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Microbial community and removal of nitrogen via the addition of a carrier in a pilot-scale duckweed-based wastewater treatment system

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HIGHLIGHTS

- The addition of a carrier does not affect duckweed growth or composition.
- The addition of a carrier improves nitrogen (N) removal in a duckweed system.
- Pyrosequencing systematically reveals the microbial community of a duckweed system.
- Abundant N-removal bacteria on the carrier biofilm contribute to improved N removal.
- An efficient N-removal duckweed system with enhanced microorganisms is established.

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ABSTRACT

Carriers were added to a pilot-scale duckweed-based (*Lemna* japonica 0223) wastewater treatment system to immobilize and enhance microorganisms. This system and another parallel duckweed system without carriers were operated for 1.5 years. The results indicated the addition of the carrier did not significantly affect the growth and composition of duckweed, the recovery of total nitrogen (TN), total phosphorus (TP) and CO_2 or the removal of TP. However, it significantly improved the removal efficiency of TN and NH_4^+-N (by 19.97% and 15.02%, respectively). The use of 454 pyrosequencing revealed large differences of the microbial communities between the different components within a system and similarities within the same components between the two systems. The carrier biofilm had the highest bacterial diversity and relative abundance of nitrifying bacteria (3%) and denitrifying bacteria (24% of *Rhodocyclaceae*), which improved nitrogen removal of the system. An efficient N-removal duckweed system with enhanced microorganisms was established.

1. Introduction

The excessive release of nutrients, such as nitrogen and phosphorus, to the environment causes serious water pollution and eutrophication. Conventional treatment systems, such as activated sludge, are the most commonly used technologies for removing nutrients from wastewater. However, the wide application of these systems in developing countries such as China is limited because of their high cost and technological complexity (Zimmo et al., 2004a). Aquatic macrophytes, such as water hyacinth, duckweed, and water lettuce, are receiving increasing attention and are being considered for their potential use in removing nutrients from wastewaters because of their high biomass yields, ease of operation and cost-effectiveness (MKandawire and Dudel, 2007).

Duckweed, which is a small floating aquatic plant within the *Lemnaceae* family, has been considered an attractive macrophyte for wastewater treatment, specifically for nutrient removal (Cheng and Stomp, 2009; Mohedano et al., 2012; Zhao et al.,

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2012). This plant has an advantage over other macrophytes used in nutrient removal because it effectively converts nutrients into valuable biomass, such as protein and starch. Duckweed biomass has been used as feed for ducks and other animals (Leng et al., 1995) due to its high protein content (more than 40%). It can also accumulate up to more than 45% of its dry weight (DW) as starch under laboratory and field conditions (Tao et al., 2013; Xiao et al., 2013), which makes it a viable feedstock for the production of bioethanol (Chen et al., 2012; Cheng and Stomp, 2009).

Nutrient uptake by duckweed is the predominant mechanism through which nutrients are removed in a duckweed system (DS) (Landesman et al., 2011). Korner and Vermaat (1998) has reported that approximately 3/4 of the total N- and P-loss could be attributed to uptake by duckweed. Additionally, microorganisms are involved in the removal of nutrients in a DS (El-Shafai et al., 2007; Korner and Vermaat, 1998; Zimmo et al., 2004a). However, the contribution of microorganisms is limited because the duckweed has a small root system and the microorganisms are easily lost with the effluent. In a previous study, we found that although duckweed had the same total nitrogen (TN) recovery rate as water hyacinth, the water hyacinth performed better with respect to the TN removal rate because it possesses a more extensive root system and a greater abundance of nitrifying bacteria in its rhizosphere (Zhao et al., 2014b). Therefore, if artificial roots (such as plastic carriers) are added to the DS to compensate for the small root system of the duckweed and to immobilize the microorganisms, the performance of the DS may improve and even surpass a water hyacinth system. Unfortunately, this type of work has seldom been reported, especially on a pilot-scale, and little is known about the microbial community and the functional microorganisms in a DS with artificial roots.

Therefore, the objectives of this work were to immobilize the microorganisms and improve the nutrient removal efficiency by adding a carrier to a DS, to investigate the microbial community of the system (including the wastewater, bottom sludge, rhizosphere of the duckweed and biofilm of the carrier) and to establish an efficient wastewater treatment system using a combination of duckweed and enhanced microorganisms.

2. Methods

2.1. Location and pilot-scale system

The experiment was performed in two parallel pilot-scale systems located 100 m east of Dianchi Lake and 30 km away from Kunming city, China (latitude 24°51′N, longitude 102°47′E, altitude 1888 m). Kunming city belongs to a subtropical plateau with a monsoon climate and has a mean monthly air temperature ranging from 7 to 20 °C and an average of 2400 h of sunlight each year.

The horizontal sections (a) and vertical sections (b) of the two parallel pilot-scale systems: the duckweed-based carrier system (DCS) and the duckweed-based system (DS) are shown in Fig. 1. Each system was made by concrete walls and consisted of a sequence of four equal ponds (P1, P2, P3 and P4 in order from inlet to outlet) in a series. Each pond covered an area of 17 m^2 (6.2 m length × 2.75 m width × 1.7 m height) and contained a volume of 25 m^3 water (1.5 m water depth). To avoid short-circuiting of the wastewater and to improve the exchange of the upper water and bottom water, the connection channels between P1 and P2 as well as P3 and P4 were at the bottom of the ponds, whereas the connection channel between P2 and P3 was at the top of the ponds (see Fig. 1b). 60 clusters of plastic elastic carrier/packing (Guangzhou green Ye environmental protection equipment Co., China) with the length of 1.3 m and a diameter of 0.15 m were evenly and symmetrically added to each pond of the DCS (see Fig. 1b) prior to the experiment.

2.2. Operation and sampling

Initially, both the DCS and DS were filled with Dianchi Lake water and local duckweed (*Lemna japonica* 0223) was collected and cultivated from nearby wastewater ponds and introduced into the eight ponds of the two parallel systems. *L. japonica* 0223 has been classified by Elias Landolt and is available on an accessible webpage in the Chengdu Institute of Biology and also on that of Rutgers University (Rutgers Duckweed Stock Cooperative, RDSC, http://www.ruduckweed.org/). The density of the inoculated duckweed was 412.5 mg fresh weight (FW)/m^2 (coverage rate of 150%). In each system, the duckweed was fed with a mixture of domestic sewage and agricultural wastewater; and 4 m^3 of the wastewater mixture was pumped into P1 of each system daily by a submersible pump, which lasted for approximately 2 h every day. To ensure that the water depth was maintained at 1.5 m, the discharge of the treated wastewater from P4 (using siphon technology) occurred simultaneously with the inflow of water into P1 (Fig. 1). The experiment was conducted over a period of 1.5 years (i.e., June 5, 2012–November 7, 2013). To ensure that a stable biofilm had formed on the surface of the carrier and the two systems were in a steady state, the data from November 7, 2012 to November 7, 2013 were selected for a year-round comprehensive comparison study between the two systems. The selected data were divided into

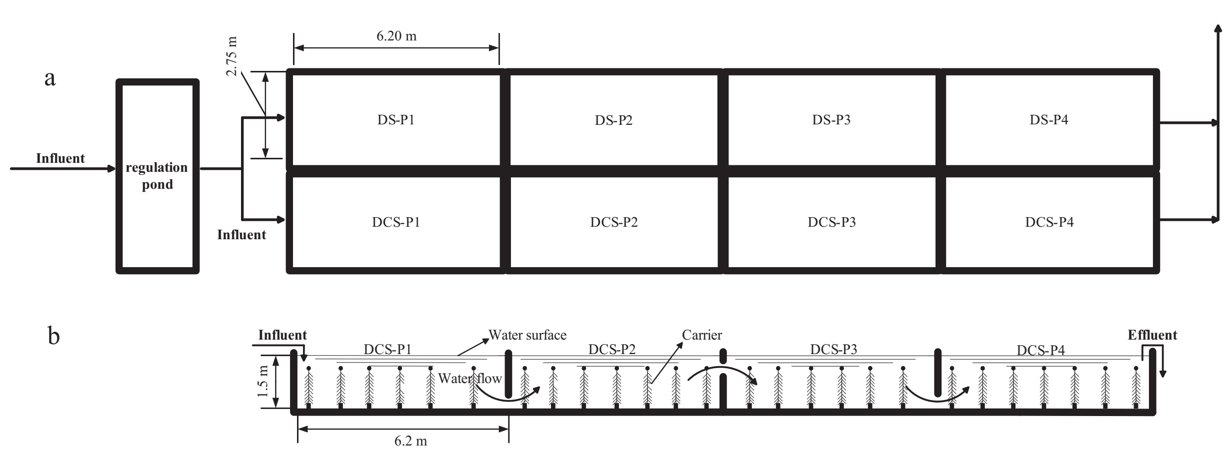

Fig. 1. The horizontal sections (a) and vertical section (b) of the wastewater treatment systems.