Short- and long-term orange dye effects on ammonium oxidizing and anammox bacteria activities

A. Val del Río, A. Stachurski, R. Méndez, J. L. Campos, J. Surmacz-Górska and A. Mosquera-Corral

ABSTRACT

The effects of orange azo dye over ammonia oxidizing bacteria (AOB) and anammox bacteria activities were tested. Performed batch tests indicated that concentrations lower than 650 mg_{orange}/L stimulated AOB activity, while anammox bacteria activity was inhibited at concentrations higher than 25 mg_{orange}/L. Long-term performance of a continuous stirred tank reactor (CSTR) for the partial nitritation and a sequencing batch reactor (SBR) for the anammox process was tested in the presence of 50 mg_{orange}/L. In the case of the partial nitritation process, both the biomass concentration and the specific AOB activity increased after 50 days of orange azo dye addition. Regarding the anammox process, specific activity decreased down to 58% after 12 days of operation with continuous feeding of 50 mg_{orange}/L. However, the anammox activity was completely recovered only 54 days after stopping the dye addition in the feeding. Once the biomass was saturated the azo dye adsorption onto the biomass was insignificant in the CSTR for the partial nitritation process fed with 50 mg_{orange}/L. However, in the SBR the absorption was determined as 6.4 mg_{orange}/g volatile suspended solids. No biological decolorization was observed in both processes.

Key words | adsorption, anammox, AOB, degradation, orange dye, partial nitritation

A. Val del Río (corresponding author)
R. Méndez
A. Mosquera-Corral
Department Chemical Engineering,
School of Engineering,
Universidade de Santiago de Compostela,
E-15782,
Santiago de Compostela
Spain
E-mail: mangeles.val@usc.es

A. Stachurski
J. Surmacz-Górska
Environmental Biotechnology Department, Faculty
    of Power and Environmental Engineering,
Silesian University of Technology,
Gliwice,
Poland

J. L. Campos
Facultad de Ingeniería y Ciencias,
Universidad Adolfo Ibáñez,
Viña del Mar,
Chile

INTRODUCTION

Dyes are compounds commonly present in the wastewater produced from industrial sectors like paper, textiles, paint or printing production. The azo dyes are the most commonly used organic dyes. They are aromatic compounds provided with a nitrogen–nitrogen double bond, which confers on them a complex structure and high molecular weight, and makes them difficult to be mineralized. Although the acute toxicity of azo dyes is slight, their potential health effects, due to their toxic by-products (e.g. aromatic amines), have been described as mutagenic and carcinogenic (Daverey et al. 2014). Therefore, the application of an efficient treatment for the removal of these compounds from the liquid effluents before their disposal into the environment is necessary.

The treatment methods, to achieve the dye decolorization in wastewater, include: physico-chemical (e.g. membranes, reverse osmosis, coagulation/flocculation); advanced oxidation (e.g. ozonation, Fenton process, electrochemical oxidation, UV/ozone/hydrogen peroxide) and

biological processes (e.g. bacterial, fungal and enzymatic degradation) (Gupta et al. 2015). Biological processes relying on specific isolated strains have been used to achieve the efficient dye removal (Sen et al. 2016). For this reason, the effect of dyes on the microbial populations, usually present in the wastewater treatment plants, has received little attention. Li & Bishop (2004) studied the removal of acid orange 7 (azo dye) in conventional wastewater treatment processes, and they observed that this dye was recalcitrant to aerobic conditions, while it was decolorised by adapted sludge under anaerobic and anoxic conditions.

The anammox (anaerobic ammonium oxidation) process has been widely studied, since the late 1990s of last century, to remove nitrogen from wastewater in a more efficient way than via the conventional nitrification–denitrification processes. The anammox bacteria are known to be very sensitive to operational conditions (temperature, pH, dissolved oxygen (DO) concentration, etc.), and they are slow-growing organisms. Furthermore, the presence

of toxic compounds can easily provoke the failure of the process, involving long recovery periods. For these reasons, an extensive amount of research work has been devoted to checking the inhibitory effect of different compounds (e.g. salts, antibiotics, heavy metals) to broaden the range of possible applications for the anammox process (Jin *et al.* 2012). However, at the moment, little attention has been paid to the effects of azo dyes over anammox based processes.

For the anammox process to take place, half of the ammonium present in the wastewater needs to be previously converted to nitrite, via the partial nitritation process under aerobic conditions. The aim of this study is to evaluate the short- and long-term effects of the presence of orange azo dye over both processes (partial nitritation and anammox) separately. Batch activity tests and continuous reactor operation were considered for this purpose. Furthermore, this research work presents the first results published about the inhibition and subsequent recovery of the anammox activity of the biomass from a sequencing batch reactor (SBR) after its exposure to the presence of orange azo dye in the feeding.

MATERIAL AND METHODS

Experimental set-up

A continuous stirred tank reactor (CSTR) of 5 L was used for the partial nitritation process (Figure 1(a)). Peristaltic pumps were used for the feeding supply and effluent discharge. DO was provided by aeration through a fine diffuser (pores of 100 μm) placed at the bottom of the reactor. The complete mixing of the liquid media was achieved by the action of a mechanical stirrer at a rotating speed of 150 ± 20 rpm. The reactor had a thermal water jacket, and the temperature was maintained at $35 \pm 1\,^{\circ}$C. The inoculum, with a specific ammonia oxidizing activity of 0.27 g NH_4^+-N per gram volatile suspended solids (VSS) per day, was taken from a nitrifying–denitrifying SBR reactor in operation. The initial biomass concentration in the CSTR was 0.043 g VSS/L. The reactor was fed with a synthetic solution composed of: 1.91–1.45 g NH_4Cl/L, 3.50 g $KHCO_3$/L, 0.05 g NaH_2PO_4/L, 0.3 g $CaCl_2 \cdot 2H_2O$/L, 0.2 g $MgSO_4 \cdot 7H_2O$/L, 0.006 g $FeSO_4$/L, 0.006 g EDTA/L and 1.25 mL/L of trace element solution according to van de Graaf *et al.* (1996).

The operation of the CSTR was divided into two stages with the operational conditions described in Table 1. In Stage I the CSTR was operated to establish the stable nitritation process by washing out the nitrite oxidizing bacteria (NOB) from the unit. In Stage II a concentration of 50 mg_{orange}/L, dissolved in the synthetic solution previously described, was continuously fed to the CSTR.

An SBR with a working volume of 5 L was used, in which the anammox process took place (Figure 1(b)). The SBR was operated in 6-hour cycles comprising the following stages: 315 min of mixing and continuous feeding, 15 min of mixing, 15 min of sedimentation and 15 min of effluent withdrawal. A set of two peristaltic pumps were used for the feeding and effluent withdrawal. A mechanical stirrer, operated at a rotating speed of 180 ± 20 rpm, maintained the complete mixture inside the reactor. A programmable logic controller (CPU 224 Siemens) controlled the actuation of the pumps, valves and stirrer. The temperature was maintained at $30 \pm 1\,^{\circ}$C by means of a thermostatic bath connected to the water jacket of the reactor. The solubilization of oxygen gas in the reactor media was avoided by continuously flushing the headspace of the reactor with argon gas. The SBR was inoculated with biomass from another laboratory anammox reactor (Fernandez *et al.*
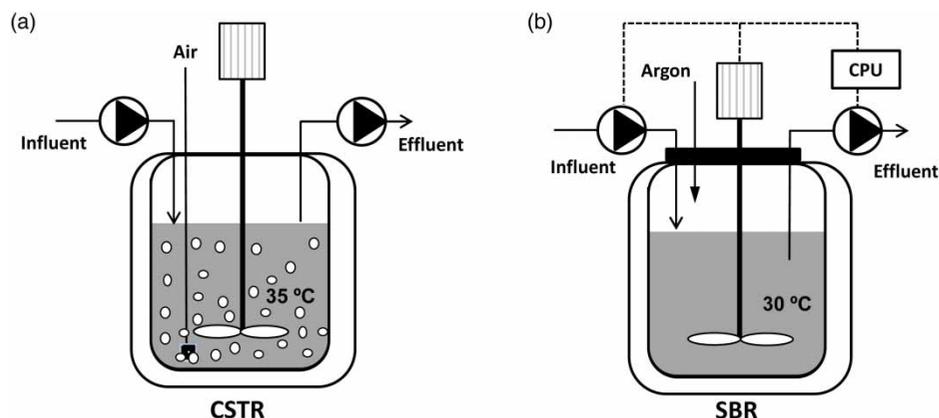

Figure 1 │ Schematic representation of the experimental set-up used: (a) CSTR for the partial nitritation process and (b) SBR for the anammox process.

Table 1 | Operational stages corresponding to the CSTR and SBR operation where the partial nitritation and anammox processes take place, respectively

	CSTR: partial nitritation		SBR: anammox	
	Stage I	Stage II	Stage I	Stage II
Operational days	0–11	12–60	0–21	22–75
NH_4^+ (mg N/L)	380	500	440	200–520
NO_2^- (mg N/L)	–	–	440	200–520
NH_4^+/HCO_3^- (mg N/mg C)	0.91	1.20	–	–
Orange azo dye (mg/L)	0	50	50	0
pH	7.5 ± 0.7	6.7 ± 0.2	8.2 ± 0.3	8.1 ± 0.2

2012). The initial biomass concentration was 4.7 g VSS/L, with a specific anammox activity (SAA) of 0.72 g N/(g VSS·d). The SAA of the inoculum was determined in triplicate according to the procedure described in Dapena-Mora et al. (2007). The feeding consisted of a synthetic solution according to Dapena-Mora et al. (2004): 0.75–2.00 g NH_4Cl/L, 1.00–2.55 g $NaNO_2/L$, 0.8 g $KHCO_3/L$, 0.05 g NaH_2PO_4/L, 0.3 g $CaCl_2 \cdot 2H_2O/L$, 0.2 g $MgSO_4 \cdot 7H_2O/L$, 0.006 g $FeSO_4/L$, 0.006 g EDTA/L and 1.25 mL/L of trace element solution according to van de Graaf et al. (1996). The feeding was supplied with 50 mg/L of orange azo dye during the first 21 operational days (Table 1).

Analytical methods

The concentrations of ammonium, nitrite, nitrate, biomass as VSS and total suspended solids were determined according to the *Standard Methods* (APHA-AWWA-WPCF 2005). Inorganic carbon concentration was measured with a Shimadzu analyser (ASI-5000-S). Orange azo dye concentration was colorimetrically determined using a spectrophotometer (Shimadzu UV-1603, UV-visible) at 480 nm. All these measurements were performed in triplicate. The pH values were measured with an electrode (Crison Instruments, S.A., 52-03) equipped with an automatic compensatory temperature device (Crison Instruments, S.A., 21-910-01). A DO probe (AQUALITYC, model OXI-921) connected to a meter (M-Design Instruments TM-3659) was used to control DO concentration in the CSTR.

Experiments of azo dye degradation

A batch experiment was performed in the CSTR by switching off the pumps for the feeding addition and effluent discharge. The potential of the ammonia oxidizing bacteria (AOB) activity to degrade the azo dye was evaluated. The initial conditions of the experiment corresponded to the steady state operational conditions of the CSTR. The evolution of the concentrations of ammonia, nitrite and azo dye was monitored for approximately 12 h by taking liquid samples each hour. The pH was monitored for the whole experiment, and when its value decreased to less than 6.5, bicarbonate was added to increase the pH and maximize the AOB activity inside the reactor. After this experiment the continuous operational conditions of the reactor were re-established.

Batch respirometric activity tests

The AOB activity was measured by respirometric tests according to López-Fiuza et al. (2002), using a bench-model oxygen meter (YSI 5300) with oxygen-selective probes (YSI 5331). Respirometric assays were performed at 35 °C and in duplicate. The sludge samples were previously washed three times with a phosphate buffer and a certain amount of orange azo dye (0, 25, 50, 130, 250 and 650 mg_{orange}/L) was added to have different concentrations inside the vials. The samples, containing approximately 1 g VSS/L, were placed in vials of 15 mL (useful volume of 10 mL). Firstly, the samples in the vials were aerated for 30 min. Then, the aeration was stopped and the evolution of the concentration of DO throughout time registered. During the first 2 min of the experiment the observed DO consumption corresponded to the endogenous activity. Then, the substrate (ammonium chloride) was injected with a micro-syringe (10 μL) to have a concentration inside the vials of 35 mg NH_4^+-N/L. The change in the slope of the curve describing the oxygen consumption in comparison with the endogenous one was used to determine the AOB activity. After each activity test, the biomass concentration (g VSS/L) inside the vials was measured. The value of the specific activity, as g NH_4^+-N/(g VSS·d), was estimated by dividing the value of the slope of the curve representing the DO consumption associated with the ammonium oxidation (after subtracting the endogenous activity) by the amount of biomass in the vial.

SAA tests

To estimate the SAA, batch assays were performed according to the procedure described by Dapena-Mora et al. (2007). The tests were performed in triplicate using vials of 38 mL (useful volume of 25 mL) at 30 °C. Firstly, the biomass samples were washed three times with phosphate

buffer and a certain amount of orange azo dye was added, to have concentrations of 0, 25, 50, 100 and 145 mg$_{orange}$/L. One vial was prepared with biomass and without substrate or azo dye addition to obtain the value of the blank. The headspace of the vials, hermetically closed, was flushed with helium gas to avoid the presence of DO. The vials were incubated at 30 °C and 150 rpm for 30 minutes to acclimate the biomass. Then the substrates were added (70 mg N/L of ammonium and nitrite, respectively), and pressure was equalized to the atmospheric one. Then, the evolution of the overpressure throughout the time was recorded using a differential pressure transducer (0–5 psi, linearity 0.5% of the full scale) manufactured by Centerpoint Electronics. After each activity test, the biomass concentration (g VSS/L) inside the vial was determined to obtain the specific activity. The value of the specific activity, as g N$_2$-N/(g VSS·d), was estimated by dividing the value of the slope of the curve representing the N$_2$ gas production consumption associated with the anammox activity (after subtracting the value of the blank) by the amount of biomass in the vial.

Calculations

The theoretical concentration of dye in the effluent of the reactor (C) throughout the time (t), assuming that no reaction takes place, was determined with Equation (1) when continuous feeding of orange azo dye was applied, and with Equation (2) when orange azo dye feeding was stopped:

$$C = C_{fed} \cdot \left(1 - \exp\left(\frac{-t}{HRT}\right)\right) \qquad (1)$$

$$C = C_{fed} \cdot \exp\left(\frac{-t}{HRT}\right) \qquad (2)$$

where C_{fed} is the dye concentration in the feeding (mg$_{orange}$/L), t is the operational time (d) from the beginning of the experiment and HRT is the hydraulic retention time (d) fixed in the reactor. The curve obtained for the theoretical concentration was compared with the curve of experimental data (concentration measured in the effluent throughout the time). The value of the area between both curves, as mg$_{orange}$/(L·d), was then divided by the concentration of solids in this operational time (g VSS/L) to obtain the quantity of dye absorbed or desorbed.

The percentage of anammox activity remaining was calculated with Equation (3) using the values obtained by batch

activity tests (SAA) and referred to the activity of the inoculum (SAA$_0$), considered as the highest value.

$$\%SAA = \frac{SAA}{SAA_0} \cdot 100 \qquad (3)$$

RESULTS AND DISCUSSION

Effect of orange azo dye on the partial nitritation process

The operation of the CSTR lasted 60 days and was divided into two stages. The first one was the start-up stage (0–11 days) to promote the development of the partial nitritation process. For this purpose, the ammonium to bicarbonate (NH$_4^+$/HCO$_3^-$) ratio in the feeding was fixed at 0.91 g N/g C and the temperature at 35 °C. The ammonium concentration in the feeding was 380 mg NH$_4^+$-N/L and the HRT was 1.5 days, which corresponded to an applied nitrogen loading rate (NLR) of 0.25 g N/(L·d). In this stage, the nitrite concentration in the effluent increased progressively from 54 (day 1) to 253 mg NO$_2^-$-N/L (day 11) while the nitrate concentration was almost depleted (Figure 2). After 11 operational days, the achieved percentage of ammonium oxidation to nitrite was 66%, without significant nitrite oxidation to nitrate. These results indicate the absence of NOB activity, despite the DO concentration being maintained higher than 6 mg O$_2$/L.

Only between days 15 and 28 was a slight increase of nitrate concentration in the effluent observed due to the NOB proliferation in the biomass grown as a biofilm on the reactor walls. Then, regular cleanings of the walls (every 2–3 days) were performed to avoid this problem, and no more nitrate accumulation was observed. Furthermore, the NOB activity, checked regularly by batch
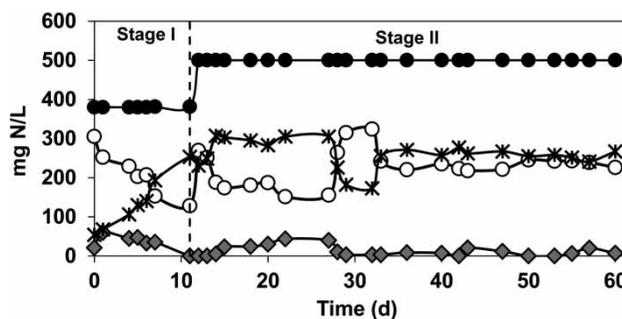

Figure 2 | Profile of nitrogen compound concentrations in the CSTR: NH$_4^+$ in the influent (●), and NH$_4^+$ (○), NO$_2^-$ (✳) and NO$_3^-$ (◆) in the effluent.

respirometric assays, was very low (approximately 0.014 ± 0.002 g NH_4^+-N/(g VSS·d)).

In Stage II the ammonium concentration in the influent was increased to 500 mg NH_4^+-N/L with the objective of restricting the ammonium oxidation to 50%, desirable to obtain a nitrite to ammonium (NO_2^-/NH_4^+) ratio of 1 g NO_2^--N/g NH_4^+-N. Furthermore, the HRT was decreased to 1.2 days, which corresponded with an increase of the applied NLR to 0.42 g NH_4^+-N/(L·d). The first 3 days after these changes, the NO_2^-/NH_4^+ ratio was close to 1 g NO_2^--N/g NH_4^+-N, but later it increased to 1.6 g NO_2^--N/g NH_4^+-N. For this reason, on day 28 the HRT was diminished to 0.8 days. Consequently, the ammonia concentration in the effluent increased and the nitrite concentration decreased, obtaining an NO_2^-/NH_4^+ ratio of approximately 0.5 g NO_2^--N/g NH_4^+-N. This decrease of the HRT provoked a washout of the biomass, as the solids concentration inside the reactor decreased from 0.110 g VSS/L (day 28) to 0.075 g VSS/L (day 33). As a counteractive measure, on day 33, the HRT was increased to 1.0 day to improve the retention of the AOB. After this change, an adequate NO_2^-/NH_4^+ ratio (1.12 ± 0.05 g NO_2^--N/g NH_4^+-N) was obtained in the effluent, suitable for a subsequent anammox process.

In this Stage II, orange azo dye was continuously supplied to the feeding at a concentration of 50 mg$_{orange}$/L. It took 3 days for the concentration in the effluent to reach a value similar to that in the feeding. Furthermore, the theoretical (calculated with Equation (1)) and experimental values throughout the time coincided. This coincidence indicates that the complete mixture inside the reactor was adequate and that the absorption of orange azo dye by the biomass was insignificant. The difference between the orange azo dye concentration in the influent and effluent was approximately $2.1 \pm 0.9\%$ for the whole operational period, irrespective of HRT changes. This difference can be considered statistically insignificant. Therefore, the orange azo dye was not biologically decolorized or removed by adsorption in the partial nitration CSTR.

To check the absence of biological decolorization by AOB activity an experiment of azo dye degradation was performed inside the CSTR at the end of the operational period (day 61) (Figure 3(a)). The initial concentrations of nitrogen compounds were 285 mg NH_4^+-N/L and 210 mg NO_2^--N/L. At the beginning of the test, the pH was 6.62, and after 1 hour it decreased to 6.42 due to the bicarbonate consumption by the AOB activity, which was 0.25 g NH_4^+-N/(L·d). Therefore, bicarbonate was added (650 mg HCO_3^-/L) to increase the pH to approximately 7.9. Due to
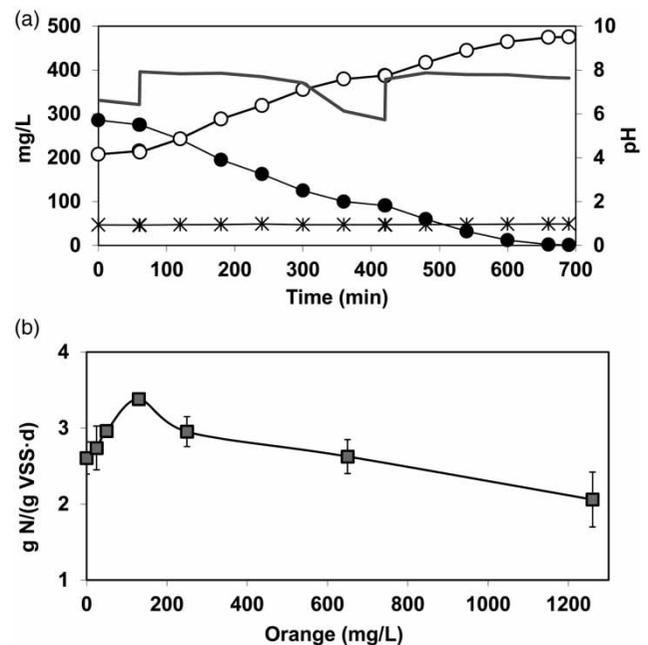

Figure 3 │ (a) Batch experiment inside the CSTR on day 61 of operation: ammonium (•), nitrite (○) and orange dye (∗) concentrations and value of pH (–). (b) Respirometric activity tests for CSTR biomass on day 61 at different orange dye concentrations.

this bicarbonate addition, the ammonium oxidizing rate increased (Figure 3(a)) to 0.91 g NH_4^+-N/(L·d) and the pH slowly started to decrease (0.12 pH unit/hour) until minute 300. Then the pH strongly decreased (0.84 pH unit/hour), which indicated that bicarbonate concentration was depleted and, consequently, the ammonium oxidation rate was slow. Again bicarbonate was added (390 mg HCO_3^-/L) in minute 420 to increase the ammonium oxidation rate to 0.71 g NH_4^+-N/(L·d). The dye concentration remained at 50 mg$_{orange}$/L during the whole experiment (Figure 3(a)), despite the promotion of a high ammonium oxidation activity. This test showed that AOB and/or the intermediates formed during the ammonium oxidation do not have the ability to decolorize the orange azo dye.

The continuous addition of azo dye concentrations of 50 mg$_{orange}$/L did not exert a significant effect on the AOB activity of the biomass in the CSTR, which was maintained around 3.10 g NH_4^+-N/(g VSS·d). Furthermore, the biomass concentration in the CSTR increased during the whole operational period from 0.043 g VSS/L (day 0) to 0.112 g VSS/L (day 60), except in Stage II due to the low HRT. Batch activity tests were performed to support this observation.

The batch AOB activity at different orange azo dye concentrations (from 0 to 650 mg$_{orange}$/L) was determined using

the biomass collected on day 61 from the CSTR. The obtained results (Figure 3(b)) indicated a stimulatory effect, of tested azo dye concentrations, on the AOB activity. The maximal measured activity was 3.40 g NH_4^+-N/(g VSS·d) for the experiment with 130 mg_{orange}/L. Previously, He & Bishop (1994) reported that the orange azo dye inhibits all stages of the nitrification process, although they observed that NOB were more sensitive than AOB. Furthermore, these authors stated that the orange azo dye inhibits the activity but does not repress the growth of the nitrifying bacteria. Later, Li & Bishop (2002) observed, by batch activity tests, that the nitrifying activity of biomass grown as biofilm was inhibited by concentrations from 1 to 25 mg_{orange}/L. However, all these research works were performed with biomass which was not previously exposed to the orange azo dye. In the present study, the biomass used to perform the batch AOB activity tests, at different orange azo dye concentrations, was previously exposed, for 50 days, to concentrations of 50 mg_{orange}/L. Therefore, AOB have enough time to presumably adapt to the presence of the applied orange azo dye concentration without loss of activity.

Effect of orange azo dye on the anammox process

A set of SAA tests were performed with the anammox biomass used to inoculate the anammox SBR to check the short-term effect of different orange azo dye concentrations. The results obtained (Figure 4) indicated that for concentrations lower than 25 mg_{orange}/L no inhibition occurred, while at 50 mg_{orange}/L the anammox activity was 83% (inhibition of 17%), with no significant differences at higher orange azo dye concentrations. These tests served to predict the loss of activity expected during the anammox SBR operation with 50 mg_{orange}/L, and to design an operational strategy to avoid the potential failure of the process.

The anammox SBR was operated for 75 days and the orange azo dye was supplied during the first 21 days at a concentration of 50 mg_{orange}/L (Stage I). The nitrogen removal efficiency was 70–80% during the overall operational period (Figure 5(a)). The large efficiencies measured in the SBR can be attributed to the fact that the applied food to microorganisms (F/M) ratio (g N/g VSS·d) was lower than the maximal specific capacity of the system (Figure 5(b)), corresponding to the value of the SAA checked by batch tests. For an appropriate operation of the anammox reactor the F/M ratio must be lower than the SAA of the biomass. This operational strategy was followed during the whole operational time of the SBR.

The concentration of dye in the effluent was lower than in the influent for the first 10 days of operation. Furthermore, the experimental concentrations of orange azo dye in the effluent remained under the theoretical ones (calculated with Equation (1)) during the whole Stage I, which indicated the possible occurrence of the absorption process. The calculated absorption was 6.4 mg_{orange}/g VSS. After 4 days of dye addition, a decrease of 33% in the maximal anammox activity was observed (from 0.72 to 0.49 g N/(g VSS·d)). At the same time, nitrite concentration in the effluent slightly increased from a not detectable value

Figure 5 │ Profiles of different parameters in the anammox SBR. (a) Concentrations (mg N/L) of NH_4^+ plus NO_2^- in the influent (•), NH_4^+ in the effluent (∗), NO_2^- in the effluent (▲) and NO_3^- in the effluent (○); percentage of nitrogen removal (–). (b) Maximal SAA (♦), applied F/M ratio (◇) and solids concentration inside the reactor (∗).
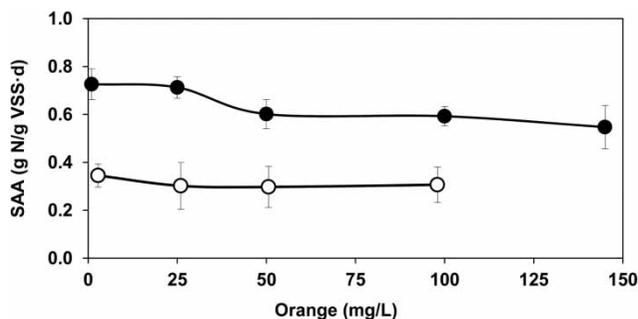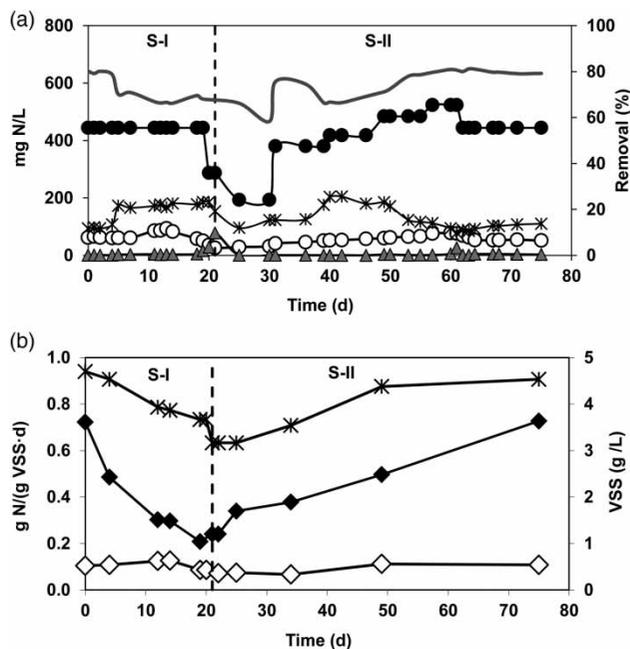
Figure 4 │ Values of SAA obtained at different orange azo dye concentrations for the biomass collected from the SBR on: day 0 (•) and day 14 (○) of operation and determined by batch activity tests.

to 2.6 ± 0.3 mg NO_2^--N/L. After 12 days of operation the anammox activity decreased by approximately 58% (SAA value of 0.30 g N/(g VSS · d)). Since nitrite concentration in the effluent increased up to around 80 mg NO_2^--N/L on day 20, the inlet nitrogen concentration was decreased to avoid the progress of the inhibitory effect on ana- mmox bacteria. Simultaneously, the orange azo dye addition was stopped (Stage II) to promote the recovery of the process. When no nitrite concentration was measured in the effluent (day 31), the inlet nitrogen con- centrations of ammonium and nitrite were increased to the initial values.

These results showed that the percentage of remaining anammox activity was 7%, 69% and 100% of the initial activity (SAA_0) after 4, 28 and 54 days from the suppression of the orange azo dye addition in the feeding, respectively. Furthermore, the experimental concentrations of orange azo dye in the effluent remained higher than the corre- sponding theoretical values (calculated with Equation (2)) in the first days of Stage II, which indicated the occurrence of dye desorption. The desorption process took place for 9 days after the dye removal from the feeding and was cal- culated as 27.2 mg_{orange}/g VSS. This means that the anammox process activity can be recovered in a reasonable period of time after exposure to orange azo dye. Although, there are no other studies about the effect and recovery of anammox bacteria activity in relation to orange azo dye, the studies with other toxic compounds report long periods for recovery of the anammox bacteria activity. For example, Zhang et al. (2015) proposed a novel strategy to accelerate the recovery of anammox activity after exposure to Cu(II) based on the use of a pre-treatment with EDTA washing combined with ultrasonic irradiation. With this strategy, they needed 64 days to recover the com- plete anammox activity inside the reactor.

In Stage I slow decrease of biomass concentration from 4.70 to 3.93 g VSS/L was observed in the SBR, due to its washout in the effluent, with an average solids concen- tration of 27.7 ± 7.0 mg VSS/L, and the biomass spent in the SAA experiments. This loss of biomass was not compen- sated for by the growth due to the inhibitory effect of the added orange azo dye. In Stage II, without dye addition, the biomass concentration increased progressively due to biomass growth associated with the recovery of the ana- mmox activity (Figure 5(b)).

The long-term inhibitory effect of the orange azo dye over anammox bacteria was estimated by measuring the SAA of biomass acclimated to the orange azo dye. SAA was determined for a biomass sample collected

from the SBR on day 14 of operation. The SAA of this sample was measured at different orange dye concen- trations (from 0 to 100 mg_{orange}/L) and compared to the values obtained for non-adapted biomass (sample from day 0) (Figure 4). The results showed that the SAA was lower than that of the non-adapted biomass for all the tested dye concentrations (Figure 4), indicating a deterio- ration of the biomass activity due to the long-term exposure to the dye. Furthermore, the values of SAA for adapted biomass remained practically constant indepen- dently of the orange azo dye concentrations. No stimulating effect was observed, contrary to what occurred with the AOB.

The mechanism of the gradual SAA inhibition is not known but its decrease could be correlated with the observed azo dye adsorption. This hypothesis is based on the fact that the initial activity of the biomass was recovered after the suppression of the addition and desorption of the orange azo dye.

CONCLUSIONS

- Biological decolorization of orange azo dye was not poss- ible by AOB or anammox bacteria.
- The absorption of orange azo dye by the anammox bio- mass, in operation in an SBR, was 6.4 mg_{orange}/g VSS.
- A concentration of 50 mg_{orange}/L provoked a 15% increase in the AOB activity and an inhibitory effect on the anammox bacteria activity: scarce for short-term (15–20%) but significant for long-term exposure (58% in 12 days).
- The recovery of the anammox activity after a long-term exposure to azo dye concentrations of 50 mg_{orange}/L was possible after the suppression of this compound addition in the feeding of the SBR.

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