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Simultaneous nutrient removal and biomass/lipid production by *Chlorella* sp. in seafood processing wastewater

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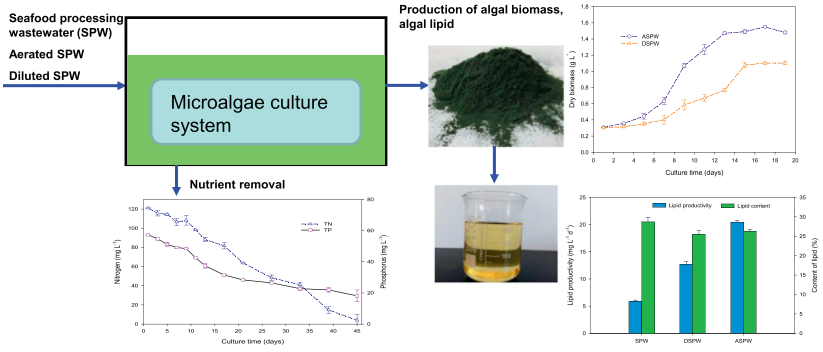
HIGHLIGHTS

- Seafood processing wastewater (SPW) was used to produce algal biomass/lipid.
- NH_3-N concentration in SPW increased rapidly in the first few days of cultivation.
- Aeration pretreatment could reduce the amount of toxic unionized ammonia in SPW.
- Aerated SPW supported the largest algal biomass and lipid productivity.
- Assimilation of microalgae removed only a small fraction of phosphorus in wastewater.

GRAPHICAL ABSTRACT



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ABSTRACT

Microalgae cultivation in wastewater has received increasing attention in recent years due to its many advantages. In this work, microalgae were cultured in seafood processing wastewater (SPW) for algal biomass and lipid production as well as nutrient removal. The biomass yield of *Chlorella* sp. achieved in the batch cultivation was 896 mg L^{-1}, indicating that SPW contains a certain amount of nutrients which can be used for the growth of microalgae. However, the maximum specific growth rate of *Chlorella* sp. cultured in SPW throughout the whole cultivation period was only 0.040 d^{-1}, suggesting that the growth of algal cells was inhibited during the culture process. High concentration of unionized ammonia in the SPW was found to be a factor inhibiting the growth of *Chlorella* sp. Aerated SPW (ASPW) and diluted SPW (DSPW) proved to be better culture media than SPW without pretreatment. The maximum specific growth rates of *Chlorella* sp. cultured in ASPW and DSPW during the culture interval were 0.156 and 0.091 d^{-1}, respectively. Aeration pretreatment of SPW reduced the amount of toxic unionized ammonia, while most of the nutrients were retained in the wastewater. Therefore, higher biomass productivity (77.7 mg L^{-1} d^{-1}) and higher lipid productivity (20.4 mg L^{-1} d^{-1}) of microalgae were achieved in ASPW. Additionally, improved nutrient removal rates from ASPW were also achieved due to the faster growth of microalgae. The average nutrient removal rates in ASPW during the whole cultivation period were 4.98 and 1.91 mg L^{-1} d^{-1} for nitrogen and phosphorus, respectively.

1. Introduction

Microalgae have emerged as an important biomass resource for the production of animal feeds, health products, pharmaceuticals and

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biofuels because of their high productivity and neutrality towards natural environments (Mata et al., 2010; Vigani et al., 2015; Katiyar et al., 2017). The photosynthesis of microalgae can potentially exceed 10%, which is 10–50 times greater than that of terrestrial plants (Rosenberg et al., 2011). Compared with plant-based crops, microalgae can also be adapted to a wider variety of water resources such as saline waster, brackish water and wastewater (Gao et al., 2015; Hom-Diaz et al., 2017). Hence, the commercial large scale production of algae is predicted to be a solution to the worldwide food and energy shortage. However, nowadays the costs of the production of algal biomass are still too high (Salama et al., 2017). A major cause of high algal biomass production costs is the large consumption of nutrients and water resources (Leite et al., 2013; Arbib et al., 2017). Nutrient expenses comprise the major part of the cost of algal biomass production (Christenson and Sims, 2011; Norsker et al., 2011). In addition, microalgae culture also requires a large amount of water, which comprises 10%–20% of the total cost of algae production (Subhadra, 2011; Sander and Murthy, 2010).

To make microalgae culture more cost-effective, many studies have suggested the combination of algal biomass production with wastewater treatment (Christenson and Sims, 2011; Sahu et al., 2013; Chen et al., 2015; Arias et al., 2017; Gao et al., 2018). Such a method would reduce the reliance on chemical fertilizers and generate environmental benefits by cleaning the wastewater (Bilanovic et al., 2012; Chen et al., 2015; Khiewwijit et al., 2018). After the cultivation of microalgae, algal cells should be harvested from wastewater by some methods, such as chemical or mechanical methods to realize the use of microalgae (Christenson and Sims, 2011). At the same time, it can also prevent ecological contamination such as algal bloom caused by the loss of the algal cells to the environment.

To date, a variety of wastewater types, such as municipal wastewater (Wang et al., 2010; Sacristán et al., 2013), livestock wastewater (Franchino et al., 2016) and aquaculture wastewater (Gao et al., 2016), have been successfully utilized for the production of microalgae. The growth of microalgae in some kinds of wastewater was even faster than that in special microalgae culture media such as BG11 (Liu et al., 2013; Luo et al., 2016).

At present, most studies on microalgae cultivation in industrial wastewater emphasize the removal of toxic pollutants such as metal ions and toxic organic compounds rather than nutrient removal and algal biomass production (Vílchez et al., 1997; De-Bashan and Bashan, 2010; Chen et al., 2015). There have been few studies involving microalgae production using industrial wastewater, i.e., microalgae cultivation in carpet mill wastewater (Chinnasamy et al., 2010), electric factory effluent (Su et al., 2011) and meat processing wastewater (Lu et al., 2015).

In recent years, large amounts of seafood processing wastewater (SPW) have been generated from the seafood industry. As a result, the potential pollution of these wastewaters has received increasing attention (Sohsalam et al., 2008; Sridang et al., 2008). For instance, high nutrient contamination in SPW could contribute to eutrophication in receiving water bodies. This type of wastewater must be properly treated before discharge. To date, some conventional biological treatment methods, such as membrane bioreactors and constructed wetlands, have been successfully used in the process of SPW treatment (Sohsalam et al., 2008; Sridang et al., 2008). These methods, although effective, do not allow the recycling of nutrients present in wastewater (de Godos et al., 2009). Therefore, microalgae cultivation was proposed as an alternative method for the treatment of SPW in this study.

SPW usually contains high concentrations of nutrients, indicating that SPW could be an alternative nutrient source for the cultivation of microalgae. However, according to some previous research, a high concentration of nutrients in the wastewater, especially unionized ammonia, may inhibit the growth of microalgae (Abeliovich and Azov, 1976; Källqvist and Svenson, 2003; Yuan et al., 2011; Park et al., 2010; Franchino et al., 2013). As a result, high-strength ammonia wastewater should be pretreated before use to increase the growth rate of microalgae (Fenton and Huallacháin, 2012). Several previous studies have confirmed that dilution has an impact on the algal biomass production and nutrient removal from several agricultural wastewaters (Canizares et al., 1993; Luo et al., 2016; Wang et al., 2012). Luo et al. (2016) cultured *Coelastrella* sp. in swine wastewater diluted with distilled water and found that 40% wastewater was optimal for the cultivation of microalgae, at which nutrient removal and productivity of biomass and lipids were maximized.

Although dilution is an effective method to promote the growth of microalgae in high-strength ammonia wastewater, this operation requires a large amount of water and reduces the nitrogen concentration in the wastewater. In addition to dilution, aeration was used to pretreat SPW in this study. By aeration, part of the ammonia in SPW could be translated to nitrite or nitrate through nitrification. Therefore, the ammonia concentration of the wastewater could be reduced, and most of the nitrogen could be kept in the wastewater. Subsequently, SPW, diluted SPW (DSPW) and aerated SPW (ASPW) were utilized to culture microalgae. The main purposes of this study were (Administration, 2002) to evaluate the feasibility of microalgae cultivation in SPW for algal biomass production and nutrient removal and (Arbib et al., 2017) to compare the microalgae growth and nutrient removal in SPW, DSPW and ASPW.

2. Materials and methods

2.1. Algal strain and pre-culture

The algal strain used in this study was *Chlorella* sp., which was obtained from the Culture Collection of Algae, Institute of Hydrobiology, Chinese Academy of Sciences. Algal cells were pre-cultured in 1000 mL flasks containing 200 mL of BG11 medium under stationary conditions at 25 °C with continuous white fluorescent light illumination (135 μmol photon m^{-2} s^{-1}) and shaking at 120 rpm.

2.2. Wastewater

SPW used in this study was collected from a sewage pool of Jinyuan aquatic foods Co., Ltd. (Zhoushan, China). Then part of the collected SPW was diluted with distilled water to prepare DSPW, and another part of SPW was aerated with air for five days to prepare ASPW. After that, SPW, DSPW and ASPW were all filtered using 0.45 μm pore size GF/C glass microfiber filters (Whatman Co.) to remove particles and microorganisms such as bacteria, fungi and microalgae. The values of the principal chemical compound concentration of SPW, ASPW and DSPW were summarized in Table 1.

2.3. Experimental procedure

First, the filtered SPW was used for the batch culture of microalgae. *Chlorella* sp. cells in logarithmic growth phase were collected by centrifugation (8000 rpm, 15 min), and then seeded to 500 mL flasks

Table 1
Main characters of the seafood processing wastewater (SPW), aerated seafood processing wastewater (ASPW) and diluted seafood processing wastewater (DSPW) (mean ± SD, n = 3).

Items	SPW	ASPW	DSPW
TN (mg L^{-1})	121.07 ± 0.82	94.80 ± 1.56	45.38 ± 2.75
TP (mg L^{-1})	57.32 ± 0.34	45.89 ± 5.49	23.19 ± 0.46
TAN (mg L^{-1})	117.22 ± 1.40	39.29 ± 2.14	39.14 ± 1.50
NO_2^--N (mg L^{-1})	0.73 ± 0.12	3.21 ± 0.12	0.29 ± 0.00
NO_3^--N (mg L^{-1})	6.99 ± 1.11	47.84 ± 1.65	3.42 ± 0.55
COD (mg L^{-1})	1220.8 ± 119.4	295.1 ± 18.3	553.6 ± 71.4
pH	7.92 ± 0.21	7.14 ± 0.17	7.53 ± 0.19
Salinity (%)	0.54 ± 0.06	0.47 ± 0.04	0.17 ± 0.03