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Nitrification in sequencing batch reactors with and without glucose addition at 11 °C

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Abstract

Nitrification was investigated in two laboratory-scale sequencing batch reactors (SBRs) at 11 °C, one with glucose addition (G-Reactor) and the other without (N-Reactor). The characteristics of nitrification and the distribution of ammonia oxidizing bacteria (AOB) within activated sludge flocs in the two reactors were examined. A high specific nitrification rate existed in the N-Reactor, while a high volumetric nitrification rate existed in the G-Reactor. The proportion of AOB enriched in the N-Reactor was about three times that in the G-Reactor. The activated sludge flocs in the N-Reactor had a larger floc size and a higher biomass density than in the G-Reactor. AOB were mainly in a form of clusters in the activated sludge flocs in the N-Reactor, but were well dispersed throughout the flocs in the G-Reactor.

Keywords: Ammonia oxidizing bacteria; Fluorescence *in situ* hybridization; Glucose; Heterotrophs; Nitrification

1. Introduction

Discharging wastewater containing ammonium nitrogen into water bodies can be toxic to fish, cause eutrophication and decrease the dissolved oxygen concentration in the receiving waters. Therefore, it is desirable to remove nitrogen from wastewaters prior to discharge. A typical biological nitrogen removal system comprises: (i) nitrification in aerobic conditions followed by (ii) denitrification in anoxic conditions. Nitrifying autotrophic bacteria carry out nitrification in two steps—nitritation and nitratation. In the nitritation step, ammonium is oxidized to nitrite by ammonia oxidizing bacteria (AOB), and in the nitratation step, nitrite is oxidized to nitrate by nitrite oxidizing bacteria (NOB). During denitrification processes, nitrate and/or nitrite are reduced to N_2 by denitrifiers.

The factors that can affect the nitrification kinetics include: organic carbon and nitrogen concentrations, microorganism populations including the ratio of heterotrophs to autotrophs, and environmental conditions, e.g., temperature. The carbon/nitrogen (C/N) ratio is a controlling factor in determining the heterotroph/autotroph population ratio in wastewater treatment systems [1–4] and nitrification is inhibited at high C/N ratio conditions. In single-reactor activated sludge systems (where all biological processes occur in one reactor) or biofilm systems, heterotrophic carbonaceous oxidation takes precedence over autotrophic nitrification, and nitrification rates decrease as the concentration of biodegradable carbon increases [5,6]. In addition, microorganisms are in forms of clusters in activated sludge flocs and mass transfer limitation could exist in the inner part of the flocs. Therefore, the distribution of microorganisms within activated sludge flocs is an important factor controlling the related bio-kinetics. Nitrification is more affected by substrate limitation, since substrate is consumed by heterotrophs first, than by mass transfer limitation in activated sludge flocs [7]. The rate of the nitrification process increases with the increase of temperature, roughly doubling for each 10 °C rise. The optimal temperature for nitrification was found to be in the range of 30–35 °C and nitrification was adversely affected at low temperatures [8–10]. However, in Ireland, nitrification occurs in wastewater treatment plants at the ambient temperature of approximately 11 °C, on average. Therefore, it is necessary to investigate nitrification at low temperatures like 11 °C.

In order to alleviate the adverse effects of high C/N ratios and low temperatures on nitrification, techniques like bioaugmentation and biofilm systems have been applied to improve

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nitrification [11–15]. These techniques are based on separation of the sludge retention time (SRT) from the hydraulic retention time (HRT). However, it is not rare in practice that nitrification fails under bio-augmentation conditions or in biofilm systems. This is partly due to the lack of a thorough understanding of the nitrification process like the characteristic of AOB and interactions between AOB and heterotrophs.

Therefore, the aims of the present study were to: (i) enrich nitrifiers and evaluate nitrification at 11 °C in two sequencing batch reactors (SBRs), one treating synthetic wastewater with high C/N ratios due to the addition of glucose and the other treating synthetic wastewater with C/N ≈ 0 without glucose addition; and (ii) examine the distribution of AOB within activated sludge flocs in the two SBRs.

2. Materials and methods

2.1. Nitrification enrichment

Two identical 2-l laboratory-scale SBRs were used to enrich nitrifiers. One SBR was fed with wastewater with the addition of glucose (G-Reactor) and the other SBR (N-Reactor) without glucose addition. The two SBRs were operated at 11 °C in a temperature-controlled room. There were five operational cycles per day and each cycle comprised the following phases: aerobic react (210 min, including a 20-min fill phase), settle (50 min) and draw/idle (28 min). A programmable timer was used to control the action of the operational sequence of SBRs. Oxygen was supplied by air pumps during the aerobic react phase and dissolved oxygen concentrations in the bulk water were maintained above 2 mg l^{-1}. pH ranged from 7.2 to 8.0 in both reactors during the aerobic phase. One liter of synthetic wastewater was fed and treated in each cycle, providing a HRT of 9.6 h. Mixed liquor (100 ml) was removed from the G-Reactor once a day, resulting in a SRT of 20 days. The investigation on the G-Reactor included three 20-day stages: the C/N ratios were 1.5, 0.7 and 0.4 in Stages I, II and III, respectively (Table 1). In the N-Reactor, the SRT of 20 days was maintained during the first 10 days (Stage I), but the biomass concentration was found to decrease rapidly due to the low biomass growth rate, so no mixed liquor was removed after Day 10. The later experiment on the N-Reactor was divided into another two stages: Stages II and III. Stage II lasted for 30 days with an influent NH_4-N of 57 mg l^{-1} and Stage III lasted for 20 days with an influent NH_4-N of 105 mg l^{-1}. Both SBRs were seeded with activated sludge taken from the Tuam Municipal Wastewater Treatment Plant, Galway, Ireland.

The added glucose, ammonium-N and $NaHCO_3$ in the synthetic wastewater and operational conditions are given in Table 1. The other components contained in the synthetic wastewater fed to both reactors included: 200 mg l^{-1} of $Na_2HPO_4{\cdot}12H_2O$, 12 mg l^{-1} of yeast extract, 200 mg l^{-1} of $KHCO_3$, 200 mg l^{-1} of $MgSO_4{\cdot}7H_2O$, 8 mg l^{-1} of $FeCl_3{\cdot}6H_2O$, 12 mg l^{-1} of $CaCl_2{\cdot}6H_2O$ and 8 mg l^{-1} of $MnSO_4{\cdot}H_2O$. The synthetic wastewater contained 17.3 mg P l^{-1}.

2.2. Test of the oxygen utilization rate (OUR)

The OUR was examined using batch experiments at 11 °C. Activated sludge samples were taken from both reactors at the end of Stage III, centrifuged at 3900 rpm for 10 min and washed twice to remove soluble nitrogen. The samples were resuspended in solutions (200 ml) with a composition that was the same as the synthetic wastewater but without any nitrogen and carbon. The mixed liquors were aerated for 30 min before the OUR tests commenced.

The OUR was measured using a Clark type O_2 micro-sensor (tip diameter of 10–15 μm) [16]. An experimental set-up consisted of a two-channel picoammeter, an analogue digital (A/D) converter, profiling-software (Profix 3.09, Unisense A/S) and a computer which was used for data collection. The OURs (three replications in each testing) of the activated sludge samples were measured sequentially for the background (mixed liquor without the addition of carbon and nitrogen), after the addition of NO_2-N (10 mg l^{-1}), NH_4-N (10 mg l^{-1}) and glucose (20 mg l^{-1}). In these batch experiments, the concentration of mixed liquor volatile suspended solids (MLVSS) was controlled around 1 g l^{-1}.

2.3. Analytical methods

Mixed liquor suspended solids (MLSS) and MLVSS were determined according to standard methods [17]. NH_4-N, NO_2-N and NO_3-N were tested using a nutrient analyzer (Konelab, USA) and the floc size was measured using a Mastersizer (Malvern Instrument, UK).

To measure the density of the activated sludge flocs, 10 ml of mixed liquor was centrifuged at 3900 rpm for 10 min and the supernatant was removed. The volume of solid biomass remain-

Table 1
Operational strategies used for the N-Reactor and the G-Reactor

	N-Reactor			G-Reactor		
	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III
Glucose-C (mg l^{-1})	–	–	–	80	80	80
NH_4-N (mg l^{-1})	57	57	105	52	110	203
$NaHCO_3$ (mg l^{-1})	520	520	520	520	520	1040
SRT (days)	20	–	–	20	20	20
Glucose-C/NH_4-N	0	0	0	1.5	0.7	0.4
Duration (days)	10	30	20	20	20	20