

Enhanced nitrogen removal in SBRs bypassing nitrate generation accomplished by multiple aerobic/anoxic phase pairs

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Abstract A lab-scale SBR was used for the study of nitrogen removal from a synthetic wastewater with an ammonium-nitrogen concentration of 50 mg/L. The *react* phase of the reactor operation was divided into three sets of consecutive aerobic and anoxic periods with a duration ratio of 1:3 (20 min aerobic and 1 h anoxic phase). Under these operating conditions, nitrogen removal was achieved via nitrite i.e. no nitrification (oxidation of nitrite to nitrate) and hence no denitrification (reduction of nitrate to nitrite) was taking place in the aerobic and anoxic phase, respectively. This was attributed to the suppression of the nitrite-oxidizers activity due to the short aerobic phase duration. This presumption was supported by the ever decreasing amount of nitrate-nitrogen generated in the react phase during the transient, even when the activated sludge of the reactor was supplemented with additional nitrite-oxidizers. On the other hand, denitrification was mainly based on stored carbon sources, as long as the organic carbon (provided in the form of acetate) was never accumulated during the anoxic/anaerobic *fill* phase of the reactor operation.

Keywords Denitrification; nitrification; nitrogen removal; sequencing batch reactor (SBR)

Introduction

Biological nitrogen removal from wastewaters is an essential treatment to avoid unpleasant conditions for the natural receivers. Among the problems occurring in the receivers, caused by the various nitrogen forms (ammonium-, nitrite- and nitrate-nitrogen) are fish toxicity, eutrophication and oxygen depletion. Nitrogen removal consists of ammonium-nitrogen oxidation to nitrite (*nitrification*) and finally to nitrate (*nitrification*) and reduction of produced nitrate-nitrogen to gas nitrogen, sequentially via nitrite, nitric oxide and nitrous oxide (denitrification).

Because nitrite-nitrogen is an intermediate compound in both processes (nitrification and denitrification), the aim, in recent years, was focused on the removal of nitrogen via nitrite rather than nitrate. This is justified by the several advantages compared to the typical procedure, including: (1) 40% reduction of the COD demand during denitrification, (2) 63% higher rate of denitrification, (3) 25% reduction of oxygen demand for nitrification (oxidation of ammonium to nitrite-nitrogen) compared to complete oxidation to nitrate and (4) 300% lower biomass yield during anoxic growth (Turk and Mavnic, 1986, 1987; Abeling and Seyfried, 1992).

It is obvious that the crucial parameter in nitrogen removal via nitrite is the elimination of nitrate production during the nitrification process. On the other hand, it seems almost unattainable to exclude the proliferation of nitrite-oxidizers, and hence the production of nitrate, due to their synergistic way of growth with the ammonia-oxidizers. However, there are several factors that cause inhibition of the nitrite-oxidizer growth, leading thus to nitrite accumulation during nitrification. High free ammonia (FA) concentration (Abeling and Seyfried, 1992), combined with high pH values (Cecen and Gonenc, 1994), or with high – over 25°C – temperature (Balmelle *et al.*, 1992), or even with low dissolved oxygen (DO) concentration (Cecen and Gonenc, 1994), lag-time when changing from anoxic to aerobic

conditions (Turk and Mavinic, 1986), free hydroxylamine (FH) concentration (Yang and Alleman, 1992) as well as addition of selective chemical inhibitors (e.g. NaClO_3) for nitrification, are some such factors that have been proposed. Therefore, in order to achieve effective nitrogen removal via nitrite, based on the use of one of the above factors alone or a combination of them, the process should be designed to (a) make use of simultaneous and/or alternating nitrification/denitrification periods in the same reactor, (b) maintain low DO concentration during aeration, (c) induce contact of the sludge with high concentration of free ammonia and/or free hydroxylamine, (d) keep high pH values, (e) maintain the reactor temperature near or above 25°C and (f) add hydroxylamine or other nitrification inhibitor in the reactor (Hyungseok *et al.*, 1999).

However, high nitrite build-up may be risky for the effective removal of nitrogen in a wastewater treatment plant. Any possible residue of nitrite-nitrogen in the effluent, due to insufficient denitrification, would be harmful for human health due to its high toxicity and mutagenicity. Therefore, nitrogen removal via nitrite must be accompanied by far-reaching denitrification to ensure low level of nitrite-nitrogen concentration in the effluent of the plant. However, denitrification is often limited by the lack of available organic carbon source in the anoxic basin to sustain high denitrification rate. Consequently, it is common practice to introduce an external organic carbon source (such as methanol) in the anoxic basin, leading thus to an increase in the operational cost of the plant. The aforementioned technique is a major drawback of the separate sludge post-denitrification systems. In order to overcome this problem, the most recently installed systems are of the single sludge pre-denitrification type. This means that their principle of operation is the recirculation of the nitrified mixed liquor to the anoxic basin or the utilization of appropriate aeration patterns in the tanks, in order to secure the existence of anoxic zones or periods. In this way, denitrification is based on the organic load of the wastewater itself or on the stored/endogenous carbon sources of the sludge.

In recent years, Sequencing Batch Reactors (SBRs) have attracted great interest for wastewater treatment, because of their simple configuration (all necessary processes are taking place time-sequenced in a single basin). Nitrification and denitrification are achieved in a SBR by temporal alternation of aerobic and anoxic periods, while the separation of treated wastewater and microorganisms is accomplished by ceasing aeration and/or mixing at the end of the process cycle (Irvine and Davis, 1971). Due to its operational flexibility, it is quite simple to increase its efficiency in treating wastewater by changing the duration of each phase rather than adding or removing tanks in continuous flow systems.

In the present work, the SBR reactor was thought to be perfectly suited for achieving nitrogen removal via nitrite, due to its inherent characteristic of establishing alternating aerobic and anoxic periods in the same basin. This selection is also encouraged by the aforementioned suggestions of Hyungseok *et al.* (1999). Surveying the above mentioned factors for inhibition of nitrate production, the lag time of nitrite-oxidizers, when changing from anoxic to aerobic conditions, was thought to be possibly exploitable in an SBR system: a frequent switching between aerobic and anoxic periods was applied in the reactor, in order to suppress nitrite oxidation and promote the growth advantage of ammonia-oxidizers over the nitrite-oxidizers. On the other hand, the use of temperature variation is questionable, because fairly disparate observations can be found in the literature, such as those of Ford *et al.* (1980) which suggest that the optimal temperature range is between 30 and 36°C . Therefore, the reactor temperature was adjusted to 25°C , which was thought to be a rather representative value, especially for warm climates, as is the case for Greece. Moreover, elevated pH values which are thought to correlate with FA and/or FH concentration are rather unlikely in well buffered systems or typical domestic wastewater where the pH range is between 7.0 and 7.5 (Metcalf and Eddy, 1991). However, the DO concentration

is a more crucial parameter in systems where simultaneous nitrification/denitrification (SND) takes place (v.Munch *et al.*, 1996).

On the other hand, the availability of carbon sources in order to sustain acceptable denitrification rates was considered of major importance. Therefore, wastewater inflow during the *fill* phase of reactor operation occurring under anoxic/anaerobic conditions, in order to promote carbon storage by the microorganisms. This would have an advantageous effect on reactor performance, as the stored carbon would be available for denitrification during subsequent anoxic periods.

Materials and methods

SBR reactor

Experiments were carried out using a lab scale SBR reactor with an operating volume of one litre. The reactor was seeded with sludge from the aerobic basin of the Wastewater Treatment Plant of the University of Patras. An air pump and a glass diffuser provided sufficient aeration of the mixed liquor while mixing was accomplished via a magnetic stirrer and a stirring bar at the bottom of the reactor. Temperature was maintained at $25 \pm 0.2^\circ\text{C}$ with a temperature P-controller (Shimaden) using an electrical resistance, a thermocouple and a U-shaped tube connected with tap water supply. Sterilized synthetic wastewater was introduced into the reactor, using a variable speed pump, through a UV chamber, to protect the feed from microbial contamination. Effluent was discharged by gravity through a sole-noid valve. Digital timers controlled the operation of the system.

Synthetic wastewater

The synthetic wastewater was prepared in separate Duran bottles and sterilized at 121°C by wet sterilization for half an hour. The feed contained acetate as the sole organic carbon source (150 mg C/L) and ammonium-nitrogen (50 mg N/L) at a C:N ratio of 3:1. A combination of potassium hydrogen phosphate (K_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) was used both to buffer the mixed liquor pH in the range of 7.2–7.3 and to provide a phosphorus source for the sludge. Sodium hydrogen carbonate (NaHCO_3) was added in excess, to ensure that the nitrification process was not limited by alkalinity. The detailed composition of the synthetic wastewater and the trace elements are shown in Tables 1 and 2.

Experimental procedure

In general, a typical SBR cycle includes five distinct phases, namely *fill*, *react*, *settle*, *draw* and *idle*. In the present work, during the *fill* phase, 0.67 litres of synthetic wastewater was introduced into the reactor, increasing the liquid volume from 0.33 litres (minimum level)

Table 1 Synthetic wastewater composition

Substance	Concentration	
$\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$	0.85	g/L
$(\text{NH}_4)_2\text{SO}_4$	0.235	g/L
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2	g/L
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.026	g/L
K_2HPO_4	5	g/L
KH_2PO_4	3	g/L
NaHCO_3	0.7	g/L
Trace elements	0.1	mL/L

Table 2 Trace elements composition

Substance	Concentration	
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.786	g/L
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	5	g/L
$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	12.609	g/L
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	4.05	g/L
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	4.398	g/L
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	2.453	g/L
KI	0.75	g/L
H_3BO_3	3	g/L
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	5	g/L
EDTA	5	g/L

to 1 litre (maximum level). The *fill* phase was taking place under anoxic/anaerobic conditions, providing only mixing of the reactor content. The *react* phase consisted of six alternating aerobic and anoxic phases of 20 min and 1 h respectively, followed by one last aerobic phase of 15 min duration. Aeration and agitation of the reactor ceased during the *settle* phase and the sludge was allowed to settle under quiescent conditions. During the *draw* phase, the clarified supernatant was withdrawn by gravity through a solenoid valve from a fixed port at the minimum liquid level. In the present work, no solids' wasting was implemented and hence no control of the Solids Retention Time (SRT) was made.

Assessment of the SBR reactor performance was undertaken at regular intervals. During an SBR cycle, samples were collected from the reactor at the beginning of the operation cycle, the end of *fill*, each *aerobic* and *anoxic* phase, as well as from the effluent after the *draw* phase. The mixed liquor sample was centrifuged for 5 min at 4,000 rev/min (Econospin, DuPont Co.) and the supernatant was filtered through a 0.2 μm pore size filter. The filtrate was analyzed for ammonium-, nitrite- and nitrate-nitrogen concentration according to the phenate method (APHA, 1989) for the first and by ion chromatography (DX300, Dionex Corp.) for the latter two. Several measurements for pH (pH 537, WTW G.m.b.H) and mixed liquor suspended solids (APHA, 1989) were taken during the SBR operation cycle. When the specific nitrification and denitrification rates remained practically constant, the reactor was considered to have reached a quasi steady state. At that time, the sampling intervals during an SBR cycle were more frequent in order to obtain a detailed profile of the reactor performance during a typical cycle. During such a cycle, measurements of filtered organic carbon (as acetate) by ion chromatography and dissolved oxygen (DO-meter RL450, Russell) were performed, apart from the regular ones already mentioned.

Results

The reactor was operated for almost three months under the operating conditions described above (6 hours cycle duration). The patterns for the nitrogen forms at the beginning (ammonium) and at the end (ammonium, nitrite and nitrate) of each one of the three aerobic phases in the last fifty days of the reactor operation are shown in Figure 1. As is clearly

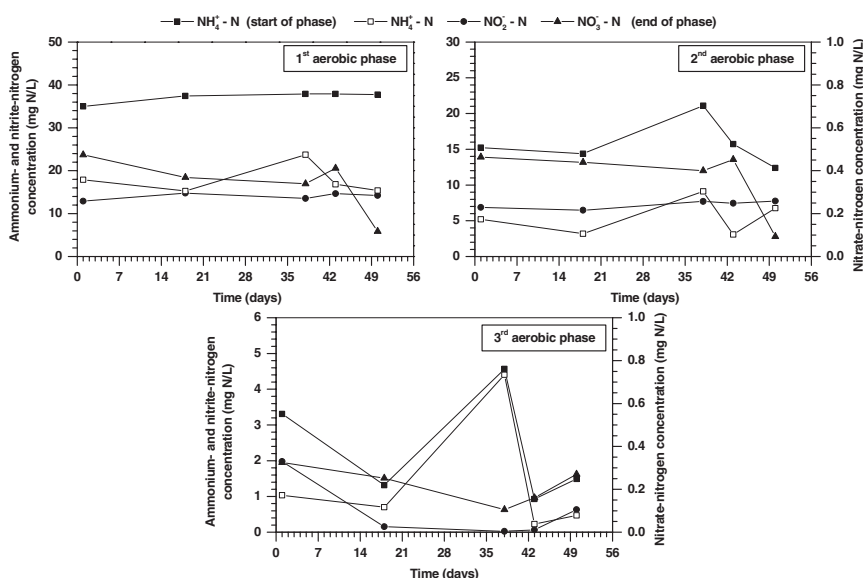


Figure 1 Daily variations of ammonium-, nitrite- and nitrate-nitrogen at the three aerobic phases

seen, the concentration of nitrate-nitrogen at the end of an aerobic phase never exceeded 0.5 mg/L, throughout the experimentation period, whereas oxidized nitrogen exclusively consisted of nitrite-nitrogen. This observation can be attributed to either the suppression of nitrite-oxidizers activity by the short aerobic phase duration or to the depletion of nitrite-oxidizers population from the sludge. The latter seems not to be the case due to the high value of the sludge age (almost 30 days) and hence the most likely explanation is the former one. In any case, the nitrite-nitrogen produced in each aerobic phase was completely eliminated during each anoxic phase of the SBR operation.

Furthermore, it is worth noting that no accumulation of acetate-carbon took place during the anoxic/anaerobic *fill* phase of the reactor, throughout the experimentation period. This observation was clarified by conducting individual batch experiments with a portion of the SBR sludge (data not shown) which revealed that the acetate-carbon was completely eliminated under anaerobic conditions. The total nitrogen concentration in the effluent of the system was 0.73 ± 0.95 mg/L. The Volatile Suspended Solids (VSS) concentration in the reactor at the end of the *fill* phase was around 10.0 ± 0.46 g/L and in the effluent of the system was 112 ± 28 mg/L.

A typical cycle of the reactor operation is shown in Figure 2. During the anoxic/anaerobic *fill* phase no accumulation of carbon is observed, while in contrast ammonium-nitrogen reaches its maximum concentration at the end of this phase. During the aerobic phase, the ammonium-nitrogen concentration rapidly decreases, while there is a corresponding increase of the nitrite-nitrogen concentration. In addition, the Dissolved Oxygen (D.O.) concentration increases to a maximum value at the end of the aerobic phase. In the subsequent phase, a rapid reduction of the nitrite-nitrogen and a small elimination of the ammonium-nitrogen take place. Similar observations are made for the next two sets of aerobic and anoxic phases, ending up in the complete removal of ammonium- and nitrite-nitrogen at the end of the *react* phase. Throughout the operating cycle, however, the nitrate-nitrogen concentration remained under 0.2 mg/L and is not presented in the figure.

In order to verify that the activity of the nitrite-oxidizers population is repressed by the operating conditions, a portion of a nitrite-oxidizers culture, corresponding to 1% of

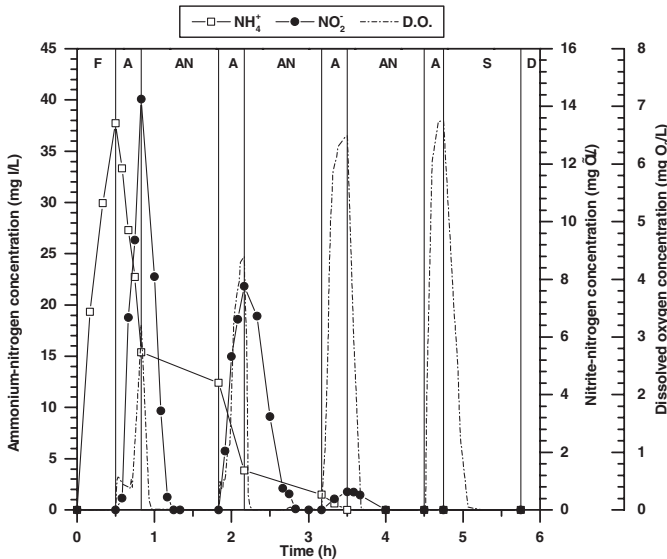


Figure 2 Concentration profiles of ammonium-nitrogen, nitrite-nitrogen and dissolved oxygen during an operating cycle. (F: *fill* phase; A: *aerobic react* phase, AN: *anoxic react* phase; S: *settle* phase; D: *draw/idle* phase)

the mixed liquor VSS concentration, was introduced to the reactor. The performance of the reactor, in terms of nitrogen forms concentrations, is presented in Figure 3 (in a similar way to that of Figure 1). As seen in Figure 3, a constantly decreasing concentration of nitrate-nitrogen was observed at the end of each aerobic phase during the experimentation period.

A more distinct insight into the reactor performance was obtained by estimating the sum of nitrite-, nitrate- and total oxidized nitrogen concentration produced in all aerobic phases during an SBR cycle. It was found that the nitrate-nitrogen concentration and hence the *nitrification* (oxidation of nitrite to nitrate) ability of the sludge decreases during the experimentation period, accompanied by a corresponding increase in nitrite-nitrogen concentration. *Nitrification* depression can be attributed to the operating conditions imposed. In Figure 4, the concentration profiles of ammonium-, nitrite- and nitrate-nitrogen are shown for the operation cycle on the day that the reactor was seeded with nitrite-oxidizers and at the end of the experimentation period. Although the nitrogen removal efficiency was almost the same for both cases (greater than 95%), there is a qualitative difference. On the first day nitrate-nitrogen was produced in each aerobic phase,

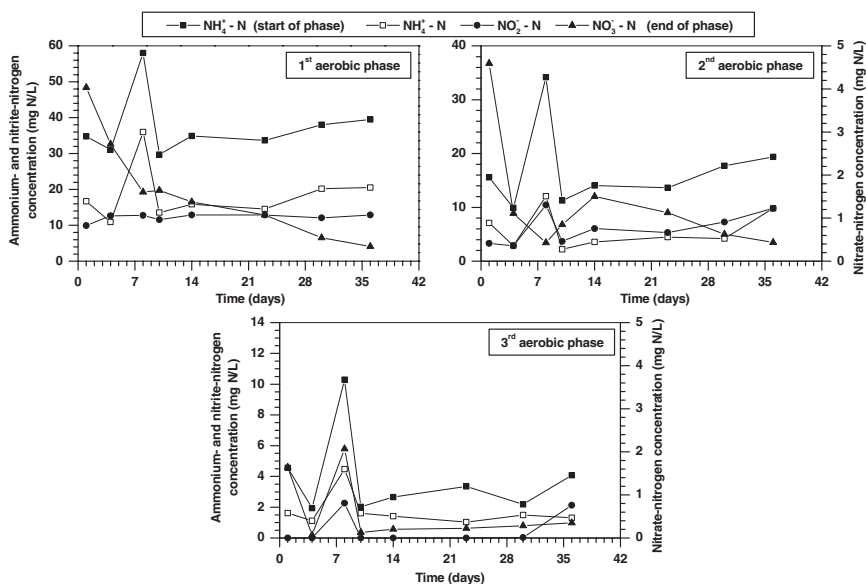


Figure 3 Daily variations of ammonium-, nitrite- and nitrate-nitrogen at the three aerobic phases after the seeding of the reactor with nitrite-oxidizers

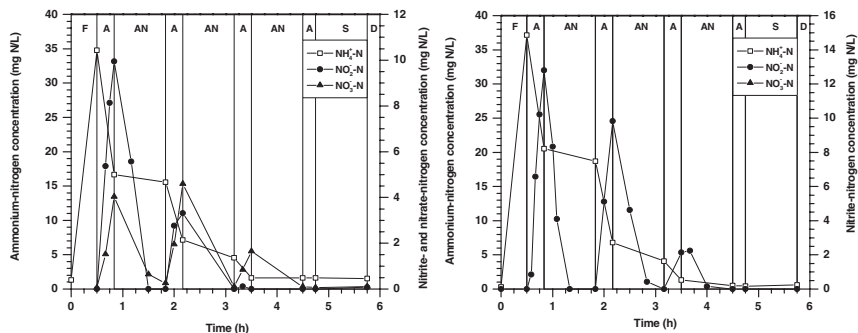


Figure 4 Concentration profiles of ammonium-, nitrite- and nitrate-nitrogen during an operation cycle on the first (left) and the last (right) day of the experimentation period after the seeding of SBR with nitrite-oxidizers. (F: fill phase; A: aerobic react phase, AN: anoxic react phase; S: settle phase; D: draw/idle phase)

whereas on the last day its production never exceeded 0.5 mg/L (therefore, its concentration profile is omitted in the second figure).

Conclusions

- *Nitrification* (oxidation of nitrite to nitrate) and hence *denitrification* (reduction of nitrate to nitrite) bypass is feasible through implementation of appropriate multiple successive aerobic/anoxic phase pairs in a Sequencing Batch Reactor!
- *Nitrification* depression is attributed to the operating conditions implemented (alternation of aerobic and anoxic conditions during *react* phase) and to the short duration of aerobic phase (20 minutes). Effective oxygen transport plays an essential role in this.
- High nitrogen removal efficiency ($98.0 \pm 1.6\%$) is achieved through implementation of an operating strategy with 6 hours cycle duration and three pairs of aerobic:anoxic phases in a phase ratio of 1:3 (20:60 minutes) for synthetic wastewater with acetate-carbon and ammonium-nitrogen influent concentrations of 150 mg/L and 50 mg/L, respectively. Nitrogen removal is attributed to nitrogen assimilation for bacteria growth (26.8%) and to nitrification/denitrification.
- Nitrogen removal is accomplished without any additional supply of organic carbon during anoxic phases in order to sustain denitrification. This fact can be associated with the absence of dissolved carbon accumulation during the anoxic/anaerobic *fill* phase. Carbon absorption (or adsorption) during the *fill* phase and its subsequent usage for denitrification, during anoxic phases, seems to be occurring.

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