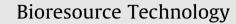
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Comparison of the removal of COD by a hybrid bioreactor at low and room temperature and the associated microbial characteristics

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ABSTRACT

To improve the efficiency of wastewater treatment and characterize the microorganism communities, microorganisms were cultured and concentrated in hybrid bioreactors at a low temperature (\sim 4 °C, low-temperature hybrid bioreactor, LTHB) and room temperature (\sim 25 °C, room-temperature hybrid bioreactor, RTHB). The performance of the LTHB and RTHB in terms of COD removal efficiency, dehydrogenase activity and functional diversity of microbial communities were evaluated. The results show COD removal efficiency increased gradually over time from 39.76% to 66.27% for LTHB and fluctuated between 81.85% and 94.78% for RTHB. The dehydrogenase activity and microbial activity in LTHB was higher than those in RTHB, implying that microorganisms cultured at low temperature had higher activities and adaptabilities than those cultured at room temperature. This study suggests that hybrid bioreactors can treat wastewater at both low and room temperatures and provides valuable insight into the adaptation processes of the microorganisms during temperature changes.

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1. Introduction

Currently, aquatic ecosystems suffer from two severe problems, the limitation of water resources and water pollution. The latter is increasingly critical in many countries due to improper economic development patterns (Agrawal, 1999; Azizullah et al., 2011; Zhao et al., 2010). It is both urgent and necessary to establish environmentally benign and cost-effective measures to eliminate water pollution.

Biological wastewater treatment based on microorganisms or their aggregates is a widely applied environmental-friendly method in wastewater treatment and can be divided into aerobic and anaerobic treatment processes according to the relationship between microorganisms and oxygen (Chan et al., 2009). It can also be divided into activated sludge and biofilm treatment processes based on microbial suspension or fixation in treatment facilities (Fadi, 1999).

The pollution level of organic matter in water is often evaluated in terms of chemical oxygen demand (COD) removal efficiency. Similarly, COD removal efficiency is often used for reflecting the level of wastewater treatment (Wu et al., 2011b). To date, many treatment processes have high COD removal rates at room or high temperature (LaPara and Alleman, 1999; Wu et al., 2011a). The performance of most treatment processes however, is negatively affected by low temperature conditions which often result in a deterioration of process performance (Nachaiyasit and Stuckey, 1997).

In practice, the long-term studies of COD removal by biological wastewater treatment in low temperature conditions are often limited due to alternating seasons. This leads to the majority of these studies being conducted at ~15 °C conditions (Elmitwalli et al., 2002; Mahmoud et al., 2004). Wastewater is still however, discharged in winter when the temperature is often lower than ~15 °C. In addition, there has been more research focusing on low-temperature anaerobic biological wastewater treatments than aerobic biological wastewater treatments, which implies that studies of aerobic biotechnology to remove COD at less than ~15 °C are important.

It has been reported that the performance of most biological wastewater treatment measures based on microorganisms and their aggregates could be affected by temperature (Collins et al., 1978). Therefore, some studies have explored ways to improve the performance of biological wastewater treatment based on microorganisms and their aggregates. For example, some researchers have tried to change operating conditions like hydraulic retention time (HRT) or adding some measure of pre-treatment or post-treatment to improve COD removal efficiency at low temperature (Elmitwalli et al., 2002; Feng et al., 2008; Mahmoud et al.,



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2004). It is well known that microbial properties such as microbial biomass and activities during the process of biological wastewater treatment are closely related to COD removal efficiency (LaPara et al., 2001). Therefore, in order to improve the COD removal efficiency at low temperatures, it is more practical to investigate microbial characteristics.

Most previous research has focused on either the enzyme activity or diversity of microbial communities to reflect microbial characteristics (Goel et al., 1998; Upton et al., 1990). Currently, dehydrogenase activity is often applied to assess microbial activity because the oxidation and decomposition of organic matter in treatment facilities often take advantage of microorganism dehydrogenation (Yang et al., 2002). Mature Biolog Microplate technology is also used for reflecting the diversity of microbial communities because it can reliably estimate and analyze diversity and characteristics of microbial communities (Kaiser et al., 1998). There have been some investigations into the relationship between removing pollution and microorganism characteristics at room temperature (Park and Lee, 2005), but few at low temperature such as 4 °C.

The objectives of this study are to (1) develop a highly effective and environmentally benign aerobic biotechnology to remove COD from wastewater, (2) compare the COD removal efficiency of the proposed hybrid bioreactor at low (4 °C) and room (25 °C) temperature conditions, and (3) explore the microorganism characteristics (dehydrogenase activity and functional diversity of microbial communities) during low and room temperature wastewater treatment. The results of this study will provide a promising biotechnology to remove COD matters at low (4 °C) and room (25 °C) temperature conditions, present valuable information about microbial characteristics during low temperature wastewater treatment processes as well as provide technical support for culturing and fostering microorganisms in low temperature wastewater biological treatment systems.

2. Methods

2.1. Experimental design

The process flow of the hybrid bioreactor is described in Fig. 1. The experimental system consisted of three parallel cylindershaped hybrid bioreactors (total and useful volume: 2.0 and 1.8 L, respectively), operated at either 4 or 25 °C. The volume of original activated sludge (which was collected from the aeration tank of a domestic sewage treatment plant) was 600 mL in each bioreactor. A refrigerator was used for storing the synthetic wastewater at 4 °C. The chemical composition of the synthetic wastewater was as follows: CH₃COONa (0.4670 g/L), NH₄Cl (0.1528 g/L), KH₂PO₄ (0.0300 g/L), MgSO₄·7H₂O (0.0226 g/L), CaCl₂·2H₂O (0.0028 g/L), FeCl₃·6H₂O (0.0002 g/L). This study began in November 2010 and ran until April 2011. The hybrid bioreactors were put into thermostat incubators, and kept at one of two constant temperatures $(4 \pm 1 \text{ and } 25 \pm 1 \degree \text{C}$ for LTHB and RTHB respectively). Each hybrid bioreactor was operated at a cyclic time of 12 h with a COD feed of $500 \pm 50 \text{ mg/L}$. The times for filling, reaction, settling and discharging were 15, 600, 90 and 15 min, respectively. One liter of the synthetic wastewater was added during each cycle. The dissolved oxygen (DO) was controlled at more 2 mg/L to maintain the activity of the activated sludge. To avoid sludge bulking, the volume of activated sludge was kept between 600–700 mL and the excess sludge removed before the next filling. The activated sludge in the LTHB was obtained through cooling the activated sludge gradually from 25 to 15 °C (over14 - days) and from 15 °C to 4 °C (over 30 days).

2.2. Analytical methods

The pH and DO of the synthetic wastewater was determined using a pH meter (PHS-3CT, Q/SMSB1-2005) and DO analyzer (JPJB-608, Q/YXLG155), respectively. The COD was determined according to the standard potassium dichromate digestion method (GB11914-89) provided by the Environmental Protection Administration of China (Wei, 2002). The dehydrogenase activity was measured using a modified triphenyl tetrazolium chloride (TTC) method (Klapwuk et al., 1974) where the sodium sulfide was the reducing agent and toluene the extracting agent. One enzyme activity unit was expressed as the amount of enzyme required to oxidize 1 mL of activated sludge suspension (33 g/L) to 1 µg triphenyl tetrazolium formazan (TPF) in 1 h.

The process to determine the functional diversity of microbial communities was as follows. Community-level substrate utilization was assayed at three temperatures (4, 15, 25 °C) using the commercially available Biolog™ ECO Microplates (Hayward, CA, USA). The plate contained an array of 96 wells and 31 types of carbon sources. The wells contained a redox-sensitive tetrazolium dye (oxidation indicator) which would turn purple as a result of respiratory electron transport in metabolically active cells (Balser and Wixon, 2009). Therefore, plate color was directly proportional to respiratory activity. For all samples, 50 mL aliquots were used for each Biolog and 150 µL aliquots were added into each well of every Biolog™ ECO Microplate, and analyzed according to Guckert (Guckert et al., 1996). Plates were incubated at 4, 15 and 25 °C and color development (590 nm) was evaluated using a Biolog Microplate Reader every 24 h for seven days (168 h).

2.3. Data analysis

Statistical Package for the Social Sciences (SPSS) Version 16.0 was used for Principal Component Analysis (PCA) and variance analysis (ANOVA). *p* was set at 0.05 for all analyses.

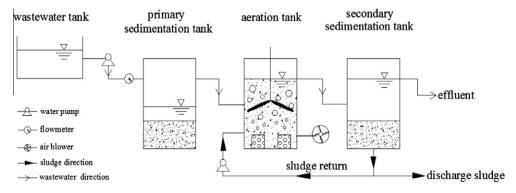


Fig. 1. Process flow of the hybrid bioreactors.

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