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NITRIFICATION AT LOW OXYGEN CONCENTRATIONIN BIOFILM REACTOR

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ABSTRACT: In a fully stirred biofilm reactor, a nitrification process at low dissolved oxygen (DO) concentration is suggested. A synthetic wastewater containing 250 mg NH4-N/L was used to feed the reactor. Throughout the course of more than 110 days of operation, a steady nitrite accumulation was attained in the effluent; NO2-N:(NO2-N + NO3-N) in the effluent reached >90% under 0.5 mg DO/L. Ammonium was entirely transformed, and the output had NH4-N levels as low as 5 mg/L. After only two days, an abrupt rise in the DO concentration in the reactor caused the entire conversion of ammonia and nitrite to nitrate. Nitrite buildup was once more triggered by a drop in DO content.

INTRODUCTION

The most effective method for removing nitrogen from water and wastewater is biological treatment. Aerobic autotrophic nitrifying bacteria first convert ammonium to nitrate in this process. Under anoxic conditions, heterotrophic denitrifying bacteria then convert nitrate to nitrogen gas. In biological wastewater treatment facilities, oxygen and organic carbon must be supplied to act as electron acceptor in nitrification and electron donor in denitrification, respectively. Ammonium conversion occurs during nitrification in two phases1986, 1987; Chen et al. 1991; Abeling and Seyfried1992):

- 25% Oxygen requirement reduction in nitrification
- 40% Organic carbon requirement reduction in denitrifi-cation
- Higher denitrification rate
- Less biomass production

These qualities are particularly advantageous for nitrogen-rich wastewaters with low organic carbon levels, such as landfill leachate, sludge dewatering discharges, and some industrial wastewaters. Indeed, in this case, an external

$$NH_4^+ + \frac{3}{2} O_2 \rightarrow NO_2^- + H_2O + 2H^+ \quad (1)$$

$$NO_2^- + \frac{1}{2} O_2 \rightarrow NO_3^- \quad (2)$$

carbon source has to be added to complete denitrification (Ber- net et al. 1996). Noting the benefits of shortcut nitrification- denitrification, many researchers tried to obtain consistent ni- trite accumulation in nitrification, which is the key prerequisite

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There are two distinct populations involved, ammonia and nitrite oxidizers, which use ammonia and nitrite as electron donors, respectively. The primary species that oxidise ammonia are Nitrosomonas, Nitrosospira, and Nitrosococcus, while those that oxidise nitrite are Nitrobacter and Nitrospira. It is generally accepted that ammonium and nitrite oxidation always occur simultaneously. Typically, the only byproduct of nitrification is nitrate. Yet occasionally, nitrite might be found in the wastewater. This data suggests that the ammonium and nitrite oxidation reactions might be distinct. Denitrification may directly decrease nitrite to form a process known as a "shortcut" nitrification-denitrification (Voets et al. 1975; Alleman 1985). When compared to the standard biological nitrogen removal method (nitrate as the end product in nitri- fication and as the electron acceptor in the following denitri- fication), the nitrite route has several advantages (Turk and for a successful shortcut nitrogen removal process. From a biological viewpoint, two ways have been approached: (1) Separating ammonium oxidizers from nitrite oxidizers by us- ing a pure culture of *Nitrosomonas* (Kokufuta et al. 1988) or by application of a ‘‘selection pressure’’ as in the SHARON process (Hellinga et al. 1998); and (2) restricting nitrite oxi- dizers to grow in a nitrifying mixture by substrate inhibition (Turk and Mavinic 1989; Chen et al. 1991; Abeling and Sey- fried 1992) or dissolved oxygen (DO) control (Garrido et al. 1997a; Picioreanu et al. 1997).

A nitrite buildup in nitrification can, of course, be obtained in pure *Nitrosomonas* cultivation. However, it is very expen- sive and impossible to use in practice. The theory support for the SHARON process is that ammonia oxidizers grow more quickly than nitrite oxidizers under high temperature. It is pos-sible to retain ammonia oxidizers and washout nitrite oxidizers at a specific retention time. SHARON is only suitable for wastewaters that originally are of high temperature, since it is cost-prohibitive to heat large quantities of wastewater.

Inhibition is a common method for bacteria selection and is used in microbiology and biotechnology research. Based on the results reported by Anthonisen et al. (1976), nitrite oxidiz- ers are more sensitive than ammonia oxidizers to free am- monia (FA) and free nitrous acid (FNA). If pH in the reactor is increased (higher FA) or lowered (higher FNA), nitrite ox- idizer inhibition will occur. Turk and Mavinic (1989) carefully designed an experimental system to try to elucidate inhibition theory. Five completely mixed reactors, in series, were used to form a plug-flow configuration. The first one was operated in a denitrification mode in which pH was high and FA con- centration was controlled below the threshold of ammonia ox- idizer inhibition and over that of nitrite oxidizers. When the sludge passed through the first reactor, nitrite oxidizers would be expected to be inhibited; thus, nitrite would accumulate in the following nitrifying reactors. The results showed that ni- trite accumulation cannot be maintained indefinitely since ni- trite oxidizers appear capable of tolerating ever-increasing lev-els of FA. Therefore, it seems difficult, if not impossible, to obtain long-term nitrite accumulation via the substrate inhi- bition route.

DO concentration is an important factor for nitrification (Stenstrom and Poduska 1980). Jayamohan et al. (1988) showed that continuous nitrification under low DO leads to a high nitrite accumulation. Nitrite oxidizers have been shown to be more sensitive to oxygen than ammonia oxidizers. Han- aki et al. (1990) found that 80% of influent ammonium was converted to nitrite in a suspended growth reactor with no sludge retention, under 0.5 mg DO/L. A 50% nitrite accu- mulation with a very high nitrogen loading rate (5 kg $N \cdot m^{-3} \cdot day^{-1}$) was carried out in a biofilm airlift suspension re- actor under 1 to 2 mg DO_2^{-1} (Garrido et al. 1997a).

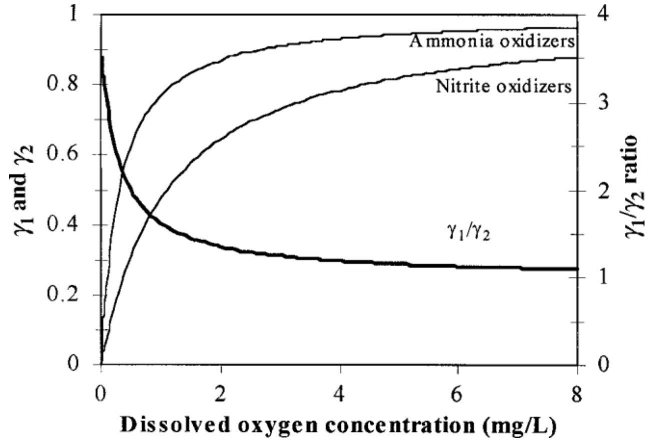

FIG. 1. Evolutions of $v1$ **=ˆ**

$\hat{\mu}\mathbf{1}/\mu\mathbf{1} = \boldsymbol{CO}/(\boldsymbol{k}\mathrm{NH}^{+}, \mathrm{O} + \boldsymbol{CO}),$ **v2 =** $\mu\mathbf{2}/\mu\mathbf{2} = \boldsymbol{CO}/(\boldsymbol{k}\mathrm{NO}^{-}, \mathrm{O} + \boldsymbol{CO})$, **and** $\gamma = \gamma 1/\gamma 2 = (k\mathrm{NO}^{-}, \mathrm{O} + CO)$ 2

An interesting follow-up to these studies is to determine if complete nitrite accumulation could be obtained in a biofilm reactor under lower DO concentrations. A biofilm system could enhance the advantage of ammonia oxidizers toward nitrite oxidizers. In this paper, a competition theory between these two populations in the biofilm, under low DO, is pro- posed. Subsequently, experimental works have been carried out at a laboratory scale to check the validity of this theory. Results are presented and discussed.

COMPETITION THEORY

Pure nitrifying bacteria cultivation shows that there exists an oxygen affinity difference between *Nitrosomonas* and *Ni- trobacter* (Laanbroek and Gerards 1993). If DO is a limiting substrate, the competition between *Nitrosomonas* and *Nitro- bacter* will occur. It is generally agreed that a double-Monod model can be used to describe nitrifiers' growth for nitrogen

$(k\mathrm{NH}^{+}, \mathrm{O} + CO)$ at Dissolved Oxygen Concentrations Varying between 0 and 8 mg/L and with $(k\mathrm{NH}_4^{+}, \mathrm{O}_2$ = 0.3 mg O_2/L and $k\mathrm{NO}_2^{-}, \mathrm{O}_2$ = 1.1 mg O_2/L (Wiesmann 1994)

which reflects the variation of growth ratios between ammonia oxidizers and nitrite oxidizers under different DO concentra- tions. Fig. 1 shows the changes of $\gamma 1$ $\gamma 2$, and γ with $K\mathrm{NH}_4^{+}$, O_2 = 0.3 mg O_2/L and $K\mathrm{NO}_2^{-}$, O_2 = 1.1 mg O_2/L (Wiesmann 1994) in a range of DO concentrations from 0 to 8 mg/L. It is clear that low DO will benefit ammonia oxidizers for competition with nitrite oxidizers, especially when DO is <1 mg/L. Both growth rates of ammonia oxidizers and nitrite oxidizers will, of course, decrease under low DO concentrations. However, the nitrite oxidizers' growth rate decreases more than ammonia oxidizers'. Therefore, ammonia oxidation rate should surpass nitrite oxidation and nitrite accumulation should occur. The decrease of oxidation rate can be compensated by the large quantities of biomass in a biofilm reactor. It must be noted

and oxygen, both of which are substrates (Jayamohan et al.

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that this phenomenon is due to the fact that < 1988)

$$\mu 1 = \mu m 1 \frac{C_{NH^+}}{K_{NH^+} + C_{NH^+}} \frac{C_O}{K_{NO^-}, O + C_O} \quad (3)$$

which has been widely observed (Siegriest and Gujer 1987; Jayamohan et al. 1988; Stevens et al. 1989; Laanbroek and Gerards 1993; Sheintuch et al. 1995).

In a biofilm system, the fast-growing bacteria tend to occupy the surface of the biofilm (Wanner and Gujer 1986). This feature should favor ammonia oxidizers since, under low DO/

$$\mu 2 = \mu m 2 \frac{C_{NO^-}}{K_{NO^-} + C_{NO^-}} \frac{C_O}{K_{NO^-}, O + C_O} \quad (4)$$

NH_4-N ratio in the bulk phase, oxygen will be the limiting substrate, only available at the surface of the biofilm because of the mass transfer limitation (Denac et al. 1983). The ratio of ammonia oxidizers to nitrite oxidizers should increase with the growth of the biofilm until the whole surface of biofilm is colonized by ammonia oxidizers. The evolution of the ecology

The growth rates for both strains under DO saturation, $\mu 1$, and $\mu 2$, are

$$\mu 1 = \mu m 1 \frac{C_{NH^+}}{K_{NH^+} + C_{NH^+}} \quad (5)$$

$$\mu 2 = \mu m 2 \frac{C_{NO^-}}{K_{NO^-} + C_{NO^-}} \quad (6)$$

in biofilm should be the disappearance of nitrite oxidizers and growth of ammonia oxidizers, at least on upper layers. Cor- respondingly, nitrate in the effluent should decrease and nitrite should increase at the same time in a continuous flow system.

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Two new parameters, $\gamma 1$ and $\gamma 2$, are defined to describe the percentage of maximum growth rate for *Nitrosomonas* (and other ammonia oxidizers) and *Nitrobacter* (and other nitrite oxidizers) under different DO concentrations

$$C_O$$

At final biological steady state, the ammonium in the influent should be completely converted to nitrite. The use of a biofilm reactor could, therefore, prevent the process from a possible adaptation of nitrite oxidizers to low DO concentration as ob- served by Laanbroek et al. (1994).

$$\gamma 1 = \hat{\mu} 1 / \mu 1 =$$

$$\gamma 2 = \hat{\mu} 2 / \mu 2 =$$

Dividing (7) by (8), we get2

$$\frac{K_{NH^+,O} + C_O}{C_O}$$

$$\frac{K_{NO^-,O} + C_O}{C_O}$$

(7)

(8)

EXPERIMENTAL METHODS

Experimental Setup

Fig. 2 presents a schematic of the experimental setup. The reactor used in the experiment was a 5 L reactor. The working volume was 4 L, in which about 0.1 m^2 as stainless bars was used as support for nitrifying bacteria. The stirring rate was

$$\gamma = \gamma 1 / \gamma 2 =$$

(9)500 rpm. The temperature in the reactor was maintained at 25$K_{NH^+,O} + C_O$

± 1°C while pH in the reactor was carefully controlled be-

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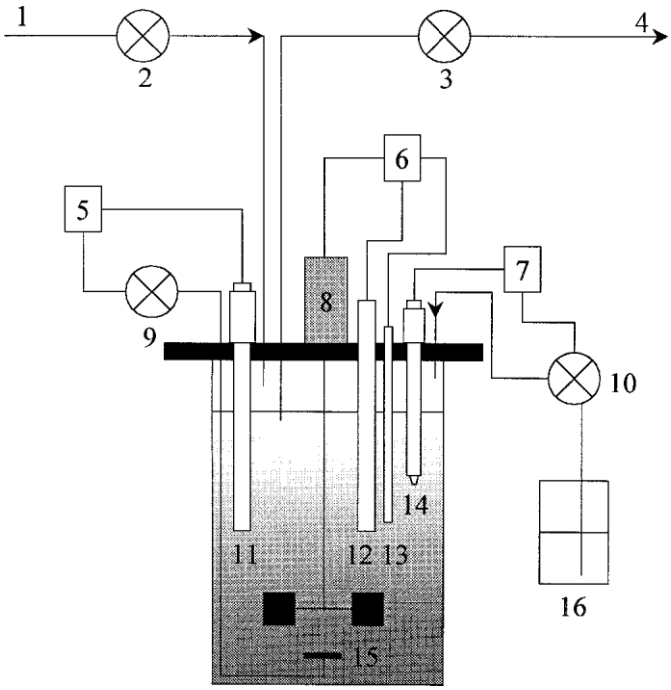

FIG. 2. Experimental Setup: 1, Influent; 2 and 3, Pumps; 4, Ef- fluent; 5, Oxymeter; 6, Mixing and Temperature Control; 7, pH Meter; 8, Motor; 9, Air Compressor; 10, Pump; 11, Dissolved
Oxygen Probe; 12, Heating Baffle; 13, Temperature Sensor; 14, pH Electrode; 15, Air Supply; 16, $NaHCO_3$ Solution

tween 7.0 and 7.5 by addition of $NaHCO_3$. Air was suppliedto the vessel by an air compressor connected to an oxymeter fitted with a dissolved oxygen probe to control DO concentra- tion in the liquid phase. During a short period of 15 days, air was replaced by O_2:argon (21:79) gas in order to determinegas composition.

Reactor Operating Conditions

The reactor was seeded with a municipal nitrifying sludge and fed with a synthetic industrial medium (Tables 1 and 2).

Startup and Operation of Process

The reactor ran initially, for >1 month, under high DO (>50% of saturation) to ensure complete nitrification and sta- ble nitrifying biofilm (Phase 1). A thick biofilm, with a brown-ish-pink color and a filamentous aspect, developed in the re- actor on every stainless surface, even though strong mixing was applied.

Subsequently, the DO concentration in the reactor was re- duced to 0.5 mg/L (range fluctuating between 0.4 and 0.6 mg/ L) and maintained (Phase 2). The changes in nitrogen com- position in the effluent were checked to evaluate the evolution of the process. After more than 100 days of low DO operation, the reactor was disturbed for 7 days with transient high aera- tion (Phase 3). Then, the reactor was operated at conditions previously applied in Phase 2 (Phase 4). Finally, the hydraulic retention time (HRT) was changed to 2.0 and 1.5 days (Phases 5 and 6, respectively) to investigate the system stability. The operating conditions are presented in Table 3.

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Analytical Methods

Ammonia was determined by the titrimetric method after distillation, using a Büchi apparatus [American Public Health Association (APHA) (1992)]. Nitrate and nitrite were analyzedby an ion chromatography system using conductivity detection(APHA 1992). Separation and elution of the anions were car-

TABLE 1. Composition of Synthetic Wastewater Used inExperiment

Chemical formula(1)	Measure(2)
NH_4Cl	250 mg/L (as NH_4–N)
K_2HPO_4	350 mg/L
$NaHPO_4$	350 mg/L
Mineral solution[a]	1 mL/g NH_3–N

[a] Composition given in Table 2.

TABLE 2. Composition of Mineral Solution[a]

Chemical compound(1)	Measure(g/L) (2)
$CaCl_2 \cdot 2H_2O$	7.34
$MgCl_2 \cdot 6H_2O$	25.07
$FeCl_3 \cdot 6H_2O$	4.8
$MnCl_2 \cdot 4H_2O$	1.03
$ZnCl_2 \cdot 2H_2O$	0.01
$CuCl_2 \cdot 2H_2O$	0.112
$NaMoO_4 \cdot 2H_2O$	0.0025

[a] 1 mL for 1 g ammonium nitrogen.

TABLE 3. Reactor Operating Conditions

Phase (1)	Time (days) (2)	Influent NH_4–N (mg/L) (3)	HRT (days) (4)	DO in reactor[a] (5)
1	15	250	3.0	High
2	110	250	3.0	Low
3	7	250	3.0	High
4	20	250	3.0	Low

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5	5	170	2.0	Low
6	15	125	1.5	Low

[a] High DO = about 5 mg/L, >50% of saturation; low DO = 0.4–0.6 mg/L.

ried out on an IonPac AS12A analytical column, utilizing acarbonate/bicarbonate eluant and autosuppression technology. Integration was done using a PC fitted with Peaknet software. Suspended solids (SS) and volatile suspended solids (VSS) were analyzed according to standard methods (APHA 1992). SS in the biofilm were determined after removing the biofilm from the support into distilled water; it was then filtered and dried at 105°C.

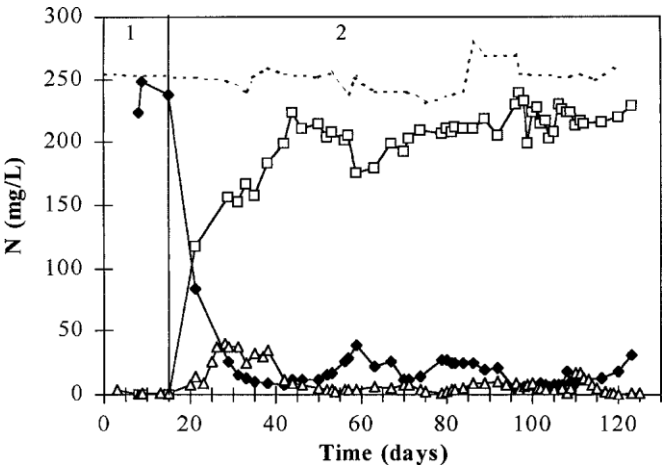
Gas composition (CO_2, O_2, H_2, N_2O, and N_2) was evaluated by gas chromatography using a Hayesep 80-100 mesh column, a molecular sieve column, and a katharometer detector (argon carrier). NO was also measured by gas chromatography but was detected using a molecular sieve column and helium as a gas vector on a Shimadzu GC14A (Patureau et al. 1996).

RESULTS AND DISCUSSION

The experimental results are presented in a time course in Figs. 3 and 4.

Phase 1

The objective of this period was to obtain complete nitrifi- cation in the reactor and develop a stable nitrifying biofilm in the support medium. A complete nitrification was obtained af- ter 1 month of operation. NH_4 – N concentration in the effluent was <1 mg/L. NO_3 – N in the effluent had nearly the same value as NH_4 – N in the influent. A thin biofilm layer could be seen with the naked eye. At this point, the nitrogen loading rate in the reactor was 0.083 kg NH_4 –N/m^3 ·day and estimated



L after 35 days (50 days from the beginning). Therefore, the ammonium oxidation rate increased to >0.081 kg NH_4 – N/m^3 ·day, which is quite similar to the value obtained in ox- ygen nonlimiting conditions.

The nitrite oxidation rate decreased from 0.083 to 0.010 kg NO_2 –N/m^3 ·day. DO concentration is the only parameter that has been modified compared with Phase 1. This change had only a slight effect on the ammonium oxidation rate but caused a sharp decrease of the nitrite oxidation rate. Assuming that this decrease occurred at too small a time scale to be explained by a change in the population ratio between ammonium and nitrite oxidizers, it can be

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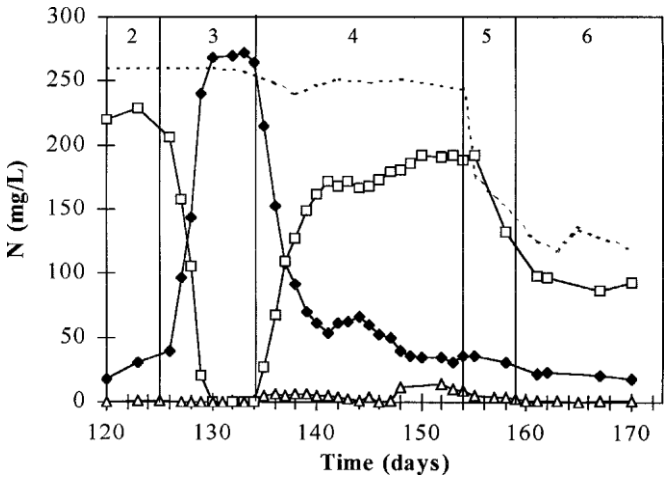
theorized to be caused by a decrease of the specific nitrite oxidation rate. Since, in the same con- ditions, no decrease of the ammonium oxidation rate occurred, it can be tentatively concluded that K_{NH_3,O_2} is much lower than $K_{NO_2^-,O_2}$ [(3) and (4)]. Indeed, ammonia and nitrite oxidation

FIG. 3. Variations of Nitrogen Composition during Phases 1 and 2 of Experimental Period: ---------- = NH4–N Influent; Δ = NH4–N Effluent; ▫ = NO2–N Effluent; ♦ = NO3–N Effluent

rates can be seen as growth rates of the corresponding nitri- fying microorganisms. It is therefore possible to estimate γ_1, γ_2, and γ at a DO concentration of 0.5 mg/L, according to (7)–(9)



$$\gamma_1 = \frac{\hat{\mu}_1}{\mu_1} = \frac{0.081}{0.120} = 0.976 \quad \text{and} \quad \gamma_2 = \frac{\hat{\mu}_2}{\mu_2} = \frac{0.010}{0.083}$$

Therefore

$$\gamma = \frac{\gamma_1}{\gamma_2} = \frac{0.081}{0.012} = 6.75$$

FIG. 4. Variations of Nitrogen Composition during Phases 3–6 of Experimental Period: ----------= NH4–N Influent; Δ = NH4–N Effluent; ▫ = NO2–N Effluent; ♦ = NO3–N Effluent

to 3.3 g NH_3–N/m^2·day. Ammonia and nitrite oxidation rates

This value is high compared with Fig. 1, likely because in our system the difference between ammonia and nitrite oxi- dizers' affinities for oxygen is increased by oxygen-diffusion limitation in the biofilm.

If oxygen limitation is the main factor responsible for nitrite accumulation, inhibition of nitrite oxidizers by free nitrous acid (HNO_2) could also play a role in this phenomenon. In- deed, FNA has been shown to inhibit nitrite oxidation at very low concentrations (Anthonisen et al. 1976). Even if pH in the bulk water was kept at values preventing production of FNA, a pH decrease could occur in the nitrifying biofilm, as has been observed by other authors (Szwerinski et al. 1986; Zhang and Bishop 1996) and considered in a recent model of nitri- fying biofilms (Flora et al. 1999a,b). The pH gradient was shown to depend on the mole ratio HCO_3^-:O_2. When this ratio is <3, the maximum pH decrease could be 1.4–1.6 (Zhang

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were about 0.083 kg N/m ·day.

Phase 2

The objective of this period was to investigate how the ni- trifying biofilm evolved under low DO and if stable nitrite accumulation could be obtained. When DO in the reactor was decreased, nitrate concentration in the effluent decreased and nitrite accumulated at the same time, indicating that evolution in the biofilm took place. The evolution developed slowly due to low growth rate of *Nitrosomonas*. After 15 days, the ratio of $NO_2 - N$ to the sum of $NO_2 - N$ and $NO_3 - N$ (h) in the ef- fluent reached more than 80% and nitrate nitrogen concentra- tion in the effluent was <25 mg/L. Subsequently, h was main- tained over 90% and sometimes >95%. Long-time operation suggested that nitrite accumulation in the biofilm system was both stable and durable (Fig. 3).

During the evolution, the oxidation activity of ammonia ox- idizers declined as predicted, due to the low growth rate under low DO concentration. Ammonium in the effluent rose grad- ually up to the highest point (40 mg/L), corresponding to an ammonium oxidation rate of 0.070 kg $NH_4 - N/m^3$ ·day. Sub- sequently, the oxidation activity recovered gradually, most likely due to the growth of *Nitrosomonas* and/or other am- monia oxidizers. $NH_4 - N$ in the effluent decreased to <5 mg/

and Bishop 1996). At a concentration of $NO_2 - N$ of 200 mg/L and a pH value of 6.0, FNA concentration can be calculated as follows (Anthonisen et al. 1976):

$$\text{FNA as HNO}_2\ (\text{mg/L}) = \frac{46}{a} \times \frac{NO_2\text{–}N}{K} \quad \text{with } K = e^{(-2{,}300/273+°C)} \frac{2^{14}}{a} \times 10^{pH}$$

Therefore, FNA = 1.48 mg/L. This value belongs to the range of inhibitory FNA concentrations given by Anthonisen et al. (1976). This FNA effect added to the competition for oxygen could explain why a very good conversion of ammonia to nitrite was obtained.

Phase 3

To test the stability of the process and its robustness toward perturbations, from Day 126, the DO was regulated to 50% of saturation. As a consequence, $NO_3 - N$ concentration increased very quickly in the reactor. After 2 days, all ammonium fed, as well as nitrite present in the reactor, were oxidized to ni- trate. This result clearly shows that nitrite oxidizers were al- ways present in the biofilm. At low DO concentrations, nearly all oxygen was consumed by *Nitrosomonas* and/or other am- monia oxidizers. *Nitrobacter* and/or other nitrite oxidizers were outcompeted for oxygen; thus, their activity decreased.

As soon as the DO level in the reactor was high enough, a full nitrite oxidation resumed.

Phase 4

The objective of this period was to investigate if the nitrite accumulation can be recovered after the process disturbance. When DO in the reactor decreased once again to 0.5 mg/L, nitrite in the effluent increased once again. After 6 days, h reached 80%. Compared with Phase 2, the second evolution proceeded more quickly. Also, $NH_4 - N$ in the effluent did not increase as previously observed. In fact, Phase 3 was so short that nitrite oxidizers did not have enough time to grow on the surface of the biofilm. Therefore, the reactor returned to the original state very quickly. It should be noted that the reactor did not recover completely after being disturbed again and h

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decreased by 10%.

Phases 5 and 6

The objective of this period was to study the effect of HRT on nitrite accumulation at a constant ammonia loading rate. When HRT in the reactor was shortened from 3 days to 2, and then to 1.5 days, both ammonium and h in the effluent did not change. This result can be explained by the fact that the active biomass was in the biofilm and the suspended growth population is negligible; suspended solids concentration in the effluent was between 5 and 10 mg/L. Total biomass in the reactor at the end of the experiment was 3.04 g VSS. The biofilm production rate can be estimated to about 0.05 g VSS·(g NH_4 – N removed)$^{-1}$ — which is lower than yield con- stants given in the literature for ammonia oxidizers— generally around 0.14 – 0.18 g·g^{-1} (Stevens et al. 1989; Wiesmann 1994; Sheintuch et al. 1995).

Nitrogen Losses

Nitrate and nitrite would possibly be reduced to gases, such as NO, N_2O, or N_2, in suspended sludge or biofilm under low DO or anoxic conditions, even in the absence of organic car- bon. Indeed, Kuai and Verstraete (1998) recently showed that oxygen-limited autotrophic nitrification-denitrification could occur. It was found from our results that only about 5% of

croprobe should help us to check the hypothesis of pH gradient inside the biofilm.

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influent nitrogen was not recovered in the effluent. In order to confirm if nitrogen gas species were produced, aeration by O2:argon was carried out over 15 days (Phase 2). Contrary to the results reported by other authors (Goreau et al. 1980; Garrido et al. 1997b), there was no NO or N2O detected and only a very small fraction of N2 (<5% of ammonium nitrogen fed).

CONCLUSIONS

Nitrification under low DO was studied in a completely mixed biofilm reactor for >170 days. The results showed that it is possible to control nitrite oxidation by acting on compe- tition for oxygen between ammonia and nitrite oxidizers' spe- cies. The bacterial competition probably influenced the biofilm evolution. As soon as the system is stabilized, $NO_2 – N$:($NO_2 – N + NO_3 – N$) ratio can reach 90% or more. Nitrite accumu- lation can be sustained in a biofilm reactor through controlling DO and was probably favored by free nitrous acid inhibition inside the biofilm. After disturbing the biofilm with high DO concentration, nitrite accumulation recovered very quickly and maintained 90% of previous activity. Therefore, nitrite accu- mulation is stable and durable. From a fundamental point of view, the use of molecular tools, such as tRNA monitoring techniques (Amann et al. 1998), could be useful to confirm the hypothesis about the competition between ammonia and nitrite oxidizers in the biofilm. Likewise, the use of a pH mi-

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APPENDIX II. NOTATION

The following symbols are used in this paper:

C_{NH^+} = ammonium nitrogen concentration (mg/L);
C_{NO^-} = nitrite nitrogen concentration (mg/L);
C_O = dissolved oxygen concentration (mg/L);
K_{NH} = saturation constant of ammonia oxidizers for am-monium nitrogen in Monod model (mg/L);

Stenstrom, M. K., and Poduska, R. A. (1980). ''The effect of dissolved oxygen concentration on nitrification.'' *Water Res.*, 14, 643–649.

$K_{NH,O}$ = saturation constant of ammonia oxidizers for oxygen in Monod model (mg/L);

Szwerinski, H., Arvin, E., and Harremoës, P. (1986). ''pH-decrease in nitrifying biofilms.'' *Water Res.*, 20(8), 971–976.

K_{NO^-} = saturation constant of nitrite oxidizers for nitrite ni-trogen in Monod model (mg/L);

Turk, O., and Mavinic, D. S. (1986). ''Preliminary assessment of a short-cut in nitrogen removal from wastewater.'' *Can. J. Civ. Engrg.*, Ottawa, 13, 600–605.

$K_{NO^-,O}$ = saturation constant of nitrite oxidizers for oxygen in Monod model (mg/L);

$\gamma = \gamma 1/\gamma 2 = (K_{NO^-,O} + C_O)/(K_{NH,O} + C_O)$;

Turk, O., and Mavinic, D. S. (1987). ''Benefits of using selective inhi-bition to remove nitrogen from highly nitrogenous wastes.'' *Envir. Technol. Letters*, 8, 419–426.
Turk, O., and Mavinic, D. S. (1989). ''Maintaining nitrite buildup in a system acclimated to free ammonia.'' *Water Res.*, 30, 1383–1388.
Voets, J. P., Vanstaen, H., and Verstraete, W. (1975). ''Removal of nitro- gen from highly nitrogenous wastewaters.'' *J. Water Pollution Control Fedn.*, 47, 394–397.
Wanner, O., and Gujer, W. (1986). ''A multispecies biofilm model.'' *Bio- technol. Bioengrg.*, 28, 314–328.
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$\gamma i = \hat{\mu} i/\mu i$;

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h = nitrite-nitrogen fraction: $NO_2–N:(NO_2–N + NO_3–N)$;

μ_{m1} = maximum specific growth rate of ammonium oxidiz-ers (h^{-1});

μ_{m2} = maximum specific growth rate of nitrite oxidizers (h^{-1});

μ_1 = specific growth rate of ammonium oxidizers at sat-uration DO concentration (h^{-1});

μ_2 = specific growth rate of nitrite oxidizers at saturation DO concentration (h^{-1});

$\hat{\mu}_1$ = specific growth rate of ammonium oxidizers (h^{-1}); and

$\hat{\mu}_2$ = specific growth rate of nitrite oxidizers (h^{-1}).