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Suppression of anaerobic ammonium oxidizers under high organic content in high-rate Anammox UASB reactor

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ABSTRACT

The effect of organic matter on the nitrogen removal performance of anaerobic ammonium oxidation (Anammox) process was investigated in an upflow anaerobic sludge blanket (UASB) reactor fed with nitrogen loading rate of 13.92 kg N m^{-3} day^{-1} at an HRT of 0.83 h. Mass balance showed that the heterotrophic denitrification prevailed in the UASB reactor, and became the dominant reactions when high influent COD/NO_2^-–N ratios of 2.92 were applied. The Anammox bacterial growth was significantly suppressed by denitrifying communities under high organic matter content due to the weaker competition for nitrite (electron acceptor) and living space. Long-term operation of the Anammox UASB reactor under relatively high organic content resulted in weak recovery performance.

1. Introduction

Anaerobic ammonium oxidation (Anammox) is a promising biological nitrogen removal technology first discovered in a pilot-scale denitrifying reactor (Mulder et al., 1995). It involves the autotrophic oxidation of ammonium to dinitrogen gas using nitrite as electron acceptor under anaerobic conditions eliminating the requirements of aeration and exogenous carbon sources (Strous et al., 1999). Thus, it proves to be a cost-effective and an attractive option for nitrogen removal from wastewaters. Recently, it has been successfully applied to treat ammonium-rich wastewater such as reject effluents and tomato processing wastewater (van der Star et al., 2007). The first full-scale Anammox reactor was the Dokhaven-Sluisjesdijk wastewater treatment plant built in Rotterdam (NL) in 2002 treating sludge dewatering effluent from a Sharon (single reactor high activity ammonia removal over nitrite) process (van der Star et al., 2007). The maximum nitrogen removal rate reached was as high as 9.5 kg N m^{-3} day^{-1} (van der Star et al., 2007) far higher than the values obtained by the conventional nitrification–denitrification process (lower than 0.5 kg N m^{-3} day^{-1}) as reported by Jin et al. (2008).

The autotrophic Anammox microbes are characterized by a very slow growth rate and low cellular yield (Strous et al., 1998). Additionally, the presence of organic matter is not suitable for the growth of Anammox bacteria (Jetten et al., 1999; Chamchoi et al., 2008; Isaka et al., 2008; Molinuevo et al., 2009). For that reason, the Anammox process was mainly used for the treatment of wastewaters with low C/N ratio (Jetten et al., 1999, 2005; Furukawa et al., 2009; Vázquez-Padín et al., 2009). Unfortunately, some wastewaters such as swine (Ahn et al., 2004; Molinuevo et al., 2009), aquaculture (Lahav et al., 2009) and monosodium-glutamate manufacturing (Yang et al., 2005; Jia et al., 2007) ones contain high organic content as well as high ammonium concentration. So the problems associated with the high organic content should be carefully considered if Anammox is applied for their treatment. Though, the effects of organic matter on performance of Anammox process have already been reported by some researchers (Güven et al., 2005; Chamchoi et al., 2008; Kartal et al., 2008; Isaka et al., 2008), most of the researchers elucidated the effect of low organic content on the process. Chamchoi et al. (2008) found that COD concentration over 300 mg L^{-1} in the wastewater could inactivate or even eradicate Anammox communities when three UASB reactors were operated at nitrogen loading rates below 0.1 kg N m^{-3} day^{-1}; while Molinuevo et al. (2009) observed that COD concentration up to 292 mg L^{-1} led to complete inhibition of Anammox reaction. The information on the behavior of Anammox process operated under relatively higher organic content and high nitrogen loading rate is limited. The specific objective of this study was to probe the effect of high organic content after initial operation of an Anammox UASB reactor achieving high nitrogen removal rate (higher than 9.5 kg N m^{-3} day^{-1}).

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2. Methods

2.1. Synthetic wastewater

Sterilized water was used for this study. Ammonium, nitrite and organic matter were supplemented to mineral medium in the form of $(NH_4)_2SO_4$, $NaNO_2$ and sucrose, respectively. The composition of the mineral medium was (g L^{-1} except for trace element solution): NaH_2PO_4, 0.05; $CaCl_2{\cdot}2H_2O$, 0.3; $MgSO_4{\cdot}7H_2O$, 0.3; $KHCO_3$, 1.25; $FeSO_4$, 0.00625; EDTA, 0.00625 and 1.25 mL L^{-1} of trace element solution. The trace element solution contained (g L^{-1}): EDTA, 15; H_3BO_4, 0.014; $MnCl_2{\cdot}4H_2O$, 0.99; $CuSO_4{\cdot}5H_2O$, 0.25; $ZnSO_4{\cdot}7H_2O$, 0.43; $NiCl_2{\cdot}6H_2O$, 0.19; $NaSeO_4{\cdot}10H_2O$, 0.21; $NaMoO_4{\cdot}2H_2O$, 0.22 and $NaWO_4{\cdot}2H_2O$, 0.05 (adapted from van de Graaf et al., 1996).

2.2. Anammox bioreactor

Owing to its high solid retention and mass transfer rate (Chamchoi et al., 2008), upflow anaerobic sludge blanket (UASB) reactor was used for this study. The reactor had an inner diameter of 50 mm with a working volume of 1.1 L. The influent was purged with 95% Ar–5% CO_2 in order to maintain anaerobic conditions. To avoid the growth of phototrophic organisms and the related oxygen production, the reactor was covered completely by a PVC material (1 mm thickness) to prevent penetration of light (van der Star et al., 2008). Internal temperature was set at 35 ± 1 °C according to Tsushima et al. (2007). Influent pH was always controlled at 6.8–7.0 by dosing hydrochloric acid (Tang et al., 2009). The synthetic wastewater was pumped into the bottom of the reactor and the gas production was recorded using a wet-gas meter.

2.3. Inoculum

The reactor was inoculated on day 0 with 0.6 L settled Anammox granular sludge obtained from a lab-scale Anammox reactor (Tang et al., 2009). The Anammox seed granules had a diameter of 2.51 ± 0.91 mm, with the specific Anammox activity of 0.072 kg NH_4^+–N kg VSS^{-1} day^{-1} (Tang et al., 2009). After inoculation, the initial sludge concentration inside the UASB reactor was about 19.5 g VSS L^{-1}.

2.4. Specific Anammox activity assays

Completely closed vials with a total volume of 120 and 100 mL of liquid volume were used to perform the Anammox batch assays. At the beginning of the experiment, the biomass concentration was about 1 g VSS L^{-1}. The pH was fixed at 7.5 and the temperature was maintained at 35 ± 1 °C. Gas and liquid phases were purged with argon to remove O_2. Initial NH_4^+–N and NO_2^-–N concentrations were 70 mg L^{-1} each. The maximum specific Anammox activity (MSAA) was estimated from the maximum slope of the curve indicated by the decrease of ammonium and biomass concentrations in the vials with the passage of time (Dapena-Mora et al., 2004).

2.5. Experimental procedure

The overall reactor operation was divided into three phases. During Phase I (days 1–193), the UASB reactor was operated without addition of organic matter aiming to achieve high nitrogen removal rate (higher than 9.5 kg N m^{-3} day^{-1} as reported by van der Star et al. (2007)). The influent nitrite concentration was kept 240 mg N L^{-1} throughout the phase considering that influent nitrite concentration higher than 280 mg N L^{-1} was inhibitory to the Anammox process in a continuously-fed fixed bed reactor (Isaka et al., 2007). When the nitrite conversion efficiency higher than 95% was maintained for at least 3 days, the hydraulic retention time (HRT) was shortened progressively. The influent ammonium concentration was relatively provided in excess in order to obtain higher nitrite removal efficiency according to Tsushima et al. (2007).

The effects of the organic matter on the ammonium conversion efficiency were investigated during Phase II (days 194–275) when the increments of organic matter were added to the UASB reactor in addition to the influent ammonium and nitrite concentrations of 240 mg N L^{-1} each and the HRT of 0.83 h. The influent COD concentration was gradually increased from 50 mg L^{-1} but stopped when the ammonium conversion efficiency decreased to 5%, which was considered to be a condition with little or even no Anammox activity. Each step was operated for at least 5 days after the influent COD concentration was increased.

In Phase III (days 276–351), the recovery performance of the UASB reactor was investigated in the presence of high COD concentration by supplementing nitrite as electron acceptor. The influent ammonium and organic matter concentrations were maintained constant at 240 mg N L^{-1} and 700 mg COD L^{-1}, respectively, and the HRT was set at 0.83 h. The influent nitrite concentration was incrementally increased by 40 mg N L^{-1} per step.

2.6. Analytical methods

The influent and effluent samples were collected on daily basis and were analyzed immediately. Water samples were analyzed according to the standard methods for the examination of water and wastewater (APHA, 1998). The analyzed parameters included ammonium, nitrite, nitrate, sulfide, chemical oxygen demand (COD) and pH. The biomass concentration was observed as total suspended solids (TSS) and volatile suspended solids (VSS). The ammonium and nitrite were analyzed using colorimetric method, nitrate was analyzed through ultraviolet spectrophotometry, COD through closed reflux method, and sulfide was analyzed using iodometric method. Gaseous composition (N_2, CH_4, CO_2 and H_2S) was analyzed through chromatography (Tremetrics Model 9000, USA) with a TCD detector and a Hayesap Q (80/100) column. Column, injector and detector temperatures were 35, 120 and 120 °C, respectively. The helium carrier gas had a flow rate of 30 mL min^{-1} (Ahn et al., 2004).

2.7. Transmission electron microscopy (TEM)

Sludge samples from the reactor were fixed in 2.5% glutaraldehyde solution and left in a refrigerator at 4 °C overnight. Then they were fixed with 1% osmium acid for 1–2 h after cleansing with phosphate buffer solution (0.1 M, pH 7.0). Subsequently, the samples were dehydrated through a graded series of 50%, 70%, 80%, 90% and 100% ethanol. After fixation and dehydration, samples were treated with pure acetone for 20 min. Then they were treated with a mixture of coating agent and acetone (V/V: 1/1, V/V: 3/1). Subsequently, the samples were infiltrated by pure coating agent and left overnight at 70 °C. Ultra thin sections of 70–90 mm size were obtained by Reichert microtome. They were stained with lead citrate solution and uranyl acetate in 50% ethanol saturated solution for 15 min, respectively. At last, the samples were observed by the transmission electron microscope (JEOL JEM-1230, Japan).

2.8. Scanning electron microscopy (SEM)

Morphology characteristics of the biomass specimens were observed using SEM model JEOL JSM-5600LV. The samples for SEM were obtained from the UASB reactor by using a sterile blade. The biomass specimens were scraped from the inner and outer layers of the granules by a sterile blade, respectively, and then fixed