B−49 Rapid Preservation and Reactivation of Anaerobic Ammonium Oxidizing Bacteria

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1. Introduction

Anaerobic Ammonium Oxidation (anammox) bacteria can oxidize ammonium (NH_4^+) using nitrate (NO_2) as the electron acceptor under anoxic condition, resulting in the production of nitrogen (N_2) (1). Anammox has revolutionized treatment of waste streams highly concentrated in NH_4-N, due to lower oxygen and external carbon source demands (2, 3). On the other hand, integration of anammox into common wastewater treatment system is still a challenge due to its slow growth rate with doubling time up to 11 days (4, 5) resulting in slow startup for the treatment processes (6, 7, 8).

The presence of viable anammox is paramount to the successful start-up of anammox process for wastewater treatment. In order to minimize start-up time of new treatment system, inoculation with preserved anammox biomass could be one possible way. Several techniques are available for the preservation of microbial culture e.g. refrigeration, freezing (-20ºC to -200ºC), lyophilization (freeze-drying), or any combination of these (9). While, these techniques have successfully preserved cultures of variety of micro-organisms, yet very few researches have been conducted on preservation of anammox. Vlaeminck *et al.* (10) demonstrated that long-term storage of anammox biomass obtained from OLAND process under sub-zero temperature (2 months at -20ºC) caused irreversible inactivation of the anammox. Rothrock *et al.* (11) could not reactivate anammox preserved during 4 month at -60ºC even with use of skim milk media and glycerol as cryoprotectants. However, they found that pre-freezing with liquid nitrogen (-200ºC) was a necessary step for successful long-term preservation of anammox via lyophilization. Later, Magri *et al.* (12) preserved anammox biomass via the PVA gel entrapment procedure through freezing included sub-zero temperature of -8 ºC during 17 hour but severely hindered the anammox activity. Recently, Heylen *et al.* (13) introduced a protocol for long-term cryopreservation of anammox method using dimethyl sulfoxide (DMSO) as a cryoprotective agent (CPA). Unfortunately, lyophilization and cryopreservation with CPA is difficult and seems to be impracticable at mass scale. Therefore, more easy, effective and rapid technique for the long-term preservation, storage and reactivation of anammox bacteria is still needed.

The goal of this study was to investigate simplest, rapid and effective methods for preservation and later reactivation of anammox biomass which can be easily doable for bulk storage anammox biomass. In this research, aggregated and immobilized anammox biomass were stored at different temperature i.e. -80ºC, 4ºC and room temperature. Specific anammox activity (SAA) of stored anammox biomass was determined by measuring $^{29}N_2$ production rate. Subsequently, 90 days stored biomass, at room temperature, was immobilized in PVA and sodium alginate gel and inoculated in small bench scale reactors.

2. Material and Methods

In this research, aggregated and immobilized anammox biomass, taken from Tsushima *et al.* (14), immersed in various nutrient feeds were stored at -80ºC, 4ºC and room temperature (**Table 1**).

Table 1: Anammox biomass storage conditions

S. No.	Storage Description	Biomass type	Temp (ºC)
1	Aggregated biomass pallet	Aggregated Biomass	-80ºC
2	Immobilized gel beads	Immobilized Biomass	-80ºC
3	Nutrient media[1]	Aggregated Biomass	4ºC
4	Nutrient media with molybdate[2]	Aggregated Biomass	4ºC
5	KNO_3[3] stored at 4ºC	Aggregated Biomass	4ºC
6	Nutrient media [4]	Aggregated Biomass	4ºC
7	Nutrient media with molybdate[4]	Aggregated Biomass	4ºC
8	Nutrient media[1]	Immobilized Biomass	4ºC
9	Nutrient media with molybdate[2]	Immobilized Biomass	4ºC
10	KNO_3 stored at 4ºC [3]	Immobilized Biomass	4ºC
11	Nutrient media[1]	Aggregated Biomass	ambient
12	Nutrient media with molybdate[2]	Aggregated Biomass	ambient
13	Nutrient media (same as S. No. 3) with Penicillin G	Aggregated Biomass	ambient
14	KNO_3[3] stored at room temperature	Aggregated Biomass	ambient

[1]$(NH_4)_2SO_4$ and $NaNO_2$ 5mM each;

[2]Sodium Molybdate (3mM) to inhibit sulphate reducing bacteria;

[3] KNO_3 media will be used and refrigeration temperature of 4ºC (10);

[4]Samples are kept without changing of media;

Later, specific anammox activity (SAA) of stored anammox biomass was determined, by measuring $^{29}N_2$ production rate as described by Oshiki *et al.* (15), after 45, 90 and 150 days of preservation. Further, anammox biomass, stored at room temperature for 90 days, was entrapped in polyvinyl alcohol (6%, w/v) and sodium alginate (2%, w/v) solution. Immobilized gel beads were inoculated in small bench scale reactors. These reactors were fed with synthetic wastewater containing NH_4 and NO_2 and nitrogen removal rates were ascertained.

3. Results and Conclusions

It was revealed that aggregated biomass stored at -80ºC recovered SAA after 45 days. However, SAA was not observed after 90 and 150 days of storage. In addition, storage of immobilized anammox biomass at -80ºC was not proved as successful **(Fig. 1).**

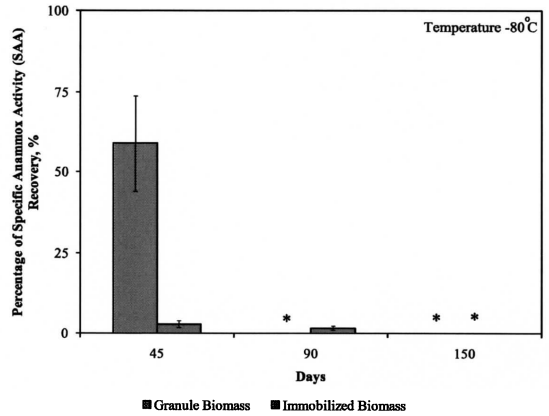

Fig. 1: Per cent recovery of specific anammox activity (SAA) of biomass stored at -80ºC after 45, 90 and 150 days. Anammox activity was based on ^{29}N-N_2 production rate from ^{15}N-ammonia and ^{14}N-nitrite. (*) represents no activity was observed. Error bars indicate the range of standard deviation (SD) derived from triplicates.

About 89% SAA recovery was obtained after storing aggregated biomass at 4ºC for 45 days. However, storage for more than 45 days at 4ºC was not a successful technique **(Fig. 2).**

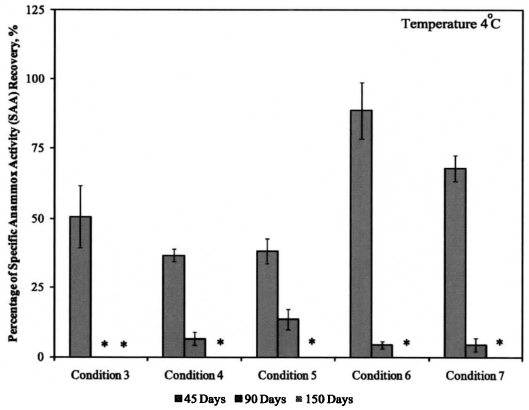

Fig. 2: Per cent recovery of specific anammox activity (SAA) of granular biomass stored at 4ºC after 45, 90 and 150 days. Anammox activity was based on ^{29}N-N_2 production rate from ^{15}N-ammonia and ^{14}N-nitrite. (*) represents no activity was observed. Error bars indicate the range of standard deviation (SD) derived from triplicates.

Preservation of immobilized biomass can also be a choice since about 60 and 37% activity was recovered while storing at 4ºC after 45 and 90 days, respectively **(Fig. 3).**

While storage at room temperature, granular anammox biomass showed excellent SAA recovery of about 96, 92 and 65% after storage for 45, 90 and 150 days, respectively **(Fig. 4).**

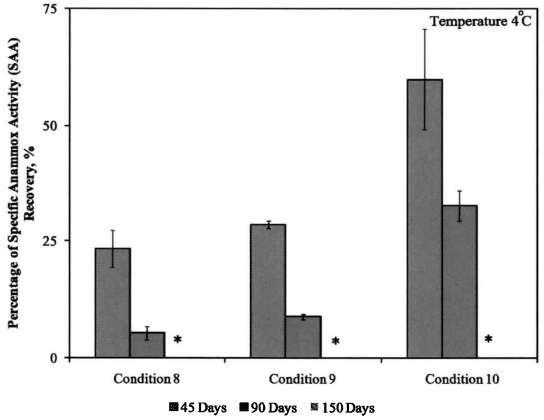

Fig. 3: Per cent recovery of specific anammox activity (SAA) of immobilized biomass stored at 4ºC after 45, 90 and 150 days. Anammox activity was based on ^{29}N-N_2 production rate from ^{15}N-ammonia and ^{14}N-nitrite. (*) represents no activity was observed. Error bars indicate the range of standard deviation (SD) derived from triplicates.

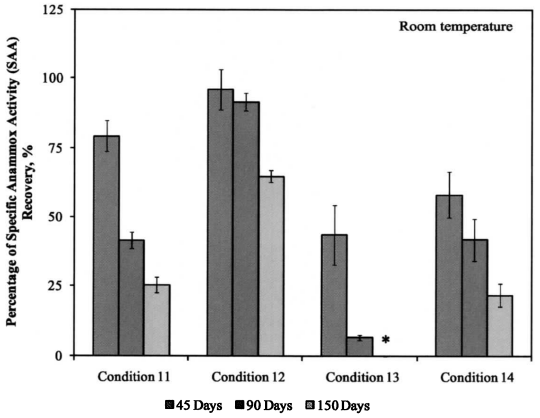

Fig. 4: Per cent recovery of specific anammox activity (SAA) of granular biomass stored at room temperature (15 ~ 25ºC) after 45, 90 and 150 days. Anammox activity was based on ^{29}N-N_2 production rate from ^{15}N-ammonia and ^{14}N-nitrite. (*) represents no activity was observed. Error bars indicate the range of standard deviation (SD) derived from triplicates.

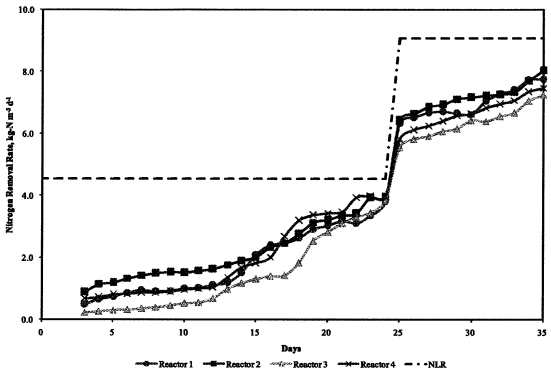

Fig. 5: Nitrogen removal rate (NRRs) of four reactors. Hydraulic retention time was 0.4 hours and nitrogen loading rate was maintained at 4.5 and 9.0 kg-N m^{-3} d^{-1} in each reactor. Temperature of these reactors was 37℃.

Each reactor showed the same nitrogen removal performance and ultimately achieved substantial NRR of 7 kg-N m^{-3} d^{-1} after 35 days of operation **(Fig. 5)**. Each anammox reactor perform equally well regardless of their different preservation conditions.

This study concluded that maximum anammox activity can be preserved while storing anammox biomass at room temperature. Storing anammox biomass without freezing not only preserve anammox activity for longer period of time but also save energy which is required while storage at freezing temperature. This simple and rapid storage technique can be easily applied for mass scale storage of anammox biomass. Storage of immobilized anammox biomass at room temperature should also be tested in future.

Further, early startup can be achieved by applying immobilization technique to preserved anammox biomass. Start-up time of full-scale anammox reactor can be reduced by inoculating immobilized anammox biomass initially. Though further investigation on optimum concentration of anammox biomass inside immobilized gel beads may be helpful to reduce initial required amount of anammox biomass.

4. References

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