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## Microalgae cultivation and nutrients removal from sewage sludge after ozonizing in algal-bacteria system



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#### ABSTRACT

The feasibility of growing algae in concentrated wastewater generated from sludge ozonation for simultaneous nutrients removal and biomass production was studied. The effects of bacteria addition into microalgae on nutrients removal, biomass yield and settleability, the growth rate of algae and concentrations of extracellular polymeric substances (EPS) and soluble microbial products (SMP) were investigated. The results showed that the growth rate of algae in algal-bacteria system (0.2182) was improved than in algae-only system (0.1852), while both of them are comparable with others reported previously. And the addition of bacteria enhanced COD, NH<sub>4</sub><sup>+</sup>-N, TN and TP removal rate by 23.9  $\pm$  3.3%, 27.7  $\pm$  3.6%, 16.6  $\pm$  1.8% and 14.9  $\pm$  2.2%, respectively. And 32.8  $\pm$  0.7% of the TN and 50.3  $\pm$  1.8% of the TP were recycled from ozonated sludge-supernatant (OSS) being absorbed into algal-bacterial biomass. The algal-bacteria system also demonstrated advantages on biomass settleability and heavy metals removal. Finally, the mechanism involving matter exchange and algal-bacteria system on OSS treatment in this study were discussed through evaluation of nutrients, SMP and EPS contents, nitrogen and phosphorus balance.

#### 1. Introduction

The environmental impacts caused by sewage sludge produced during wastewater treatment have aroused extensive attention. In addition to secondary pollution caused by persistent organic pollutants, pathogens and heavy metals in the excess sludge, the cost for residual sludge disposal is about 25-60% of the overall expense of running a wastewater treatment plant (Zhang et al., 2009). To reduce the excess sludge production and improve the sludge stability while realizing resource utilization, numerous methods of sludge treatment have been researched for the past few years, including thermal hydrolysis (Wilson and Novak, 2009), ultrasonic process (Guo et al., 2011), mechanical disintegration (Kampas et al., 2007), ozonation (Braguglia et al., 2012), alkaline treatment (Cai et al., 2004) and et al. Among these techniques, sludge ozonation is a promising technology, which can not only decompose a variety of refractory organic compounds in sludge improving the biodegradability of sludge, but also produce non-toxic by-products during the treatment process reducing 50-100% of the waste sludge generation (Yan et al., 2009a).

Many studies have shown the effectivity and sustainability of sludge ozonation. Sakai et al. (1997) reported that the decrement of sludge reached 66% after ozonation. Zhang et al. (2009) found that the amount of volatile solid and total solid reduced by 45.7% and 49.1% through ozonation, respectively. Zhang et al. (2016) also indicated that ozonation improved the sludge's settleability and decreased the relative hydrophobicity of sewage sludge by 19.2  $\pm$  6.4%. Besides, sludge ozonation combined with other systems (e.g., anoxic/oxic process, sequenced batch reactor) have been reported with a sludge reduction of 40-100% (Chu et al., 2009a). It was also reported that the phosphorus, nitrogen, carbon and metals from sewage sludge were released into the liquid phase by ozonation (Chu et al., 2009b). The ozonated sludgesupernatant (OSS) contains high concentrations of nitrogen, phosphorous, chemical oxygen demand (COD) and metals (Semblante et al., 2017), when it is returned back to the sewage biological treatment process may result in an excess load for the sewage treatment system, leading to a reduce of processing efficiency and an increased concentrations of nitrogen, phosphorus and metals in the effluent (Chu et al., 2009a; Yan et al., 2009b). Thus, if nitrogen and phosphorus of the OSS can be recycled, COD and metals can be removed before it is returned back to sewage treatment process, which can not only recover the nutrients of nitrogen and phosphorus, but also reduce the excess load of the sewage biological treatment system.

It's worth noting that the application of microalgae-based processes in wastewater purification is attractive for their strong ability to remove

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nutrients and uptake metals from wastewater, and their low cost to produce microalgal biomass that can be utilized for producing biofuels or fertilizer (Van Wagenen et al., 2015). In this process, microalgae provide the O<sub>2</sub> needed for bacteria respiration, while bacterial supply the CO2 required by microalgae for photosynthesis (Munoz and Guieysse, 2006). And the nitrogen and phosphorus in the wastewater are also effectively removed via assimilation into algal-bacterial biomass (Decostere et al., 2016). Numerous studies have shown the symbiotic relationship between algae and bacteria in the effective wastewater disposal and high biomass yield (Sun et al., 2017; Xie et al., 2018). Recently, many researchers have investigated the ability of different types of wastewater for supporting algae growth, such as anaerobically digested effluents (Yang et al., 2017), second clarifier effluent (Su et al., 2012, 2016), urine and anaerobically treated black water (de Wilt et al., 2016), concentrated municipal wastewater (Li et al., 2011; Zhou et al., 2011), and so on. The OSS which contains more nutrients, such as nitrogen, phosphorous and COD than ordinary wastewater, also can be provided as a potential substrate for algae cultivation to producing energy while removing nutrients. So far, however, rare studies about the feasibility of growing algae in OSS have been reported. Besides, the effect and mechanism of algae on nutrients and heavy metals removal as well as the advantage of the algal-bacteria symbiosis system to OSS is still unknown. Furthermore, this study is a new and indirect method to recycle nutrients from sewage sludge.

The main objective of this study was herein to (1) evaluate the operational feasibility of removing nutrients from sewage sludge for microalgae cultivation after ozonation, (2) explore the effects of the added bacteria in the microalgae cultivation batch reactors on nutrients removal efficiency, growth rate of algae and settleability of biomass fed with the OSS, and (3) investigate the mechanisms of matter exchange and algal-bacteria symbiosis for in this study. It is anticipated that this paper would not only be able to provide a new approach for sludge reduction and nutrients recycling but also have some referential value to high-density culture of microalgae.

#### 2. Materials and methods

#### 2.1. Ozonation treatment of sewage sludge

The raw sludge in this study was acquired from the secondary sedimentation tank of Taiping wastewater treatment plant (WWTP, Harbin, China). After being screened (3.8 cm aperture) to remove impurities, the sewage sludge was precipitated for 5 h to thicken sludge and then stored at 4 °C before further use (in 48 h). The characteristics of the sewage sludge are shown in Table S1. The sludge was then ozonized by an ozonation system including an ozonation sludge reactor, an ozone generator and an excess ozone absorption equipment, which was described in details in our previous research (Zhang et al., 2016). In short, 3000 ml sewage sludge were transferred into the ozonation reactor and ozonized by 50.4 mg  $O_3$ /g SS for 80 min, during which 100 ml sludge mixture were taken out from the reactor every 10 min, and another100 ml thickened sewage sludge were supplemented into the ozonation sludge reactor at the same time to insure the ozonation system running continuously (Zhang et al., 2016). The consumption of the ozone was calculated according to (Zhang et al., 2016). Then the ozonized sludge taken out from the reactor were centrifuged at 5000 rpm for 5 min and then the supernatant was used for microalgae cultivation, and the residual sludge were transferred into a membrane bioreactor for further treatment. The above tests were all performed in duplicate at room temperature.

#### 2.2. Experiment operation

Before the experiment, algae and bacteria (activated sludge) were cultured for enrichment. The algae inoculum was acquired from the second clarifier wall of the Taiping WWTP, and cultivated in BG11(without sterilization) until the algae grew to exponential phase (the initial mixed liquor suspended solids (MLSS) concentration was around 300 mg/L). Then the cultivated algae were centrifuged at 4000 rpm for 5 min, and the sediment was used as algal inoculum (microscopic observations revealed that Scenedesmus sp. was the dominant genus). Meanwhile, the activated sludge inoculum was obtained from the secondary sedimentation tank of Taiping WWTP. After two weeks' acclimation, the MLSS of seed sludge was about 15,000 mg/L as the bacterial inoculum.

Three stirred batch photobioreactors were set up, each was made of glass (30 cm in depth and 16 cm in diameter), and the working volume was 5 L. They were operated with only algae, both algae and bacteria and only bacteria, and named A system, AB system and B system, respectively. These batch reactors were set up to study the effects of the added bacteria on the performance of microalgae cultivation, nutrients removal and corresponding mechanism in OSS.

The initial MLSS concentration was 1500 mg/L and the ratio of algae and sludge in AB system was 1:3 (w/w). All batch reactors were applied to treat OSS and operated for 10 days. The A system and AB system were irradiated under the 2500 lx in the inner wall of the reactors with 12 h light-12 h dark cycle per day (from 5:00–17:00). The B system was covered with silver paper to prevent the reactor from illuminating. A magnetic stirring bar (80 rpm) was used to maintain constant mixing and avoid algae sedimentation. The above batch tests were also conducted in duplicate at room temperature.

#### 2.3. Methods of analysis

Before the illumination period was ended in 2 h each day, the pH and dissolved oxygen (DO) were tested by a pH meter (FE20, INESA, China) and a DO meter (Oxi3210, WTW, Germany), respectively. The samples used for experiment measurements were taken from the center of the batch reactors per day after 4 h of illumination period.100 ml samples in AB and A system were firstly transferred to 100 ml graduated cylinder to evaluate the biomass settleability as described by Su et al. (Su et al., 2012) and the TSS samples were pipetted every 10 min from a point 10 mm below the surface of the liquid in the cylinder, which procedure lasted for 1 h. The MLSS, MLVSS, NH4<sup>+</sup>-N, NO3<sup>-</sup>-N, NO<sub>2</sub>-N, TN, TP and COD in the samples were determined according to Standard Methods (CEPB, 2002). At the end of the tests, nitrogen and phosphorus in biomass was calculated according to Su et al. (2012). The concentration of heavy metals was monitored by an inductively coupled plasma optical emission spectrometry (Perkin Elmer optima 5300DV, PERKINELMER, China). The extraction and measure method of extracellular polymeric substances (EPS) and soluble microbial products (SMP) were measured according to the procedures described by Tian et al. (2015).

#### 2.4. Algal growth determination

In this paper, Chlorophyll a (Chl-a) content was used to characterize the growth of algal biomass (Lee et al., 2015) and measured as described by Tang et al. (2016). Five reasonably defined phases are usually used to characterize algae growth in batch culture, which are lag, exponential, declined, stationary and death phase, respectively. Generally, the growth rate (k) is determined from the exponential phase according to the equation:

$$\ln N = \ln N_0 + kt \tag{1}$$

where  $N_0$  and N stand for the biomass content at the start ( $t_0$ ) and any given time (t) respectively.  $N_0$  and k can be determined through linear regression according the straight line from a plot of lnN against t.

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