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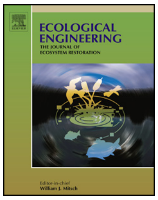

Continuous microalgae cultivation in aquaculture wastewater by a membrane photobioreactor for biomass production and nutrients removal

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ABSTRACT

An efficient continuous microalgae cultivation process for biomass production and nutrients removal from aquaculture wastewater was developed using a membrane photobioreactor (MPBR). *Chlorella vulgaris* and *Scenedesmus obliquus* were firstly batch cultured in aquaculture wastewater. *C. vulgaris* showed better performance with the specific growth rate of $0.17\,d^{-1}$ and was continuously cultivated in MPBR. The average volumetric biomass productivity in the MPBR operated at HRT of 1 day was $42.6\,mg\,L^{-1}\,d^{-1}$, which was 5.8-fold larger than that achieved in batch cultivation in flask. Advanced nutrients removal from aquaculture wastewater was also achieved in MPBR. The average reduction in TN and TP was 86.1% and 82.7%, respectively, after stabilization. The corresponding effluent concentration was below 1.30 and $0.12\,mg\,L^{-1}$ for TN and TP, respectively. Unionized ammonia, which is usually toxic to aquatic animals, was also effectively removed in MPBR, with effluent concentration below $0.002\,mg\,L^{-1}$.

1. Introduction

In recent years, land based intensive aquaculture has developed quickly all over the world to meet the increasing demand of aquatic food consumption. However, the continuous development of intensive aquaculture also has brought some problems, especially the issue of wastewater disposal (Mook et al., 2012; Piedrahita, 2003; Tovar et al., 2000; Martins et al., 2010; Chen et al., 2015). The wastewater discharged from the intensive aquaculture is usually concentrated with nutrients (nitrogen, phosphorus), which mainly come from fish excreta and feed residue (Crab et al., 2007). To date several biological and chemical methods have been successfully used in the process of removing theses nutrients to obtain a satisfactory quality of aquaculture effluent such as the common biological nitrification/denitrification process to remove nitrogen (Van Rijn, 1996; Boley et al., 2000) and chemical precipitation process to remove phosphorus (Ebeling et al., 2003). These methods, although effective, are less environmentally friendly since they produce chemical waste or sludge as by-product, which are usually considered to be pollutions of the environment.

Photoautotrophic microalgae can effectively transform the inorganic nutrients, CO_2, H_2O and other substances into organic compounds such as protein, carbohydrate, lipid and other ingredients through photosynthesis. Some studies have demonstrated that growing microalgae in aquaculture wastewater is possible (González-López et al., 2013; Michels et al., 2014; Guo et al., 2013; Van Den Hende et al., 2014; Nasir et al., 2015). And the cultivation of microalgae in aquaculture wastewater offers the combined advantages of nutrients removal and simultaneously production of algal biomass, which can be used for the production of valuable products such as aquaculture feed, health products and biofuels (Joseph et al., 1988; Michels et al., 2014). But, at present microalgae cultivation is not yet a competitive method of nutrients removal in intensive aquaculture industry mainly because of the slow growth rate of microalgae in aquaculture wastewater. Thereby a long hydraulic retention time (HRT) is usually needed for nutrients uptake. Some previous studies of batch microalgae cultivation in aquaculture wastewater showed that most of the nutrients could be removed by the assimilation of microalgae cells (Guo et al., 2013; Ji et al., 2013; Nasir et al., 2015). But it should also be noted that the time of

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batch cultivation in these studies was rather long (about 10 days). Consequently, a large reactor volume will be required in the actual project.

In photobioreactor high concentration of microalgae and high loading of nutrients are usually necessary to maintain a high growth rate of microalgae. Since the concentration of nitrogen and phosphorus in aquaculture wastewater is far below that in the traditional cultivation media such as BG11, a high supply flow rate of aquaculture wastewater is necessary to support high nutrients loading. But this will also lead to the wash out of microalgae cells in the conventional photobioreactor, in which HRT and biomass retention time (BRT) usually were not independently controlled (Tang et al., 2012; Marbelia et al., 2014).

Recently, a new design of photobioreactor equipped with submerged membrane module which allows independent control of the HRT and BRT was proposed (Singh and Thomas, 2012; Honda et al., 2012; Marbelia et al., 2014; Bilad et al., 2014; Gao et al., 2014). This way, higher microalgae productivity and nutrients removal rate may be obtained when the reactor operated with large supply rate of culture medium (Honda et al., 2012; Marbelia et al., 2014; Gao et al., 2014). The independent control of HRT and BRT is also necessary for the production of concentrated microalgae biomass (Marbelia et al., 2014; Gao et al., 2015). Therefore, the concentration of algal biomass in the reactor can be free from the influent nutrients concentration and the growth rate of the microalgae cells. This is conducive to both algal biomass production and nutrients removal especially when low-strength wastewater such as aquaculture wastewater was used for microalgae cultivation. Although several studies have investigated microalgae culture in aquaculture wastewater in a batch cultivation mode (Guo et al., 2013; Ji et al., 2013; Nasir et al., 2015). A study of using submerged membrane filters in photobioreactor for concentrated microalgae cultivation in aquaculture wastewater is needed, and the membrane photobioreactor (MPBR) performance including algal biomass production and nutrients removal should be investigated in detail.

In this study, two microalga species were firstly cultured in batch mode to evaluate the growth rate and biomass productivity of microalgae in aquaculture wastewater. Subsequently, continuous flow MPBR seeded with the best performance species was then operated to evaluate the biomass productivity and nutrients removal efficiency from aquaculture wastewater of this process.

2. Materials and methods

2.1. Microalgae and wastewater

Two algal species, *Chlorella vulgaris* and *Scenedesmus obliquus* from the Culture Collection of Algae, Institute of Hydrobiology, Chinese Academy of Sciences were used as inocula in this study. Algal cells were pre-cultivated in 1000 mL flasks with BG11 medium under stationary condition at 25 °C, continuous white fluorescent light illumination (about 12,000 Lux) and shaking at 100 rpm. The aquaculture wastewater used in this study was collected from an aquaculture rearing tank of *Penaeus vannamei Boone* located in Zhoushan, China, and letting it stand overnight. Then the clear supernatant was collected and used for the experiment. The average values of the principal chemical compound concentration of the clear supernatant were summarized in Table 1. The salinity of this wastewater was 2.8%.

2.2. Batch cultivation

The microalgae cells in logarithmic growth phase were collected by centrifugation (8000 rpm, 15 min), and then seeded to 1000 mL flasks containing 800 mL aquaculture wastewater. For each algal species, three replicate flasks were used, and the average biomass concentration was about 0.025 g L^{-1}. All the flasks were placed in a shaker operated at 100 rpm and 25 °C under continuous white fluorescent light illumination (about 12,000 Lux). During the culture interval, algal biomass concentrations were measured daily to investigate the biomass productivity in aquaculture wastewater of *C. vulgaris* and *S. obliquus*.

Table 1
Characteristics of the aquaculture wastewater used in this study. Values are in mg L^{-1} except the pH.

Compound	mean ± SD	Compound	Mean ± SD
TAN	4.24 ± 0.38	TN	6.81 ± 0.68
BOD_5	8.5 ± 1.9	TP	0.42 ± 0.05
NO_2^--N	0.13 ± 0.07	pH	7.78 ± 0.31
NO_3^--N	2.00 ± 0.23		

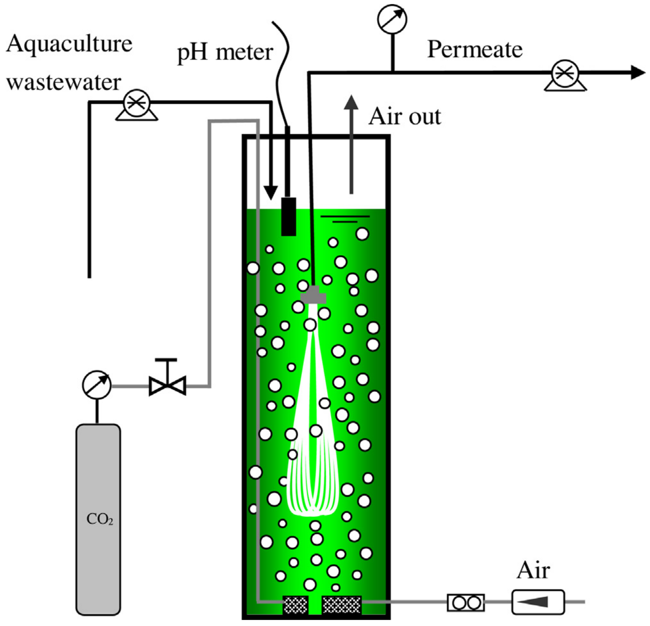

Fig. 1. Schematic diagram of the lab-scale MPBR.

2.3. Lab-scale MPBR

The lab-scale cylindrical MPBR with an internal diameter of 7.2 cm was constructed in transparent plexiglass (Fig. 1). The total and working volume of the reactor were 5 and 4 L, respectively. A polyvinylidene fluoride (PVDF) hollow-fiber MF membrane module that used as a solid–liquid separator was submerged in the middle of the reactor. The pore size of the membrane was 0.1 μm and the effective area of membrane surface in the module was 0.05 m^2. Two LED lamps with a red/blue light ratio of 4:1 were placed at a distance of 2 cm from the surface of the reactor. The power of each LED lamp was 9 w. At the bottom of the reactor two gas distributors were installed. Air was pumped into the reactor through one of the distributors to form bubbles which provided agitation in the column and no other mixing was used. Pure CO_2 (99.9%) from a pressurized cylinder was injected into the reactor through another distributor to adjust the pH value in the reactor.

2.4. Continuous cultivation in MPBR

The fastest-growing microalga species in the study of batch cultivation was exploited again in continuous cultivation in three parallel MPBRs. The microalgae cells in logarithmic phase were collected and then were seeded to the reactors to give an average inoculum of about 0.41 g L^{-1}. After inoculation, aquaculture wastewater was continuously supplied to the reactors as cultivation medium with HRT of 1 day. Membrane permeate was intermittently withdrawn by suction pumps, which operated in 12-min on/3-min off cycle. For the entire duration of the experiments, the maximum light intensity on the surface of the reactor was about 9000 Lux. Air was feed to the reactors at a constant rate of 0.5 L $\min^{-1}$. All the reactors were located indoor and the temperature was maintained at 25 ± 2 °C, and the pH of the culture liquor was in the range 6.8–7.2.

2.5. Analyses

The dry biomass concentrations of microalgae both in batch cultivation and continuous cultivation were determined gravimetrically. Algal cells were centrifugally collected (7000*g*, 10 min, 4 °C), and then freeze dried and weighed (Liu et al., 2013). During the continuous cultivation experiment, water samples were taken daily from the inflow and outflow of the MPBRs to evaluate the water quality. Only samples from the inflow were filtered with a 0.45 μm filter before analysis. Determination of total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), total nitrogen (TN), total phosphorus (TP), BOD_5 was carried out according to Chinese standard analytical methods for the examination of water (State Environmental protection Administration of China, 2002). Water pH was directly measured by a pH analyzer (PHB4, REX, China). At the end of the experiment, nutrients content of the microalgae biomass was analyzed to study the nutrients balance in MPBR. The nitrogen content of the microalgae biomass was calculated as the difference between TN and soluble nitrogen of the culture liquor in the reactors. The phosphorus content of the microalgae biomass was also calculated according to above method.

3. Results and discussion

3.1. Microalgae growth in aquaculture wastewater

Fig. 2 shows the growth curves of microalgae during the batch cultivation with aquaculture wastewater. No lag phases were observed in the two microalgae growth curves, suggesting that these two freshwater microalgae could adapt well in this saline aquaculture wastewater. Similar growth patterns, with 6 days of exponential growth phase followed by stationary phase were presented both for *C. vulgaris* and *S. obliquus*. It can be calculated that the biomass productivities in the exponential growth rate for *C. vulgaris* and *S. obliquus* were 7.3 and 6.2 mg L^{-1} d^{-1} (Table 2), respectively. These biomass productivity values were far less than that achieved in other wastewaters containing higher nutrients such as municipal sewage and swine feedlot wastewater (Table 2), indicating that the nutrients level in aquaculture wastewater is insufficient to maintain high algal biomass productivity in batch culture mode.

Continuous supply of aquaculture wastewater can maintain higher nutrients loading for microalgae production. But this will also lead to the loss of the algal cells, which is suspended in the water. Tang et al. (2012) reported that the changes of the dilution rate of the continuous flow photobioreactor had a significant effect on the biomass concentration and biomass production of microalgae. In this study, the average specific growth rate in the first 6 days were 0.17 d^{-1} for *C. vulgaris* and 0.15 d^{-1} for *S. obliquus* (calculated from the data in Fig. 2). It could be calculated that the generation times of *C. vulgaris* and *S. obliquus* in batch cultivation in this study were 4.07 and 4.62 days, respectively. Given that the growth rate of microalgae in continuous cultivation is similar to that in batch cultivation, and then a long HRT of about 5 days is needed to keep the microalgae from washing out of the continuous flow photobioreactor. Consequently, a photobioreactor with very large volume is then required.

Moreover, as Fig. 2 presents, the increased biomass concentrations of the microalgae through the culture interval were very low. The biomass growth concentrations in the exponential growth phase for *C. vulgaris* and *S. obliquus* were 0.044 and 0.037 g L^{-1}, respectively. This would result in a very high cost of harvesting. Therefore, it can be concluded that, without independent control of HRT and BRT, aquaculture wastewater with low concentration of nutrients cannot be used as a suitable medium for microalgae cultivation.

3.2. Algal biomass production in MPBR

As *C. vulgaris* showed better performance in terms of biomass productivity and specific growth rate in the batch cultivation study, it was exploited again in MPBR for continuous biomass production and nutrients removal from aquaculture wastewater with HRT of 1 day. The filtration effect of the membrane module submerged in MPBR can enable the reactor to operate with continuous supply and without the wash out of microalgae cells, therefore concentrated microalgae cultivation can be achieved in MPBR (Honda et al., 2012; Gao et al., 2014). In this study, as Fig. 3 presents, dry biomass concentration of *C. vulgaris* in the reactors kept increasing through the culture interval. The average volumetric biomass productivity in the MPBR was found to be 42.6 mg L^{-1} d^{-1}, which was 5.8-fold larger than that achieved in exponential phases in batch cultivation with the same alga strain and wastewater (Table 2). This volumetric biomass productivity was also higher or similar to that achieved in batch cultivation in past studies using municipal, industrial or poultry breeding wastewater (Sacristán de Alva et al., 2013; Gao et al., 2014; Liu et al., 2013; Chinnasamy et al., 2010), although the nutrients concentration in these wastewaters were much higher than that in aquaculture wastewater used in this study (Table 2). Therefore, higher volumetric biomass productivity and concentrated cultivation in low nutrient wastewater such as aquaculture wastewater were achieved in MPBR.

3.3. Nutrients removal in MPBR

The removal of nutrients from aquaculture wastewater in MPBR was also investigated through the continuous culture interval. As shown in Fig. 4, effluent TN and TP concentration of the reactors initially declined in the first 4 days and then remained at lower levels in the rest of the cultivation. The average reduction of nutrients achieved in the MPBR was 86.1% for TN and 82.7% for TP after stabilization, and the corresponding effluent concentration was below 1.30 and 0.12 mg L^{-1} for TN and TP, respectively. This reduction of nutrients from aquaculture wastewater is comparable to that achieved in batch cultivation in past studies (Guo et al., 2013; Nasir et al., 2015). Although a much longer HRT of above 10 days was used in these previous studies (Table 3). This indicated that the HRT of microalgae-based wastewater treatment process can be effectively reduced with the utilization of MPBR. Then a much smaller reactor volume is required for the microalgae-based wastewater treatment system. Also, the influent rate of the reactor can be dramatically enhanced due to shorter HRT. Therefore, high nutrients loading with diluted wastewater was achieved in MPBR by installing the submerged membrane filter in the reactor as a solid-liquid separator. This is conducive for the improvement of both the biomass production and the nutrients removal rate of the reactor (Honda et al., 2012; Gao et al., 2014).

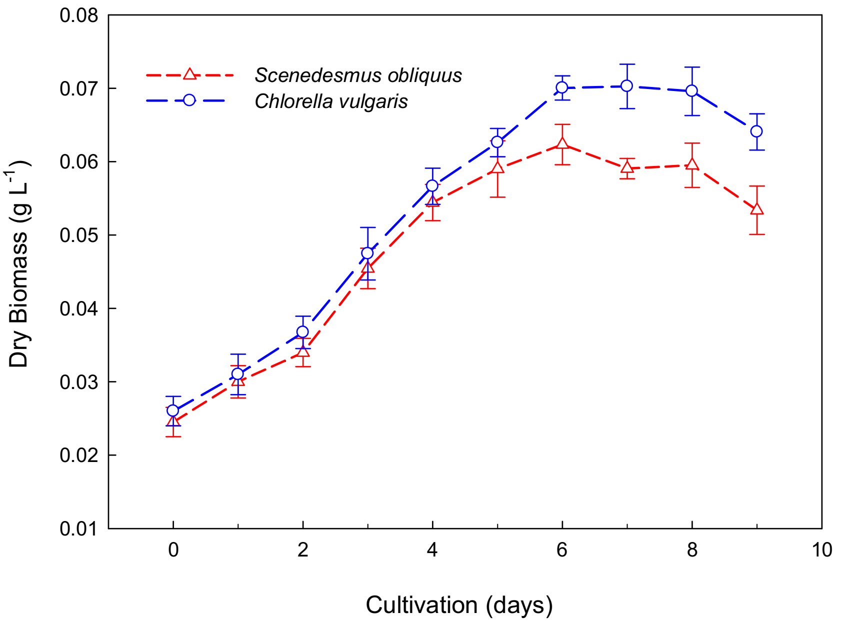

Fig. 2. Algal growth curves of batch cultivation with aquaculture wastewater (mean ± SD, n = 3).

Table 2
Comparison of the cultivation conditions and biomass production with the previous studies.

Study	Wastewater type	Nutrients concentration ($mg\,L^{-1}$)		Alga cultivation	Alga strain	Biomass Productivity ($mg\,L^{-1}\,d^{-1}$)
		Nitrogen[a]	Phosphorus[b]			
This study	Aquaculture	6.81	0.42	Batch culture in PBR	*Chlorella vulgaris*	7.3[f]
This study	Aquaculture	6.81	0.42	Batch culture in PBR	*Scenedesmus obliquus*	6.2[f]
This study	Aquaculture	6.81	0.42	Continuous culture in MPBR	*Chlorella vulgaris*	42.6[g]
Sacristán de Alva et al. (2013)	Municipal	62.8[c]	9.5[e]	Batch culture in flask	*Scenedesmus acutus*	73.7
Gao et al. (2014)	Treated sewage	19.12	1.24	Batch culture in PBR	*Chlorella vulgaris*	10.36
Liu et al. (2013)	Swine feedlot	348.2	26.62	Batch culture in flask	*Botryococcus braunii*	32.9
Chinnasamy et al. (2010)	Carpet mill	32.6–45.9[d]	5.47–13.83	Batch culture in flask	*Chlorella saccharophila*	23

[a] Total nitrogen (TN) unless specified.
[b] Total phosphorus (TP) unless specified.
[c] Ammonia nitrogen + organic nitrogen + nitrate nitrogen.
[d] Total Kjeldahl nitrogen (TKN).
[e] Orthophosphate phosphorus (PO_4^{3-}-P).
[f] Biomass productivity of exponential phases in the first 6 days.
[g] Biomass productivity of the whole continuous culture interval in MPBR.

Table 3
Comparison of the operation conditions and nutrients removal performance of the treatment of aquaculture wastewater with the previous studies.

Study	Nutrients concentration in influent ($mg\,L^{-1}$)		Treatment method	HRT	Nutrients reduction (%)	
	Nitrogen[a]	Phosphorus[b]			Nitrogen	Phosphorus
This study	6.81	0.42	Microalgae cultivation in MPBR	1.0 d	86.1	82.7
Guo et al. (2013)	2.39	0.21[d]	Batch cultivation of microalgae	14 d	87–95	98–99
Nasir et al. (2015)	0.91[c]	2.6[d]	Batch cultivation of microalgae	14 d	72.1–94.3	63.1–92.2
Shi et al. (2011)	13.9	0.08	Constructed wetland	0.4–1.0 d	66.8	23.8
Klomjek and Nitisoravut (2005)	19.5–24.3[c]	7.8–8.8	Constructed wetland	2.0–5.0 d	18.0–65.3	12.2–40.5
Sharrer et al. (2007)	50.7–68.4	19.2–57.2	Membrane biological reactor (MBR)	40.8 h	91.8–95.5	65.2–96.1

[a] Total nitrogen (TN) unless specified.
[b] Total phosphorus (TP) unless specified.
[c] Ammonia nitrogen (NH_3-N).
[d] Orthophosphate phosphorus (PO_4^{3-}-P).

The nutrients reduction from aquaculture wastewater achieved in this study is also comparable to that achieved in traditional treatment processes such as constructed wetland and membrane biological reactor (MBR) with similar HRTs (Table 3). In addition, the algal biomass generated from the treatment of MPBR can be utilized for the production of biofuel, animal feeds, etc. Therefore, it can be deduced that MPBR is a competitive technology for the treatment of aquaculture wastewater.

Usually, nutrients removal processes in photobioreactor mainly include microalgae assimilation and chemical processes such as

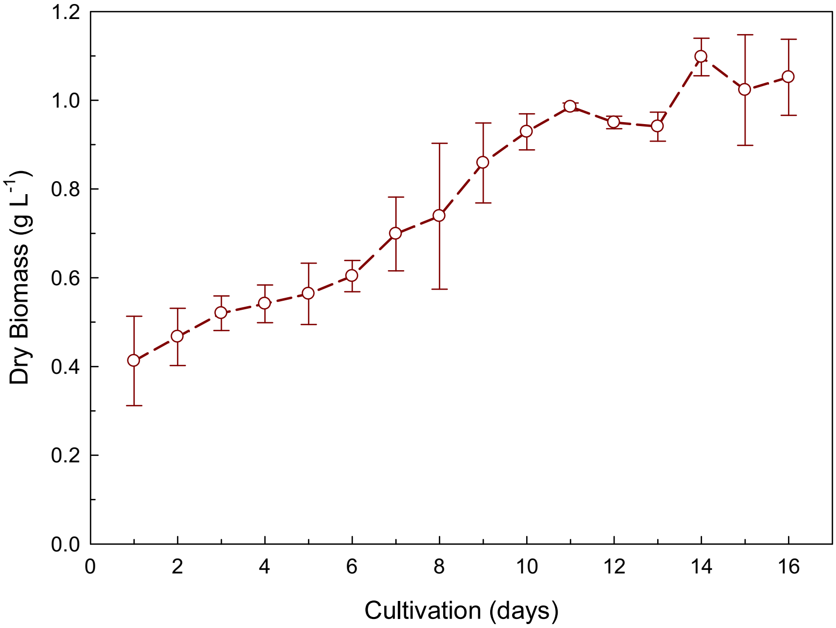

Fig. 3. Algal growth curves of continuous cultivation in MPBRs (mean ± SD, n = 3).

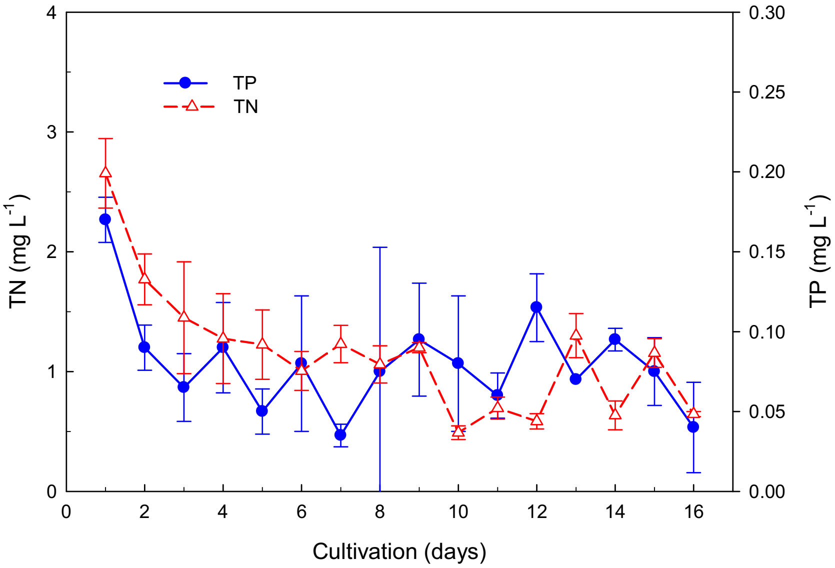

Fig. 4. Effluent concentrations of TN and TP of the MPBRs during the continuous culture interval (mean ± SD, n = 3).

chemical precipitation of phosphates and ammonia volatilization. Both of the chemical processes are enhanced by increasing the culture pH. As reported in previous studies, chemical processes could be considered as insignificant for the nutrients removal when the culture pH was kept around 7 (Ruiz-Marin et al., 2010; Gao et al., 2015). In addition, a neutral pH is also suitable for microalgae growth. In our case, the culture pH was in the range 6.8–7.2. Then, nutrients assimilation by microalgae cells could be considered as the main way of nutrients removal in this study. This hypothesis can be illustrated by the study of nutrients balance in the MPBR. At the end of cultivation, microalgae cells in the reactors were harvested to analyse their nutrients contents (mg g^{-1}, dry biomass). The average nutrients content of the microalgae biomass from the MPBRs was 12.7 ± 1.48% for N and 0.79 ± 0.12% for P, respectively. Based on the nutrients removal rate and biomass productivity (Table 2) in the reactors, it could be calculated that 93.3% of the eliminated N and 97.1% of the eliminated P during the operation period were assimilated by the microalgae cells.

The N:P ratio in the aquaculture wastewater used in this study (34.9 ± 6.3 on average) was considerably higher (more N rich) than the Redfield ratio (16:1) (Takahashi et al., 1985). Therefore, P could be considered as the limiting nutrient, and then was deeply reduced by the assimilation of algal cells. As presented in Fig. 4, in most of the culture time the average TP concentration in the effluent of the reactors was below 0.1 mg L^{-1}, and even sometimes it could be reduced to undetectable levels. Thus, nearly complete removal of P from the aquaculture wastewater was achieved in the MPBR.

Different from phosphate, inorganic nitrogen compounds usually are regarded as more toxic to aquatic animals (Lin and Chen, 2001; Schuler et al., 2010). Moreover, among the different inorganic nitrogen compounds, it has been established that ammonia (unionized) and nitrite are more toxic than nitrate (Tilak et al.,

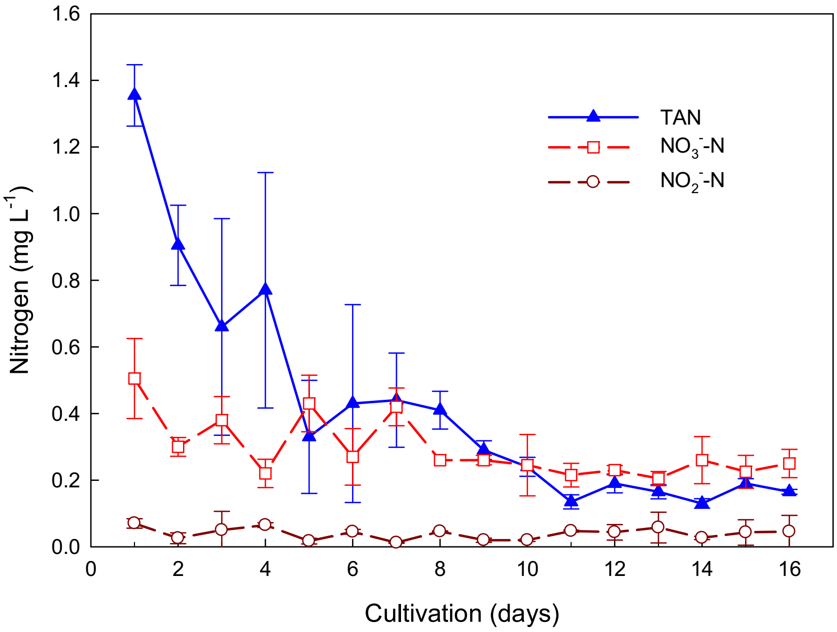

Fig. 5. Evolution of inorganic nitrogen concentrations in the effluent of MPBRs during the continuous culture interval (mean ± SD, n = 3).

2002; Alonso and Camargo, 2003). Tilak et al. (2002) studied the acute toxicity of different inorganic nitrogen compounds to the fish Catla catla, and the result indicated that the median lethal concentration (LC50) values for 24 h of unionized ammonia, nitrite and nitrate were 0.036, 117.43 and 1484.08 mg L^{-1} in continuous flow test respectively.

Concentration of unionized ammonia in the wastewater can be calculated as a function of pH, temperature and TAN by the expression (Anthonisen et al., 1976):

$$[NH_3\text{-N}] = \frac{TAN \times 10^{pH}}{K_b/K_w + 10^{pH}}$$

$$\frac{K_b}{K_w} = e^{(6344/273+t)}$$

where [NH_3-N] and TAN are the concentration of unionized ammonia nitrogen and total ammonia nitrogen in the wastewater respectively. In this study, based on the TAN concentration and pH of the aquaculture wastewater, it could be calculated that the NH_3-N concentration in the untreated wastewater was in the range of 0.023–0.041 mg L^{-1}. This value of NH_3-N concentration may be harmful to aquatic animals and also exceeded the national water quality standard (0.02 mg L^{-1}) for fisheries in China.

Microalgae cultivation in MPBR showed better performance of ammonia removal in this study. As shown in Fig. 5, compared to NO_3^--N, TAN in the effluent could be reduced to a lower value of concentration, although TAN was the main nitrogen forms in the influent (Table 1). And then it could be calculated that the effluent NH_3-N concentration of the reactors was also reduced to low values of below 0.002 mg L^{-1}. Thus, nearly complete removal of unionized ammonia from the aquaculture wastewater was achieved in the MPBR. This indicated that ammonia was the better nitrogen source than nitrate for the *C. vulgaris* species used in this study, and then could be deeply decreased by the assimilation of microalgae cells. Usually different algal species have different optimal nitrogen source (Li et al., 2008; Liu et al., 2013; Gao et al., 2014). Considering the greater toxicity of unionized ammonia to aquatic animals, selecting microalga species which preferentially utilize ammonium is needed for the treatment of aquaculture wastewater.

The levels of NO_2^--N concentration in the effluent of the reactors were very stable, and remained below 0.07 mg L^{-1} during the culture interval (Fig. 5). These levels of NO_2^--N concentration were well below the toxic levels for aquatic animals (Tilak et al., 2002; Alonso and Camargo, 2003).

4. Conclusions

This study demonstrates the potential of continuous microalgae cultivation in aquaculture wastewater for biomass production and nutrients removal by MPBR. Compared to batch cultivation in flask, continuous cultivation in MPBR achieved much higher volumetric biomass productivity of microalgae. In addition, MPBR could effectively remove most of the nutrients and nearly all the unionized ammonia from the aquaculture wastewater with a short HRT of only 1 day, which was well below that used in some previous batch cultivation studies. Thus, the nutrients removal rate of microalgae cultivation in aquaculture industries can be effectively enhanced with the use of MPBR.

According to our study, MPBR showed good performance both in microalgae biomass production and nutrients removal when low strength aquaculture wastewater was supplied as culture medium. The MPBR will be one of the most promising advanced treatment technologies to remove nutrients from diluted wastewaters such as aquaculture wastewater. In addition, MPBR is also a useful technology for concentrated microalgae cultivation with diluted wastewater.

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References

Alonso, A., Camargo, J.A., 2003. Short-term toxicity of ammonia, nitrite, and nitrate to the aquatic snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca). Bull. Environ. Contam. Toxicol. 70, 1006–1012.

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., Srinath, E.G., 1976. Inhibition of nitrification by ammonia and nitrous acid. J. Water Pollut. Contr. Fed. 48, 835–852.

Bilad, M.R., Arafat, H.A., Vankelecom, I.F., 2014. Membrane technology in microalgae cultivation and harvesting: a review. Biotechnol. Adv. 32, 1283–1300.

Boley, A., Müller, W.R., Haider, G., 2000. Biodegradable polymers as solid substrate and biofilm carrier for denitrification in recirculated aquaculture systems. Aquacult. Eng. 22, 75–85.

Chen, S., Yu, J., Wang, H., Yu, H., Quan, X., 2015. A pilot-scale coupling catalytic ozonation–membrane filtration system for recirculating aquaculture wastewater treatment. Desalination 363, 37–43.

Chinnasamy, S., Bhatnagar, A., Hunt, R.W., Das, K.C., 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresour. Technol. 101, 3097–3105.

Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., Verstraete, W., 2007. Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270, 1–14.

Ebeling, J.M., Sibrell, P.L., Ogden, S.R., Summerfelt, S.T., 2003. Evaluation of chemical coagulation–flocculation aids for the removal of suspended solids and phosphorus from intensive recirculating aquaculture effluent discharge. Aquacult. Eng. 29, 23–42.

Gao, F., Yang, Z.H., Li, C., Wang, Y.J., Jin, W.H., Deng, Y.B., 2014. Concentrated microalgae cultivation in treated sewage by membrane photobioreactor operated in batch flow mode. Bioresour. Technol. 167, 441–446.

Gao, F., Yang, Z.H., Li, C., Zeng, G.M., Ma, D.H., Zhou, L., 2015. A novel algal biofilm membrane photobioreactor for attached microalgae growth and nutrients removal from secondary effluent. Bioresour. Technol. 179, 8–12.

González-López, C.V., Cerón-García, M.C., Fernández-Sevilla, J.M., González-Céspedes, A.M., Camacho-Rodríguez, J., Molina-Grima, E., 2013. Medium recycling for *Nannochloropsis gaditana* cultures for aquaculture. Bioresour. Technol. 129, 430–438.

Guo, Z., Liu, Y., Guo, H., Yan, S., Mu, J., 2013. Microalgae cultivation using an aquaculture wastewater as growth medium for biomass and biofuel production. J. Environ. Sci. 25, 85–88.

Honda, R., Boonnorat, J., Chiemchaisri, C., Chiemchaisri, W., Yamamoto, K., 2012. Carbon dioxide capture and nutrients removal utilizing treated sewage by concentrated microalgae cultivation in a membrane photobioreactor. Bioresour. Technol. 125, 59–64.

Ji, M.K., Abou-Shanab, R.A., Kim, S.H., Salama, E.S., Lee, S.H., Kabra, A.N., Lee, Y.S., Hong, S., Jeon, B.H., 2013. Cultivation of microalgae species in tertiary municipal wastewater supplemented with CO_2 for nutrient removal and biomass production. Ecol. Eng. 58, 142–148.

Joseph, C.W., Raymond, P.G., John, R.B., 1988. Photobioreactor design: mixing, carbon utilization, and oxygen accumulation. Biotechnol. Bioeng. 31, 336–344.

Klomjek, P., Nitisoravut, S., 2005. Constructed treatment wetland: a study of eight plant species under saline conditions. Chemosphere 58, 585–593.

Li, Y., Horsman, M., Wang, B., Wu, N., Lan, C.Q., 2008. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. Appl. Microbiol. Biotechnol. 81, 629–636.

Lin, Y.C., Chen, J.C., 2001. Acute toxicity of ammonia on *Litopenaeus vannamei* Boone juveniles at different salinity levels. J. Exp. Mar. Biol. Ecol. 259, 109–119.

Liu, J., Ge, Y., Cheng, H., Wu, L., Tian, G., 2013. Aerated swine lagoon wastewater: a promising alternative medium for *Botryococcus braunii* cultivation in open system. Bioresour. Technol. 139, 190–194.

Marbelia, L., Bilad, M.R., Passaris, I., Discart, V., Vandamme, D., Beuckels, A., Muylaert, K., Vankelecom, I.F.J., 2014. Membrane photobioreactors for integrated microalgae cultivation and nutrient remediation of membrane bioreactors effluent. Bioresour. Technol. 163, 228–235.

Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P., d'Orbcastel Roque, E., Verreth, J.A.J., 2010. New developments in recirculating aquaculture systems in Europe: a perspective on environmental sustainability. Aquac. Eng. 43, 83–93.

Michels, M.H., Vaskoska, M., Vermuë, M.H., Wijffels, R.H., 2014. Growth of *Tetraselmis suecica* in a tubular photobioreactor on wastewater from a fish farm. Water Res. 65, 290–296.

Mook, W.T., Chakrabarti, M.H., Aroua, M.K., Khan, G.M.A., Ali, B.S., Islam, M.S., Hassan, M.A., 2012. Removal of total ammonia nitrogen (TAN), nitrate and total organic carbon (TOC) from aquaculture wastewater using electrochemical technology. Rev. Desali. 285, 1–13.

Nasir, N.M., Bakar, N.S.A., Lananan, F., Hamid, S.H.A., Lam, S.S., Jusoh, A., 2015. Treatment of African catfish *Clarias gariepinus* wastewater utilizing phytoremediation of microalgae, *Chlorella* sp. With *Aspergillus niger* bio-harvesting. Bioresour. Technol. 190, 492–498.

Piedrahita, R.H., 2003. Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. Aquaculture 226, 35–44.

Ruiz-Marin, A., Mendoza-Espinosa, L.G., Stephenson, T., 2010. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresour. Technol. 101, 58–64.

Sacristán de Alva, M., Luna-Pabello, V.M., Cadena, E., Ortíz, E., 2013. Green microalgae *Scenedesmus acutus* grown on municipal wastewater to couple nutrient removal with lipid accumulation for biodiesel production. Bioresour. Technol. 146, 744–748.

Schuler, D.J., Boardman, G.D., Kuhn, D.D., Flick, G.J., 2010. Acute toxicity of ammonia and nitrite to Pacific White Shrimp, *Litopenaeus vannamei*, at low salinities. J. World Aquacult. Soc. 41, 438–446.

Sharrer, M.J., Tal, Y., Ferrier, D., Hankins, J.A., Summerfelt, S.T., 2007. Membrane biological reactor treatment of a saline backwash flow from a recirculating aquaculture system. Aquacult. Eng. 36, 159–176.

Shi, Y., Zhang, G., Liu, J., Zhu, Y., Xu, J., 2011. Performance of a constructed wetland in treating brackish wastewater from commercial recirculating and super-intensive shrimp growout systems. Bioresour. Technol. 102, 9416–9424.

Singh, G., Thomas, P.B., 2012. Nutrient removal from membrane bioreactor permeate using microalgae and in a microalgae membrane photoreactor. Bioresour. Technol. 117, 80–85.

State Environmental protection Administration of China, 2002. Methods for Water Analysis, fourth ed. Environment Science press, Beijing.

Takahashi, T., Broecker, W.S., Langer, S., 1985. Redfield ratio based on chemical data from isopycnal surfaces. J. Geophys. Res. 90, 6907–6924.

Tang, H., Chen, M., Simon Ng, K.Y., Salley, S.O., 2012. Continuous microalgae cultivation in a photobioreactor. Biotechnol. Bioeng. 109, 2468–2474.

Tilak, K.S., Lakshmi, S.J., Susan, T.A., 2002. The toxicity of ammonia, nitrite and nitrate to the fish, *Catla catla* (Hamilton). J. Environ. Biol. 23, 147–149.

Tovar, A., Moreno, C., Mánuel-Vez, M.P., Garcíía-Vargas, M., 2000. Environmental impacts of intensive aquaculture in marine waters. Water Res. 34, 334–342.

Van Den Hende, S., Beelen, V., Bore, G., Boon, N., Vervaeren, H., 2014. Up-scaling aquaculture wastewater treatment by microalgal bacterial flocs: from lab reactors to an outdoor raceway pond. Bioresour. Technol. 159, 342–354.

Van Rijn, J., 1996. The potential for integrated biological treatment systems in recirculating fish culture—a review. Aquaculture 139, 181–201.