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Effect of COD/N Ratio on Growth and Adaptation of Nitrifying Bacteria

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Abstract

Acclimation of nitrifying bacteria using inorganic carbon source (bicarbonate) and organic carbon source (bicarbonate + glucose) was performed in this study. Sequential batch reactors were inoculated by activated sludge (mixed culture of heterotrophic and nitrifying bacteria), and fed by synthetic wastewater contain high concentration of ammonium (1000 $mg \cdot L^{-1}$). Adapted nitrifying bacteria, only using bicarbonate as carbon source, caused nitrification at a rate of 41 $mg\ L^{-1}\ h^{-1}$. The second reactor, which using bicarbonate and glucose as carbon source due to high C/N ratio resulting high growth of heterotrophic bacteria and result revealed a minor effect on nitrifying bacteria, and complete removal of ammonium occurred.

Keywords: Wastewater, nitrification, acclimatization, heterotrophic bacteria

1. Introduction

Inappropriate discharge of wastewater containing ammonia causes eutrophication in water bodies and toxicity to aquatic culture [1]. Biological nitrogen removal as a promising method for eliminating ammonia from wastewater generally occurs in two steps, namely, nitrification and denitrification[2]. Nitrification is known as a controlling step that consists of two consecutive familiar reactions, nitritation (Eq. (1)) and nitratation (Eq. (2)), which are carried out by autotrophic bacteria in two main groups — ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), respectively [3].

First step: $NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O$ (1)

Second step: $NO_2^- + 0.5O_2 \rightarrow NO_3^-$ (2)

The efficiency and sustainability of nitrification processes depend on the activities of nitrifying bacteria, and the rate of nitrification is affected by many factors, such as nitrogen concentration, organic carbon, and microorganism population, including the ratio of heterotrophs to autotrophs, sludge age, and environmental conditions (e.g., temperature and pH) [4]. Among different controlling factors, the substrate concentration (carbon/nitrogen, C/N) determines the heterotroph/autotroph population ratio in such wastewater treatment systems as single reactor activated sludge, SBR, and biofilm systems. When the concentration of organic carbon is high (high ratio of C/N), heterotrophic bacteria dominate the denitrifying bacteria, thereby reducing the nitrification rate [5]. Therefore, researchers have attempted to determine the C/N ratio, which acts as a limiting factor in the nitrification process. As a result, full nitrification of sewage treatment plants occurs at a loading rate lower than 0.15 g-COD g-VSS L^{-1} d^{-1} [6]. Dinçer & Kargi (2001) reported that a C/N ratio less than 0.25 is essential to carry out effective nitrification. Furthermore, Okabe et al.(1996) found that a C/N ratio of 1.5 has unfavorable effects on the performance of the nitrification process in a biofilm system [7]. Wu. G. et al. (2008) investigated the effect of different C/N ratios (1.5, 0.7, and 0.4) on the enrichment of nitrifying bacteria in a nitrification reactor as well as in a nitrification and organic removal reactor. Their results demonstrated potential enrichment in both reactors but that the specific nitrification rate in the nitrification reactor was higher than that in the other reactor. Researchers have recommended using two biological units, with the nitrification process occurring separately in the second unit, to overcome the above-described shortcomings [6, 8]. However, although a separate unit for nitrification may enhance the potential of nitrification, it increases the initial capital and maintenance cost of the entire procedure. Other researchers have thus focused on the development of energy-saving nitrogen removal systems and increasing the nitrification rate by applying cost-effective processes in the treatment of reject water [9], such as SHARON [10], Anammox, and a combination of the two [11].

Partial nitrification (PN) occurs via AOB according to Eq. (1) limit the growth of NOB and enrich AOB, which cause nitrite accumulation [2, 9, 12]. Savings of 25% in aeration costs using low concentrations of dissolved oxygen only to enrich AOB and a reduction of 40% of the external carbon source needed during denitrification by limiting NOB in PN have been reported [13, 14].

This study evaluated the role of the C/N ratio as a controlling factor in the nitrification process. In addition, the possibility of partial nitrification as a cost-effective process was investigated during enrichment of nitrifying bacteria with a high concentration of ammonium.

2. Materials and methods

2.1. Experimental set-up and feeding

Tow laboratory-scale sequencing batch reactors (SBRs) with a working volume of 5 L and sequencing stages of 23 h reaction, 50 min settling, 5 min decanting, and 5 min filling have been used in this study (Figure1). The operating conditions in this research are summarized in Table 1.

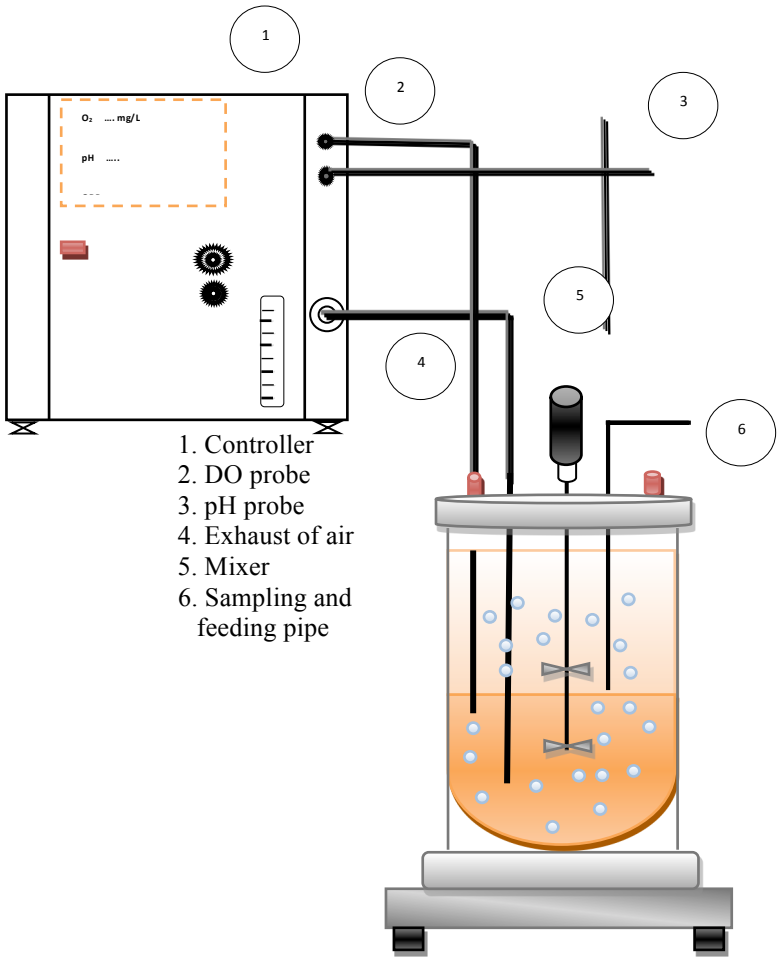

Figure 1. Schematic diagram of the experimental apparatus

Activated sludge from an wastewater treatment plant (WWTP) in Pantai Dalam, Kuala Lumpur, Malaysia with an initial mixed-liquor suspended solid (MLSS) concentration of 2 $g \cdot L^{-1}$ was inoculated in two sequencing batch reactors. The reactors were fed with synthetic wastewater consist of different C/N ratio with 1000 $mg \cdot L^{-1}$ of $(NH_4)_2SO_4$ as a source of nitrogen; 200 $mg \cdot L^{-1}$ KH_2PO_4; 3000 $mg \cdot L^{-1}$ $NaHCO_3$ and 1 ml/L trace elements: MgSO4 (60mg/L), EDTA (10mg/L), ZnSO4.7H2O (2.2mg/L), CoCL2.6H2O (3.2mg/L), MnCl2.4H2O (10.2mg/L), CuSO4.5H2O (0.22mg/L), (NH4)6Mo7O24.4H2O (2.2mg/l), CaCl2.2H2O (1.1mg/L), FeSO4.7H2O (10mg/L), H3BO3 (0.3mg/L), NiSO4.6H2O (1mg/L).

Table 2. Operational conditions of the SBRs

Parameters	R1	R2
Temperature (ºC)	30±0.5	30±0.5
DO (mgL^{-1})	>3	>3
HRT(h)	48	48
Cycle (h)	24	24
MLSS (mgL^{-1})	2000	2000
MLVSS (mgL^{-1})	1650	1650
pH	7.6± 0.3	7.6± 0.3
C/N	0	500
Volumetric exchange rate (%)	50%	50%

3. Analytical methods

The samples were analyzed for determination of ammonium, nitrate, and nitrite concentrations by using an Advanced Compact IC 861 (Metrohm® Ltd., Herisau, Switzerland) ion chromatograph (IC) and guard column. A digital controller monitored the process temperature, pH, DO, and ORP continually. In addition, the MLSS and MLVSS were determined following standard methods [15]. By adjusting pH, temperature, and the remaining concentration of ammonium, FA and FNA concentrations were calculated according to Equations (3) and (6) [9].

$$FA(NH_3, mgL^{-1}) = \frac{17}{14} \times \frac{[NH_4^+] \times 10^{pH}}{e^{\left[\frac{6344}{(273+T)}\right]} + 10^{pH}} \quad (3)$$

$$FNA(HNO_2, mgL^{-1}) = \frac{46}{14} \times \frac{NO_2^-}{e^{\left[\frac{-2300}{(273+T)}\right]} \times 10^{pH}} \quad (4)$$

4. Results and discussion

In the first stage, the external carbon source was established as C/N ratio 0 for R1 and 0.5 for R2 by glucose. During this period, Figure 2 shows that in the first cycle, the NH_4^+–N concentration decreased from 1000 mg/L to 0 mg/L at 144 h in R1. Reactor R2 showed higher efficiency in

reducing ammonium at the beginning of the process, which may be due to fast activation of process by heterotrophic bacteria. However, the complete ammonium removal occurred after 156 h. Figure 2 illustrates that acclimatization of nitrifying bacteria at C/N = 0 according to the goal of this study (24 h) was observed in R1 after 11.5 days during 4 cycles. On the other hand, complete nitrification was observed in R2 after 12 days. The ammonium reduction was less in R2 than R1, but the accumulation of nitrite was different, which can be due to glucose addition. The presence of organic carbon in R2 can support the growth of heterotrophs and was able to inhibit the activity of nitrifying bacteria [16].

The inhibition by FA and FNA takes place at different concentrations, which means that the AOB and NOB were able to acclimatize to high FA and FNA concentrations. However, the determination of threshold inhibition values of FA on NOB have been somewhat inconsistent until now [17]. In this study, by maintaining some controlling parameters constant, such as DO, pH, temperature, and concentration of ammonium, the effects of FA and FNA concentrations were considered as the main strategy to inhibit NOB activity for the performance of PN process. In both reactors R1 and R2, the FA and FNA concentrations gradually decreased due to the increase of ammonium removal at the first cycle of the biological oxidation process of ammonium. At subsequent cycles, especially at the second stage of process, the concentration of FA and FNA was approximately 70 $mg \cdot L^{-1}$ and 0.2 $mg \cdot L^{-1}$, respectively.

Therefore, the accumulation of NO_3^- -N, approximately 18 $mg \cdot L^{-1}$ at first cycle of process, was negligible, which shows that the ammonium oxidizing reactions were dominant due to higher concentration of FA and FNA than the threshold reported by Anthonisen et al. (1976) (AOB was inhibited from 10 mg-FA/L to 150 mg-FA/L, and the inhibition of NOB began at a concentration of 0.1 mg-FA/L to 1 mg-FA/L).

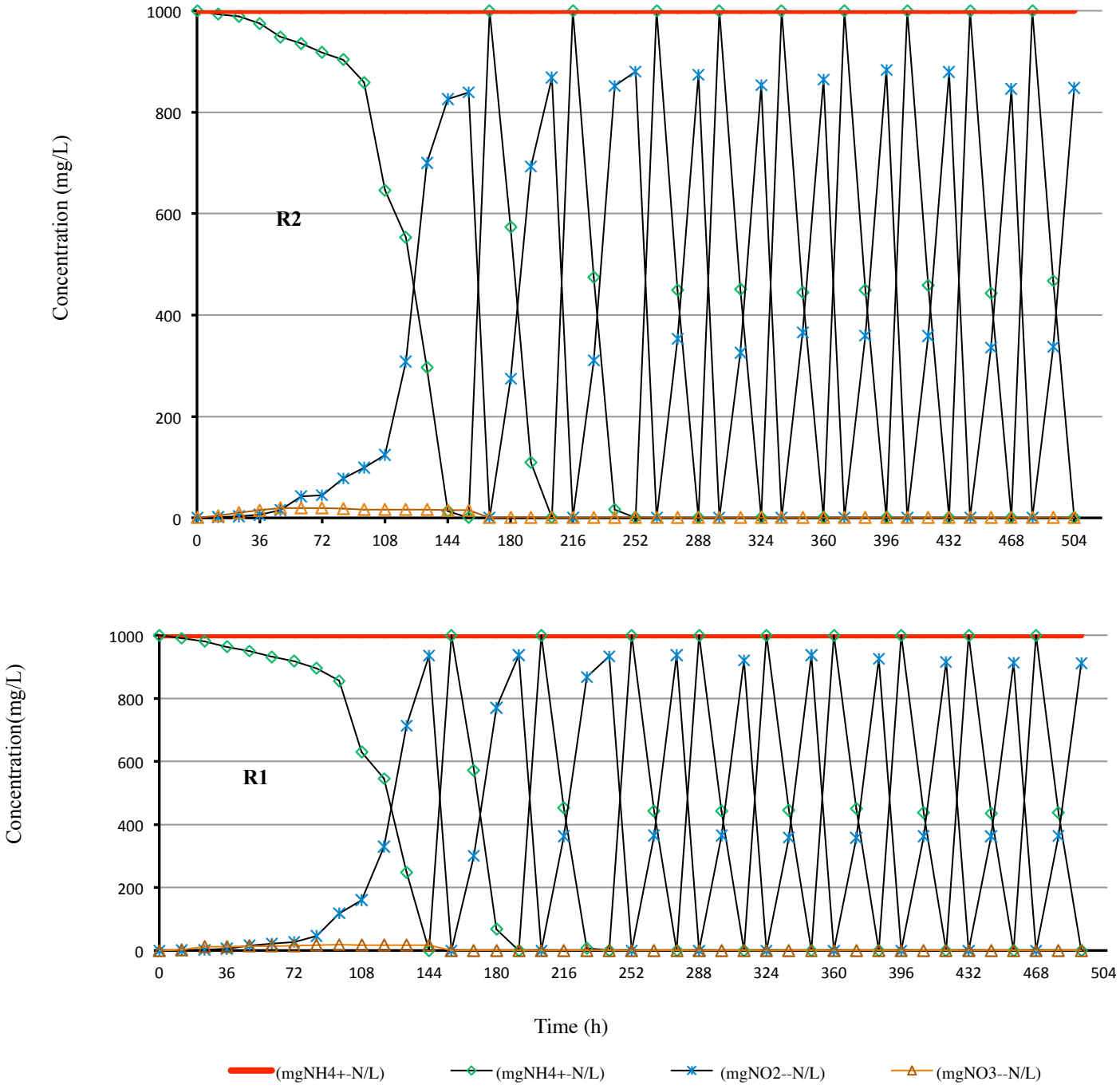

Figure 2. Nitrification profile during acclamatization of HA- AOB without COD (R1), and with COD(R2).

The result depicted that NO_2-N/ (NO_2-N +NO_3-N) ratio is as high as in the previous study, and a comparison of them is shown in Table 3. The rate of ammonium removal was 41.66 mg NH_4^+ -

$NL^{-1}h^{-1}$. However, regarding the rate in the beginning of the process, the first cycle in R2 was less than R1, and complete removal of ammonium in R2 was conducted after R1, but the result for both reactors show comparable ammonium removal rate. This means that the rate of ammonium removal was not affected strongly by the presence of heterotrophic bacteria.

Table 3. The comparison of results in this research with previous studies

Type of reactor	(MgNH4+-N/L)	NO2-N (%)	(MgNH4+-N/d L)	PH	DO	HRT (Day)	NH4+-N/L Removal %	References
CSTR (PN)c	534.5±55	84.8	-	7-7.5	>1	16	86.2±9.7	[9]
AS (PN)d	610	65	-	6.4-8.9	0.7	5.7h	98	[13]
AS (PN)	500	75	-	7.8	1.4	-	95	[18]
SBR	1000	90-100	1000	7.6±0.3	>3	48	100	Present study

5. Summary

The study shows a successful enrichment of high activity-AOB in both reactors (with and without glucose) with suitable capacity of PN at high ratio of NO_2^--N/(NO_2^--N+NO_3^--N). The results confirmed that the rate of ammonium removal was not affected strongly by the presence of heterotrophic bacteria resulting complete removal of ammonium at both reactors.

6. Acknowledgement

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