Bioresource Technology 99 (2008) 8273–8279

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Evaluation of oxygen adaptation and identification of functional bacteria composition for anammox consortium in non-woven biological rotating contactor

Sitong Liu [a], Fenglin Yang [a,*], Yuan Xue [a], Zheng Gong [a], Huihui Chen [a], Tao Wang [a], Zhencheng Su [b]

[a] Key Laboratory of Industrial Ecology and Environmental Engineering, MOE, School of Environmental and Biological Science and Technology, Dalian University of Technology, Dalian 116024, PR China
[b] Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, PR China

ARTICLE INFO

Article history:
Received 3 February 2008
Received in revised form 4 March 2008
Accepted 4 March 2008
Available online 24 April 2008

Keywords:
Anammox
Nitrosomonas eutropha
Dissolved oxygen
Bacteria composition
Biological rotating contactor

ABSTRACT

In this study, the anammox consortium was found to adapt to the wastewater containing dissolved oxygen (DO), as the DO was gr\adually increased. Batch tests indicated the maximum aerobic ammonium oxidizing activity of the consortium was 1.38 mmolNH_4^+—N(g VSS)$^{-1}$ day^{-1}, which played key roles in the oxygen consumption process; the maximum anaerobic ammonium oxidizing activity was slightly decreased after long-term oxygen exposure, but only from 21.23 mmolNH_4^+—N(g VSS)$^{-1}$ day^{-1} to 20.23 mmolNH_4^+—N(g VSS)$^{-1}$ day^{-1}. Microbiological community analysis identified two strains similar to *Nitrosomonas eutropha* were responsible for oxygen consumption, which were able to exist in the autotrophic anaerobic condition for long periods and protect anammox bacteria *Planctomycetales* from the influence of oxygen. Microbiological composition analysis showed *Nitrosomonas* and *Planctomycetales* approximately accounted for 10% and 70% of the bacteria, respectively. The possibility of cultivation anammox consortium in presence of DO will lead to substantial savings of energy and resource in the industrial application.

1. Introduction

Anammox is a new technology developed recently in which ammonium is converted to N_2 with nitrite as electron acceptor under anoxic condition (van de Graaf et al., 1996). Compared with the conventional biological nitrogen removal process, it is a novel, promising, low-cost alternative (Abma and Schultz, 2007; van Loosdrecht and Jetten, 2001; Tsushima et al., 2007) and has many advantages, e.g., no requirement for external carbon sources, low oxygen demand, minimized surplus sludge and reduced CO_2 emissions. The investigation of anammox is an important revolution in the nitrogen removal theory and has a huge application foreground in the nitrogen removal technology.

The anammox process is a technique which can treat with wastewater contained much ammonium and little organic material, such as sludge digestor effluents. However, the stringent metabolism conditions and extremely slow growth rate of the anammox bacteria have restricted their application to pilot-scale plants (Strous et al., 1998), although some significant works have been successfully done to apply anammox process on actual industrial wastewater (Waki et al., 2007; Dong and Tollner, 2003). The anammox reaction is easily inhibited by oxygen and nitrite. Very low oxygen levels (>0.04 mg L^{-1}) inhibit reversibly (Strous et al., 1998) and high nitrite concentrations (>100 mg L^{-1}) inhibit irreversibly (Strous et al., 1999) the anaerobic ammonium oxidizing activity. The influence of oxygen on the anammox process has been investigated in several experiments and the results indicated that only when all the oxygen was removed from the reactor by vigorously flushing with inert gases, the conversion of ammonium and nitrite resumed, thus indicating that the anammox activity in these enrichment cultures is only possible under strict anoxic conditions (Jetten, 2001).

In the view of applicability on semi-industrial scale, the anammox consortium must cope with the variable and harsh conditions of wastewater treatment compared with the optimal laboratory condition. Efforts to enlarge the application range of anammox process in industrial utilization have been done over the past years. For example, the combination of anammox and denitrification processes was studied aiming to treat high-ammonium wastewater contained COD (Chamchoi et al., 2007), and then the possibilities of cultivating anammox consortium under low ammonium-fed (Pathak et al., 2007) or low temperature conditions (Dosta et al., 2007) were also investigated. Up to day, one of other problems remained to be solved in practical utilization of anammox process is the adaptation of dissolved oxygen (DO) contained in the influent or reactor, namely the possibility of cultivating anammox consortium under oxygen conditions, which should be evaluated for the industrialization of anammox process in order to save high cost in the maintenance of absolutely anaerobic condition.

* Corresponding author. Tel.: +86 411 8470 6172; fax: +86 411 8470 6171.
E-mail address: anammox@163.com (F. Yang).

0960-8524/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2008.03.006

A promising strategy to avoid the DO influence is to enrich some oxygen-consumed bacteria for creating the anammox's anaerobic niches. However, the cultivation of anammox consortium was often in strictly autotrophic, oxygen-absence conditions. Even though the DO can be supplied in the influent, the concentration is much lower than that in some other processes equipped with oxygen-offered devices. So under this oxygen-lack condition, it is difficult to successfully enrich such oxygen-consumed bacteria and make them take action in the anammox consortium. Actually, there is few kinds of bacteria which can sustain the nutrition lack condition of the anammox consortium for a long time and the diversity of the anammox consortium composition is lower compared with that of some other biological nitrogen removal processes.

Whereas, the consistent presence of some oxic nitrifiers in the anammox reactors (Jetten et al., 1999) or anammox biofilms (Schmid et al., 2000) confirms that although nitrifiers may not be enriched under anoxic conditions, they can at least survive. Moreover, it has been known that many of the aerobic ammonium oxidizers, such as some strains belonging to *Nitrosomonas*, are facultatively anaerobic (Kuenen and Jetten, 2001) and have anaerobic metabolisms. They can use a variety of electron donors (hydrogen, pyruvate, and ammonium) for the reduction of nitrite (Poth and Focht, 1985; Schmidt and Bock, 1998; Bock et al., 1995) under anaerobic condition and produce NO, N_2O and N_2 (Poth and Focht, 1985; Poth, 1986). In sharp contrast to the anammox, they have more versatile metabolism. The highest anaerobic ammonium oxidizing activity is 25 times lower than that of anammox ($55\ nmol NH_4^+—N(mg\ protein)^{-1}\ min^{-1}$) (Kuenen and Jetten, 2001), but high enough to survive in prolonged periods of oxygen-absence. For the multiplicate metabolism, the possible long-term existence of some *Nitrosomonas* strains in anammox consortium has the potential to provide the biotechnological foundation to facilitate the DO adaptation of the anammox consortium.

In this work, we have targeted the DO questions for anammox consortium in feasibility studies with wastewater in laboratory scale. The objectives of this paper were to assess the DO adaptation of anammox consortium and the corresponding nitrogen removal performance. DO levels might also have a definite impact on the functional microbial community structure in the reactor, so in this study, members of the functional bacteria were detected and enumerated using the recent developing molecular techniques such as polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) and fluorescence in situ hybridization (FISH). The possibility of DO adaptation in cultivation anammox consortium will lead to substantial savings of energy and resource to the treatment of high-ammonium wastewater.

2. Methods

2.1. Non-woven rotating biological contactor reactor

The non-woven rotating biological contactor (NRBC) reactor configuration used in the experiment is given in Fig. 1. Non-woven porous polyester coated with a pyridinium-type polymer (Japan Vilene, US patent 5,185,415; 1993) was used as the discs to enhance the attachment performance for its good adsorption characteristics. Ten discs were mounted (10 cm diameter of one disc, 0.5 cm thickness, 1 cm interspace) on a horizontal shaft by fixing with stainless steel. The discs were rotated at 0.5 rpm to mix the substrate with 100% submergence of the disc surface area. The cylindrical reactor equipped with a thermostatic jacket (maintained at 35 °C) made of perspex has an effective volume of 1.7 L. The reactor and feed vessels were all sealed tightly in order to maintain anaerobic condition and covered to protect the bacteria from light and algal growth. The hydraulic retention time (HRT) was fixed at 6 h by a peristaltic pump.

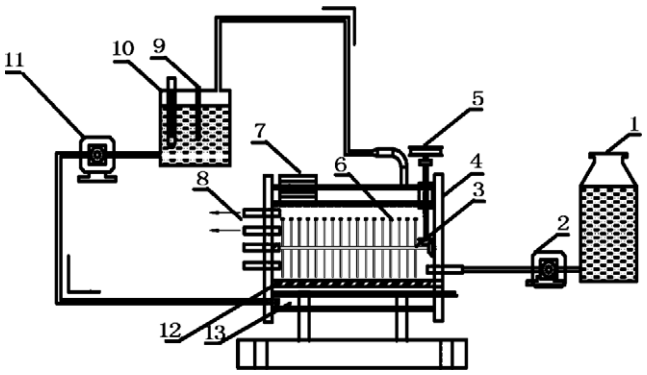

Fig. 1. Scheme of the NRBC reactor: (1) influent tank; (2) influent pump; (3) horizontal shaft; (4) rotating contactor; (5) rotating device; (6) disc; (7) opening for sample collection; (8) outlet; (9) heater tank; (10) thermometer; (11) cycling pump; (12) aerating hole; (13) thermostatic jacket.

2.2. Synthetic wastewater and inoculum

The synthetic wastewater fed to the NRBC reactor in this experiment mainly contained ammonium and nitrite in the form of $(NH_4)_2SO_4$ and $NaNO_2$, the amounts of which varied depending on the applied load. The composition of the mineral medium was as specially described by van de Graaf et al. (1996). The pH of the synthetic wastewater was adjusted to 8.0 ± 0.1 by 1 M HCl and 1 M Na_2CO_3 before providing to the reactor. The anammox consortium for inoculation was taken from a laboratory scale anammox up-flow column reactor (Furukawa et al., 2003; Liu et al., 2008). The initial biomass concentration in the reactor was about 0.8 g VSS L^{-1}.

2.3. Analytical procedures

The concentrations of nitrogen compounds were measured according to standard methods, as set out by the American Public Health Association (APHA, 1995). NH_4^+—N and NO_2^-—N were measured colorimetrically, NO_3^-—N was measured spectrophotometrically. TN was determined by the TOC analyzer equipped with a total nitrogen-measuring unit (TOC-VCPH, Shimadzu). The pH measurement was done using a digital, portable pH meter, DO measurement was done using a digital, portable DO meter (YSI, Model 55, USA). The volatile suspended solids (VSS) were determined to calculate the biomass concentration according to the standard methods (APHA, 1995).

2.4. Operation of the NRBC system

The experiment was divided into two periods. In period I (day 0–50), the reactor was operated under absolute anaerobic condition by flushing nitrogen gas in the influent. In period II (day 50–100), the system was turned to oxygen condition, namely the oxygen concentration in the influent was gradually increased by flushing nitrogen gas with gradual decreasing fluxes, until no need of any nitrogen gas. Samples were taken every 2 days and analyzed for NH_4^+—N, NO_2^-—N, NO_3^-—N and total nitrogen (TN). At the beginning of the experiment and at the end of each the period, the compositions of the biofilm were analyzed with PCR-DGGE and FISH technologies.