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Exogenous organic carbon as an artificial enhancement method to assist the algal antibiotic treatment system

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ABSTRACT

The study established an integrated view to evaluate the effect of exogenous organic carbon (EOC) on removal rate of cefradine and amoxicillin by *Microcystis aeruginosa*. Meanwhile, the toxicity control assessment of effluents was also investigated on the rotifer (*Brachionus calyciflorus*) during the algal treatment. The results indicated that glucose and NaAc could be consumed as EOC which enhanced the treatment efficiency of cefradine from 27.11% to 85.19% and amoxicillin from 14.70% to 58.20%, while the toxicity of the reaction products could be controlled at a similar level as the antibiotic itself. Moreover the algal treatment and nutrition regulation could also be applied for artificial wastewater and significantly promote the removal rate with respective increases of 47.04% and 48.75% for cefradine and amoxicillin, respectively.

1. Introduction

Due to the wide range of effects in diseases treatment, and in promoting the growth of livestock and poultry, antibiotics are extensively used in human and veterinary medicine. But in most cases, besides tiny amounts being absorbed, most of the antibiotics are excreted into the environment compartments, i.e., sewage and surface water (Bound and Voulvoulis, 2004; Chu et al., 2015). Because of incomplete treatment, improper disposal and high bioconcentrated, antibiotics possess the quality of highly persistent pollutants (Bergheim et al., 2015; Sun et al., 2016). Antibiotic residues have been shown to affect the biomass and activity of microbial, and induce generations of resistance genes in the environment medium (Li et al., 2016a; Manaia et al., 2016). Previous survey demonstrated that antibiotics have a significantly impact on aquatic organisms in the matter of survival, growth and reproduction (Ji et al., 2012; Lai et al., 2009).

Although several municipal sewage treatment techniques have been applied to control antibiotics wastewater, such as physical treatment, chemical treatment and biological treatment, the disadvantages and problems of the traditional treatments should be studied (Binelli et al., 2015; Reboleiro-Rivas et al., 2015). These processing methods will result in larger energy demand, high operating costs and the introduction of antibiotic resistant bacteria (Elmolla and Chaudhuri, 2011; Jimenez et al., 2015; Karthikeyan et al., 2012; Lin et al., 2015). In previous studies, microalgae has been proved to a dominant role in the removal of target pollutant, such as nitrogen or phosphorus compounds, heavy metals and pharmaceutical and personal care products (PPCPs) (Guo et al., 2016; Usha et al., 2016; Wang et al., 2009). Compared with activated sludge, algae are not the target organism of the antibiotics and therefore would not introduce relevant resistance genes (ARGs), which create a major environmental pollution (Gulkowska et al., 2008). Meanwhile, after the treatment, excess algal cells could be reused in biofuels, carbon fixation, and biochemical products, which avoid secondary pollution after the treatment (Da Rós et al., 2012; Nozzi et al., 2013; Rosgaard et al., 2012).

M. aeruginosa, belonging to cyanophyta, is one of the dominant species of algae bloom and widely distributed in nature. Previous literature suggests that antioxidant responses of *M. aeruginosa* to antibiotics have been observed (Qian et al., 2012a, 2012b). This species was tolerant and still kept growth capacity under the

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impact of antibiotics (Liu et al., 2012a, 2015). Our previous study also found that the density of *M. aeruginosa* increased within 12 h when the concentrations of cefradine were below 3.0 mg/L (Chen and Guo, 2012). Additionally, the inhibitory effect of amoxicillin on the growth of *M. aeruginosa* was not obvious (Gonzalez-Pleiter et al., 2013; Liu et al., 2014). On this basis, *M. aeruginosa* could be considered as an algal treatment material to remove antibiotics. Previous studies mostly focused on the growth and toxicity responses of *M. aeruginosa* under a long-term exposure to antibiotics, including the changes of algal density and intracellular protein (Liu et al., 2016; Shang et al., 2015). For green algae, there are several good applications to treat antibiotics, while studies on the removal of antibiotics by *M. aeruginosa* are still limited. But, antibiotics removal efficiency usually performed species-dependent. For instance, 68.73% of amoxicillin was treated after a 12 h treatment by *Chlorella pyrenoidosa*, while the removal rate of cefradine was only less than 10% for 24 h under the same conditions (Li et al., 2015). Thus, due to the advantages of *M. aeruginos*a in cell size, rapid growth, nitrogen and phosphorus consumption, the organism could be viewed as a potential bio-material in algal treatment. Previous study pointed out that *M. aeruginos*a could remove some antibiotics while it is comparatively low in efficiency. 32.9% of spiramycin and 33.6% of amoxicillin could be removed even after a 7-days treatment (Liu et al., 2012b).

Thus, to overcome this problem and find economical and practical approaches to obtain profit from the harmful algae, artificial enhancement to improve the remove efficiency should be considered. Nutrition regulation, as an important and widely used artificial enhancement method, has been developed in recent years. The exogenous organic carbon (EOC) has been applied to influence the metabolism of microorganisms and therefore promotes the removal on the difficult-decomposition organic contaminants, such as antibiotics, fungicides and hypnotics (Dawas-Massalha et al., 2014; Onesios et al., 2009). Nitrogen and carbon sources not only maintain a certain amount of biomass production as the important substrates, but also act as the electron donor on the active site of target compounds, and stimulate the removal of the selected PPCPs (Tran et al., 2009). Previous study suggested that the removal efficiency of lincomycin increased by nearly 60.0% when glucose as an external carbon source was added (Li et al., 2016b). However, relevant researches of EOC are mostly limited on bacteria, whether the algal removal efficiency of the target compounds could be improved by the EOC has not been reported.

In the present study, bloom algal species *M. aeruginosa* (FACHB-315), a non-microcystin-producing strain, was used to treat two widely used antibiotics, amoxicillin and cefradine. Two kinds of EOCs, glucose and NaAc, were added with the aim to enhance the algal treatment efficiency, which compared with the published results. In fact, after algae treatment, the toxicity of effluents on aquatic organisms deserves more attention. The cyanophycean toxin was detected and aquatic toxicity assessment was also applied. Therefore, the removal capacity and effluent toxicity control were evaluated to test the following hypothesis: (1) addition of glucose and NaAc could be used as an effective artificial enhancement method for the algae *M. aeruginosa* to improve the removal capacity of these two target antibiotics both in a short term (6 h) and a long term (24 h); (2) the toxicity could be under control after the algal treatment; (3) the algal treatment and EOC could also be applied for artificial wastewater and in a larger scale reactor.

2. Materials and methods

2.1. Chemical and analytical method

The antibiotics amoxicillin (>98% purity) and cefradine (>98% purity) used in this study were purchased from Yabang investment holding group CO., LTD. The concentrations of antibiotics were determined by high-performance liquid chromatography (HPLC) equipped using an Inertsil ODS column (4.6 mm × 150 mm, 5 μm), with the mobile phase of target antibiotics as follows: water-acetonitrile-3.86% sodium acetate-4% glacial acetic acid (862: 120: 15: 3) for cefradine, 0.05 mol/L potassium dihydrogen phosphate (pH = 5.0) - acetonitrile (97:3) for amoxicillin. The flow rate was 1.00 mL/min with ambient temperature and the wavelength of the ultraviolet detector be set to 254 nm. At the same time, the mobile phase of NaAc was water (pH = 2.15)-methanol (90:10) with 0.5 mL/min of flow rate at 60 °C, and the detection was implemented at the wavelength of 210 nm (Shimadzu SPD-10A).

2.2. Mass culture of M. aeruginosa

M. aeruginosa (FACHB-315) was purchased from Freshwater Algae Culture Collection in Institute of Hydrobiology, Chinese Academy of Sciences. Under aseptic conditions, *M. aeruginosa* was inoculated in during the algae growth period. All algae were cultivated in an intelligent light incubator at 25 ± 1 °C under 4000 lux illumination on the photoperiod 12:12 (L: D). Conical flasks were shaken two times a day and randomly replace the position to avoid the precipitation.

2.3. The treatment of antibiotics under different algal density

A certain volume of *M. aeruginosa* in the logarithmic growth phase were centrifuged and re-suspended in a small amount of BG-11 medium and then diluted with the cefradine solution of 50 mg/L. The initial algal density was about 1, 10, and 50 × 10^6 cells/mL, respectively (Group C-1, C-2, C-3) that showed in Table 1, and each group had three replications. The entire treatment was carried out at 25 ± 1 °C in a light-dark interval of 12:12 h (4000 lux). The concentrations of the residual antibiotic were measured by HPLC at the given time during the algal treatment process. The samples were collected before and after the algal treatment, respectively, and used for the acute toxicity test. The experimental design for amoxicillin removal (Group A-1, A-2, A-3) was all the same as above.

2.4. The effect of EOC on the treatment efficiency

The target antibiotic (50 mg/L) was treated by *M. aeruginosa* of 10 × 10^6 cells/mL with glucose or NaAc as the organic carbon source. The treatments of cefradine with the external carbon source were divided into six groups (Group C-4 to C-9), and each group had three replications. The concentrations of glucose were 5, 50 and 500 mg/L, respectively (Group C-4, C-5 and C-6, shown in Table 1), and the concentrations of NaAc were 5, 50 and 500 mg/L, respectively (Group C-7, C-8 and C-9, shown in Table 1). For amoxicillin, the experimental design was all the same as cefradine, divided into six groups (Group A-4 to A-9, shown in Table 1). The experimental conditions, the sampling time and the measurement indexes were all the same as 2.3. Additionally, *M. aeruginosa* were added into the filter-sterilized artificial wastewater (the characteristics of the artificial wastewater were shown in Table S1) with an initial algal density was about 10 × 10^6 cells/mL to verify the feasibility of the above algal treatment and EOCs. In a larger scale reactor (4 L), amoxicillin and glucose were selected as one kind of the target antibiotics and EOCs, respectively in the artificial wastewater.