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SHORT COMMUNICATION

Alkalinity and dissolved oxygen as controlling parameters for ammonia removal through partial nitritation and ANAMMOX in a single-stage bioreactor

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Abstract The oxidation of ammonia to dinitrogen through partial nitritation and anaerobic ammonium oxidation (ANAMMOX) in a single-stage bioreactor is based on suppressing the nitratation process. The single-stage process operated on a laboratory-scale fixed film bioreactor achieved ammonia removal of 0.7 kg NH_4-N/(m^3 day) at 4 h hydraulic retention time (HRT) by controlling the nitratation process through a 'three-way control mechanism' comprising control of electron donor (nitrite), electron acceptor (oxygen) and carbon source (bicarbonate). The control of alkalinity and dissolved oxygen (DO) concentrations in feed to maintain an alkalinity to ammonia ratio of less than 8 and DO loading of less than 0.06 mg O/(mg N day), respectively, was necessary for inhibiting nitratation and enhancing partial nitritation and ANAMMOX. Therefore, feed alkalinity along with DO concentrations are critical controlling parameters in a single-stage biological process for nitrogen removal.

Keywords Alkalinity · ANAMMOX · DO · Nitritation · Nitrogen removal

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Introduction

Biological nitrogen removal (BNR) in wastewater treatment is mostly carried out by multi-step microbial processes. Conventionally, treatment for wastewaters bearing high ammonia and high organics (chemical oxygen demand, COD) usually comprises two to three stages: the first stage for COD removal, the second for ammonia removal and the third for nitrate removal. Nitrification takes place in the second stage after most of the organics or COD in the wastewater have been removed in the first stage. Denitrification, in the third stage, is a heterotrophic process and needs organic carbon source, thus making the process more complicated. Because of high operation and maintenance cost, technologists are now looking for 'single-sludge single-reactor' systems that preferably have low or nil oxygen requirements. Completely autotrophic nitrogen removal over nitrite (CANON), oxygen-limited autotrophic nitrification–denitrification (OLAND) and simultaneous nitrification, ANAMMOX & denitrification (SNAD) are such 'single-sludge single-reactor' systems based on partial nitritation and ANAMMOX which are applied in wastewaters having low C/N ratio [1–3]. These processes attempt to combine the last two stages of conventional treatment into a single stage wherein the wastewater would be high in ammonia and low in COD concentrations. Principally, the compact reactor configuration in the single-stage CANON or OLAND process is cost effective due to lower oxygen demand, but technically, operational control is more sensitive than the two-stage stable high-rate ammonium removal over nitrite (SHARON)–ANAMMOX process [4].

Intricate control of process parameters is required to maintain balance between aerobic (ammonium-oxidizing bacteria, AOB, and nitrite-oxidizing bacteria, NOB) and anaerobic groups of ammonia oxidizers (ANAMMOX

bacteria) [5]. To achieve a single-stage biological nitrogen removal (SBNR) process, the activity of NOB has to be inhibited without affecting the activities of AOB and ANAMMOX bacteria. These three groups of microorganisms are closely interlinked with common electron donor and acceptors. Many workers have reported that by controlling the concentration of DO and nitrite, partial control of NOB activity could be achieved [4, 6–9]. NOB competes with AOB and ANAMMOX bacteria for DO and nitrite, respectively. In the absence of nitrite in wastewater, NOB depends directly on AOB for their source of electron donor. By limiting DO concentration, AOB consume available DO for nitrite production. Hence, under this condition, NOB experiences two-way limitations, initially in terms of electron donor (nitrite) and later in terms of electron acceptor (oxygen). Similarly, all the three groups of chemolithotrophic microorganisms also require inorganic carbon source for their cell growth [2]. By controlling bicarbonate alkalinity, the process of elimination of NOB can be further fine-tuned by a 'three-way control mechanism'. In the CANON and OLAND processes, control of nitratation is achieved by limiting DO. The effect of alkalinity on such single-stage systems has not been reported so far.

The aim of this study was to investigate the feasibility of performing the SBNR process in a laboratory-scale fixed film bioreactor system and to assess the effect of alkalinity on the SBNR process in association with limiting DO.

Materials and methods

Experimental setup

A 0.5-l-capacity glass reactor with an effective volume of 0.43 l was used for the study. It was packed with uniformly sized pieces of refractory bricks (0.2- to 0.3-cm diameter) as support media for biomass. The 2-l capacity influent tank had detachable DO and pH probes (Multimeter PCD 650, Eutech) for monitoring DO and pH, respectively. A peristaltic pump (SciQ 323 pump, Watson Marlow) was used for delivering feed into the reactor at a predetermined and constant flow rate. The effluent was collected in an effluent collection tank.

Synthetic wastewater

The experiments were conducted with simulated wastewater as per our previous study [6] and no organic carbon was added. Ammonia was added as $(NH_4)_2SO_4$ in the required concentration for feed preparation (Fig. 1). Under ambient conditions, DO in the feed was always present in the range of 4.0–4.5 mg/l, and external aeration was not provided for maintaining specific DO concentration in the bioreactor.

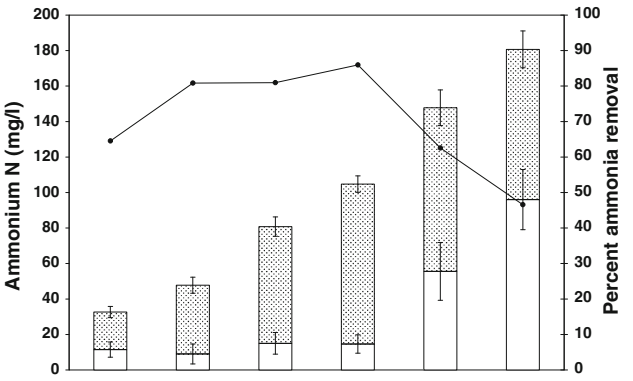

Fig. 1 Percentage ammonia removal at different influent ammonia concentration between day 1 and 214 at a fixed HRT of 4 h. The *whole bars* represent influent ammonia loading and *white bars* represent residual ammonia loading. *Line plots* (*filled circles*) represent percentage ammonia removal. *Error bars* indicate the standard deviation

Seed biomass

The seed biomass was obtained from mixing biomass from two lab-scale reactors, one from an oxygen-limited, ammonia-oxidizing fixed film reactor [6] and another from a laboratory-scale ANAMMOX reactor treating ammonia under anaerobic conditions for more than 300 days (Bagchi et al., personal communication). The AOB and NOB populations in the fixed film bioreactor were confirmed through fluorescence in situ hybridization (FISH) assay using 5′ fluorescein tagged Nsm 156 and Nit 3 probes. The anaerobic ANAMMOX culture was reddish-brown in colour, microgranular in appearance with a mean diameter of 10–20 μm. ANAMMOX bacteria was confirmed through positive hybridization of the biomass with 5′ fluorescein tagged Amx 820 and Pla 46 probes confirming presence of *Candidatus "Brocadia anammoxidans" and Candidatus "Kuenenia stuttgartiensis"* species as well as the broad group of *Planctomycetales* in the reactor, respectively (data not shown).

Process operation

The seed biomass had suspended solids (SS) and volatile suspended solids (VSS) values of 25.5 g/l and 19.5 g/l, respectively. The HRT was maintained at 4 h. During the study period, the liquid temperature varied between 25 and 34°C.

Analytical methods

Ammonia was estimated by a colorimetric method according to standard methods [10]. Nitrate and nitrite

were analysed by an ion chromatography system (Metrohm) using conductivity detection. Separation and elution of anions were carried out on an IonPac 250A anion column, utilizing carbonate/bicarbonate eluent and autosuppressor technology. DO and pH were measured by using a multimeter (PCD 650, Eutech). Alkalinity was measured by a titrimetric method as per standard methods [10]. The experimental results were statistically analysed by using Minitab 15. The same software was used for configuring the three-dimensional contour plots.

Results and discussion

The laboratory-scale fixed film bioreactor reactor was operated for 214 days with increasing ammonia loading rate. The operational parameters are given in Table 1 while the performance of the process is depicted in Fig. 1. The maximum ammonia removal rate of 0.7 kg NH_4-N/(m^3 day) and specific ammonia removal rate (K) of 0.13 kg NH_4-N/(kg VSS day) were achieved at highest ammonia loading of 1.32 kg NH_4-N/(m^3 day). The nitrogen conversion rate was comparable to other single-stage nitrogen removal processes (Table 2). During the initial 100 days of reactor operation, on average, 62.5% of ammonia was oxidized to nitrate. Nitrate generation reduced to 0–6 mg NO_3-N/l after 150 days (Fig. 2). The ratio of nitrate generated to ammonia oxidized was 0.81 ± 0.3 during the peak nitrate generation period (initial 100 days) indicating nitratation activity.

During nitrification, 7.14 g of alkalinity is consumed per gram of N oxidized [11]. Hence, an alkalinity consumption (alkalinity to ammonia consumption) ratio of 7 or more is considered as an indicator of nitratation, which is not desirable in the SBNR process. The two-dimensional contour plot in Fig. 3a also confirms suppression of nitratation at an alkalinity to ammonia consumption ratio of less than 8.0. The maximum ammonia removal of 117.3 mg

Table 1 Characteristics of the SBNR process

Parameter	Magnitude
Test period (days)	1–214
Influent ammonia concentration (mg NH_4-N/l)	29–200
Ammonia load [kg NH_4-N/(m^3 day)]	0.25–1.32
Ammonia removal [kg NH_4-N/(m^3 day)]	0.08–0.7
Ammonia removal (%)	34–99
Nitrogen gas generation [kg NH_4-N/(m^3 day)]	0–0.74
Specific ammonia load [kg NH_4-N/(kg VSS day)]	0.016–0.1
Specific ammonia removal [kg NH_4-N/(kg VSS day)]	0.007–0.065
Maximum specific ammonia removal rate (K) [kg NH_4-N/(kg VSS day)]	0.13
Hydraulic retention time (h)	4.0

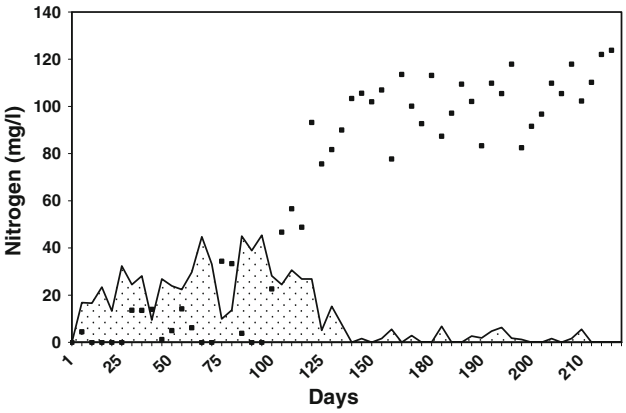

Fig. 2 Evolution of nitrogen gas generation in the SBNR process. The *dotted area* represents the nitrate generation and the *filled squares* represent nitrogen generation

Table 2 Comparison of maximum nitrogen loading rate and nitrogen removal rate of different single- and two-stage reactors based on partial nitritation and ANAMMOX processes

Process	Reactor type	NLR_{max} [kg N/(m^3 day)]	NRR_{max} [kg N/(m^3 day)]	Reference
OLAND	SBR	0.13	0.05	[1]
CANON	SBR	0.131	0.075	[2]
CANON	SBR	0.22	0.12	[8]
SNAD	NRBC	0.69	0.48	[3]
SBNR	FFB	1.32	0.7	This study
OLAND	RBC	0.716	0.7	[9]
SHARON–ANAMMOX	CSTR + SBR	1.2	0.75	[14]
CANON	MABR	0.87	0.77	[4]
CANON	Gas lift	5.5	1.5	[13]

SBR sequential batch reactor, *NRBC* non-woven rotator biological contractor, *FFB* fixed film bioreactor, *RBC* rotator biological contractor, *CSTR* continuous stirred tank reactor, *MABR* membrane aerated bioreactor, NLR_{max} maximum nitrogen loading rate, NRR_{max} maximum nitrogen removal rate

NH_4-N/l occurred at an influent alkalinity to ammonia ratio of 3.4. This value corresponds with the theoretical alkalinity consumption of 3.6 mg/mg NH_4^+-N oxidized in the CANON process [1].

In the present study, in order to limit nitratation, external aeration was not provided. The dissolved oxygen present in the influent, in the range of 4–4.5 mg/l, was the only source of oxygen in the reactor system. As per the stoichiometry of nitrification, for oxidation of 1 mg of NH_3-N to NO_3-N, 4.56 mg of oxygen is required [7]. In the absence of external aeration, a steady DO concentration in the reactor was not ensured. Hence, only 4.0–4.5 mg of DO was available in the feed to oxidize 29–200 mg of NH_3-N. As feed ammonia concentration varied, the applied DO load per unit ammonia concentration also varied. The performance data indicated that DO loading of more than 0.06 mg O/(mg N day) favoured nitratation (Fig. 3b). Hence, a DO concentration of less than 0.06 mg O/(mg N day) in bulk liquid within the reactor is desirable for the SBNR process. In the CANON process, a DO consumption of 0.21–0.36 mg O/mg N has been reported which is higher than that in the present study (Table 3). The result is in accordance with our previous observations where partial nitrification was achieved under oxygen-limiting condition [6]. Similarly, nitrate generation to ammonia oxidation ratio during the 175–214th days of operation ranged from nil to 0.07, which is lower than the reported ratio of 0.26 for the CANON process [4, 8]. The

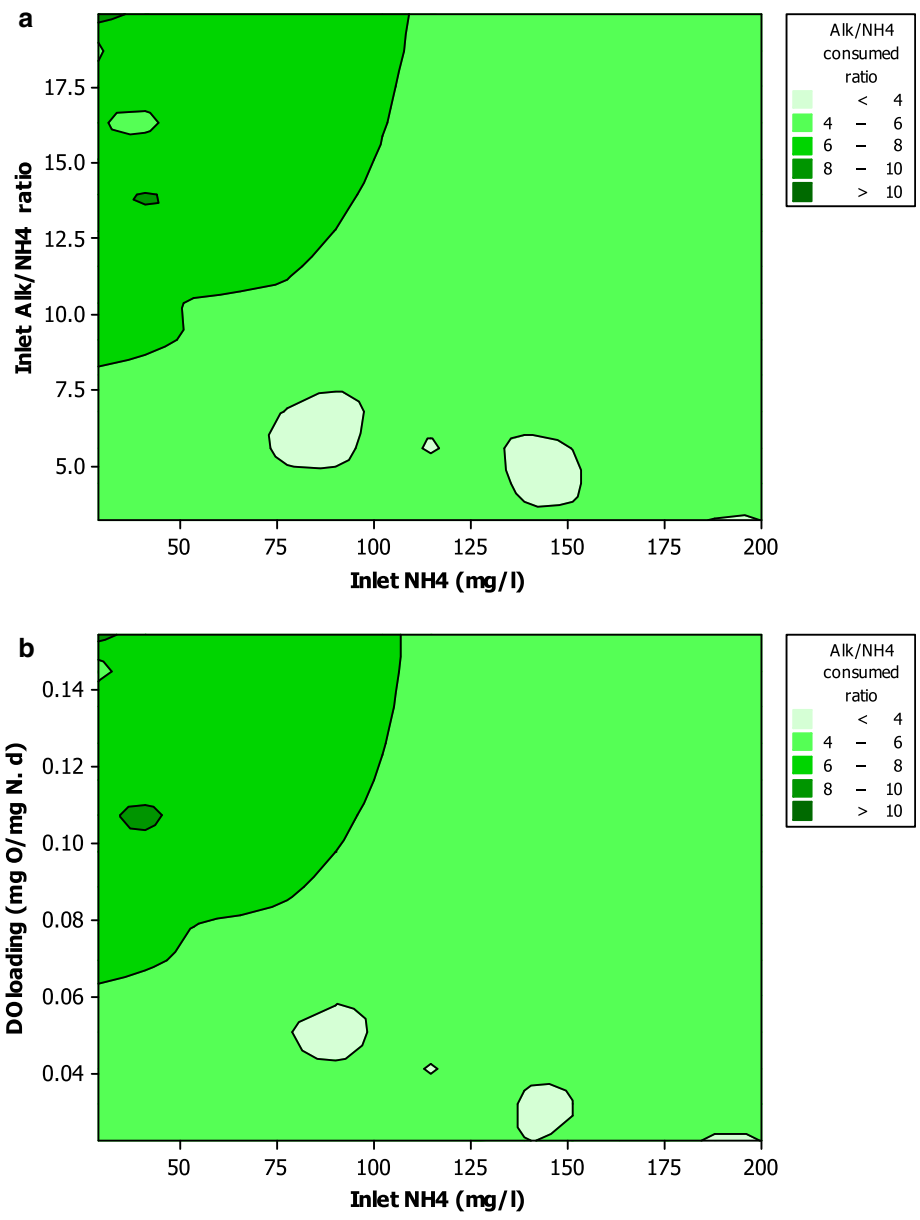

Fig. 3 Two-dimensional contour plots of the two-factor interaction models for consumed alkalinity to ammonia ratio: **a** showing effect of inlet alkalinity to ammonia ratio and influent ammonia and **b** showing effect of DO loading and influent ammonia

Table 3 DO requirement in different biological nitrogen removal processes

Process	Oxygen requirement (mg O/mg N)	Reference
Nitrification–denitrification	4.6	[7]
Partial nitrification	3.43	[8]
SHARON–ANAMMOX	1.9	[15]
CANON	0.21–0.36	[12]
SBNR	0.06	This study
ANAMMOX	Nil	[13]

observed oxygen requirement is incompatible with the established mechanism of the CANON process based on stoichiometric calculations. Further studies are needed to understand the mechanism of low nitrate generation in this hitherto unknown process.

In single-stage biological nitrogen removal process, the aerobic NOB must be kept out of competition. As the ammonia concentration was increased in the reactor, the aerobic AOB utilized ammonia by consuming available oxygen and alkalinity, and thereby out-competed the aerobic NOB by limiting oxygen and bicarbonate concentrations. This is reflected in increased nitrogen removal efficiency and reduced nitratation with time (Fig. 2). Under oxygen-limited conditions, nitrite oxidizers had to compete for oxygen with the aerobic AOB and for nitrite with anaerobic ammonia oxidizers [2]. Similarly, stiff competition exists between all the three groups of chemolithotrophic nitrogen-removing bacteria for inorganic carbon source. The presence of all three groups of bacteria in the SBNR sludge was confirmed during the start-up of the experiment through FISH assay. By introducing alkalinity-limited conditions, the nitrite oxidizers face yet another stress of limited carbon source. The limitation of substrates (oxygen and nitrite), coupled with limited availability of carbon source, keeps the nitrite-oxidizing bacteria from competition. Therefore, feed alkalinity and DO can be used as controlling parameters for biological nitrogen removal in a single-stage bioreactor system. Such single-stage BNR systems have potential applications in the treatment of nitrogenous wastewater having low C/N ratio such as in municipal leachate, landfill rejects, effluents from fertilizer industries, etc.

Conclusion

Single-stage biological nitrogen removal based on partial nitritation and ANAMMOX has been successfully achieved in a laboratory-scale fixed film bioreactor. Ammonia removal of 0.7 kg NH_4-N/(m^3 day) was achieved at maximum ammonia loading of 1.32 kg NH_4-N/(m^3 day). The study revealed that by maintaining the feed alkalinity to ammonia ratio of less than 8 along with limiting DO concentration, the SBNR process can be favoured by inhibiting nitrite-oxidizing bacteria. A DO loading of less than 0.06 mg O/(mg N day) was essential for maintaining the SBNR process. The DO loading needed for partial nitritation could be easily achieved in the wastewaters without requiring external aeration, leading to economy in the operational cost.

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