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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		

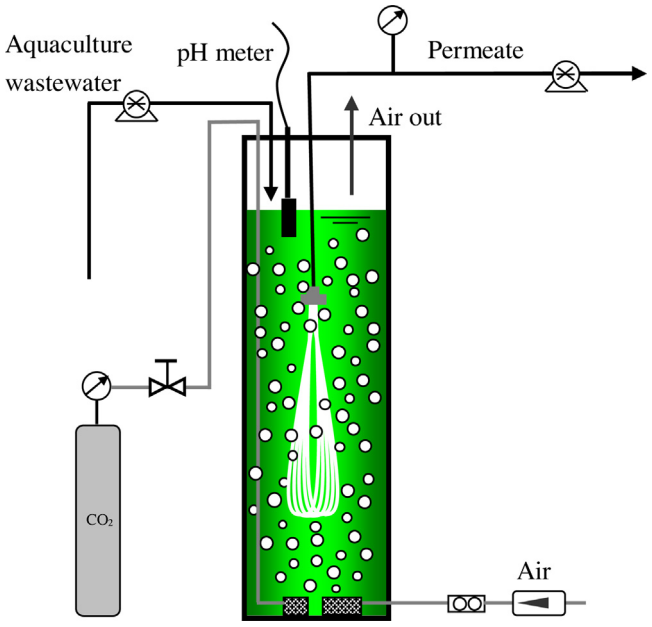

Fig. 1. Schematic diagram of the lab-scale MPBR.

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