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Simultaneous removal of organic matter and nitrogen by a heterotrophic nitrifying–aerobic denitrifying bacterial strain in a membrane bioreactor

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HIGHLIGHTS

- *Bacillus methylotrophicus* L7 was inoculated into a membrane bioreactor solely.
- This single bioreactor was applied for treating artificial sewage aerobically.
- A simultaneous removal of COD and TN was achieved efficiently.
- This was different from traditional O/A system and SBR.

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ABSTRACT

A heterotrophic nitrifying–aerobic denitrifying bacterial strain, *Bacillus methylotrophicus* L7, was inoculated solely into a submerged membrane bioreactor (MBR) for continuous treatment of artificial sewage. The running conditions were also optimized for improvement of the treatment efficiency. The results indicated that inoculation of this single strain in a single reactor under constant aerobic conditions resulted in simultaneous removal of organic matter and nitrogen, in striking contrast to traditional aerobic nitrification–anaerobic denitrification treatment system and the sequencing batch reactor (SBR) systems. The optimal running conditions for the MBR were dissolved oxygen (DO) 4.5 mg/L, pH 7.5, loading ammonia <100 mg/L, and C/N ratio 3.5. Under these conditions, the removal percentages of chemical oxygen demand (COD), NH_4^+–N, and TN as high as 96%, 77.5% and 53%, respectively, were achieved without nitrite accumulation.

1. Introduction

Chemical oxygen demand (COD), reflecting organic matter content, and nitrogen content are two key parameters monitored during wastewater treatment. The most efficient method for removal of nitrogen from wastewater is based on the processes of nitrification (by autotrophic nitrifiers) and denitrification (by anoxic denitrifiers). Nitrifiers convert ammonia to nitrite and then to nitrate. Denitrifiers reduce nitrate to nitrite and then to N_2 (Joo et al., 2005). Organic matter interferes with the activity of nitrifiers (Kulikowska et al., 2010) but is necessary for denitrifiers. Oxygen is required for the growth of nitrifiers but is toxic to denitrifiers (Lloyd et al., 1987). Because of these opposing tolerances to organic matter and oxygen, it is impossible to achieve simultaneous removal of these two components in a single bioreactor under constant aerobic conditions. Removal strategies therefore require separate treatment systems and precise control of treatment conditions. Nitrification by autotrophic bacteria that have slow growth rates requires a long retention time of flowing wastewater in the reactor (Jetten et al., 1997; Muller et al., 2003). These factors all tend to increase the cost of wastewater treatment.

An increasing number of studies have focused on new biological nitrogen removal technologies. One exciting development is the discovery of "heterotrophic nitrifying–aerobic denitrifying" bacteria. Bacteria of this type have been isolated from many different environments; examples include *Arthrobacter* sp. (Verstrae and Alexande, 1972), *Alcaligenes faecalis* No. 4 (Joo et al., 2005), *Bacillus* sp. strains (Yang et al., 2011), *Pseudomonas* sp. (Wan et al., 2011), and *Bacillus methylotrophicus* L7 (Zhang et al., 2012). In comparison with traditional methods, nitrogen removal by heterotrophic nitrification and aerobic denitrification has several advantages: (i) The utilization of organic substrates and tolerance to oxygen by these bacteria are compatible, making it possible to achieve the simultaneous removal of organic matter and nitrogen via simultaneous nitrification and denitrification (SND) in a single reactor (Third

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et al., 2005). (ii) The process of denitrification counters the acidification caused by nitrification and tends to maintain a stable pH in the reactor. (iii) The diversity of possible substrates and products of heterotrophic nitrification facilitates mixed culture with a variety of bacterial strains and expands the scope of possible applications (Marazioti et al., 2003).

Recent studies of heterotrophic nitrifying–aerobic denitrifying bacterial have focused on substrate removal, accumulation of intermediates, and production of gaseous nitrogen compounds (Wan et al., 2011; Zhang et al., 2012). The characteristics and optimal reaction conditions of these bacteria were investigated in sequencing batch flask culture (Kulkarni, 2013). However, no study to date has addressed the removal of organic matter and nitrogen during the continuous sewage treatment via SND by these bacteria.

Previously, the heterotrophic nitrification–aerobic denitrification abilities of *B. methylotrophicus* L7 were described (Zhang et al., 2012). In the present study, these abilities of strain L7 were further assessed and its potential of simultaneous removal of organic matter and nitrogen was also investigated, by inoculating the strain into a membrane bioreactor designed for continuous wastewater treatment. The results suggest a basis for an alternative nutrient removal method for domestic wastewater.

2. Methods

2.1. Bacterial strain and culture condition

The bacterial strain *B. methylotrophicus* L7 was cultured in LB broth at 30 °C for 24 h. Cells were harvested by centrifugation at 9000×g for 30 min, washed twice with sterile saline solution, and the cell mass was resuspended in sterile saline solution to 3000 mg/L (wet weight) for use as the inoculum in a membrane bioreactor.

2.2. Membrane bioreactor and startup and operating conditions

The laboratory-scale submerged membrane bioreactor (MBR) with a total effective volume of 29 L (Fig. 1) was fitted with a polypropylene hollow fiber membrane module (average pore diameter ~0.2 μm; Hangzhou Kaihong Membrane Technology Co. Ltd., Hang Zhou, China) was used. Polypropylene spherical suspended fillers were used as a biological carrier, with a filling rate of 30% (v/v). A continuous flow of wastewater was provided by a delivery pump installed between the storage tank and the reaction tank. A fully aerobic operation throughout the experimental period was ensured by the introduction of compressed air via aerator pipes at the bottom of the reactor. The air bubbles provided oxygen for biological needs and a hydrodynamic scouring effect to reduce membrane fouling (Chang et al., 2011).

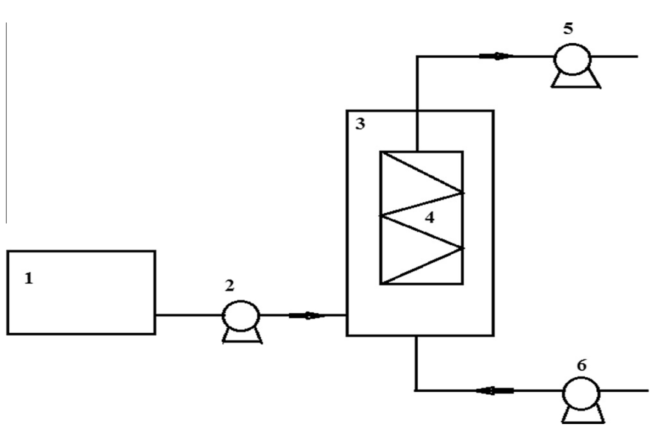

Fig. 1. Schematic diagram of the membrane bioreactor (MBR) system. (1) Storage tank; (2) delivery pump; (3) MBR; (4) membrane model; (5) air pump; (6) suction pump.

Table 1
The quality of artificial wastewater.

Items	COD (mg/L)	NH_4^+–N (mg/L)	TP (mg/L)	pH
Value	1000	120	7.8–9.4	7.5

For startup, the reactor was inoculated with 6 L of strain L7 cell suspension prepared as above and run at room temperature. The influent was controlled to hydraulic retention time (HRT) 24 h, dissolved oxygen (DO) 3.5–4.5 mg/L, and pH 7.5, ammonia content 30–40, and 150–300 mg/L. The startup period was approximately 30 days.

For continuous treatment of artificial wastewater after startup, the running conditions were: temperature 25–30 °C, HRT 24 h; DO 4.5 mg/L; influent pH 7.5, influent NH_4^+–N 120 mg/L, C/N ratio 3.5, or otherwise stated.

2.3. Artifical wastewater

Artificial wastewater, used as an imitation of sanitary, was composed of glucose, peptone, KH_2PO_4, NH_4Cl and tapwater (Table 1), plus appropriate trace amounts of calcium chloride, copper sulfate, and magnesium sulfate.

2.4. Analytical methods

Bacterial growth was determined by monitoring the optical density at 600 nm (OD_{600}) with a spectrophotometer (model UV-7200, UNICO, Shanghai, China). NO_2^-–N level was determined by *N*-(1-naphthyl)-1, 2-diaminoethane dihydrochloride spectrophotometry (Mahmood et al., 2009). Ammonia level was determined by Nessler assay (Zhang, 2009). Total nitrogen (TN) content was determined by peroxydisulfate oxidation with a UV spectrophotometric method (Ebina et al., 1983). COD was determined using a COD instrument COD (model CTL-12, Chengde Huatong Environmental Protection Equipment Co. Ltd., Chengde, China). DO was determined with a DO meter (model JPSJ-605Shanghai Precision & Scientific Instrument Co., Ltd, Shanghai, China). pH was measured with a pH meter (model PB-10, Sartorius, Germany).

3. Results and discussion

3.1. Reactor startup

During the 30-day startup period, the removal percentages of COD, ammonia, and total nitrogen (TN) increased gradually to 80.0%, 66.7% and 48.6%, respectively, on day 30 (data not shown). These findings indicate that a successful startup was achieved, according to the criteria of Trigo et al. (2006) and Xue et al. (2008).

3.2. Factors that affected the removal efficiency

To improve the treating performance of the MBR, several factors as described below were evaluated for their effects on the removal efficiency of COD and nitrogen.

3.2.1. Effect of DO

DO was decreased gradually from 6.0 to 1.5 mg/L during a period of 72 days (Fig. 2). A stable, high average COD removal percentage (85%) was maintained throughout this period (Fig. 2A), indicating a good biological degradation capability with consequent high effluent quality. In contrast, the removal percentages