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Ecological characteristics of seeding sludge triggering a prompt start-up of anammox

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HIGHLIGHTS

- Pre-inoculation to three external MBRs harvested the different initial seedings.
- Initial relative abundance and concentration trigger a prompt start-up of anammox.
- A high initial concentration and even anammox population benefits the start-up.

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ABSTRACT

Anammox start-up can be limited by the availability of seeding biomass in some areas. Previous studies have listed suitable alternative seeding sludge for anammox start-up such as anaerobic digestion sludge and conventional activated sludge (CAS), the ecological reasons behind has long been ignored. In this study, the inherent ecological factors that trigger a prompt start-up of anammox were identified, focusing on the initial relative abundance and concentration of anammox bacteria. An external membrane bioreactor was utilized as an enriching tool due to its suitability of retaining cells. Results revealed that a high initial concentration of anammox bacteria benefitted the start-up, meanwhile an even community seeding sludge (Gini coefficient < 0.25) gained a more than three-time higher anammox activity compared to the uneven one (Gini coefficient > 0.5). The discovery reminds to select the seeding sludge that is ecologically appropriate rather than to only care for the type of sludge in general.

1. Introduction

Anaerobic ammonium-oxidizing bacteria (anammox bacteria) belong to the *Planctomycetes* that are capable of autotrophically oxidizing ammonium with nitrite as electron acceptor (Kuenen, 2008). After having been discovered over 20 years, anammox has reached to a stage of full-scale application, treating ammonium rich wastewater with low demand of energy and cost (Joss et al., 2009; Kartal et al., 2010). Due to the slow-growing characteristics of anammox bacteria, the start-up from activated sludge is time-consuming and hence is not recommended (van der Star et al., 2007). Although lab-scale studies from different continents have reported successful start-up of anammox inoculated with local sources (Kieling et al., 2007; Park et al., 2010; Tang et al., 2011), considering the huge demand of anammox seed and the lack of experiences on efficiently enrichment, the full-scale application is still limited by the availability of anammox biomass in some areas (Araujo et al., 2011). Even for the cases where anammox seed were available, the mixed-in of high percent of extra sludge (Park et al., 2010) would decrease the initial concentration of anammox bacteria, possibly bringing uncertainties to start-up.

The comparison study based on previous experiences (Joss et al., 2009; Park et al., 2010; van der Star et al., 2007) indicates that the sole anammox start-up seeded with partial/all anammox biomass is faster than the one with complete conventional activated sludge (CAS). The probable reasons may be that anammox seed has: (1) higher relative abundance of anammox bacteria (higher proportion in a given community with the unit of %), and/or (2) higher anammox bacteria concentration (with the unit of gene copies per milliliter). Theoretically, a high abundance means being (one of) the dominant species in a microbial community. Previous studies have pointed that it is important to limit the growth of anammox competitors, for example ammonium-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and denitrifying bacteria (Dapena-Mora et al., 2004; Tao et al., 2011). Hence, it is conceivable that a seeding community with less proportion of anammox competitors would have a faster start-up. On the other hand, given the fact that high

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concentration of anammox bacteria will also present a high anammox activity (in kg N m^{-3} d^{-1}) (Hu et al., 2010; Shen et al., 2011), the high concentration of anammox bacteria in seeding sludge may also lead to a quick start-up. However, there is very limited experimental proof to these hypotheses that are based on comparison studies. From an ecological perspective, details concerning the characteristics of the favoring seeding sludge for anammox start-up are also scarce to date.

Membrane bioreactors (MBRs) combines suspended biomass with filtration membranes. From an engineering perspective, MBRs are ideal for localized and decentralized sewage treatment. From a microbiological perspective, since MBRs are able to (almost) completely keep biomass from washing-out, they are feasible tools for enriching slow-growing microbes such as methane-oxidizing sulfate-reducing microorganisms (Meulepas et al., 2009b), methanotrophic archaea (Meulepas et al., 2009a) and anammox bacteria (van der Star et al., 2008). In this study, high retention capability was used to identify the ecological factor of seeding sludge that triggers a prompt anammox start-up. Two ecological factors (relative abundance and bacterial concentration) are compared through a successful manipulation of initial community structure of the seeding sludge.

2. Methods

2.1. EMBR reactor

External MBRs (EMBRs) were used to start the anammox process, which is quite different from the previous studies that used submerged EMBRs (Suneethi and Joseph, 2011; Wang et al., 2009). An EMBR would circumvent the potential break of the strictly anaerobic environment caused by replacement of membrane module, which would benefit the start-up from conventional sludge. Although most biomass was returned to EMBRs after washing in buffer, the loss of some biomass during the replacement was still inevitable (about 150–200 mg volatile suspended solids (VSS) lost) due to their adhesive attachment to the membrane surface with the help of their metabolic products. Three EMBRs had the same size (volume of 3.0 L and 80% as effective volume) and configuration. The equipped membrane module had an effective volume of 0.19 L with a core of 15 sheets of polyethylene hollow fiber membrane (Mitsubishi Rayon Co., Ltd. Tokyo, Japan; mean pore size 0.4 μm). The bulk liquor was completely mixed by a mechanical stirrer (150 rpm). The EMBRs were covered with opaque fabrics to prevent phototrophic conversions. The EMBRs were fed with synthetic medium that has been described previously by Tao et al. (2011). The three reactors shared the same operational conditions (Table 1).

2.2. Seeding sludge and Pre-inoculation

EMBR1 was inoculated with about 10 g of VSS of CAS (described as ***control sludge***) from an anoxic tank of a full-scale wastewater treatment plant (WWTP) located in Harbin, China. The CAS was not washed using anammox medium before inoculation because the settlability of it was unsatisfied (SVI ~ 155 mL/gMLSS), which could be due to relatively low temperature when sampled (16 °C). So too much suspended biomass, which could play an important role in this study, could be washed out during washing process.

EMBR2 was firstly inoculated with the same type and amount (10 g VSS) of CAS as EMBR1. Then a 10 days pre-inoculation was applied (Fig. 1A) in order to achieve a microbial community with the similar concentration of anammox biomass to EMBR1 (the same order of magnitude in gene copies per milliliter biomass)

Table 1
Operational conditions.

Operational parameters	Value and unit
Temperature	33 ± 1 °C
pH of Influent	8.0 ± 0.3
pH in Reactor	7.3 ± 0.4
DO of influent	<0.08 mg L^{-1}
DO in reactor	<0.02 mg L^{-1}
TOC in reactor (stable stage[a])	0.5–11 mg L^{-1}
HRT	2 d
Retention time of sludge in the membrane module[b]	1.1 min
Effective life-span of the membrane[c]	23–40 d

[a] The stable stage means the time after day 60, when the fluctuations of TOC and VSS in the EMBRs were little.
[b] Since the effective volume of the membrane tube was 188 mL and the flow rate of recycling pump was 170 mL/min, the sludge stayed in the tube for about 1.1 min.
[c] The period from the start to the appearance of severe membrane fouling, by when the permeate flow rate could not match the influent flow rate. This value is relevant to both biomass concentration and bacterial metabolism.

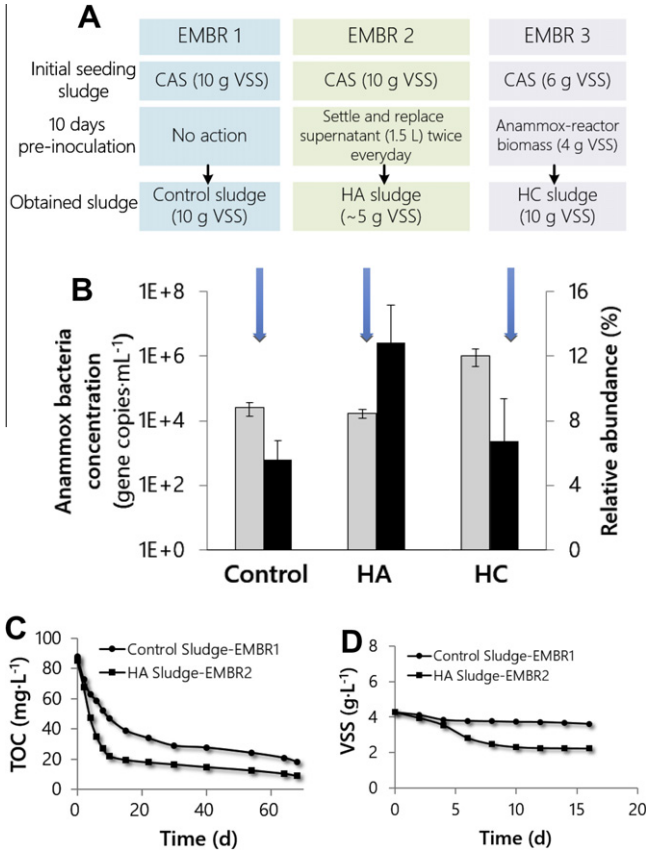

Fig. 1. Pre-inoculation to each EMBR and its result. (A) Operation scheme of the 10-day pre-inoculation process, CAS stands for conventional activated sludge. (B) Anammox bacteria concentration and the relative abundance after pre-inoculation, grey bars stand for the anammox bacteria concentration based on qPCR results and the black bars show the relative abundance based on T-RFLP analysis, error bars indicate the range of data from the triplicated tests. (C) The variation of total organic carbon (TOC) concentrations of EMBR1 and EMBR2. (D) The variation of the volatile suspended solids (VSS) concentrations of the sludge from EMBR1 to EMBR2.

but higher anammox abundance (percentage of anammox bacteria out of the total species). For every 12 h during pre-inoculation, EMBR2 was shut down for 30 min until most sludge settled and then the supernatant was moved out and replaced by new anammox medium (Fig. 1A). Since the medium was organic-matter free, the total organic carbon (TOC) concentration in EMBR2 rapidly decreased from 80 down to 20 mg L^{-1} and kept low (Fig. 1C). Thus,