) were calculated by Eqs. (8) and (9), respectively:

$$\text{RP}\left(\%\right)=\frac{\left(C_0-C_t\right)}{C_0} \tag{8}$$

$$\text{RR}\left(\text{mg L}^{-1}\text{ day}^{-1}\right)=\frac{\left(C_0-C_t\right)}{\left(t-t_0\right)} \tag{9}$$

where C_t and C_0 are the concentration at time t and the beginning of cultivation, respectively.

2.6. Statistics analysis

All the experiments were conducted in duplicates and average values with standard deviations were reported. One-way ANOVA using SPSS software (version 11.0) with $P < 0.05$ was carried out for data validation.

3. Results and discussion

3.1. Microalgae growth and biomass productivity

The variation of biomass concentration during the 15 d cultivation is depicted in Fig. 1. It was observed that no lag phase occurred during cultivation, demonstrating that *S. platensis* could be rapidly adapt to the raw piggery wastewater in all treatments. A significant increase in biomass concentration in all treatments was observed after 3 d incubations. At the end of cultivation, the maximum biomass concentration reached 1.28, 1.65 and 1.12 g L^{-1} for 0$FeSO_4$, 0.05$FeSO_4$ and 0.10$FeSO_4$, respectively, suggesting that moderate ferrous sulfate supplement could facilitate biomass growth. Excessive ferrous sulfate, however, had a negative impact on *S. platensis* growth, which could be attributed to the limitation effect on the photosynthesis rate of *S. platensis* [22]. It was shown in Fig. 1 that no decay phase was observed since the experiment lasted for only 15 d. Compared to maximum biomass concentration, biomass productivity and specific growth rate are more important indicators with practical significance to evaluate the scale-up potential of microalgae biomass production. Thus, the biomass productivity and specific growth rate of every treatment were calculated and listed in Table 2. According to Table 2, the biomass productivity and specific growth rate of *S. platensis* in 0.05$FeSO_4$ and 0.10$FeSO_4$ were 98.67 mg L^{-1} d^{-1}, 0.1348 d^{-1}

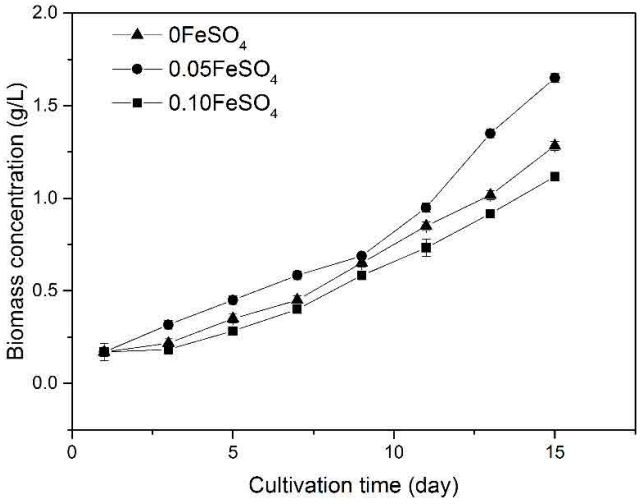

Fig. 1. Biomass concentration during incubation.

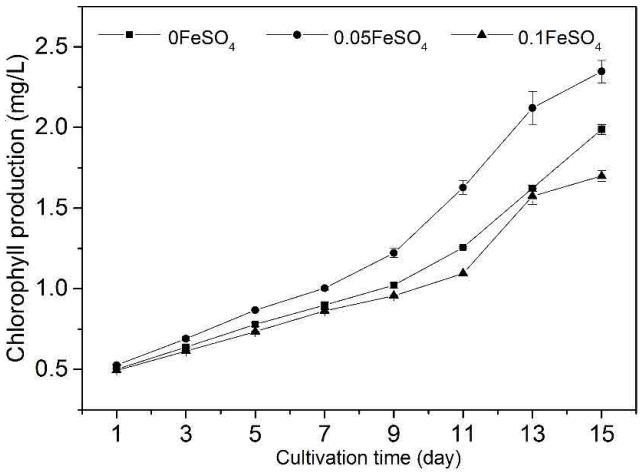

Fig. 2. Variations chlorophyll content in the biomass during incubation.

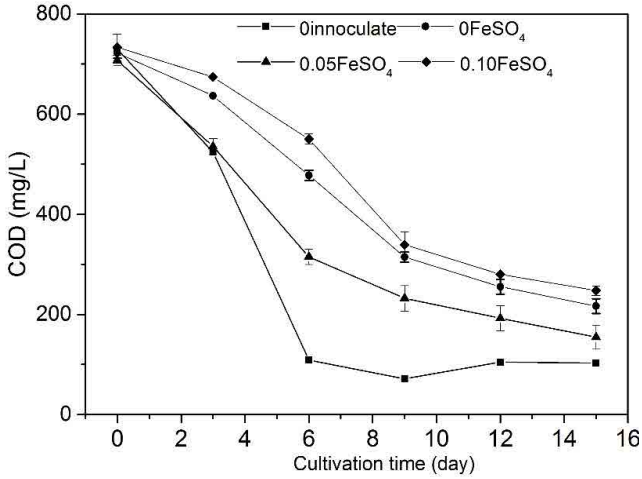

Fig. 3. Variation of COD during incubation.

and 63.11 mg L^{-1} d^{-1}, 0.1515 d^{-1}, respectively, compared to 74.22 mg L^{-1} d^{-1} and 0.1255 d^{-1} of $0FeSO_4$, respectively. In addition, it is clearly illustrated in Fig. 2 and Table 2 that chlorophylls productivity slightly increased with 0.05 g L^{-1} $FeSO_4$ supplementation in the raw piggery wastewater, which could be explained by the increased synthesis rate of b-aminolevulinic acid, and b-aminolevulinic acid, which facilitate chlorophylls accumulation in microalgae grown in substrates with more iron available [23]. Nevertheless, the inhibition effect emerged in the treatment of $0.10FeSO_4$. Therefore, appropriate ferrous sulfate loading should be carefully selected to achieve higher *S. platensis* biomass productivity and chlorophyll production.

3.2. Nutrients removal

Overloaded discharge of nutrients (nitrogen and phosphorus) can easily lead to eutrophication of natural water body [24]. On the other hand, carbon, nitrogen and phosphorous are valuable nutrients for microalgae growth. Raw piggery wastewater contains a high concentration of carbon, nitrogen and phosphorous, which has great potential to be used as an alternative to the conventional culture medium (normally chemical fertilizers) for massive *S. platensis* biomass production. In this study, variations of COD, TN, and TP concentration in piggery wastewater vs. cultivation time are plotted in Figs. 3–5, respectively. As shown in Fig. 3, continuous reduction of COD concentration in the initial 9 d of cultivation were observed in all treatments, which could be ascribed to the assimilation of indigenous bacteria in the piggery wastewater (based on the obvious COD reduction in the 0inoculate) and respiration by the *S. platensis*. After this, however, COD concentration decreased much slower in all treatments, especially for the control (0inoculate), in which COD slightly increased after a minimum value occurred. This phenomenon could be attributed to the production of extracellular substances by the indigenous microorganisms [25]. At the end of 15 d cultivation, the maximum COD removal percentage and removal rate achieved was 85.87%

Table 2
Specific growth rate and biomass productivity

Treatments	Biomass productivity (mg L^{-1} d^{-1})	Specific growth rate (d^{-1})	Chlorophyll productivity (mg L^{-1} d^{-1})
$0FeSO_4$	74.22 ± 0.024	0.1255 ± 0.0015	0.134 ± 0.0000
$0.05FeSO_4$	98.67 ± 0.024	0.1348 ± 0.0015	0.156 ± 0.0046
$0.10FeSO_4$	63.11 ± 0.024	0.1515 ± 0.0015	0.113 ± 0.0023

and 41.76 mg L^{-1} d^{-1}, 70.01% and 36.82 mg L^{-1} d^{-1}, 78.13% and 36.82 mg L^{-1} d^{-1}, and 66.26% and 32.39 mg L^{-1} d^{-1} for 0inoculate, $0FeSO_4$, $0.05FeSO_4$ and $0.10FeSO_4$, respectively, which were higher than that reported by Cheng [26], who cultivated *Scenedesaceae* sp. in diluted swine breeding effluent and obtained COD removal percentage of only 37.1%. The better performance of COD removal efficiency in the current work could result from lower ammonium concentration and turbidity of raw piggery wastewater employed.

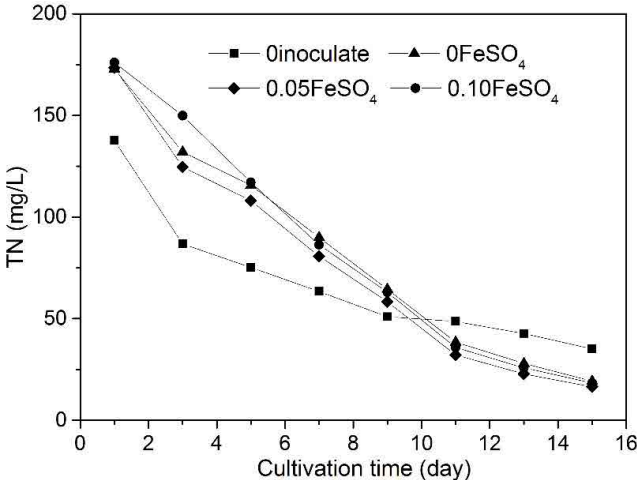

Fig. 4. Variation of TN during incubation.

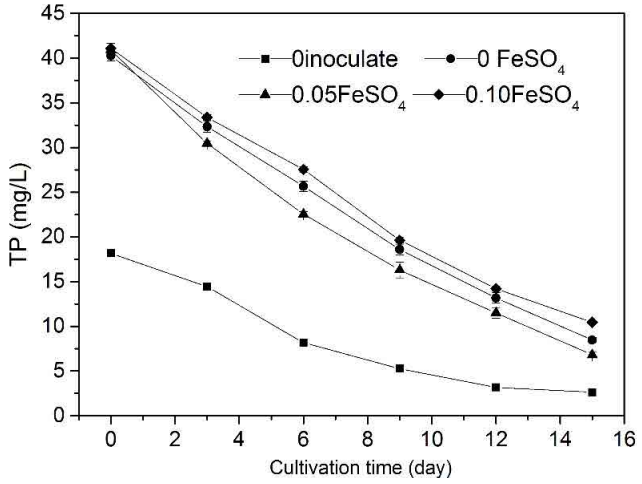

Fig. 5. Variation of TP during incubation.

High ammonium loading can inhibit the growth of microalgae, and high turbidity can reduce light penetration to algal cell surface leading to lower photosynthesis activity [27,28]. Lower COD removal percentage in treatments with *S. platensis* inoculated compared to that of the control (0inoculate) might be caused by the generation of extracellular organic substances during the *S. platensis* growth and degradation of dead *S. platensis* cells into solubilized organic carbons by indigenous bacteria in the raw piggery wastewater.

Chemical structure and concentration of nitrogen source in the culture medium can significantly affect microalgae growth and the chemical composition of microalgae biomass [29]. Variations of TN concentration in the raw piggery wastewater during *S. platensis* cultivation were determined and are illustrated in Fig. 4. It was evident that nitrogen concentration in the raw piggery wastewater continuously reduced during *S. platensis* cultivation in all treatments. Reduction of nitrogen concentration in the piggery wastewater during *S. platensis* cultivation could be attributed to assimilation by *S. platensis* and air stripping caused by fierce aeration to the wastewater of high alkalinity (pH > 9.0 as indicated in Fig. 6). By the end of 15 d cultivation of *S. platensis*, TN concentration in the raw piggery wastewater decreased by 74.49%, 88.83%, 90.47%, and 89.15% in 0inoculate, $0FeSO_4$, $0.05FeSO_4$ and $0.10FeSO_4$, respectively. Higher TN removal percentage in microalgae inoculated treatments compared to 0inoculated demonstrated that *S. platensis* growth in the piggery wastewater could enhance nitrogen removal. However, no dramatic difference was observed with different strengths of $FeSO_4$ supplementation on the nitrogen removal percentage. Regarding nitrogen removal rate, much higher value (10.24–10.50 mg L^{-1} d^{-1}) was achieved in the treatments with *S. platensis* inoculated, in comparison with only 6.84 mg L^{-1} d^{-1} for no *S. platensis* inoculated, suggesting the removal efficiency of nitrogen from the raw piggery wastewater was significantly enhanced by the uptake of *S. platensis*.

Phosphorous is another essential nutrient in microalgae metabolic processes, such as signal transduction, energy conversion and photosynthesis [30]. The variation of TP concentration in the raw piggery wastewater during *S. platensis* cultivation was shown in Fig. 5. According to Fig. 5, a notable reduction of TP concentration ($P < 0.05$) could be observed after the 15 d cultivation of *S. platensis*. According to Johansson et al. [31], the decrease of phosphorous in wastewater was induced by two mechanisms: Microorganisms absorption and chemical precipitation. In this work, it can speculate that phosphorus removal during biomass production possibly combined both mechanisms since the culture

Table 3
Nutrient removal percentage

Treatments	COD	NH_3–N	TP	TN
	Removal percentage (%)			
0inoculate	85.87 ± 0.00	100 ± 0.00	79.29 ± 0.00	74.49 ± 0.07
$0FeSO_4$	70.02 ± 1.58	100 ± 0.00	78.69 ± 0.00	85.86 ± 0.48
$0.05FeSO_4$	78.15 ± 3.11	100 ± 0.00	83.39 ± 0.60	91.34 ± 0.33
$0.10FeSO_4$	66.21 ± 2.55	100 ± 0.00	74.59 ± 0.01	85.82 ± 0.32

Table 4
Nutrient removal rate

Treatments	COD	NH_3–N	TP	TN
	Removal rate (mg L^{-1} d^{-1})			
0inoculate	41.76 ± 0.00	not detected	2.11 ± 0.00	6.84 ± 0.05
0$FeSO_4$	33.70 ± 0.26	18.17 ± 0.26	2.13 ± 0.00	9.89 ± 0.00
0.05$FeSO_4$	36.81 ± 0.91	17.88 ± 0.39	2.27 ± 0.00	10.56 ± 0.06
0.10$FeSO_4$	32.39 ± 2.42	17.61 ± 0.26	2.04 ± 0.03	10.07 ± 0.04

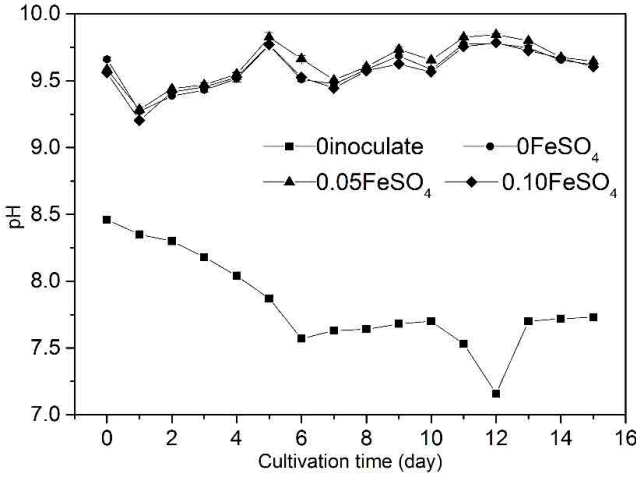

Fig. 6. pH variation during incubation.

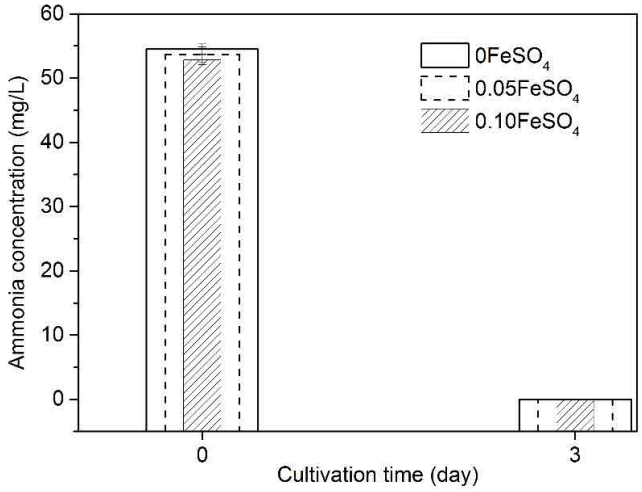

Fig. 7. Variation of NH_3–N concentration during incubation.

pH remained above 9.0 during the experiment (as indicated in Fig. 6) and an obvious increase of biomass concentrations was obtained (Fig. 1).

The variation of ammonium concentration vs incubation time is depicted in Fig. 7. No significant difference in ammonia removal rate in all treatments was observed, due to the volatility of free ammonia and strong mixing of the raw piggery wastewater caused by aeration. By the end of the cultivation, all ammonia in the wastewater was removed, which was similar to the result reported by Wang et al. [28].

3.3. Chemical composition of biomass

It is generally recognized that protein content is one of the most critical indicators in algal biomass nutrition value evaluation [32]. Thus, in the present research, the protein content in the harvested biomass was determined. The results are demonstrated in Fig. 8. It can be observed from Fig. 8 that protein content in the obtained biomass ranged from 45.31%–55.15% on a dry weight basis at all treatments with *S. platensis* inoculation. The protein contents in this study were slightly lower than the results reported by Raoof et al. [33], in which, the protein content of *Spirulina* sp. biomass cultivated in Zarrouk's medium [16] and formulated medium were between 58.25% and 63.3%. This could be the result of different components of culture medium, especially the nitrogen content and bioavailability in the culture medium, which had a great effect on the biosynthesis of protein. Furthermore, indigenous bacteria in the raw piggery wastewater can also compete with nutrients with *S. platensis*, leading to lower nitrogen availability for protein synthesis by *S. platensis*.

Carbohydrate content is another pivotal index for algal biomass energy potential assessment. Moreover, carbohydrate content can directly reflect the carbon fixing capacity of microalgae when it is applied in flue gases or organic carbon abundant wastewater bioremediation. In this work, carbohydrate contents in the biomass obtained were in the range of 8.53%–12.32% on a dry weight basis as indicated in Fig. 8. Ferrous sulfate supplementation could notably enhance carbohydrate synthesis ($P < 0.05$), this tendency was contrary to that of protein, which might result from sufficient carbon sources in the raw piggery wastewater as well as removal efficiency of organic carbon substances was enhanced by ferrous sulfate addition. Nevertheless, these values were dramatically lower than that of other microalgae species cultivated in similar wastewater, suggesting that *S. platensis* is not an ideal candidate microalgae species for carbon mitigation in industrial exhausts and COD removal in wastewater treatment [34,35]. Whereas for lipid content in the obtained *S. platensis* biomass, no remarkable differences were observed in all treatments (10.42%–11.49%). The lipid contents in the biomass of this study were higher than that achieved by Li et al. [36] (less than 7%) but lower than the results reported by Chang [37] (17.2%–19.8%), who used human urine as a medium to cultivate *Spirulina* sp. Iron content in the resulted biomass was 1.31 and 2.03 mg g^{-1} on

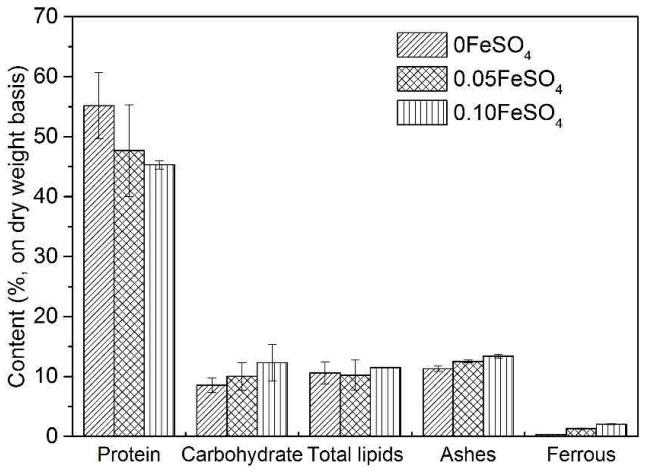

Fig. 8. Biochemical composition of harvested biomass.

biomass dry weight basis, for $0.50FeSO_4$ and $0.10FeSO_4$ treatment, respectively, which were higher than that of $0FeSO_4$ (0.34 mg g^{-1}) ($P < 0.05$). This phenomenon was the result of physical biosorption and assimilation effect between Fe^{2+}/Fe^{3+} and microalgae cells [38,39]. Higher iron content facilitates the obtained biomass to be an iron supplement ingredient for animal feed. Ash contents of harvested algal biomass ranged from 11.31%–13.41%, which was attributed to inorganic substances contained in the biomass.

4. Conclusions

Based on the results in the present work, nutrient removal and protein abundant biomass could be achieved by cultivating *S. platensis* in raw piggery wastewater. Supplementation of ferrous sulfate could significantly increase *S. platensis* growth promotes the accumulation of chlorophylls and iron in *S. platensis* biomass. Moreover, nitrogen and phosphorus removal efficiencies were also enhanced by ferrous sulfate addition in the piggery wastewater. These results indicated that the coupling of nutrition value-added biomass production and nutrients bioremediation by cultivating *S. platensis* in raw piggery wastewater is technically feasible. Ferrous sulfate supplementation with moderate strength is a promising approach to increase the productivity and nutritive value of *S. platensis* biomass, which can facilitate to reduce microalgae-based protein production cost. However, the effect of ferrous sulfate on the metabolic pathway of *S. platensis* remained to be specified. Risks regarding the contamination of unwanted compounds like heavy metals, pathogens, etc. on the produced *S. platensis* biomass, if used as animal feed additive should be addressed, and techno-economic issues in scaling up of the proposed process are required to be thoroughly evaluated in the subsequent work.

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