Design, application and verification of a novel system utilizing bacteria and microalgae to treat swine farm wastewater and produce value-added biomass

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Abstract: Swine farm wastewater is extremely harmful to the environment if not treated before it is discharged. In this study, a system was developed and optimized for testing the high levels of organic matter in swine farm wastewater utilizing a microalgae/bacteria co-culture combined with a novel closed-loop extraction and dilution process. Importantly, the system produces biomass that also could be harvested and used in value-added applications. The efficacy of biomass as a biofertilizer was demonstrated by using a model plant of *Arabidopsis*. In addition, the analysis of biomass indicates that it also has potential as a source for biofuel. After a 20-d cultivation period, a yield of biomass was achieved to 2.063 g /L of wastewater. The highest removal rates recorded in steady state conditions were: 13.8 mg/L·d of Total Nitrogen (TN); 11.5 mg/L·d of Ammonia Nitrogen (NH_4^+-N); 24.8 mg/L·d of Chemical Oxygen Demand (COD); and 16.9 mg/L·d of Total Phosphorus (TP). After cultivation, the composition of the biomass was analyzed on a dry basis; the major components were protein (44.9%), lipids (24.6%), carbohydrates (19.9%), Chlorophyll-A (2.75%), Chlorophyll-B (1.66%), and carotenoids (0.57%). This biomass was diluted with water (5% by weight) and used as a biofertilizer to grow *Arabidopsis*. The results showed that the average root and stem lengths of *Arabidopsis* were 43.0% and 55.0% longer compared to those of the control group. Additionally, the number of leaves and the maximum leaf length increased by 30.2% and 39.7%; and the fresh and dry leaf weights increased by 44.0% and 33.7%, respectively. These results demonstrate the efficacy of this system for treating swine farm wastewater whilst simultaneously producing a value-added microalgae/bacteria biomass. This paper also demonstrated the use of biomass as a fertilizer for cultivating a value-added crop and, based on the compositional analyses, propose that the biomass could be used as a raw material for biofuel production due to its high lipid content of 24.6%. By constructing a microalgae/bacteria symbiosis system, Swine farm wastewater can be treated as resources utilizing producing value-added biomass with demonstrated efficacy as a biofertilizer.

Keywords: bacteria-microalgae, swine wastewater, environmental impact, value-added biomass, biofertilizer
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1 Introduction

As a unicellular microorganism, microalgae have received much attention from many researchers, due to their unique advantages of organic carbon and CO_2 fixation, water quality purification, high biomass accumulation, and no competition with arable land [1-3]. More importantly, microalgal biomass can also be used to produce high value-added products such as biofuels[4-6], biofertilizers[7] and animal feed[8]. However, the cost of cultivating microalgae on an industrial scale is high due to the amount of water and nutrients required. For example, producing 1 kg of biodiesel directly from microalgae biomass would consume 3726 kg of water, 0.33 kg of nitrogen, and 0.71 kg of phosphate[9]. Many studies have successfully demonstrated that combining microalgal cultivation with wastewater treatment can not only purify the wastewater but also substantially reduce the cost of microalgal culture[9-11]. In addition, many studies have reported that the nutrient composition in animal wastes is similar to the traditional culture medium for

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algal growth[3,12].

Additional studies have demonstrated that algal cells grown in wastewater from animal farming are not only rich in nutrient salts, such as nitrogen and phosphorus, but also rich in proteins, polysaccharides, pigments, and multiple mineral elements, which offer good potential for resource recovery[13,14]. Microalgal biomass fertilizers can produce plant growth hormones, polysaccharides, antimicrobial compounds, and other metabolites which can promote plant growth, while improving soil fertility. Currently, the use of fertilizers in crops is dominated by inorganic chemical fertilizers, which can be harmful to human health and have the potential to disrupt the ecological balance in the long-term[15]. Hence, it is advantageous to develop biofertilizers that can meet agricultural needs without damaging the ecosystem[16,17].

It can be difficult to use highly concentrated animal manure wastewater directly to culture microalgae, because the high concentration of some pollutants in the wastewater can inhibit the growth of microalgae and affect the conversion of pollutants as well as the accumulation of biomass[18]. Swine wastewater contains high levels of suspended solids and high NH_3-N concentration, which can both inhibit the growth of microalgae and poison them, resulting in the death of microalgae cells in severe cases[19,20]. The main strategy adopted in the current study to address these issues for the cultivation of microalgae is to co-culture bacteria and dilute the swine wastewater[21]. Principally, the bacteria microbial community in swine wastewater treatment was involved in nitrogen, phosphorus and COD digestion, uptake/removal[22]. A combination of these steps in a sequential order is used for enhanced nutrient removal[23]. In addition, Liu et al.[24] found that after an 8-fold dilution of swine wastewater, the growth of *Chlorella* was superior to growth in BG11 medium. After dilution, the microalgae were able to remove 84.95% of the NH_3-N and 92.89% of the TP. This study intended to establish and implement a new co-culture of bacteria and microalgae system to utilize efficiently swine manure wastewater with culture broth recycling, while also producing value-added biomass that could be used as a fertilizer so as a source for biofuel.

In recent years, researchers have begun to recycle culture broth after biomass harvesting to reuse the water and nutrients in the culture medium. For example, the culture broth (permeate) left after the removal of algal biomass by membrane filtration, was used to culture *Chlorella vulgaris*, and reduce fresh water use by 77%[25]. Deng et al.[26] constructed a recirculating system for daily recycling of harvested culture broth in which the algal grew well and, at the end of the culture period, the accumulative biomass ranged from 1.68 to 3.47 g/L, and the daily productivity ranged from 234.1 to 532.2 mg/L·d. In addition, at 4-fold and 6-fold dilution of the wastewater, the algal exhibited the highest production of carbohydrates, proteins, and lipids at 76.4, 257.2 and 183.7 mg/L·d, respectively.

Upon review of the literature, to enable the normal growth of *Chlorella* in swine wastewater, our research used swine wastewater to culture exogenous bacteria first, and then culture *Chlorella* in the broth with a reduced dilution ratio of 3-fold forming a co-culture system. The culture broth recycle system was also established to recover and reuse as the culture solution. The harvested, partial biomass was then utilized in the growth studies of *Arabidopsis*. The main objective of this study was to examine the feasibility of this system for the cultivation of *Chlorella vulgaris* (UTEX 2714) and to verify that the biomass could be used as an organic fertilizer to improve plant growth. Walls[27] used the algal bacteria system composed of algal algae and wild fungi to treat urban sewage, and found that 100% of total nitrogen and 96% of phosphate could be removed, and 2.74 g/L biomass could be accumulated.

2 Materials and methods

2.1 Wastewater source and pretreatment

The swine wastewater used in this study was collected from a swine farm in the Pinggu District of Beijing, China. The wastewater was centrifuged (4000 r/min, 10 min at 25°C, Sigma 3-18KS, Made in Germany) and left to stand for 24 h. The supernatant was collected and characterized for water quality (see Table 1) and stored at 4°C for the experiment.

Table 1 Characterization of water quality after pretreatment of swine farming wastewater

Parameter	Wastewater quality
Chemical oxygen demand (COD)	5483.75±14.14 mg/L
Total phosphorus (TP)	46.48±1.72 mg/L
Total nitrogen (TN)	1708.33±16.97 mg/L
Ammonia nitrogen (NH_4^+-N)	2069.5±4.95 mg/L
pH	7.0±0.11

2.2 Microalgal strains, bacterial strains, and *Arabidopsis* growth

Collos et al.[28] have shown that Microalgae of *Chlorella* have higher ammonia tolerance and good growth. The microalgal strain (*Chlorella vulgaris*, UTEX-2714) was chosen in this experiment which was purchased from the UTEX algal stock bank at the University of Texas, USA. *Chlorella vulgaris* was cultured in tris-acetate-phosphorus (TAP) medium. The culturing conditions were set as follows: temperature 25°C; light intensity 5000 lx; 16 h light 8 h dark cycles. The cultures were grown to the logarithmic growth phase, washed three times with distilled water, then the precipitate was suspended for later use.

The preserved bacteria strains (Pseudomonas, Klebsiella, Proteus) were from previous studies by the lead author with good results on wastewater treatment. The bacteria were taken from a frozen state (–80°C), activated, grown in Lysogeny Broth (LB) liquid medium, then cultured to the logarithmic phase, washed three times using distilled water, then the precipitate was suspended for later use.

Arabidopsis thaliana seed disinfection: the appropriate amounts of seeds were put into a 1.5 mL centrifuge tube, 1 mL of 1.5% NaClO and 2 μL of 20% Triton X-100 were added, and mixed for 15 min, and washed 3-5 times with distilled water. *Arabidopsis* seeding: seeds were sown on Murashige & Skoog (MS) medium (Table 2) in an ultraclean bench, vernalized at 4°C for 2-4 d, and transferred to the incubator for light growth. The culture conditions were: temperature 22°C, light intensity of 8000lx, cycles of 16 h light, 8 h dark.

Table 2 Composition of MS medium

Reagent	Amount
MS	4.43 g
MES	0.5 g
Sucrose	10 g
Agar	8 g
pH	5.6-5.8
dH_2O	Up to 1000 mL

2.3 Experimental design of circulating system

This study established a culture system with regular circulation

of postharvest culture broth, consisting of a cultivation unit, a feeding unit, and a harvest unit (Figure 1). The cultivation unit employed a 5 L glass tank as the photobioreactor with the initial inoculation concentration of 0.1 g/L microalgae and 2.8×10^6 CFU/mL bacteria and utilized 2-3 L of swine farm wastewater. The feeding unit contained swine wastewater (SW) and distilled water which was added to the cultivation unit at regular intervals. The harvesting unit collected biomass by centrifugation and returned the supernatant to the bioreactor. Previous studies have reported that the growth of microalgae was inhibited when the NH_3-N concentration was over 200 mg/L. In this experiment, the NH_3-N concentration was much higher than 200 mg/L, thus the wastewater was pretreated using a "bacteria before algae" step. Specifically, the wastewater was initially used to culture bacteria, and after culturing for 2-3 d, depending on the remaining NH_3-N concentration, the culture broth was diluted and then inoculated with *Chlorella* to form a co-culture system. The first recycling of the culture broth was started when the algae and bacteria were co-cultured for 2 d; the cycle was repeated every 2 d; at the same time, the removal effect of nutrients, as well as the accumulation of biomass, was measured.

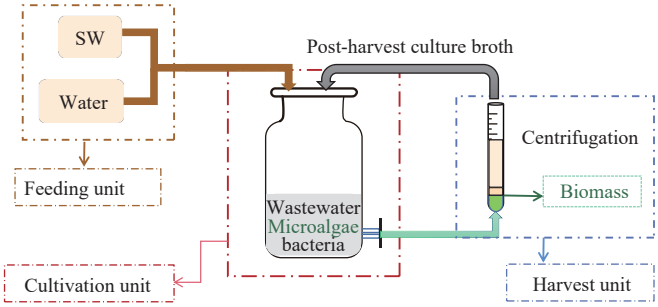


Figure 1 Schematic diagram of the recycling culture system

Two multi-cycle modes were used in this model system:

Mode 1: Constant Volume: one-sixth of the culture broth was harvested every 2 d during the bacteria and algae co-culture period. This volume was replaced with one-third coming from the wastewater and two-thirds from a combination of the distilled water and the recycled culture broth after centrifugation (see Figure 1). This protocol ensured that, during each cycle, the same amount of wastewater was added equivalent to one-third of the volume replaced. The other two-thirds were made up using the supernatant from the centrifuge along with distilled water. Due to the constant recycling, the amount of distilled water required was reduced with each cycle.

Mode 2: Constant Ammonia Nitrogen concentration: In this mode, the goal was to maintain the ammonia nitrogen (NH_4-N) concentration constant at approximately 200 mg/L. As with Mode 1, one-sixth of the culture broth was removed every two days, the biomass and culture medium are separated by centrifugation, and the obtained culture medium is returned to the culture unit for the next cycle. At the same time, according to the ammonia nitrogen level, swine wastewater and distilled water are added to the culture unit to maintain the NH_3-N concentration at a constant level (about 200 mg/L) at each 2-d cycle.

2.4 Experiment design

Arabidopsis plant experiments were set up with 3 treatment groups[29].

a) Group 1, the control group, without applying any fertilizer.

b) Group 2 utilized the harvested biomass concentrate (*Chlorella* dominated) with water content of about 95%.

c) Group 3 used the ternary composite manure nutrient solution (approx. 1 g manure dissolved in 250 mL of water).

The seedlings, grown 7 d in size, were transferred to a nursery tray, the test soil samples were all treated with vermiculite without nutrients, and the culture conditions are as described in section 2.2. Seedlings were divided into 3 treatment groups each utilizing one of the solutions above every 6-7 d. At the end of the incubation period, root length, stem length, number of leaves, maximum leaf length, and fresh and dry weights were measured.

2.5 Biomass analysis

Every day, 10 mL of the algal liquor was collected from each experimental group, into a 15 mL centrifuge tube (oven-dried in advance to constant weight M_0). The liquor was centrifuged at 4000 r/min at 4°C for 5 min (Sigma 3-18KS, made in Germany), the centrifuge tube containing the biomass pellet was placed in an oven at 105°C to constant weight (M_1), and the biomass (g/L) was calculated as follows[30].

$$L = (m_1 - m_0)/10\times10^{-3} \quad (1)$$

where, L is the biomass dry weight, g; m_0 is the empty tube mass, g; m_1 is the post-oven tube and the total mass of material, g.

2.6 Pigment determination in algae

Determination of pigments in algal cells was done using the method of Luo[31]: 10 mL of the mixed culture solution was taken into a 15 mL centrifuge tube, placed in a centrifuge at 12 000 r/min for 10 min, then separated, 10 mL absolute ethanol was added to the solid sample which was sealed with parafilm and put into a 4ºC freezer. After 24 h, the centrifuge tube was removed, centrifuged at 12 000 r/min for 5 min, and the supernatant was collected to measure the absorbance at wavelengths 470 nm, 645 nm and 663 nm, which were denoted as A470, A645 and A663, respectively. Total chlorophyll concentration (C_{a+b}), Chl-a concentration (C_a), Chl-b concentration (C_b), and total carotenoid concentration (C_x) were calculated using the following equations:

$$C_{a+b} = 20.2\times A_{645} + 8.02\times A_{663} \quad (2)$$

$$C_a = 12.21\times A_{633} - 2.81\times A_{645} \quad (3)$$

$$C_b = 20.13\times A_{645} - 5.03\times A_{633} \quad (4)$$

$$C_x = (1000\times A_{470} - 3.27\times C_a - 104\times C_b)/229 \quad (5)$$

2.7 Determination of lipid content and fatty acid composition in microalgae

Determination of lipid content[32]: About 0.2 g of the prepared biomass was added to a chloroform/methanol solution (2:1 by volume), followed by sonification of the solution using an ultrasonic cell disruptor for 10 min in an ice bath, then centrifuged at 4000 r/min by refrigerated centrifuge (4°C) for 10 min. The supernatant was collected, and the process was repeated twice. The supernatant was then used for lipid determination. Lipid-free precipitation was used for protein and polysaccharide assays. The resulting supernatant was added to an equal volume of 0.1% NaCl solution and allowed to stand for 15 min after mixing vigorously, the chloroform layer on the bottom was blown dry with nitrogen gas and put in an oven at 60°C to constant lipid dry weight of biomass.

The lipid content was calculated as follows:

$$\text{Lipid content}(\%) = (m_1 - m_2)/m_3 (6) \quad (6)$$

where, m_1 is the mass of the centrifuge tube blown with nitrogen to a constant weight mass, g; m_2 is the empty centrifuge tube mass, g; m_3 is the weight of the dry biomass, g.

Lipid composition analysis: Before the analysis of fatty acids, the samples were first subjected to a trans-esterification reaction[33]. The lipids obtained above were trans-esterified by adding 10 mL of a mixture of methanol, concentrated H_2SO_4, and chloroform (ratio of 4.25:0.75:5.0 by volume) in a 90°C water bath for 90 min to obtain fatty acid methyl esters. After the esterification reaction was finished the chloroform layer to be tested was collected. The composition of lipids was analyzed by GC-MS. The method used was an improved version of that reported by Li et al.[34] The programmed temperatures included: initial column temperature of 80°C, after holding for 2 min, raised to 250°C at 4°C/min, holding for 2 min. The carrier gas was set at a flow rate of 1 mL/min; Split ratio of 25:1, and an injection volume of 1 μL. Finally, the chromatographic data were recorded and integrated using Agilent data analysis software. The obtained compounds were searched against the NIST mass spectral database to identify the specific fatty acids in the samples, and the relative contents of each fatty acid methyl ester were determined by using peak area normalization.

2.8 Carbohydrate and protein determination in microalgae

The carbohydrate and protein extraction methods used were those reported by Luo[35]. Carbohydrate determination was performed by the phenol sulfuric acid method. Protein was determined using the Bradford method[36].

2.9 Physicochemical characteristics analysis

Chemical Oxygen Demand (COD) values were determined spectrophotometrically with rapid digestion; Ammonia nitrogen values were determined spectrophotometrically using nanodrop reagent; Total Nitrogen (TN) values were determined by UV spectrophotometry; Total Phosphorus (TP) values were determined spectrophotometrically with ammonium molybdate.

2.10 Statistical analysis

Using GraphPad Prism 8.0.1, SPSS 2015, and origin8.0 software for data processing. The experimental results were obtained by averaging the three tests.

3 Results and discussion

3.1 Biomass accumulation profile analysis

The biomass accumulation under the recycling system is shown in Figure 2. The cycle started after 2 d of co-cultivation between *Chlorella* and bacteria and repeated three times during Phase I (the first 11 d). From the results of Phase I, the biomass accumulation was unstable, and a decline was observed when repeating the second and third cycles. One possible reason is that the concentrations of nutrients, especially NH_3-N (more than 200 mg/L), in the whole system after newly added wastewater could inhibit *Chlorella* growth. Therefore, after the end of the third cycle (day 11), additional algal was supplemented in the wastewater in the bioreactor, see Figure 2 "algal treatment" stage, and the results showed that the accumulation of biomass was significantly increased after algal supplementation. Importantly, it increased from 0.856 g/L to 1.712 g/L at the end of the third cycle after 4 d of culture, indicating that the concentration of algal was also particularly important when microalgae were cultured in the wastewater. Consequently, the concentration of ammonia and nitrogen was maintained at a similar level (200 mg/L) at the start of Phase II, and the highest that the biomass yield could reach was 2.063 g/L by the end of five cycles within Phase II. Based on these results, it can be concluded that *Chlorella vulgaris* (UTEX 2714) can achieve the highest biomass (2.063 g/L) at a recovery rate of 1/6 in this system.

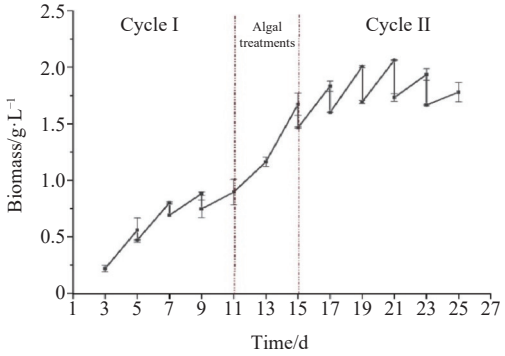


Figure 2 Biomass changes under the circulatory system

3.2 *Chlorella* chemical composition analysis

In general, the chemical composition of the microalgal biomass was dominated by carbohydrates (5%-23%), proteins (6%-52%), and lipids (7%-23%)[37]. As shown in Figure 3, after the cultivation ended, the harvested biomass was subjected to compositional analysis. The protein content of *Chlorella* accounted for 44.9% of the cell dry weight. This result was consistent with many previous studies[38]. Nitrogen is one of the main elements in algal cells, and the ammonia in the swine wastewater is a rich source of nitrogen for *Chlorella* spp. consequently, a higher amount of protein was produced[39,40]. Carbohydrates are also one of the important components in algal. In this study, after analyzing the content of the harvested *Chlorella*, carbohydrates accounted for 19.9% of the dry weight of the algae cells. This is a consequence of the high concentration of COD in swine wastewater.

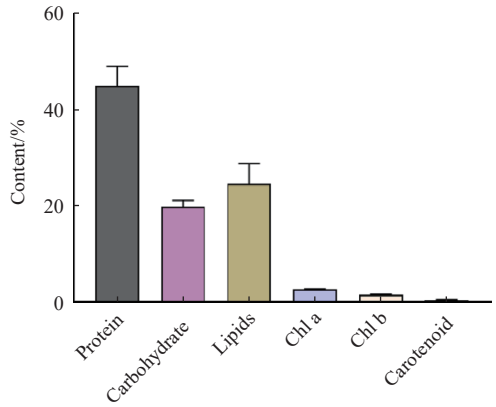


Figure 3 *Chlorella* chemical analysis Figure 4 *Chlorella* fatty acid composition

The lipid in the microalgae biomass was 24.6% in this experiment which is similar to the content in other papers[33,41]. The fractions of fatty acids in microalgae cells were analyzed (shown in Figure 4). A total of 10 different fatty acids were produced, the main ones being C16 and C18, which accounted for 96.3% of the overall fatty acid composition. Additionally, C16:0 and C18:3 were the two most abundant fatty acids in this algae, 24.6% and 32.0% of the total fatty acids, respectively. These results met the standard requirements for biodiesel preparation[42-45]. Furthermore, the percentages of saturated fatty acid (SFA), mono-unsaturated fatty acids (MUFA), and poly-unsaturated fatty acids (PUFA) were 31.6%, 24.5% and 41.4%, respectively which might be used as a feedstock for the production of biodiesel or hydrogenated for renewable diesel[46].

The most abundant pigments in common *Chlorella* are Chlorophyll-A, Chlorophyll-B, and carotenoids. These pigments

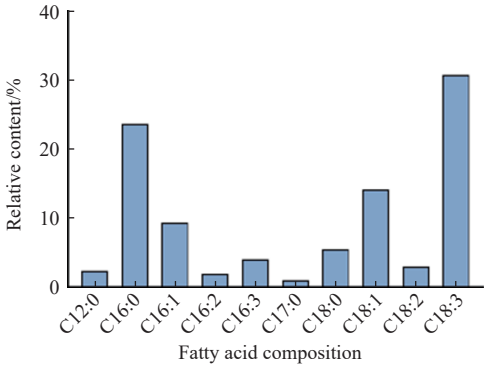


Figure 4 Chlorella fatty acid composition

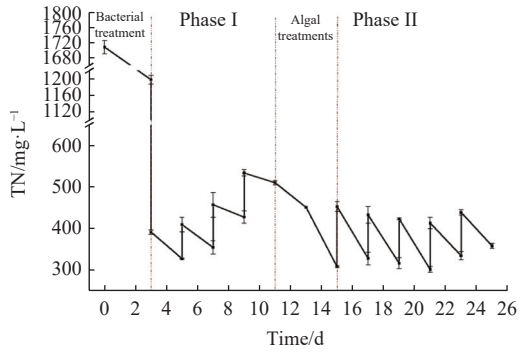
have various therapeutic effects, such as antioxidant activity, protection against retinal degeneration, regulation of blood cholesterol, and enhancement of the immune system[47]. Pigments have a wide variety of applications, ranging from food colorants, to being effective agents for disease prevention[48]. In this study, after extraction from the harvested biomass, the composition of the three pigments, Chl a, Chl b, and carotenoids, were found to be 2.75%, 1.66% and 0.57%, respectively (Figure 3), which were higher than found in previous study[10].

3.3 Analysis of nutrient removal

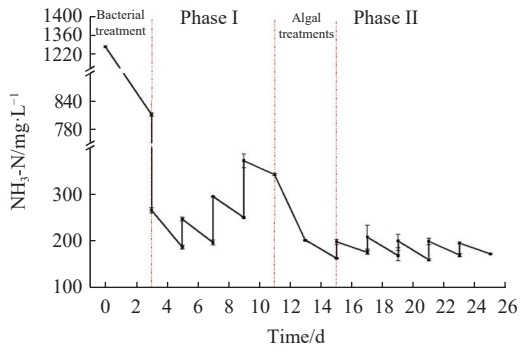
3.3.1 TN and NH_3-N removal

Figure 5 illustrates the degradation of TN (Figure 5a) and NH_3-N (Figure 5b) under the circulatory system. The whole degradation process can be divided into four parts:

1) Bacterial treatment,
2) Phase I,
3) Algal treatment, and
4) Phase II.



a. TN analysis in different time



b. NH3-N analysis in different time

Figure 5 TN and NH_3-N changes under the circulatory system

The lower panel shows that the degradation profiles of TN and NH_3-N are similar. On the 3rd day of bacterial treatment, the concentrations of TN and NH_3-N decreased from 1708.33 and 1236.75 mg/L to 1197.5 and 811.25 mg/L, respectively. *Chlorella* treatment was added to the wastewater after a 3 to 1 dilution of the bacteria culture broth, and the concentrations of TN and NH_3-N at that time were 391.87 and 266.2 mg/L, respectively, after which they were cycled every 2 d for a total of three cycles (this was Phase I).

In Phase I stage, after repeating three cycles, the concentrations of TN and NH_3-N had a consistently increasing trend with final concentrations of 511 mg/L and 342.8 mg/L, respectively (Shown in Figure 5）. The possibility is the wastewater was discharged from each cycle and replenished with the raw wastewater which could lead to the concentration of the nutrients in the culture broth increasing if the microalgae *Chlorella* cannot degrade those nutrients accordingly. To further degrade the nutrients quickly, *Chlorella* was supplemented in the wastewater and resulting in the concentrations of TN and NH_3-N 308.5 mg/L and 163.35 mg/L, respectively after 4 d of culture (2 cycles). The high concentration of NH_3-N in wastewater (≥200 mg/L) could inhibit the growth of microalgal growth as discussed earlier. Therefore, in phase II, the improved treatment approach is to maintain the NH_3-N concentration at around 200 mg/L or less. The results showed that the removal of TN and NH_3-N reaches a steady state after five cycles (10 d). TN and NH_3-N can be reduced to 312 and 170.1 mg/L, and the maximum removal rates in this stage are 13.8 and 11.5 mg/L·d, and the average removal rates are 11.5 and 6.4 mg/L·d, respectively. These results are slightly higher than those reported by Park et al.[49], who observed that the NH_4^+-N removal was only 5.2-6.5 mg/L·d when anaerobically digested from swine farm wastewater to culture anaerostipes in water. In summary, the maximum removal rates of TN and NH_3-N were 13.8 mg/L·d and 11.5 mg/L·d, respectively, could be obtained with the cycling treatment system in steady state Phase II.

3.3.2 COD reduction

Figure 6 illustrates the reduction of COD under the circulatory system. The degradation process is divided into four parts. After the stage of bacteria treatment, the COD decreased from 5483.75 to 1403.9 mg/L, and the removal rate was 1359.95 mg/L·d (1.36 g/L·d). Adding microalgae into the phase I, the wastewater was diluted to 3 to 1 of the bacteria culture broth, the concentration of COD was 482.25 mg/L. In this study, swine wastewater was used as a medium to culture *Chlorella*, which can utilize a variety of volatile fatty acids, such as acetic acid, propionic acid, and butyric acid, as organic carbon sources[10]. The content of organic carbon is directly associated with the change of COD because there was a linear relationship between COD and organic carbon in swine wastewater[50]. After algal supplementation in the third stage, the COD showed a continuous decreasing trend during the 4 d of culture, and the concentration decreased from 482.25 to 349.15 mg/L.

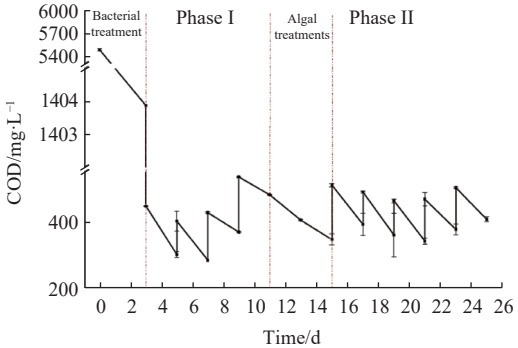


Figure 6 COD changes under the circulatory system

After entering phase II, the decreasing trend of COD is more stable, and the lowest level was achieved with a reduction to 354.5 mg/L by the end of this phase. The maximum reduction rate of COD in this stage is 13.0 mg/L·d, and the average rate is 10.6 mg/L·d in the first cycle in this stage. These results are higher than that reported by Deng et al.[40] With a cycle ratio of 1/6, their study showed that the maximum and average removal rates of COD are 1.96 and 0.94 mg/L·d, respectively. Compared with that reported by Deng et al.[40], the COD removal rate in Phase II was improved greatly. The possible reason was at this stage, the NH_4^+-N concentration was maintained at 200 mg/L and below, which did not inhibit the growth of *Chlorella*. In addition, the maximum removal of COD was 24.8 g/L·d at the end of the whole process culture.

3.3.3 TP removal

Figure 7 shows the degradation of TP under the recycling system. The whole degradation process was similarly divided into four stages. During the bacterial treatment stage, TP decreased from 46.48 to 22.88 mg/L, and the removal rate was 16.9 mg/L·d. In addition, TP changing trend in Phase I was the same as observed for nitrogen and COD. After three cycles, the concentration of TP was 8.36 mg/L. After the third stage of algal supplementation, TP decreased from 8.36 to 6.36 mg/L within 4 d at which time the removal rate was 6.0 mg/L·d. After entering Phase II, a total of five cycles were performed, the maximum removal of TP is 7.20 mg/L·d. Compared with Phase I, it was found that the degradation trend of TP, COD, and nitrogen is basically at a steady state when the NH_3-N concentration was maintained at about 200 mg/L.

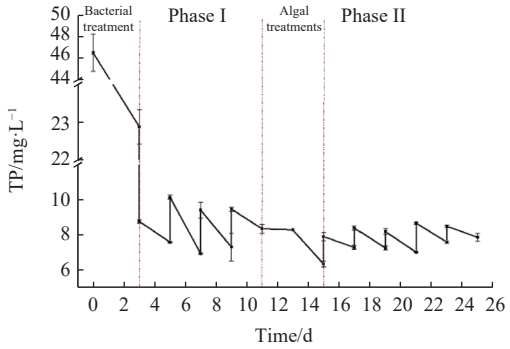
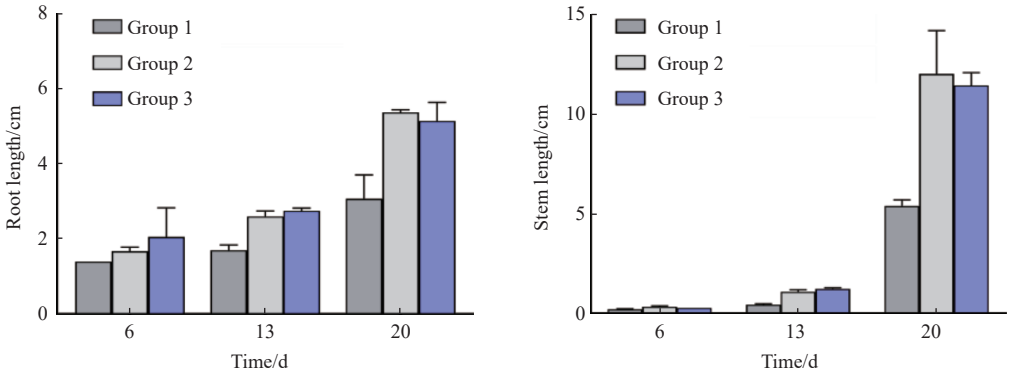


Figure 7 TP changes under the circulatory system

3.4 Biomass resource utilization

3.4.1 Bio-fertility effects of biomass concentrate on *Arabidopsis* root length and stem length after fertilization

To verify the bio-fertility of the biomass concentrate, the root length and stem length of *Arabidopsis* within 20 d were monitored, and the results are shown in Figures 8a and 8b. This shows that the root length and stem length grew similarly among 3 treatment groups. In the group 2, on the 13th day of fertilization with biomass concentrate, there was no significant effect on the root length and stem length of *Arabidopsis* (p>0.05), a result consistent with those of Yang and Saadatnia[29,51]. However, the changes in root length and stem length by biomass concentrate were evident by day 20. The relative impact degrees on root length and stem length of each treatment group were in a descending order of group 2, group 3, and group 1 at day 20. Compared with the control group1 (CK), the average root length and stem length of *A. thaliana* in treatment group 2 were 43.0% and 55.0% longer than those of the control group 1 after the end of cultivation. This indicates that *Chlorella* has accumulated a large amount of N and P-containing nutrient salts, after cultivation in the swine wastewater, which contained high concentrations of these nutrients. The harvested biomass (*Chlorella* dominate) concentrate was used as a biofertilizer to grow *A. thaliana*, and thus, released intracellular nutrients, allowing the plants to obtain more nutrients.



a. Root length b. Stem length

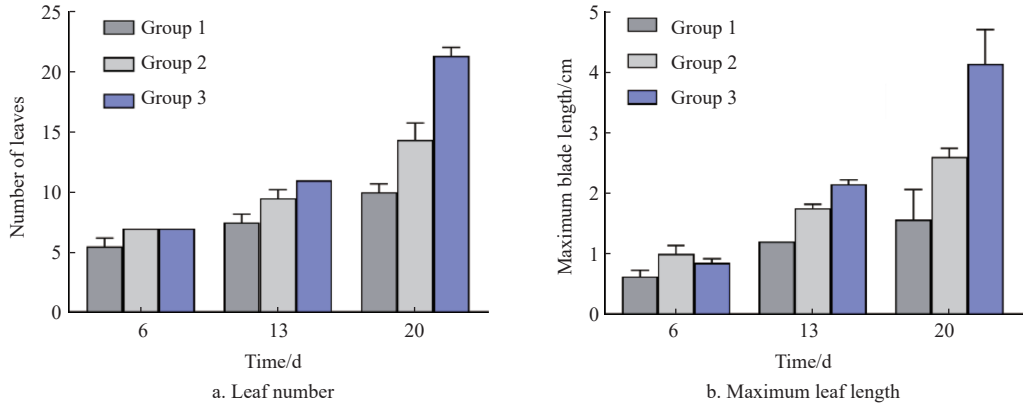
Figure 8 Root and stem lengths of *Arabidopsis* in different groups

3.4.2 Bio-fertility effects on *Arabidopsis* leaf number and maximum leaf length after biomass concentrate fertilization

To verify whether the harvested biomass concentrate contributed to the number of leaves and maximum leaf length in *Arabidopsis* thaliana, these were also monitored for 20 d, and the treatment results are shown in Figures 9a and 9b. As shown in Figure 9, the number of leaves and maximum leaf length of *Arabidopsis thaliana* grown over 20 d were in a descending order of group 3, group 2, and group 1. Initially (after 6 d), it was found that biomass concentrate did not significantly (p>0.05) enhance the leaf number and maximum leaf length in *Arabidopsis* compared with the control group 1. However, after 20 d, the biomass concentrate increased the number of *Arabidopsis* leaves by 30.2% and the maximum leaf length by 39.7%. This illustrates that the harvested biomass concentrate could promote the increase of *Arabidopsis* leaf number and maximum leaf length.

3.4.3 Bio-fertility of the fresh and dry weight of *Arabidopsis* leaves after fertilization with biomass concentrate

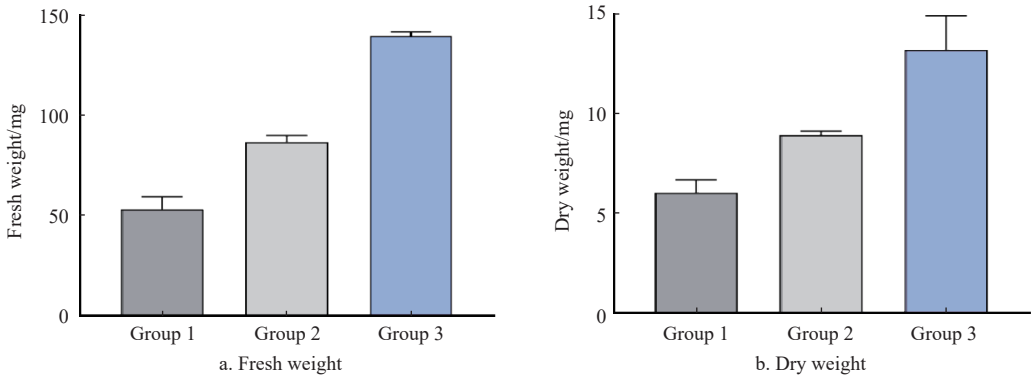
The bio-fertility effects on fresh and dry weights of *Arabidopsis* leaves were monitored when *Arabidopsis* was grown for 20 d, and the average fresh and dry weight results per plant are shown in Figures 10a and 10b. Compared with the group 1, biomass concentrate also showed no significant (p>0.05) increase in both the fresh weight and dry weight of *Arabidopsis*. As shown in Figures 10a and 10b, the fresh weight and dry weight of *Arabidopsis* in the group 2 were increased by 44.0% and 33.7% compared with the



a. Leaf number b. Maximum leaf length

Figure 9 Leaf number and maximum leaf length of *Arabidopsis* in different groups

group 1. And group 3 increased even more than group 2. This illustrates that biomass concentrate can increase the water content of *Arabidopsis* leaves and suppresses the loss of water in *Arabidopsis* leaves.



a. Fresh weight b. Dry weight

Figure 10 Effects of different treatment groups on fresh weight and dry weight of *Arabidopsis*

4 Conclusions

As a result of this work, after recycling the culture broth at a ratio of 1/6, 2.063 g/L of biomass could be accumulated at the end of the cultivation, and the highest removal rates of TN, NH_4^+-N, COD, and TP were 13.8, 11.5, 24.8 and 16.9 mg/L·d, respectively. Consequently, it was able to demonstrate the practical application of this system. The composition of *Chlorella* included mainly protein, carbohydrate, lipid, Chl-a, Chl-b, and carotenoids, were 44.9%, 19.9%, 24.6%, 2.75%, 1.66%, and 0.57% of the dry weight of the cells, respectively. The analysis of the composition of fatty acids in the lipids from the algal cells, revealed a total of 10 different fatty acids, mainly C16's and C18's; 96.3% of these fatty acids met the requirements for biodiesel feedstock.

The harvested biomass was used as a bio-fertilizer to grow *Arabidopsis*. The results showed that the average root length and stem length of *Arabidopsis* were 43.0% and 55.0% longer than those of the control group (which did not use the bio-fertilizer). Additionally, leaf number and maximum leaf length also increased by 30.2% and 39.7%; fresh and dry weights increased by 44.0% and 33.7%. These results clearly illustrated the feasibility of using *Chlorella* biomass as an effective bio-fertilizer. In this study, a system capable of handling the high levels of organic matter in swine farm wastewater was developed, tested and optimized utilizing a microalgae/bacteria co-culture combined with a novel closed-loop extraction and dilution process. The microalgae/bacteria system was used to remove the high concentration of nutrients from swine farm wastewater and obtain high value-added products. Biomass increased to 2.036 g/L, compared with 0.78 g/L found by others. Microalgal biomass fertilizers can produce plant growth hormones, polysaccharides, antibacterial compounds and other metabolites, which can improve soil fertility and quality while also promoting plant growth and meeting food needs without damaging the ecosystem.

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