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Effect of the Carbon Source on Nitrifying in an Activated Sludge System Treating Aquaculture Wastewater

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Abstract

The nitrogen in the aquaculture wastewater can have significant effects on receiving water bodies like eutrophication and ammonia toxicity to fish communities. Removing nitrogen by nitrification-denitrification can reduce the potential impact of aquaculture wastewater discharge. Nitrification is affected by different factors including dissolved oxygen, temperature, pH, alkalinity, toxicity, unionized ammonia and substrate concentration. All these parameters affect ammonia-oxidizing and nitrite-oxidizing bacterial activity.

The aim of this work is to study the effect of the carbon source on nitrifying bacterial activity in an activated sludge treating aquaculture wastewater.

An activated sludge (AS) system was set up and operated continuously for 180 days. The operation was divided into two phases. During Phase I a source of organic carbon (CH_3COONa) with a C/N level of 2.4 (operation days 1 to 101) was fed into the system, while in Phase II a source of inorganic carbon ($NaHCO_3$) with a C/N level of 16.2 was fed into the system (operation days: 102 to 180).

The maximum NH_4^+-N removal efficiency was 49.7% during the Phase I, during which the NO_3^--N and NH_3^+-N concentrations were 37.1 ± 14.0 and 2.9 ± 1.1 mg/L, respectively. In Phase II, the maximum NH_4^+-N removal efficiency was 45% and NO_3^--N and NH_3^+-N effluent concentrations were 2.8 ± 0.3 mg/L and 210 ± 49 mg/L, respectively. Ammonia- and nitrite-oxidation decreased in Phase I from 0.231 ± 0.005 mg NH_4^+/gVSS min to 0.018 ± 0.004 mg NH_4^+-N/gVSS min and in Phase II from 0.049 ± 0.011 mgNO_2^--N/gVSS min to 0.010 ± 0.002 mgNO_2^--N/gVSS min.

Keywords: nitrification, carbon source, nitrifying activity, activated sludge, aquaculture

1. Introduction

Aquaculture activities discharge effluents into the receiving aquatic ecosystem with pathogenic bacteria, therapeutic chemicals, antibiotics, metabolic products and food wastes (Cripps & Bergheim, 2000; Michael, 2003; Stewart et al., 2006). These effluents are rich in solids, organic matter and dissolved metabolites such as ammonia, urea and carbon dioxide. In particular, one kilogram of fish production discharges approximately 577 g of BOD_5 (biological oxygen demand), 90.4 g of nitrogen and 10.5 g of phosphorus (Jegatheesan & Shu, 2011). Aquaculture wastewaters rich in nitrogen content may have a significant effect on the receiving water bodies such as eutrophication and ammonia toxicity to fish communities (Boaventura et al., 1996; Jegatheesan & Shu 2011). Consequently, biological nitrification-denitrification treatment is employed to remove nitrogen from wastewater (Campos et al., 2007). In this biological process the nitrification stage can determine the performance of the entire process (Shammas, 1986). Nitrification consists of the biological oxidation of NH_4^+ to NO_3^- under aerobic conditions. First, NH_4^+ is oxidized into NO_2^- by autotrophic ammonia-oxidizing bacteria (e.g. *Nitrosomonas*). NO_2^- is then oxidized to NO_3^- by autotrophic nitrite-oxidizing bacteria (e.g. *Nitrobacter*) (Ahn, 2006; Chen et al., 2006). Equations (1) and (2) show the basic chemical conversions that occur in nitrification (Chen et al., 2006).

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

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad (2)$$

Conventional biological treatment, like activated sludge (AS), is used to remove nitrogen (Ahn, 2006; Leu et al., 2010). Such systems can remove up to 99% of NH_4^+-N operated under an ammonia loading rate (ALR) of 4 gNH_4^+-N/L·d (Antileo et al., 2002; Campos et al., 2002). Although AS systems are used for nitrification, they are not ideal for this purpose because they generate large and rapidly growing populations of heterotrophs compared to small and slow-growing populations of nitrifying bacteria (Gerardi, 2002; Campos et al., 2007). As well, nitrification is affected by a number of environmental factors including dissolved oxygen, temperature, pH, alkalinity, toxicity, unionized ammonia and substrate concentrations. Consequently, nitrification takes place with dissolved oxygen concentrations of > 2 mg/L and at temperatures between 4 and 45 °C. The temperature range determined for *Nitrosomonas* is 5 to 30 °C, while for *Nitrobacter* it is 5 to 40 °C (Gerardi, 2002). The optimal pH for nitrification is between 7.5 and 8.5. Specifically the optimal pH range for ammonium-oxidizing bacteria is 7.5-8.0, while for nitrite-oxidizing bacteria it is 7.2-7.8 (Chen et al., 2006). Complete ammonium oxidation consumes medium alkalinity and reduces pH. The alkalinity required is 7.1 mg $CaCO_3$/mg NH_4^+-oxidized (Ahn, 2006; Li & Irvin, 2007). Autotrophic nitrifying bacteria use $CaCO_3$ (CO_2 or HCO_3) as an external carbon source for growth, which is useful as a buffer to maintain medium pH (Ahn, 2006). On the other hand NH_3 concentrations of 10-150 mg/L and 0.1-1 mg/L inhibit ammonia- and nitrite-oxidizing bacteria, respectively (Anthonisen et al., 1976; Gerardi, 2002). Consequently, simultaneous growth of nitrifying and heterotrophic organisms in a single reactor results in low rates of nitrification due to the sensitivity of organic matter. Nitrification can only be successfully carried out under low chemical oxygen demand (COD) (Li & Irvin, 2007) because of which industrial operations produce wastewater with low C/N ratios to support the growth of nitrifying bacteria favored by a C/N level of < 10:1 (Campos et al., 2007; Xia et al., 2008). As well, a substrate with organic carbon (CH_3COONa) promotes the growth of heterotrophic bacteria in the system, as opposed to a substrate of inorganic carbon ($NaHCO_3$), which favors the growth of nitrifying bacteria (Austin, 1980; Gerardi, 2002).

The objective of this work is to study the effect of the carbon source on nitrifying bacterial activity in an activated sludge treating aquaculture wastewater.

2. Materials and Methods

2.1 Inoculum

The inoculum consisted of 5 g/L of volatile suspended solids (VSS) of sludge obtained from the nitrifying reactor of a fish farm in southern Chile.

2.2 Synthetic Wastewater

The influent was synthetic medium aquaculture wastewater composed of NH_4Cl - 700 mg/L, $MgSO_4$ - 0.06 g/L, NaCl - 1.0 g/L, KH_2PO_4 - 0.025 g/L. CH_3COONa - 1.5 g/L was used to study the effects of organic and inorganic carbon sources in Phase I, while $NaHCO_3$ - 20 g/L was used as a carbon source in Phase II.

2.3 Activated Sludge System

A laboratory scale system of activated sludge (AS) was set up that included an aerobic reactor (1.0 L) and a settling unit (0.4 L), both made of glass agrees with Vidal et al. (2004). Synthetic wastewater was fed into the aerobic reactor by a peristaltic pump with the flow rate adjusted to the desired hydraulic retention time (HRT) based on the net liquid volume of the reactor. The system was operated at a temperature of 18.0 ± 2.0 °C. The dissolved oxygen (DO) concentration was maintained at > 2 mg/L with an air diffusion system. The sludge was periodically recycled from the settling unit to the aerobic reactor to maintain approximately 3.0 gVSS/L. Figure 1 shows a scheme of the activated sludge system and its components.

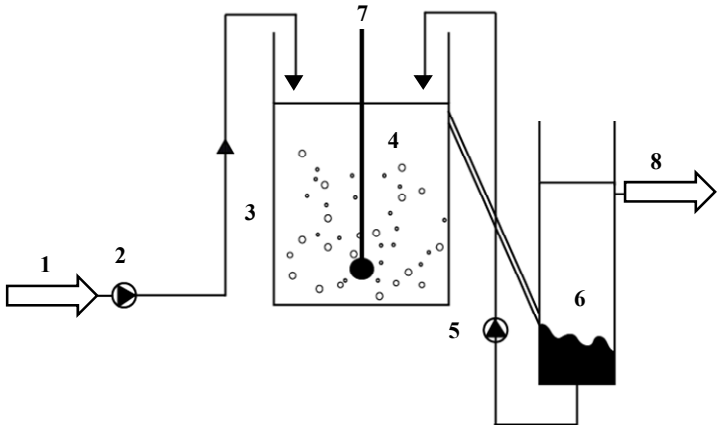

Figure 1. Schematic of activated sludge system: (1) influent, (2) pump, (3) aeration tank, (4) biomass, (5) biomass recirculation, (6) sedimentation, (7) aeration, and (8) effluent

2.4 Operational Conditions

The system was operated continuously for 180 days. The operation was divided into two phases. In Phase I a source of organic carbon (CH_3COONa) was fed into the system from days 1 to 101 at a C/N ratio of 2.4. In Phase II a source of inorganic carbon ($NaHCO_3$) was fed into the system from days 102 to 189 at a C/N ratio of 16.2. The ammonium loading rate (ALR) in the influent was maintained throughout the operation at 0.33 gNH_4^+/L·d, with a hydraulic retention time of 2.05 ± 0.19 d. Table 1 shows the characteristics of the system during the two phases of operation.

Table 1. Characteristics of influent fed to the activated sludge system during the two operating conditions

Characteristics of influent		Phase	
		I	II
Substrate	-	CH_3COONa	$NaHCO_3$
Operating time	d	0-101	102-180
C:N ratio	-	20	8
ALR	gNH_4^+/L·d	0.33	0.33
OLR	gCOD/L·d	0.36 ± 0.04	-
HRT	d	2.09 ± 0.18	2.01 ± 0.20
Sludge concentration	gVSS/ L	7.22 ± 3.81	3.47 ± 1.20
DO	mg/L	6.75 ± 1.18	5.55 ± 0.87
pH influent	-	7.11 ± 0.21	7.40 ± 0.65

Note. ALR: Ammonium Loading Rate, OLR: Organic Loading Rate, HRT: Hydraulic Retention Time, DO: Dissolved Oxygen.

The efficiency of NH_4^+-N removal was calculated throughout the operation. COD and BOD_5 removal efficiencies were evaluated only in Phase I. Efficiencies were calculated using Equation (3):

$$E(\%) = (Q_i \times C_i - Q_o \times C_o)/(Q_i \times C_i \times 100) \quad (3)$$

Where, E (%) is the removal percentage; Q is the flow rate (L/d); C is the parameter concentration (mg/L); and subindex "i" and "o" are the inflow and outflow, respectively.

NO_2^--N, NO_3^--N and NH_3 concentrations in the effluent were determined weekly and alkalinity, the oxidation reduction potential (ORP), temperature, pH, and DO were monitored daily.

Ammonium- and nitrite-oxidation were analyzed by the oxygen uptake rate (OUR) and the specific oxygen

uptake rate (SOUR) during both operating phases on days 29, 62, 118, 139, 162 and 168. As well, microorganisms were studied microscopically.

2.5 Analytical methods

Chemical oxygen demand (COD), biological oxygen demand (BOD_5), total suspended solids (TSS), volatile suspended solids (VSS) and alkalinity were measured according to standard methods (APHA-AWWA-WPCF, 1998). NH_4^+-N, NO_2^--N and NO_3^--N content were measured in filtered samples (0.45 μm) with a Merck specific spectroquant NOVA-60 kit. Free ammonia content in the effluent was estimated according the Equation (4) (Hansen et al., 1998):

$$\frac{[NH_3]}{TNH_3} = \left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T(K)}\right)}}\right)^{-1} \quad (4)$$

Where, $[NH_3]$ is the concentration of free ammonia, $[TNH_3]$ is the total ammonia concentration and T (K) is the temperature (Kelvin).

Temperature and DO were measured using a HQ-10 oxygen meter with an LDO sensor. pH and ORP were measured by a multiparameter (Oakton PC650).

Ammonium- and nitrite-oxidation were evaluated with a respirometry using a biological oxygen monitor (BOM) YSI 5,300. The system was operated with an air-tight respiration vessel fitted with a DO probe YSI 5,231. The vessel was continuously stirred and thermally controlled according to procedure of López-Fiuza et al. (2002). $(NH_4)_2SO_4$ 0.25 M and $NaNO_2$ 0.25 M were used as a degradation substrate for ammonium- and nitrite-oxidizing bacteria, respectively. The OUR was determined by linear regression from the slope obtained by plotting dissolved oxygen concentration versus time and SOUR with the SSV value used in the assay.

Microscopic examination was performed within 1 h of collection, using a Leica Microsystems microscope (model DM500).

3. Results and Discussion

Figure 2 shows the evolution of the oxidation reduction potential (ORP) and alkalinity during Phases I and II in the AS. During Phase I the C/N ratio was 2.4 with an organic carbon source meanwhile during Phase II the C/N was 16.2 with an inorganic carbon source. The average ORP value was 105.2 ± 38.7 mV and the DO concentration was 6.2 ± 1.2 mg/L. During Phase I pH in the influent was 7.1 ± 0.2 and in Phase II it was 6.9 ± 0.1, while in the reactor it was 7.7 ± 0.6 and 8.9 ± 0.4 for Phases I and II, respectively. Alkalinity increased from 81 mg$CaCO_3$/L during Phase I to a maximum of 12,000 mg$CaCO_3$/L during Phase II. Under these conditions the microorganisms observed in the reactor in Phase I were principally stalked ciliates (e.g. *Vorticella sp*). According to Gerardi (2002), these protozoa are present in relatively large numbers during rapid nitrification. In Phase II this type of protozoa was not observed.

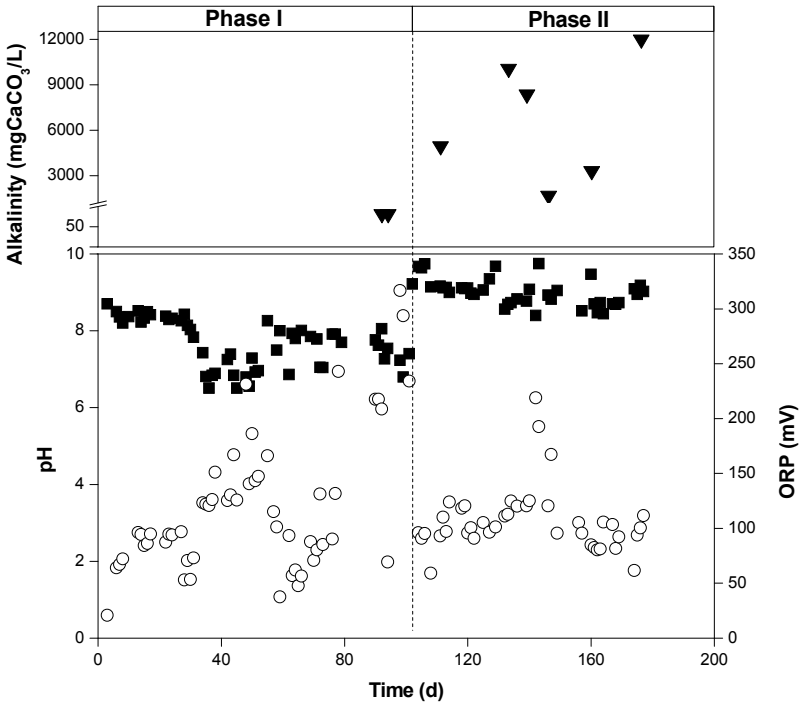

Figure 2. Evolution of alkalinity (▼), pH (■) and oxidation-reduction potential (ORP) (○) in the activated sludge system for different operating conditions

Figure 3 shows COD and NH_4^+-N removal efficiencies and Figure 4 shows the NO_2^--N, NO_3^--N and NH_3^+-N concentrations in the effluent. In Phase I COD and NH_4^+-N removal efficiencies were 84 ± 3 and 37 ± 8%, respectively, while NO_2^--N and NO_3^--N concentrations in the effluent were 27.5 ± 13.1 and 37.1 ± 14.0 mg/L, respectively. Nitrite accumulation was detected during transformation. The estimated NH_3-N concentration was 2.9 ± 1.1 mg/L. Wang et al. (2010) reported a maximum NH_4^+-N removal of 99.8% in a batch system with an organic carbon source as substrate and a C/N ratio of 4 to 10. In a simultaneous nitrification-denitrification process, nitrification was dominant at a low C/N. With a C/N ratio of 6.3 and an organic carbon source, NH_4^+-N removal was 41%, with nitrite accumulation present. The highest nitrification rate was observed with C/N at 19.7 (Chiu et al., 2007).

In Phase II, the NH_4^+-N removal was 33 ± 9%. NO_2^--N and NO_3^--N concentrations were 1.01 ± 0.02 and 3.84 ± 0.81 mg/L, respectively, and the value of NH_3-N was 210 ± 49 mg/L. In an activated sludge system with a C/N ratio of 5.7 and $NaHCO_3$ as the carbon source, full ammonia removal to nitrate was observed (Campos et al., 2007).

The efficiency of NH_4^+-N removal decreased by 4% with the switch to an inorganic substrate. Decreases of up to 99% in NO_2^--N and NO_3^--N concentrations were observed, while the value of NH_3-N increased by two orders of magnitude in Phase II.

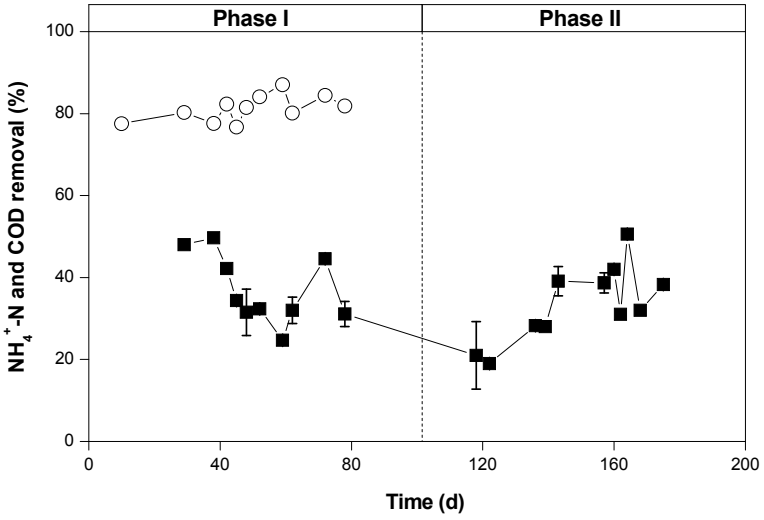

Figure 3. NH_4^+-N (■) and COD (○) removal during operation of the activated sludge system

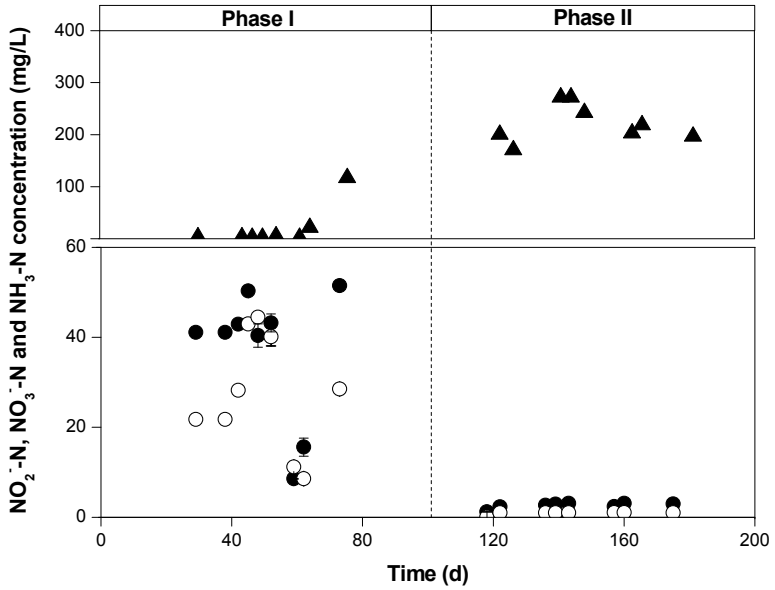

Figure 4. Evolution of NO_2^--N (○), NO_3^--N (●) and NH_3-N (▲) activated sludge effluent concentrations during operating conditions

Table 2 shows ammonia- and nitrite-oxidation and concentrations of NH_4^+-N on operation days 29 and 62 during Phase I and days 118, 139, 162 and 168 during Phase II. There was no variation in activity during the two phases.

During Phase I, ammonia-oxidization was 0.231 ± 0.005 mgNH_4^+-N/gVSS·min, nitrite-oxidization was 0.049 ± 0.001 mgNO_2^--N/gVSS·min. and NH_4^+-N removal efficiency was 31 ± 1%. During Phase II, when the carbonaceous substrate was $NaHCO_3$, ammonia-oxidization decreased by 90% to 0.018 ± 0.002 mgNH_4^+-N /gVSS·min, nitrite-oxidization decreased 76% and 0.010 ± 0.002 mgNO_2^--N/gVSS·min, and NH_4^+-N removal efficiency was 28 ± 4%.

The pH values (9.22 ± 0.29) and ammonia concentrations (208-331 mg/L) may be the main factors for the lower ammonia- and nitrite-oxidization values. Shammas (1986) showed that a pH level between 8 and 9 is optimal for nitrification and that when pH increases to 9.6 nitrification drops almost zero. In this study, during the Phase II pH values reached over 9.6, at which level nitrite was not detected in the process. These results concur with

those of Ruiz et al. (2003), who found a complete inhibition of nitrification in an AS system without nitrite accumulation at a pH level higher than 8.95. Campos et al. (2007) found a reduction from 0.4 to 0.2 mgNH_4^+-N/gVSS·min of ammonia-oxidation when the system was subjected to a pH level of 11. However, specific nitrite-oxidation remained constant at around 0.7 g NO_2-N/gVSS d when pH was recovered. Moreover, nitrate accumulation and NH_3 were in the effluent. Willke and Vorlop (1996) found a nitrite-oxidizing bacteria resistant to pH variations, indicating that activity is affected when pH is in the range 4.5 and 10, among others. In this way Suthersand and Ganczarczyk (1986) found that a pH shock reduces nitrite-oxidation by 14% less than ammonia-oxidation.

While ammonia and nitrite serve as energy sources for the microorganisms responsible for oxidation, they can inhibit biological activity in their unionized forms, NH_3 and HNO_2, respectively. The concentrations of free nitrous acid and free ammonia are functions of temperature, ph and ammonia and nitrite concentrations, respectively (Villaverde et al., 1997). The free ammonia concentration increases to basic pH while the nitrous acid concentration increases to acid pH (Gerardi, 2002).

NH_3 concentrations increase with higher temperatures (Emerson et al., 1975). From Phase I to II, the pH level increased by 1.2 points (from 7.7 to 8.9) and temperature increased by 2 degree °C (from 14.9 to 16.9 °C). Under these conditions 20% of the total ammonia in the system is in the form of NH_3 in the system. The maximum pH was 9.75 and under these conditions 64% of ammonia was NH_3 (Hansen et al., 1997). At these levels of NH_3 ammonia- and nitrite-oxidation are inhibited (Fontenot et al., 2007).

Table 2. Ammonium-oxidation in sludge, influent and effluent ammonia concentration (mg/L), and percentage reduction of total ammonia during operation of the activated sludge system

Phase	Time (d)	Ammonium-oxidation (mgNH_4^+-N/gVSS·min)	Nitrite-oxidation (mgNO_2^--N/gVSS·min)	NH_4^+-N concentration		
				Influent (mg/L)	Effluent (mg/L)	Reduction (%)
I	29	0.233 ± 0.008	0.049 ± 0.001	695 ± 10	485 ± 10	30
	62	0.229 ± 0.002	0.049 ± 0.001	700 ± 10	475 ± 10	32
II	118	0.013 ± 0.003	N. D.	750 ± 20	590 ± 20	21
	139	0.017 ± 0.001	N. D.	702 ± 10	512 ± 5	28
	162	0.020 ± 0.002	0.008 ± 0.002	695 ± 5	475 ± 5	31
	168	0.023 ± 0.001	0.012 ± 0.001	685 ± 5	465 ± 5	32

Note. N.D.: Not determined.

Table 3 shows the nitrogen mass balance. The tendency of the nitrogen balance evolution concurs with the analysis of the ammonia-oxidizing and nitrate-oxidizing bacteria. The NH_4^+-N load in the influent of the AS system during Phase I and II was 0.355 ± 0.012 g/d, while the NH_4^+-N load in the effluent was 0.502 ± 0.039 g/d. The effluent loads of NO_2^--N and NO_3^--N decreased from Phase I to Phase II, to a non-detectable level for NO_2^--N and to 92% for NO_3^--N. The effluent load increased from Phase I (0.001 g/d) to Phase II (0.135 ±0.018 g/d). NH_4^+-N removal was over 40%, in both phases.

Table 3. Evolution of nitrogen matter balance in an activated sludge system during the two operating phases

Phase	Time (d)	Input (g/d) NH_4^+-N	Reactor (gO_2/d)	Output (g/d)			
				NH_4^+-N	NO_2^--N	NO_3^--N	NH_3-N
I	29	0.347	0.596	0.242	0.011	0.021	0.001
	62	0.350	1.440	0.237	0.004	0.007	0.001
II	118	0.375	0.010	0.295	N.D.	0.001	0.122
	139	0.351	0.006	0.256	N.D.	0.001	0.165
	161	0.348	0.045	0.238	N.D.	0.001	0.133
	168	0.343	0.018	0.233	N.D.	0.001	0.120

Note. N.D.: Not Detected.

4. Conclusions

The maximum NH_4^+-N removal efficiency was 49.7% in Phase I, during which NO_3-N and NH_3-N concentrations were 37.1 ± 14.0 and 2.9 ± 1.1 mg/L, respectively. In Phase II, the maximum NH_4^+-N removal efficiency was 45% and NO_3-N and NH_3-N concentrations were 2.8 ± 0.3 mg/L and 210 ± 49 mg/L, respectively. Ammonia-oxidizing and nitrite-oxidation decreased from 0.231 ± 0.005 mgNH_4^+/gVSS·min to 0.018 ± 0.004 mgNH_4^+-N/gVSS·min in Phase I and from 0.049 ± 0.011 mgNO_2^--N/gVSS·min to 0.010 ± 0.002 mgNO_2^--N/gVSS·min in Phase II.

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