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Start-up and microbial communities of a simultaneous nitrogen removal system for high salinity and high nitrogen organic wastewater via heterotrophic nitrification

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HIGHLIGHTS

- The system provides a new way for treating high salinity organic wastewater.
- COD removal and SND was achieved efficiently in the single-stage reactor.
- Heterotrophic nitrification bacteria were identified based on PCR–DGGE.
- *Flavobacterium phragmitis* and *Paracoccus denitrificans* were the pivotal populations.
- Microbial community of salt-tolerant halophilic organism was developed successfully.

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ABSTRACT

In this study, a simultaneous nitrogen removal system for high salinity and high nitrogen organic wastewater was developed in a pressurized biofilm reactor. The result showed that under the air supply rate of 200 L h^{-1}, salinity of 3.0 ± 0.2%, organic load of 10 kg COD m^{-3} d^{-1} and nitrogen loading of 0.185 kg m^{-3} d^{-1}, the reactor started up rapidly and performed stably after 30 days operation. Meanwhile, a simultaneous COD and nitrogen removal was achieved in the single-stage reactor, with COD, NH_4^+-N and TN removal efficiency of 97%, 99% and 98%, respectively. Denaturing gradient gel electrophoresis profile demonstrated that simultaneous nitrogen removal could be achieved through heterotrophic nitrification–aerobic denitrification, and the pivotal microorganisms were *Flavobacterium phragmitis* and *Paracoccus denitrificans*. The microbial community of salt-tolerant halophilic microorganisms was developed successfully. This study can provide a more efficient and feasible solution to treat high salinity organic wastewater.

1. Introduction

With the rapid development of economy of the Three Gorges Reservoir area, a large amount of high salinity and high nitrogen organic wastewater about 3.5 million m^3 per year (Chai and Kang, 2012) is discharged by the Fuling Mustard Tuber industry. It poses a serious threat to the water environment of reservoir.

High salinity is a key problem that affects the processes of biological treatment for Fuling Mustard Tuber wastewater. In recent years, the effects of salinity on the activities of microorganism in bioreactors were studied. The studies have showed that high salinity in wastewater typically dampens the degrading enzymes and decreases the cell activity, can even cause cell plasmolysis (Rietz and Haynes, 2003), and it also has negative effects on organics removal (Pendashteh et al., 2012; Peyton et al., 2002). Generally, high salinity wastewater needs to be diluted before treating, it means the long process flow and low operation load which demand gigantic investment and high operating cost (Zhang et al., 2012). Consequently, building a microbial community of salt-tolerant halophilic microorganisms that can improve the adaptability of system to high salinity is the key to treat the high salinity wastewater.

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Table 1
Quality of the Mustard Tuber wastewater in the experiment.

Salinty % (NaCl)	COD_{cr} (mg/L)	BOD_5 (mg/L)	NH_4^+-N (mg/L)	NO_3^--N (mg/L)	TN (mg/L)	pH
3.0 ± 0.2	10,000 ± 500	4500 ± 500	175 ± 5	8 ± 1	185 ± 5	8.4 ± 0.2

How to remove the nitrogen in high salinity and high nitrogen organic wastewater is another key problem. Traditionally, the nitrogen removal processes were based on the autotrophic nitrification and heterotrophic denitrification (Khardenavis et al., 2007). But high salinity in wastewater can affect the conventional processes of ammonium oxidation, nitrification and denitrification (Liu et al., 2009; Yang et al., 2011). The traditional process of autotrophic nitrification was inhibited by organic matter. Therefore, the simultaneous removal efficiency of nitrogen and COD was low. Meanwhile, the low organic loading of autotrophic nitrification reactor can lead to large reactor volume, high investment and high operating cost. With the proposed concept of heterotrophic nitrification and aerobic denitrification (Robertson and Kuenen, 1982), heterotrophic nitrification bacteria gradually was known by the public. Comparing with the traditional nitrogen removal methods, heterotrophic nitrification has several advantages. Heterotrophic nitrification bacteria can remove COD and nitrogen simultaneously in one reactor via simultaneous nitrification and denitrification (Joo et al., 2006; Zhu et al., 2012). Heterotrophic nitrification bacteria have the ability to utilize different kinds of materials. And it is conducive to coexist with other strains (Marazioti et al., 2003). Meanwhile, some species of heterotrophic nitrification bacteria have the characteristic of high-level ammonia resistant (Taylor et al., 2009). These features of heterotrophic nitrification bacteria have great significance for treating the high salinity and high nitrogen organic wastewater. Recent studies of heterotrophic nitrification bacteria mainly focused on substrate removal, accumulation of intermediates, and the removal of COD and nitrogen via simultaneous nitrification and denitrification (Wan et al., 2011; Yao et al., 2013). But the applications of heterotrophic nitrification–aerobic denitrification in treating the high salinity wastewater have rarely been reported. Some salt-tolerant halophilic microorganisms capable of heterotrophic nitrification–aerobic denitrification have been isolated (Chen et al., 2014; Duan et al., 2015), but they could not perform well in nitrogen removal with high salinity.

In this study, a simultaneous nitrogen removal system for high salinity and high nitrogen organic wastewater via heterotrophic nitrification was developed in a pressurized biofilm reactor. During the experiment, there is no anaerobic–aerobic conditions and no excess sludge discharge in the single-stage reactor. The performances of COD and nitrogen removal were examined. Meanwhile, PCR–DGGE was used to detect the microbial diversity and community structures in reactor. This study can provide a more efficient and feasible solution to treat the high salinity and high nitrogen organic wastewater.

2. Materials and methods

2.1. Wastewater collection

Mustard Tuber wastewater for system was taken from the Huafu Mustard Tuber factory, located in Fuling, Chongqing, China. The detailed information of Sample could be found in Table 1.

2.2. Pressurized biofilm reactor and operating conditions

A pressurized biofilm reactor with a total effective volume of 70 L and diameter of 160 mm (Fig. 1) was used in this system. Polyurethane foam fillers were used as a biological carrier, with a filling rate of 40% (v/v). Inflow, provided by an elevated tank entered the recycle tank first, was then pressurized by an influent pump and oxygenated by a gas–liquid ejector. After that, it entered the pressurized biofilm reactor. The gas flow provided by the gas–liquid ejector was improved by a recycling system of effluent after oxygenating.

To start up the system, the biofilm reactor was inoculated with the sludge of 10 g/L, which was obtained from Ji Guanshi wastewater treatment plant (WWTP), Chongqing, China. There was no excess sludge discharge for reactor during the start-up period and stable circulation period. Throughout the experiment, the system was maintained with temperature of 35 ± 0.5 °C, air supply rate of 200 L h^{-1}, pressure of 0.2 MPa, organic load of 10 kg COD m^{-3} d^{-1} and nitrogen loading of 0.185 kg m^{-3} d^{-1}. And system was continuous influent/effluent with 24-h operation per day.

2.3. Microbial community analysis

2.3.1. DNA extraction

The inoculated sludge and the sludge sample after 60 cycles of stably operation were stored at −40 °C for further analysis. DNA was extracted from the sludge with a bead beater and three freeze–thaw cycles in boiling water and liquid nitrogen (Miller et al., 1999). Finally, the extraction was detected by 0.8% agarose gel electrophoresis.

2.3.2. PCR–DGGE

The V3 region of the 16S rRNA gene from the sample was amplified with the bacterial universal primer pair P3-GC (5′-CGC CCG CCG CGC GCG GCG GGC GCG GGG GCA CGG GGG GCC CCA TAC GGG AGG CAG CAG-3′) and P2 (5′-ATT ACC GCG GCT GCT GG-3′) (Muyzer et al., 1993). The PCR condition was initial denaturation for 5 min at 94 °C for 1 cycle and then 30 cycles of 94 °C for 45 s, 60 °C for 45 s and 72 °C for 90 s, followed by 1 cycle of 72 °C for 10 min. The presence of PCR products was confirmed by

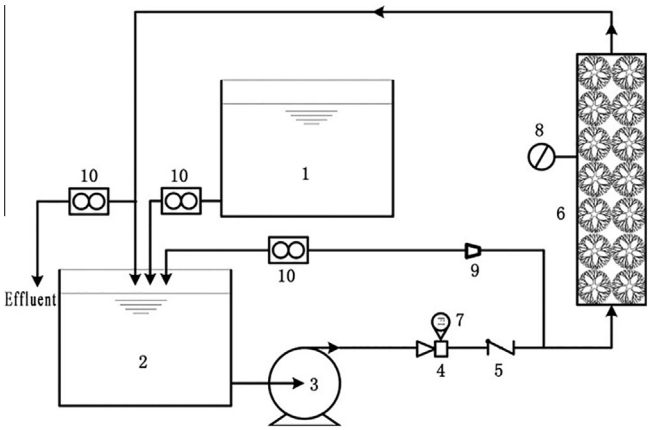

Fig. 1. Experiment equipment and flow of the pressurized biofilm reactor. (1) Elevated tank; (2) recycle tank; (3) influent pump; (4) gas–liquid ejector; (5) check valve; (6) pressurized biofilm reactor; (7) gas rotameter; (8) pressure gauge; (9) relief valve; (10) flow controller.