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RESEARCH ARTICLE

Microalgae cultivation and culture medium recycling by a two-stage cultivation system

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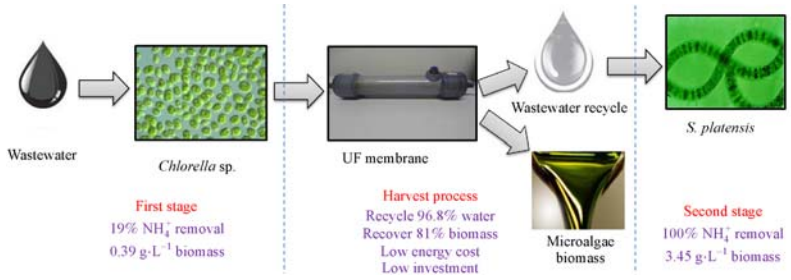
HIGHLIGHTS

- A two-stage system was designed for microalgae cultivation and nutrients removal.
- Two species of microalgae were cultivated for biomass production.
- UF costed less than centrifuge for harvesting microalgae at small scale.
- 100% NH_4^+ of the wastewater was removed and met discharge requirement.

GRAPHIC ABSTRACT



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ABSTRACT

Nutrients and water play an important role in microalgae cultivation. Using wastewater as a culture medium is a promising alternative to recycle nutrients and water, and for further developing microalgae-based products. In the present study, two species of microalgae, *Chlorella* sp. (high ammonia nitrogen tolerance) and *Spirulina platensis* (*S. platensis*, high growth rate), were cultured by using poultry wastewater through a two-stage cultivation system for algal biomass production. Ultrafiltration (UF) or centrifuge was used to harvest *Chlorella* sp. from the first cultivation stage and to recycle culture medium for *S. platensis* growth in the second cultivation stage. Results showed the two-stage cultivation system produced high microalgae biomass including 0.39 $g \cdot L^{-1}$ *Chlorella* sp. and 3.45 $g \cdot L^{-1}$ *S. platensis* in the first-stage and second-stage, respectively. In addition, the removal efficiencies of NH_4^+ reached 19% and almost 100% in the first and the second stage, respectively. Total phosphorus (TP) removal reached 17% and 83%, and total organic carbon (TOC) removal reached 55% and 72% in the first and the second stage, respectively. UF and centrifuge can recycle 96.8% and 100% water, respectively. This study provides a new method for the combined of pure microalgae cultivation and wastewater treatment with culture medium recycling.

1 Introduction

Microalgae have attracted considerable interest as a promising feedstock for biofuel production due to their high lipid content, high growth rate and greenhouse gas sequestration (Pires et al., 2012). However, during the microalgae cultivation, there are imperative factors limiting the large-scale microalgae production for biofuel, such as culture medium (nutrients and water). Nutrients use (e.g., nitrogen, phosphorus) can account for half of the cost and energy input in microalgae cultivation (Xia and Murphy, 2016). It is estimated 3726 kg water (84.1% of

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the water is discharged after harvest without recycling), 0.33 kg nitrogen and 0.71 kg phosphorous are required for producing 1 kg biodiesel (Yang et al., 2011).

Recycling culture medium is a sustainable approach to reduce cost during microalgae cultivation, especially for raceway ponds (Wang et al., 2013). Moreover, there are substantial amounts of nutrients in the harvest water that do not meet the discharge requirements (Zhu, 2015). Numerable studies have been conducted to recycle culture medium for microalgae growth (Loftus and Johnson, 2017), such as using bioflocculant as harvest method (Kim et al., 2011), developing a new growth medium (Hadj-Romdhane et al., 2012), coupling microalgae culture and harvesting in membrane photobioreactors (Bilad et al., 2014), using seawater with commercial chemicals (Sing et al., 2014). Additionally, microalgae growth can be improved by enhancing light-transfer efficiency through recycling culture medium and no additional nutrients replenishment (Huang et al., 2016).

Wastewater streams contain high nutrients, which can be a better solution to save water and nutrients for microalgae cultivation (Park et al., 2013; Zhu et al., 2013). Up to date, few researches have focused on pure cultivation of different species of microalgae using recycled wastewater. It is well-known that only with a proper nutrient concentration, microalgae can grow and multiply quickly. Our previous study showed that *Chlorella* was more tolerate to wastewater than *Spirulina* (Wang et al., 2015). Hence, *Chlorella* is usually used to treat wastewater containing more nutrients or with low dilution ratio. It was found that *Chlorella* growth was inhibited in the wastewater with an ammonia concentration of 260 $mg \cdot L^{-1}$ (Konig et al., 1987). As for *Spirulina*, with a fast growth rate, it is commonly used to treat slightly polluted wastewater with the ammonia nitrogen concentration below 40 $mg \cdot L^{-1}$ (Chang et al., 2013). In this study, a two-stage cultivation system was developed to investigate a combination of wastewater recycling and cultivation of different microalgae species.

In this work, two microalgae species were selected to treat and recycle poultry wastewater through a two-stage cultivation system. In the first-stage, *Chlorella* sp. was cultured to consume the nutrients (especially ammonia nitrogen) in wastewater to a low level. During this stage, ultrafiltration (UF) and centrifuge were investigated respectively as microalgae harvesting methods. In the second-stage, the cultivation of *S. platensis* utilized the residual nutrients in the recycled water from the first stage to gain more biomass. Therefore, to achieve the goal of high nutrients recovery and biomass production, *Chlorella* was chosen for nutrients removal in the first cultivation and *Spirulina* was chosen for biomass production in the second cultivation. The present study provides a novel method for wastewater treatment and reuse, as well as microalgae production.

2 Materials and methods

2.1 Characteristics of wastewater

The poultry wastewater was obtained from a biogas plant of Minhe Biological Technology Co., China and was collected from the permeate stream of a UF process during liquid bio-fertilizer production process. Here, this wastewater was referred as fertilizer wastewater (FW) in this study. The UF treated FW was not further disinfected prior to the microalgae cultivation. The pH of the FW (Table 1) was 8.08.

2.2 Microalgae species

S. platensis (FACHB-314) and *Chlorella* sp. (FACHB1067) were obtained from the Institute of Hydrobiology of the Chinese Academy of Sciences in Wuhan, China, and were cultivated in standard culture mediums, Zarrouk (Watanabe and Hall, 1995) and BG-11 (Rippka et al., 1979), respectively. The microalgae were cultivated at 28°C. The light intensity was 170 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and daily lighting schedule was 12 h on and 12 h off.

2.3 Experimental procedures

Based on the NH_4^+ tolerance of *Chlorella* sp. and *S. platensis*, the FW was diluted to suitable NH_4^+ concentration in a two-stage microalgae cultivation system. In the first stage, *Chlorella* sp. was cultivated in wastewater with high NH_4^+ concentration. Then *Chlorella* sp. from the first stage was harvested by centrifugation or UF. Based on the

Table 1 Characteristics of the FW

Parameters	Concentration ($mg \cdot L^{-1}$)
TP (total phosphorus)	179±3
TN (total nitrogen)	3131±319
NH_4^+ (ammonia nitrogen)	2990±114
TOC (total organic carbon)	1563±18
IC (inorganic carbon)	3039±20
COD (chemical oxygen demand)	4058±125

preliminary test of *Chlorella* sp. and *S. platensis* in the recycled culture medium, *S. platensis* was selected to be cultivated in the second stage with wastewater at low NH_4^+ concentration.

2.3.1 Microalgae cultivation in the first-stage

Our previous study demonstrated *Chlorella* sp. could be cultivated in diluted FW with NH_4^+ concentration of 125–1300 mg·L^{-1}. As such, in the first-stage, *Chlorella* sp. was cultivated in the diluted FW at an NH_4^+ concentration of 400 mg·L^{-1}. Batch experiments were conducted in 2000 mL flasks with 160 mL microalgae broth, 200 mL FW and 1240 mL distilled water. The initial dry cell weight (DCW) of *Chlorella* sp. in the culture medium was 81 mg·L^{-1}. Parafilm was used for the flasks to avoid contamination. The pH was adjusted to 7.1±0.1 using 1 M NaOH and 1 M HCl solutions. The microalgae were cultivated under the same condition as mentioned in section 2.2. Microalgae from the first cultivation stage were then harvested by centrifuge or UF.

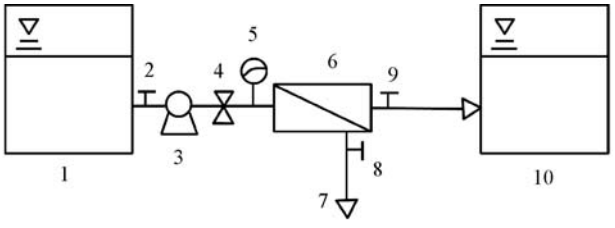
2.3.2 Microalgae harvest by centrifuge

The power of the centrifuge (3K15 Sigma Corporation) was 1010W. There were 6 tubes and each tube had a sample volume of 50 mL. The centrifugation was set for 8 min and 8000 r·min^{-1}. Supernatant was recycled for the next cultivation of microalgae in the second stage.

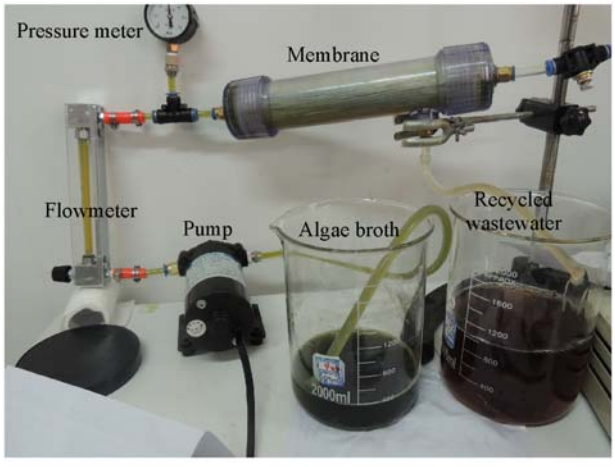
2.3.3 Microalgae harvesting with UF

The UF used hollow fiber hydrophilic polyethersulfone (PES) membranes (Chaoyu Company, Guangzhou, China). The nominal molecular weight cutoff specified by the manufacturer is 10000 Dalton. The initial flow rate of the UF membrane system was 10 L·h^{-1}. The initial pressure was 0.08 MPa and the applied pressure was controlled below 0.3 MPa. After harvesting the microalgae, UF membrane was backwashed using distilled water at a flow rate of 30 L·h^{-1}. A schematic diagram of algal harvesting system with UF membrane is shown in Fig. 1.

In the first stage, the flask was first filled with diluted FW and subsequently inoculated with *Chlorella* sp. broth. When the microalgae were in the steady growth phase in the flask (1), the control valve (2) was opened. The mixture of *Chlorella* sp. and culture medium were pumped to the UF unit (6) to separate microalgae and culture medium. Then the UF filtrate was recycled for *S. platensis* cultivation in the flask (10) via the valve (9). Once the



(a) Schematic diagram of microalgal harvesting system with membrane



(b) Process of microalgae harvesting

Fig. 1 Two-stage microalgae cultivation system with UF membrane. (a) Schematic diagram of microalgal harvesting system with membrane, 1. flask for *Chlorella* sp. cultivation, 2.8.9. control valve, 3. pump, 4. flow meter, 5. pressure gauge, 6. UF, 7. microalgae retentate, 10. flask for *S. platensis* cultivation; (b) process of microalgae harvesting.

harvest process completed, microalgae retentate (7) came from the valve (8). When the membrane was fouled, backwash would be conducted with distilled water going through valve (9) to valve (8).

2.3.4 Recycled water to cultivate microalgae

Preliminary test was performed to investigate the feasibility of using recycled wastewater for culturing *Chlorella* sp. and *S. platensis*. Wastewater and microalgae broth were inoculated in 250 mL flasks. Culture medium (BG11 or Zarrouk) was added as specified in Table 2. 0.02 $g \cdot L^{-1}$ Na_2CO_3 and 16.8 $g \cdot L^{-1}$ $NaHCO_3$ were added as the carbon sources of BG11 and Zarrouk culture medium, respectively.

2.3.5 Recycled water with different NH_4^+ concentrations to cultivate *S. platensis*

The effect of different NH_4^+ concentrations on *S. platensis* growth was further studied in the second stage. The FW was diluted with Zarrouk medium to achieve the NH_4^+ concentration of 15, 24, 30, and 60 $mg \cdot L^{-1}$, referred as NH_4^+-15, NH_4^+-24, NH_4^+-30, and NH_4^+-60 in the test runs, respectively. Inoculated with 50 mL algae broth, batch experiments were conducted with a volume of 160 mL in 250 mL flasks. Zarrouk medium was used as blank control. The condition of microalgae cultivation was the same as that of the first stage.

2.4 Analysis methods

DCW of microalgae was measured using 0.45 μm pore size glass fiber filter (Midwest Group, China) and dried overnight in an oven. Total phosphorus (TP), NH_4^+ and total nitrogen (TN) of water samples were analyzed following the Environment Protection Agency Standard Methods (SEPA, 2002). The TOC and inorganic carbon (IC) of water samples were tested by a Total Organic-Carbon Analyzer TOC-VCPN (Shimadzu Corporation Company, Japan). The pH and light intensity were monitored using a PSH-3 pH meter (Shanghai Precision and Scientific Inc., China) and a LI-250A light meter (LI-COR Inc., Canada), respectively. All the experiments were conducted in duplicates, and the reported results are the average values.

Daily productivity is calculated according to the following Eq. (1):

$$\text{Daily productivity } (g \cdot L^{-1} \cdot d^{-1}) = \frac{DCW_i - DCW_0}{t_i - t_0}, \quad (1)$$

where DCW_i and DCW_0 are the dry cell weight ($g \cdot L^{-1}$) at time t_i and t_0 (initial time), respectively. The specific growth rate (μ) in the exponential phase of algal growth is calculated as below Eq. (2) (Zhu et al., 2013):

$$\mu(day^{-1}) = \ln(d_{w2}/d_{w1})/(t_2 - t_1), \quad (2)$$

where d_{w1} and d_{w2} represent dry biomass ($g \cdot L^{-1}$) at time t_1 and t_2, respectively.

The removal quantity is calculated using the following Eq. (3):

$$\text{Removal quantity } (mg \cdot L^{-1}) = C_0 - C_i, \quad (3)$$

where C_i and C_0 are the final and initial concentration, respectively, of NH_4^+, TP and TC ($mg \cdot L^{-1}$). The removal efficiency is calculated using the following Eq. (4):

$$\text{Removal efficiency } (\%) = \frac{C_0 - C_i}{C_0} \times 100, \quad (4)$$

where C_i and C_0 are the final and initial concentration, respectively, of TN, TP and TC ($mg \cdot L^{-1}$). The volume concentration factor (C) is expressed as Eq. (5) (Huang et al., 2012):

$$C = \frac{V_0}{V_i}, \quad (5)$$

where V_0 is the initial volume of the microalgae broth before the concentration process and V_i is the final volume of microalgae broth after the concentration process. Biomass recovery rate (BRR) is calculated using the following Eq. (6) (Huang et al., 2012):

Table 2 Summary of experiments using recycled wastewater to cultivate microalgae

Tests	Algae species	Culture medium	Harvest method
C-C1	*Chlorella sp.*	BG11 medium	Centrifuge
C-C2	*Chlorella sp.*	Carbon source of BG11 medium	Centrifuge
C-M1	*Chlorella sp.*	BG11 medium	Membrane
C-M2	*Chlorella sp.*	Carbon source of BG11 medium	Membrane
S-C1	*S. platensis*	Zarrouk medium	Centrifuge
S-C2	*S. platensis*	Carbon source of Zarrouk medium	Centrifuge
S-M1	*S. platensis*	Zarrouk	Membrane
S-M2	*S. platensis*	Carbon source of Zarrouk medium	Membrane

Note: 0.02 $g \cdot L^{-1}$ Na2CO3 and 16.8 $g \cdot L^{-1}$ NaHCO3 were added as the carbon source of BG11 and Zarrouk culture medium, respectively.

$$\text{BRR} = \frac{V_i \times C_i \times 100}{V_0 \times C_0}, \quad (6)$$

where V_i and V_0 are the final and initial volume of the microalgae broth, respectively; and C_i and C_0 are the final and initial concentration of microalgae, respectively.

3 Results and discussion

3.1 Cultivate *Chlorella* sp. in the first stage

The characteristics of the diluted wastewater before and after microalgae cultivation, and dry cell weight of microalgae in the first stage are shown in Fig. 2.

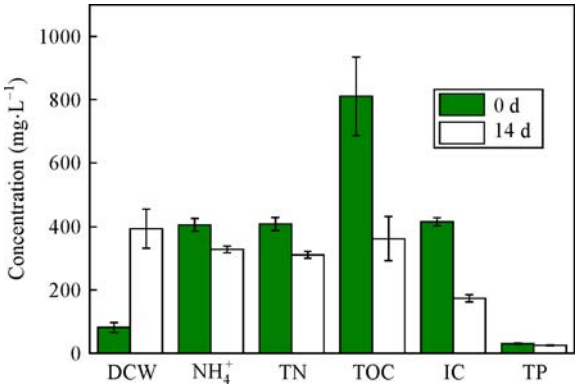


Fig. 2 Nutrients change of culture medium and DCW change of microalgae in the first stage. The error bars represent the standard deviation.

During the cultivation, the dry cell weight increased 3.8 times. The removal of TP, NH_4^+, TN, TOC, and IC were 17%, 19%, 24%, 55% and 58%, respectively. The removal of TOC and IC further implied that microalgae were cultured mixotrophically. The maximum specific growth rates of photosynthetic and heterotrophic *Chlorella* sp. were comparable, and the maximum specific growth rate of mixotrophic *Chlorella* sp. was almost equal to the sum of the former two values. Therefore, using the FW as culture medium for *Chlorella* sp. cultivation is feasible.

3.2 Comparison of harvesting microalgae by centrifuge and UF

When the microalgae reached in the plateau growth stage, they were separated in the UF unit and harvested by backwash which consumed 1500 mL of water. The results of microalgae harvested and water recycled by the UF process are shown in Fig. 3. The BRR of UF membrane was 81% (Fig. 3a), which is relatively lower than that of centrifuge (100%). About 96.8% water was recycled from

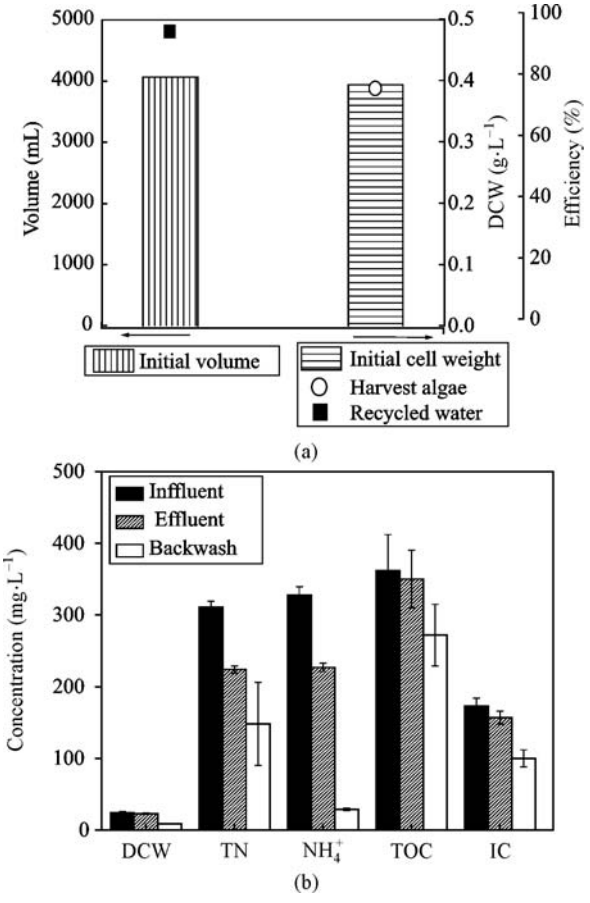


Fig. 3 Microalgae harvesting and water recycled with UF. (a) Efficiency of the harvested algae and recycled water; (b) water quality of FW, algae effluent, and membrane backwash water.

the UF process. Although centrifuge could recycle 100% of water, more water was needed for cleaning the centrifuge tubes.

The *Chlorella* sp. was condensed for 3 times and the final concentration of the microalgae reached 1.5 $g \cdot L^{-1}$ in this study. The result of this study was much lower compared with the results of Zhang et al. (2010), who enriched *Scenedesmus quadricauda* 150 times by UF membrane, and the final concentration of the microalgae reached 155 $g \cdot L^{-1}$. This difference might be due to the differences in initial concentration of dry cell weight and the harvesting conditions.

NH_4^+ concentration and TP concentration of recycled water before and after UF were 328 $mg \cdot L^{-1}$ and 24.6 $mg \cdot L^{-1}$, 227 $mg \cdot L^{-1}$ and 22.8 $mg \cdot L^{-1}$, respectively (Fig. 3b). Some microalgae could attach and be retained in the UF hollow fibers, which leads to a decrease of the nutrients in the water and causes flux decline. Membrane backwash would consume more fresh water. To avoid the usage of fresh water, it is recommended to use microalgae broth for membrane backwash from valve 2 to valve 8 as illustrated in Fig. 1.

3.3 Recycled wastewater to cultivate *Chlorella* sp.

The wastewater generated in the first stage was recycled to cultivate *S. platensis* and *Chlorella* sp. (Fig. 4).

For biomass production, there were negligible differences between centrifuge and UF ($p>0.05$), and between addition of carbon sources and culture medium ($p>0.05$). There was a significant reduction of *Chlorella* sp. growth in the second stage (a growth rate of 0.01 $g\cdot L^{-1}\cdot d^{-1}$) compared with that in the first stage (a growth rate of 0.026 $g\cdot L^{-1}\cdot d^{-1}$). Same result was also found in Zhu et al.'s study (Zhu et al., 2013). The phenomenon can be due to the reuse of supernatant as the culture medium, which may have inhibitory or toxic effects on the microalgae production (Hadj-Romdhane et al., 2013). Because the use of recycled supernatant had negative influence on the same microalgae strain, the recycled supernatant was used for cultivating a different algal species–*S. platensis*.

3.4 NH_4^+ concentration on *S. platensis* growth in the second-stage

The wastewater from the first stage was recycled to cultivate *S. platensis* (Fig. 4d). The preliminary study showed the pH of the solution remained stable while the DCW of the microalgae declined. Therefore, it presumably implied that the NH_4^+ concentration of the recycled water exceeded the tolerance of *S. platensis.* The influence of NH_4^+ concentration on *S. platensis* growth is shown in Fig. 5.

As the microalgal biomass concentration increased, the pH of all the culture mediums increased. During the growth period of *S. platensis*, IC ($CO_3^{2-}$and HCO_3^-) was consumed (Abelson and Hoering, 1961). The consumption of IC led to a decrease of the H^+ concentration and resulted in the increasing pH of culture medium. After 15 days cultivation, the DCW of *S. platensis* in all runs increased to over 2.5 $g\cdot L^{-1}$, except that of NH_4^+-60. The growth rate of *S. platensis* from high to low was: NH_4^+-15 (0.22 $g\cdot L^{-1}\cdot d^{-1}$)>$NH_4^+$-24 (0.20 $g\cdot L^{-1}\cdot d^{-1}$)>$NH_4^+$-30 (0.19 $g\cdot L^{-1}\cdot d^{-1}$)>and NH_4^+-60 (0.001 $g\cdot L^{-1}\cdot d^{-1}$), which indicates that NH_4^+ in the recycled water plays an important role in the growth of *S. platensis*. These results are comparable to that of Yuan et al. (2011) which was in the range of 0.16–0.28 $g\cdot L^{-1}\cdot d^{-1}$.

During the growth period of *S. platensis*, almost 100% of NH_4^+ in the culture medium was removed (Fig. 5c). The NH_4^+ concentration after the microalgae cultivation met the discharge standards of pollutants for livestock and poultry breeding in China (GAQSIQ,2005). The removal

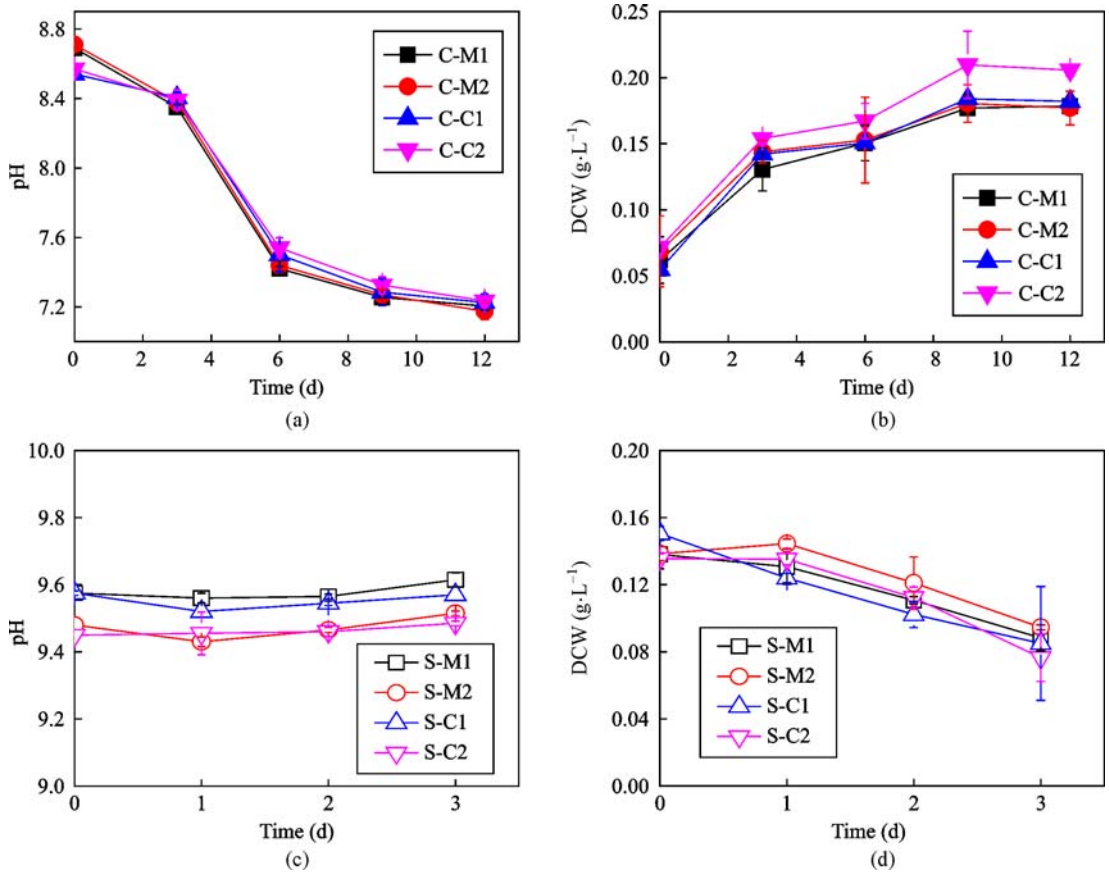


Fig. 4 Microalgae cultivation using recycled wastewater. (a) pH of the culture medium for *Chlorella* sp.; (b) DCW of *Chlorella* sp.; (c) pH of the culture medium for *S. platensis*; (d) DCW of *S. platensis*.

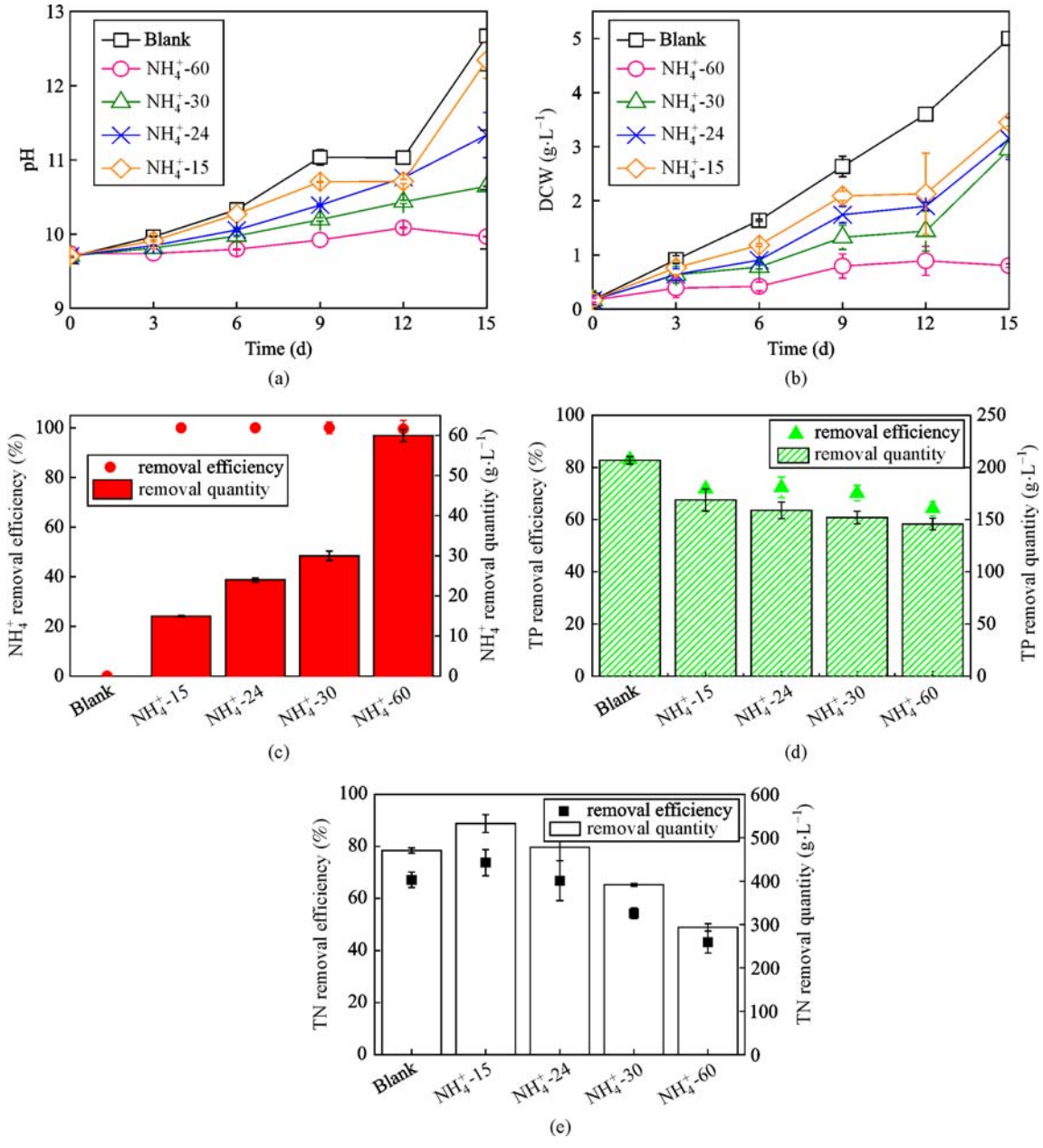


Fig. 5 Cultivation of *S. platensis* using in recycled water with different NH_4^+ concentrations. (a) pH of the culture medium, (b) the *DCW* of *S. platensis*. (c) NH_4^+ removal efficiency and removal quantity. (d) TP removal efficiency and removal quantity. (e) TN removal efficiency and removal quantity.

efficiencies were 25% higher than that in Canizares and Dominguez's study (Canizares and Dominguez, 1993). It was noticed that although *S. platensis* was inhibited in run NH_4^+-60, NH_4^+ of run NH_4^+-60 was still removed. This could be due to the higher temperature of culture medium (28°C) and a higher pH value, which favored the volatilization of ammonia from aqueous solution to air. The TP removal efficiencies ranked in the order of: Blank (83%)>NH_4^+-15 (72%)>NH_4^+-24 (71%)>NH_4^+-30 (70%)>NH_4^+-60 (64%) (Fig. 5d). The TN removal efficiency followed the same order of: NH_4^+-15 (74%) >NH_4^+-24 (67%)>NH_4^+-30 (54%)>NH_4^+-60 (43%), except that of the blank (67%) (Fig. 5e). Based on the nutrients removal efficiency and DCW, NH_4^+-15 run was most suitable for *S. platensis* growth.

3.5 Evaluation of the two-stage microalgae cultivation system

The present study provides a new approach to cultivate microalgae with recycled culture medium through a two-stage cultivation system. It has been well known that

dewatering is a major bottleneck to microalgae-based fuel production. Harvesting biomass from the algal broth was estimated contributing 20%–30% of the total cost of algal biomass production (Gudin and Thepenier, 1986). In addition to the chemical coagulation, membrane and centrifuge (Milledge and Heaven, 2013; Bilad et al., 2014a) are frequently used methods for microalgae harvesting which avoid the change in ash content during the harvest process.

There are two main components of cost during microalgae harvesting in this study: infrastructure investment and operation cost (Table 3). Because the power of the algae separation device varied from 200 W to 1000 W, energy consumption per cubic meter of dewatered biomass can vary widely from 8 kWh for centrifugation, 10–20 kWh for flotation processes, and 1 kWh for pressure filtration (Cheryan, 1998). Membrane system is relatively less expensive due to low capital and operation cost, but with a short service life. Backwash is generally needed to minimize membrane fouling, which would consume more energy and water. In this study, the investment of centrifuge is rather high, over 100 times higher than that of membrane system. Although the centrifuge can recover considerably high solid content of microalgae and has extensive service life (likely over 20 years), for smaller scale applications such as for the algae broth volume below 20,000 L, membrane is a better option in microalgae harvesting due to lower cost (Mackay and Salusbury, 1988).

In this study, the volume concentration factor of using the UF was 2, lower than the results reported by Hwang et al. (2013). This might be due to the small testing scale of the microalgae used in this study. Volume concentration factor of microalgae dewatering varies based on membrane configuration, initial concentration of microalgae and algae species (Mo et al., 2015). The harvesting efficiency of the centrifuge could be over 90%. Unit biomass production cost of centrifuge for raceway ponds, tubulars and flat panels were 1.19, 0.43, and 0.39 €·kg^{-1}, respectively (Norsker et al., 2011). Energy consumption was reduced by 82% when only 28.5% of the incoming algal biomass was harvested by centrifuge (Dassey and Theegala, 2013). Furthermore, membrane filtration can be coupled with the centrifuge using in microalgae harvesting to improve the harvesting efficiency. And this strategy would save half of energy consumption and ownership cost (Monte et al., 2018).

Table 4 shows the characteristics of different stages in the two-stage cultivation system. The removal of NH_4^+, TP, and TN at the second-stage was 81%, 32% and 59% higher compared with that of the first-stage. While, the biomass accumulation of *S. platensis* in the second-stage was 10 times higher than that of *Chlorella* sp. in the first-stage. Thus, it is important to consider the different characteristics of different algal species during microalgae cultivation. Furthermore, because different cultivation modes could also lead to a high biomass production (Farooq et al., 2013), the cultivation mode could be investigated in this two-stage cultivation system for further research. Wastewater is regarded as a free resource for

Table 3 Comparison of harvesting methods using UF, microfiltration (MF), and centrifuge

	Algae species	Infrastructure investment	Energy consumption	Operating condition (pressure, flux or flow rate)	Volume (or Scale)	Maximum concentration (or C)	Reference
UF	*S. costatum* & *H. ostrearia*	\$500 – 3500	3 – 10 kWh·m^{-3}	40 L·h^{-1}·m^{-2}	4 L	(>20)	(Rossignol et al., 1999)
UF	*Chlorella pyrenoidosa*	n.a.	n.a.	130 – 180 kPa	32 L	7.77 g·L^{-1} (11.4)	(Sun et al., 2013)
UF	Mixture algae	n.a.	n.a.	0.16 – 13.0 L·min^{-1}	0.1 L	(5 – 40)	(Petrusevski et al., 1995)
MF	*Chlorella* sp.	n.a.	n.a.	40 – 60 kPa	2 L	n.d.	(Hung and Liu, 2006)
MF	*Scenedesmus* sp.	n.a.	0.70–2.23 kWh·m^{-3}	72.4 L·m^{-2}·h^{-1}	(Pilot-scale)	(150)	(Gerardo et al., 2013)
MF	*Nannochloropsis* sp.	n.a.	0.3 – 0.7 kWh·m^{-3}	103.4 – 206.8 kPa	(Bench-scale)	>150 g·L^{-1}	(Bhave et al., 2012)
Centrifuge	n.a.	\$275000	74 kWh·m^{-3}	113560 L·h^{-1}	8509347841 L (Large-scale)	100 g·L^{-1}	(Richardson et al., 2014)
Centrifuge	*Chlorella*-like wild algae	\$2506	73 kWh·m^{-3}	2000 r·min^{-1}	(Bench-scale)	800 g·L^{-1}	(Udom et al., 2013)
Continuous centrifuge	n.a.	n.a.	62000 kWh·m^{-3}	901 L·h^{-1}	3785 L (Pilot-scale)	342.9 g·L^{-1}	(Kovalcik, 2013)
UF	*Chlorella* sp.	\$79	1.6 kWh·m^{-3}	30 L·h^{-1}	4 L (Lab-scale)	1.5 g·L^{-1} (2)	this study
Centrifuge	*Chlorella* sp.	\$9447	404 kWh·m^{-3}	8000 r·min^{-1}	4 L (Lab-scale)	750 g·L^{-1}	this study

Table 4 Evaluation of two-stage microalgae cultivation system performance

Stage	Microalgae	μ (d^{-1})	Daily productivity ($g \cdot L^{-1} \cdot d^{-1}$)	TP removal (%)	NH_4^+ removal (%)	TOC removal (%)	IC removal (%)
First stage (Section 2.3.1)	*Chlorella* sp.	0.113	0.026	17	19	55	58
Second stage (Section 2.3.4)	*Chlorella* sp.	0.089	0.010–0.011	33–36	4–26	20–46	18–29
Second stage (Section 2.3.4)	*S. platensis*	0	0	2–9	4–39	9–16	7–20
Second stage (Section 2.3.5)	*S. platensis*	0.044–0.132	0.023–0.198	24–83	82–100	7–72	28–48

microalgae cultivation. Based on the study of Fret et al. (2017), recycling wastewater to cultivate microalgae can save more than 3.5 € kg^{-1} in this study.

4 Conclusions

Culture medium and nutrients is one of the bottlenecks for the sustainable development of microalgae industry. A two-stage cultivation system with wastewater recycling was studied to reduce the cost of culture medium and nutrients and increase microalgae biomass production. This system reached 3.84 $g \cdot L^{-1}$ microalgae biomass production and the highest removal efficiencies of TOC and TP in the second stage were 72% and 83%, respectively. 100% NH_4^+ of the wastewater was removed, which can meet the discharge requirement. Compared with centrifugation, UF is a better option for microalgae harvesting in small scale applications due to the lower cost. For this system, 3.5 € $\cdot kg^{-1}$ was saved for recycle the culture medium. This system not only harvests microalgae, but also produces more microalgae biomass with higher nutrients removal. The established system can provide an alternative method for microalgae industry and wastewater treatment plant.

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