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Application of deep-sea psychrotolerant bacteria in wastewater treatment by aerobic dynamic membrane bioreactors at low temperature

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ABSTRACT

Wastewater treatment by biological processes was greatly hindered at low temperature. To improve the performance, screened deep-sea psychrotolerant strains were inoculated into an aerobic dynamic membrane bioreactor (ADMBR). The effect of bioaugmentation on reactor start-up, wastewater treatment performance, dynamic membrane formation, and membrane fouling under different temperature conditions was studied. Compared to the control reactor, the bioaugmented reactor showed a shorter start-up period, and better wastewater treatment performance at ≤ 15 °C for a sudden decrease, and ≤ 7 °C for a gradual decrease in temperature. Dehydrogenase activity in the bioaugmented reactor was enhanced at low temperature. Severe membrane fouling was not observed with bioaugmentation and the nonwoven fabric module we used was convenient to clean with tap water. Insoluble extracellular polymeric substances, especially insoluble protein were found to be responsible for membrane fouling. Fluorescence in situ hybridization (FISH) analysis displayed the dominance of two inoculated strains at the low temperature of 5 °C. This study gave evidence that bioaugmentation with psychrotolerant strains was a feasible strategy to shorten the start-up time and to improve the wastewater treatment for ADMBR at low temperature.

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1. Introduction

Temperature was very important for biological wastewater treatment because it affected biochemical reactions in many ways, including but not limited to reaction rates, reaction pathway, microorganism yields, and death rates [1]. Most studies on biological wastewater treatment have been conducted under suitable temperatures with activated sludge. However, at higher latitudes or in hilly regions, water temperature of municipal wastewater treatment plants rose and fell between 8 and 15 °C, some even lower between 5 and 8 °C. The low temperature in winter influenced the biological water treatment process severely in terms of microbial activity, substrate utilization rate, adsorption rate, and settling ability of the activated sludge [2]. It was found that nitrogen removal decreased significantly as the temperature dropped from 11.1 °C to 2.5 °C for micro-polluted raw water using a moving-bed biofilm reactor [3]. Sun et al. [4] investigated the influence of low temperature on package membrane bioreactor

operation and found that, at 4 °C or below, chemical oxygen demand (COD) and ammonia ($\text{NH}_4^+ - \text{N}$) removal rates dropped to around 15% and 40%, respectively. Concentration of total organic carbon (TOC) and total nitrogen (TN) in supernatant was much higher at 10 °C than at 20 °C in a laboratory-scale MBR for selected micropollutant treatment from synthetic municipal wastewater [5]. Low temperature was also found to have significant influence on the membrane fouling [6], microbial community and species richness [7]. Although considerable efficiency reduction was observed in many studies on MBRs treatment at low temperature, methods to improve the performance were very limited. Heat treatment for large scale wastewater at low temperature was feasible but not economical.

In general, acclimation and bioaugmentation were effective ways to take into account of improving biological wastewater treatment performance at adverse conditions. Although satisfied removal of specified target pollutant at low temperature, such as 10 °C, was achieved by cold acclimation of functional consortium [8], the long term for acclimation and constant temperature did not meet the seasonal temperature cooling in many chilly regions. On the other hand, whether the fine qualities obtained by acclimation could be genetically inherited to next generations stably, was still of much debate. Comparatively, bioaugmentation

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might be more effective to wastewater treatment at low temperature. By introducing a consortium of six denitrifying bacteria for bioaugmentation, the removal of N and P could be significantly promoted at 16.5 °C [9]. Guo et al. [10] obtained the rapid start-up and stable operation of biological processes for municipal wastewater treatment by application of bioaugmentation at 13 °C. Head and Oleszkiewicz [11] revealed the impact of sudden decrease from 20 °C, 25 °C and 30 °C to 10 °C in temperature on nitrification rate in sequencing batch reactors which bioaugmented nitrifying bacteria, and the average decreases in nitrification rates were 58%, 71% and 82% for biomass cooled to 10 °C. However, research works on wastewater treatment at changeable low temperature more close to that of the winter in chilly area were barely reported.

Selection appropriate process was also important for wastewater treatment at low temperature. Although MBRs had attracted interest for their reliability and high efficiency at low temperature in recent years [12], the high costs in the membrane modules, high energy consumption and severe membrane fouling had greatly limited their application. Compared to MBRs, the aerobic dynamic membrane reactor (ADMBR) had the advantages of economical and practical [13], high pollutant removal efficiencies, good particle rejection capacity, high critical flux through the activated sludge dynamic membrane (DM) [14], and the membrane modules was also convenient to clean with tap water, air backwashing or simple mechanical brushing without any chemical reagents [15]. In addition, the membrane could intercept the inoculated bacteria with long sludge retention time, which made the ADMBR more suitable for bioaugmentation.

In this study, two psychrotolerant strains with high pollutants removal ability and high activity at 15 °C were screened and inoculated into an ADMBR with activated sludge. Different temperature shocks, a sudden decrease in temperature (25, 20, 15, 10, and 5 °C), and a gradual decrease of temperature (15, 12, 10, 7, and 5 °C), were applied to examine the efficiency of bioaugmentation in shortening the start-up periods and the performance of wastewater treatment at low temperature. The effects of bioaugmentation on dynamic membrane formation and membrane fouling were also investigated.

2. Materials and methods

2.1. Screening and identifying psychrotolerant bacteria

Studied bacteria were screened from 20 pure strains of psychrotolerant bacteria isolated from surface sediments of Jiaozhou Bay (Shandong province, China) and provided by State Key Laboratory of Microbial Technology, Shandong University. All the bacteria were firstly activated in culture medium on a rotary shaker (200 rpm) at 15 °C for 12 h. The medium contained 0.3% (w/v) yeast powder, 1% peptone, and 0.5% NaCl. After activation, 10% (v/v) of the bacterial solution was inoculated in shake flasks containing 300 mL synthetic wastewater composed as shown in

Table 1
Composition of synthetic wastewater.

Composition	Concentration (mg/L)	Composition	Concentration (mg/L)
Glucose	150	NaCl	50
Sodium acetate	180	CaCl ₂	15
Peptone	75	MgSO ₄ · 7H ₂ O	12.5
Beef extract	50	FeSO ₄	0.3
NH ₄ Cl	100	ZnSO ₄ · 7H ₂ O	0.1
KH ₂ PO ₄	20	MnSO ₄ · 7H ₂ O	0.25
Na ₂ HPO ₄ · 12H ₂ O	7.5	CoCl ₂ · 6H ₂ O	0.025

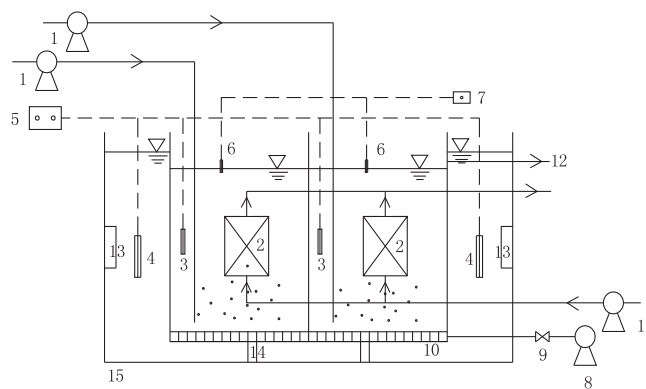


Fig. 1. Schematic diagram of the experimental set-up. (1) peristaltic pump; (2) membrane module; (3) temperature sensor; (4) temperature control meter; (5) refrigerator; (6) constant level sensor; (7) liquid level control instrument; (8) air pump; (9) air flow meter; (10) air diffuser; (11) backwash pump; (12) overflow pipe; (13) aquarium internal filter; (14) holder; (15) clean water tank.

Table 1. The flasks were then shaken at 200 rpm at 15 °C and samples were taken every 6 h. After centrifuging at 10,000 rpm for 10 min, supernatants were tested for cell density (OD600), concentrations of COD, TN, and total phosphorus (TP). Two strains with the best growth and performance were selected for the inocula of the ADMBR.

The screened strains were identified by 16S rDNA analysis described in reference [16].

2.2. Experiments

2.2.1. Experimental set-up

Fig. 1 presented a schematic diagram of the experimental set-up of two dynamic membrane bioreactors in a large water tank. The two bioreactors were parallel and independently controlled, with effective volume of 6 L. The water tank was designed to be an open temperature refrigeration system. The refrigeration system was composed of a clean water tank, a temperature control meter, an aquarium internal filter, a temperature sensor, and a refrigerator. The refrigerator and temperature control meter worked automatically to keep the target temperature detected by the temperature sensor. Water temperature in the whole tank was managed through water circulation by the aquarium internal filter. Two holders were fixed under the two reactors to keep full circulation of water in the tank.

Although colloidal, particulate organic and inorganic material in wastewater significantly influenced treatment performance in in-situ wastewater treatment, most of these were readily removed by pre-treatment systems. For the ADMBR, a kind of secondary treatment, colloidal and particulate organic matter could be produced by growth of microorganisms, but soluble chemicals were considered the most difficult target pollutants to separate [17,18]. Therefore in this study, soluble synthetic wastewater (composition as shown in Table 1) was employed as the influent.

A flat-sheet nonwoven fabric module with nominal pore size of 15 μm and filtration area of 260 cm² was submerged in each reactor. Synthetic wastewater was pumped continuously into the bottom of each reactor with a peristaltic pump. Unlike a conventional MBR, the ADMBR flow was independent of the effluent pump and filtration was driven only by a water head drop between the reactor and the effluent port. To prevent overflow, the feed pump was controlled by a level sensor to keep constant liquid level in the reactor. The aeration unit with a flow meter was placed at the side of each reactor to serve as both aeration and flow circulation force.

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