

Effect of SBR feeding strategy and feed composition on the stability of aerobic granular sludge in the treatment of a simulated textile wastewater

R. D. G. Franca, J. Ortigueira, H. M. Pinheiro and N. D. Lourenço

ABSTRACT

Treatment of the highly polluting and variable textile industry wastewater using aerobic granular sludge (AGS) sequencing batch reactors (SBRs) has been recently suggested. Aiming to develop this technology application, two feeding strategies were compared regarding the capacity of anaerobic–aerobic SBRs to deal with disturbances in the composition of the simulated textile wastewater feed. Both a statically fed, anaerobic–aerobic SBR and an anaerobic plug-flow fed, anaerobic–aerobic SBR could cope with shocks of high azo dye concentration and organic load, the overall chemical oxygen demand and color removal yields being rapidly restored to 80%. Yet, subsequent azo dye metabolite bioconversion was not observed, along the 315-day run. Moreover, switching from a starch-based substrate to acetate in the feed composition deteriorated AGS stability. Overall, the plug-flow fed SBR recovered more rapidly from the imposed disturbances. Further research is needed towards guaranteeing long-term AGS stability during the treatment of textile wastewater.

Key words | aerobic granular sludge, azo dye biodegradation, stability, textile wastewater

R. D. G. Franca
J. Ortigueira
H. M. Pinheiro
N. D. Lourenço (corresponding author)
iBB–Institute for Bioengineering and Biosciences,
Department of Bioengineering,
Instituto Superior Técnico,
ULisboa, Av. Rovisco Pais,
1049-001 Lisboa,
Portugal
E-mail: nidia.lourenco@tecnico.ulisboa.pt

INTRODUCTION

High organic loads, recalcitrant dyes and potentially toxic azo dye breakdown products have long been considered the top environmental issues associated with textile industry wastewater (O'Neill *et al.* 1999). The need for efficient and environmentally friendly dye removal processes led to the study of biological wastewater treatment, as an alternative to physical and/or chemical processes, which are expensive and create secondary pollution problems (dos Santos *et al.* 2007). The ability of bacteria to decolorize azo dyes (the dominant textile dye type) via anaerobic reduction, and to aerobically mineralize some of the resulting aromatic amines, motivated the application of anaerobic–aerobic sequencing batch reactors (SBRs) to treat textile wastewater (Sarayu & Sandhya 2012). Nevertheless, despite the success reported for the anaerobic decolorization stage (Lourenço *et al.* 2000), data on the fate of the aromatic amines during the aerobic stage, when available, revealed that most were not degraded (Van der Zee & Villaverde 2005). In this context, the novel aerobic granular sludge (AGS) (Beun *et al.* 1999; Pronk *et al.* 2015b), known for its high settleability, its resistance to toxic compounds and its potential for the co-existence of aerobic/anoxic microenvironments within

granules, has been recently applied in studies with simulated textile wastewater (Muda *et al.* 2010; Franca *et al.* 2015).

Despite the advances in AGS technology, long-term stability of aerobic granules (AG), a key factor for stable operation of AGS SBRs, is still a challenge (Lee *et al.* 2010; Zhang *et al.* 2016). A low microbial growth rate inside AG has been described as an important factor to keep AG stability (de Kreuk & van Loosdrecht 2004). Regarding the SBR hydrodynamic and aeration regimens, anaerobic plug-flow feeding followed by aerobic reaction has been associated with improved structural stability of AGS in tubular SBRs being attributed to the selection of heterotrophic microorganisms able to anaerobically convert easily biodegradable substrates to slowly biodegradable storage polymers, thus exhibiting lower growth rates in the aerobic phase (de Kreuk & van Loosdrecht 2004; Pronk *et al.* 2015a). On the other hand, when comparing with the use of an anaerobic plug-flow feeding, a stirred anaerobic phase following a fast feeding period has been suggested to improve anaerobic substrate uptake prior to the aerobic stage in small height-to-diameter ratio (H/D) reactors (non-tubular), and consequently enhance AGS stability (Rocktäschel *et al.* 2013).

The reaction stage of both SBRs used in the present work (small H/D) included an anaerobic stirred phase followed by an aerobic stage, the difference between the reactors lying in the feeding strategy. Specifically, this study assessed the effect of two feeding regimens – an anaerobic plug-flow feeding through the sludge bed and a static anaerobic feeding – on the stability of AGS in non-tubular SBRs treating a synthetic textile wastewater, namely when subjected to changes in feed composition, with a focus on azo dye biodegradation and chemical oxygen demand (COD) removal.

MATERIAL AND METHODS

Carbon source and azo dye stock solutions

Sodium acetate (analytical grade, AppliChem GmbH, Germany) and/or a starch-based sizing agent used in the cotton textile industry, Emsize E1 (Emsland-Starke GmbH, Germany), were used as carbon source. The latter's stock solution (100 g L⁻¹) was prepared by hydrolyzing Emsize E1 in alkaline conditions (Lourenço *et al.* 2000). The azo dye stock solution (5.0 g L⁻¹) was prepared by dissolving Acid Red 14 (AR14, Chromotrope FB, Sigma-Aldrich, 50% dye content) in distilled water.

Synthetic wastewater composition

The synthetic textile wastewater used as feed solution was prepared by diluting the carbon source stock solution with a nutrients and salts solution, to a COD content of 1,000 mg O₂ L⁻¹ (1.15 g L⁻¹ Emsize E1). When acetate was used as carbon source, it was directly dissolved in this

solution, to this same overall COD level. This solution contained pH buffering phosphates and nutrients to the following concentrations: 2,310 mg L⁻¹ Na₂HPO₄·12H₂O, 762 mg L⁻¹ KH₂PO₄, 143 mg L⁻¹ NH₄Cl, 22.5 mg L⁻¹ MgSO₄·7H₂O, 27.5 mg L⁻¹ CaCl₂, 250 µg L⁻¹ FeCl₃·6H₂O, 40 µg L⁻¹ MnSO₄·4H₂O, 57 µg L⁻¹ H₃BO₃, 43 µg L⁻¹ ZnSO₄·7H₂O, 35 µg L⁻¹ (NH₄)₆Mo₇O₂₄·4H₂O. All salts were analytical grade. In this feed solution the COD:N:P mass ratio was 100:3.7:37. Dye was added from the stock solution to a concentration of 40 mg AR14 L⁻¹.

SBR setup and operation

AGS from previously operated laboratory-scale SBRs was used to inoculate two 1.5-L SBRs (H/D = 2.5) after a 2.5-month storage period. The new reactors were operated for 315 days, comprising six experimental periods (A–F), characterized in Table 1. Filling was performed from the bottom of the reactors (Figure 1): as a plug-flow regimen in SBR2, allowing the feed to spread through the sludge bed; or at a peripheral site at the bottom of SBR1, the feed rapidly channeling across the settled biomass with no relevant contact between them. Both SBRs were operated in 6-h cycles including a 30-min static anaerobic feeding in SBR1 (no distribution of the feed through the sludge bed) and a 50-min static plug-flow feeding, through the sludge bed, in SBR2 (changed to 1.3-h in SBR2 from day 180 on), followed by a 1.5-h stirred anaerobic phase (changed to 1-h in SBR2 from day 180 on), 3.5-h aeration, 5-min settling, 1-min drain and idle. Mechanical mixing was provided by an anchor-like impeller at 70 rpm and by a 4-blade axial impeller at 280 rpm in SBR1 and SBR2, respectively, and aeration was supplied by air compressors via porous bottom diffusers (Figure 1).

Table 1 | Summary of the operational conditions for SBR1 (static anaerobic feeding) and SBR2 (plug-flow feeding) along the experimental periods, regarding feed composition and anaerobic phase duration in SBR2 (E1 – Emsize; Ac – Acetate; AR14 – Acid Red 14)

Period	Days	Feed composition (SBR1 and SBR2)		SBR2 anaerobic phase		F/M (gCOD gVSS ⁻¹ d ⁻¹)	
		Substrate (mg O ₂ L ⁻¹)	AR14 (mg L ⁻¹)	Filling (min)	Stirring (min)	SBR1	SBR2
A	0–157	E1 - 1000	40	50	90	0.4	0.3
B	158–164	E1 - 1000	120			0.5	0.4
C	165–172	E1 - 3000	120			1.2	0.9
A'	173–179	E1 - 1000	40			0.6	0.5
D	180–255	E1 - 1000	40	80	60	0.4	0.5
E	256–291	50% E1 + 50% Ac - 1000	40			1.1	0.5
F	292–315	Ac - 1000	40			1.8	1.0

The indicated food-to-microorganism ratio (F/M) was calculated for each period, taking into account the respective feed COD and average VSS.

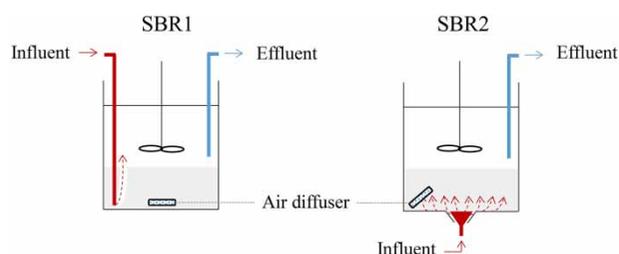


Figure 1 | Schematic representation of the SBRs used in this study employing two different feeding strategies: SBR1 had an anaerobic static feeding, followed by a stirred anaerobic phase and an aerobic stage; SBR2 had an anaerobic plug-flow feeding stage through the sludge bed, followed by a stirred anaerobic phase and an aerobic stage. The dashed arrows represent the feed flow pattern in each SBR.

The feed was supplied to the SBRs with a hydraulic retention time of 12 h. Three-fold increases in dye and Emsize (the latter together with NH_4Cl) concentrations in the SBR feed, to $120 \text{ mg AR14 L}^{-1}$ and to an organic loading rate (OLR) of $6.0 \text{ kg COD m}^{-3} \text{ d}^{-1}$, were applied on days 158–164 (period B) and 165–172 (period C), respectively (Table 1). Following a transitional period, when the original operating conditions were restored (days 173–179, period A), acetate was introduced in the feed, the carbon source being changed from 100% to 50% Emsize (days 256–291, period E), and finally to 100% acetate (days 292–314, period F). In fact, starches (such as Emsize E1) and acetic acid (acetate as conjugate base) are among the major pollutants found in textile wastewater contributing for the high organic load characteristic of these effluents, arising from desizing, scouring, washing and dyeing processes (Delée *et al.* 1998).

Analytical methods

Total suspended solids (TSS), volatile suspended solids (VSS), sludge volume index after 5 and 10 min settling (SVI_5 and SVI_{30} , respectively), COD and pH were determined through standard procedures (APHA 1995). Biomass morphological analysis employed a transmission light microscope (BA200, Motic) with a digital camera and respective software (Moticam 2, Motic). Staining of intracellular lipids was performed with Nile Blue according to Ostle & Holt (1982), visualized through fluorescence microscopy (BA410 microscope with episcopic fluorescence attachment EF-UPR-III and TRITC (Rhodamine)/Dil/Cy3 filter set, Motic).

Color removal was followed by reading the absorbance of clarified (centrifuged) samples at 515 nm (AR14 maximal absorbance wavelength in the visible region) in a UV-visible

spectrophotometer (Specord 200, Analytik Jena) against distilled water. Dye concentration in terms of color equivalents (dye-color) was determined from a calibration curve obtained with standard AR14 solutions. Dye degradation and metabolite formation were followed by reversed-phase high performance liquid chromatography (HPLC) (Franca *et al.* 2015).

RESULTS AND DISCUSSION

Biomass inventory and AGS properties

Inoculation with a 2.5-month stored AGS led to different biomass profiles in the two SBRs, with distinct settling characteristics. SBR1 presented a peak in the SVI_5 and SVI_{30} values on day 48 (177 and 98 mL gTSS^{-1} , respectively), subsequently stabilizing along period A at higher levels than those registered in SBR2 (Table 2). Although SVI was more stable in the latter during period A, the shock loads applied in periods B–C caused a sharp increase in the SBR2 SVI values, high levels being maintained throughout the subsequent period D (Table 2). As a result of this settleability shift, TSS levels decreased from 7 to 5 gTSS L^{-1} in SBR2 after period C, in contrast with SBR1, which maintained an average value of 5 gTSS L^{-1} throughout periods A–D (Table 2). Accordingly, while the sludge retention time (SRT) levels in SBR1 were mostly stable until the end of period D (9 days, on average), in SBR2, the initial average sludge age of 18 days deteriorated to 6 days after the shock loads were applied (Table 2). Subsequently, upon introduction of acetate as 50% and 100% of the carbon source load in the feed (periods E and F, respectively), biomass settleability was significantly reduced in both reactors (Table 2), reaching maximal SVI_5 and SVI_{30} values of 592 and 282 mL gTSS^{-1} in SBR1, and 400 and 255 mL gTSS^{-1} in SBR2, respectively, on day 299. This substrate switch led to an almost complete biomass washout, resulting in biomass concentrations around 1.5 gTSS L^{-1} in both SBRs. Yet, after adaptation to the new carbon source, SBR2 resumed biomass accumulation along period F, with SVI_5 and SVI_{30} levels gradually decreasing to final values of 225 and $123 \text{ mL gTSS L}^{-1}$, respectively.

Overall, the average and standard deviation values for the TSS and SVI in each experimental period (Table 2) showed that for both SBR1 and SBR2, periods A, B and C presented similar results in terms of biomass properties. In contrast, significant differences were observed in periods D, E and F for the two reactors.

Table 2 | Summary of average and standard deviation values registered for the TSS, sludge volume index after 5 and 30 min settling (SVI₅ and SVI₃₀, respectively) and SRT in each experimental period for the two SBRs

Period	SBR1			SBR2				
	TSS (g L ⁻¹)	SVI ₅ (mL gTSS ⁻¹)	SVI ₃₀	TSS (g L ⁻¹)	SVI ₅ (mL gTSS ⁻¹)	SVI ₃₀	SRT (days)	
A	4.8 ± 1.0	119 ± 20	72 ± 10	9 ± 6	7.4 ± 1.4	85 ± 11	51 ± 7	18 ± 10
B	4.8 ± 0.0	111 ± 5	72 ± 1	7 ± 1	6.3 ± 0.9	110 ± 11	64 ± 7	13 ± 3
C	5.8 ± 0.2	99 ± 8	65 ± 6	7 ± 1	7.3 ± 0.2	91 ± 2	57 ± 1	6 ± 1
D	3.9 ± 0.0	103 ± 13	64 ± 9	9 ± 6	4.6 ± 0.0	138 ± 15	85 ± 9	6 ± 4
E	5.1 ± 0.7	357 ± 123	237 ± 81	2 ± 0	4.9 ± 0.5	180 ± 46	112 ± 29	6 ± 4
F	2.0 ± 0.9	502 ± 105	310 ± 53	2 ± 1	4.4 ± 1.1	284 ± 71	179 ± 51	9 ± 6

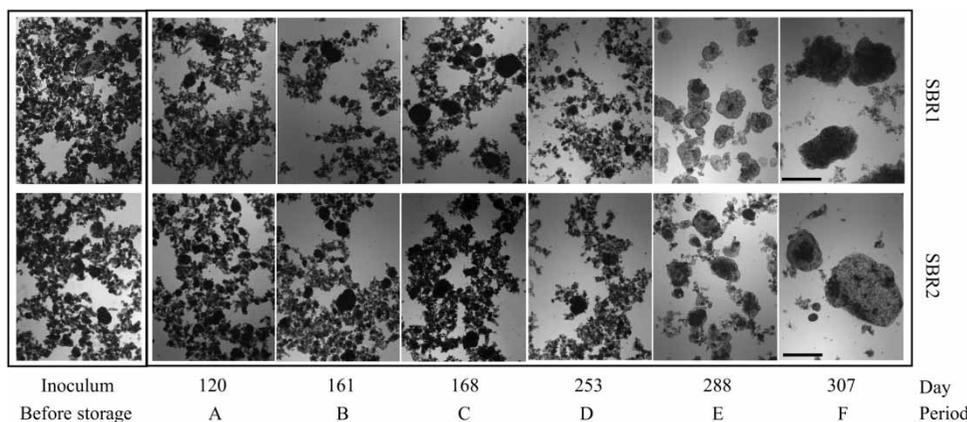
The indicated SRT corresponds to the average of the SRT values calculated for each week (weekly SRT) along the respective period.

The morphology of the AGS before the 2.5-month storage period was comparable for SBR1 and SBR2, as shown in Figure 1. Similarly, throughout this experimental run, both SBRs presented a mixture of AG, flocs and dispersed biomass, SBR2 retaining the highest fraction of AG in period A (Figure 2). The increase in carbon load (period C) induced AG size growth (to diameters <600 µm). However, in accordance with the SVI results, the shock loads apparently had a negative effect in AG integrity, as later granules gradually became smaller and less abundant (period D, Figure 2). In period E, fed with the mixed carbon source, AG morphology was significantly altered. Larger granules (diameters up to 600 µm), with a well-defined, round outline were formed (Figure 2), their morphology suggesting low density, and high EPS content. The change to acetate as sole carbon source (period F) caused AG to further increase in size (to diameters >1 mm). Moreover, the substrate change

caused a marked decrease in the flocculent sludge fraction in both reactors, as well as in the abundance of protozoa, such as rotifers, which were no longer observed in periods E–F.

The food-to-microorganism ratio (F/M) values in SBR1 and SBR2 (Table 1) follow the same tendency along periods A–D. In periods A and B, the F/M values were within the range of 0.3–0.5 gCOD gVSS⁻¹ d⁻¹. Higher values were achieved in period C (1.2 and 0.9 gCOD gVSS⁻¹ d⁻¹ in SBR1 and SBR2, respectively), when a 3-fold higher organic load was applied, subsequently decreasing upon reestablishment of the initial feed conditions (period D: 0.4 and 0.5 gCOD gVSS⁻¹ d⁻¹ in SBR1 and SBR2, respectively). Accordingly, the biomass morphology evolved similarly in the two reactors along these periods (Figure 2).

Upon introduction of acetate as carbon source (periods E and F), significant differences were observed in the biomass morphology in the two reactors (Figure 2). In fact,

**Figure 2** | Micrographs (magnification 40) from biomass samples harvested from SBR1 and SBR2 on the indicated periods (see Table 1) and respective operational days. Scale bar = 600 µm.

the F/M increased (Table 1) more significantly in SBR1 (periods E and F: 1.1 and 1.8 gCOD gVSS⁻¹ d⁻¹, respectively) than in SBR2 (periods E and F: 0.5 and 1.0 gCOD gVSS⁻¹ d⁻¹, respectively). Similar to the apparent lower density of the granules microscopically observed, these F/M values denote the deterioration in the sludge settleability, especially in SBR1.

The low average SRT levels observed (Table 2) were mainly caused by erratic, intense biomass washout episodes in the SBRs. This likely indicated the dominance of heterotrophic fast-growing organisms, which have been associated with AG instability (Pronk *et al.* 2015a). High microbial growth rates are a consequence of high residual COD levels at the start of the aeration phase (de Kreuk & van Loosdrecht 2004). Filamentous bacteria are usually associated to these operational conditions, but none were observed during this study. This might result from the starvation stage at the end of the aerobic phase, since the feast-famine regimen has been shown to prevent filamentous outgrowth in AG (de Kreuk & van Loosdrecht 2004). In addition, the shear stress caused by mechanical stirring might also have impaired the development of filamentous organisms.

Color and carbon load removal performance

High color removal yields (around 80%) were attained under anaerobic conditions in both SBRs, with no further decolorization during the aerated phase. The overall COD removal was 80%, of which up to 70% and 80% were removed anaerobically in SBR1 and SBR2, respectively. The plug-flow feeding stage of SBR2 contributed minimally for this (Figure 3(a)). Upon the dye concentration shock, the decolorization rate increased (Figure 3(b)), denoting first-order kinetics, and was further improved to 90% removal yield when the shock step in OLR was applied (Figure 3(c)). In period C, overall COD removal decreased to 68%, SBR2 removing a higher fraction anaerobically (30% versus 5% in SBR1). In addition, the 3-fold increase in feed COD (period C) caused a marked acidification of the medium during the anaerobic phase (Figure 3(c)), as compared with the typical pH profile (Figure 3(a)). This suggested a higher volatile fatty acids (VFA) production upon fermentation of the hydrolyzed starch-based substrate during the anaerobic period, and subsequent VFA consumption in the first 30 min of aeration. Upon re-establishment of the initial feed conditions, 80% COD removal was resumed in both reactors, but decolorization yields decreased to 74% and 66% in SBR1 and SBR2, respectively. Nevertheless, high color and COD removal efficiencies were resumed in

SBR2 after adaptation to the shorter stirred anaerobic phase (Table 1, Figure 3(d)), which aimed to reduce energy consumption in SBR2.

The change to 50% acetate in the feed did not affect decolorization, and even improved overall COD removal, mostly aerobically (Figure 3(e)). Upon complete replacement of Emsize with acetate, color removal dropped to 16% in SBR1 (days 299–307), being subsequently restored to regular levels from day 309 on. In contrast, color removal in SBR2 responded positively to acetate, namely during the plug-flow feeding period, reaching an overall yield of 91% (Figure 3(f)). In fact, the color profile representative of period F suggests that the anaerobic plug-flow feeding through the sludge bed can be advantageous in terms of decolorization performance when acetate is present in the wastewater as the main carbon source. In addition, the introduction of acetate caused a pH shift to higher values (Figure 3(e) and 3(f)). In fact, the pH level was stable throughout the anaerobic phase, denoting the presence of a non-fermentable substrate, the latter being rapidly consumed upon the start of aeration, causing an equally fast pH increase.

The combination of substrate diffusion and microbial consumption rates is known to determine the substrate gradient inside a biofilm and, consequently, its stability (de Kreuk *et al.* 2010). Specifically, a plug-flow hydrodynamic regimen rising through the settled bed, with high substrate concentration allows soluble, easily biodegradable substrates to penetrate the entire AG depth and be converted to storage polymers (Pronk *et al.* 2015a). Consequently, easily biodegradable COD will not be available in the subsequent SBR aerobic phase and slow microbial growth can occur down to the core of the AG, ensuring a stable AG structure. In contrast, in an anaerobic fast feeding-stirred strategy the biomass contacts with a lower substrate concentration, as a result of its dilution with the remaining supernatant from the previous SBR cycle. Thus, in this situation, substrate gradients are likely to occur in the AG, which may lead to AG instability (de Kreuk *et al.* 2010).

In addition to the feeding regimen, the reactor geometric configuration should be taken into account. In fact, Rocktäschel *et al.* (2013) noted that in reactors with a small H/D, the plug-flow feeding may develop heterogeneous flow patterns through the granular bed, leading to short contact times between the substrate and the biomass and thus to incomplete anaerobic carbon uptake during this stage. Accordingly, low COD removal occurred during the plug-flow anaerobic feeding of SBR2, with most of the anaerobic COD removal being achieved during the subsequent stirred anaerobic phase, where better mass

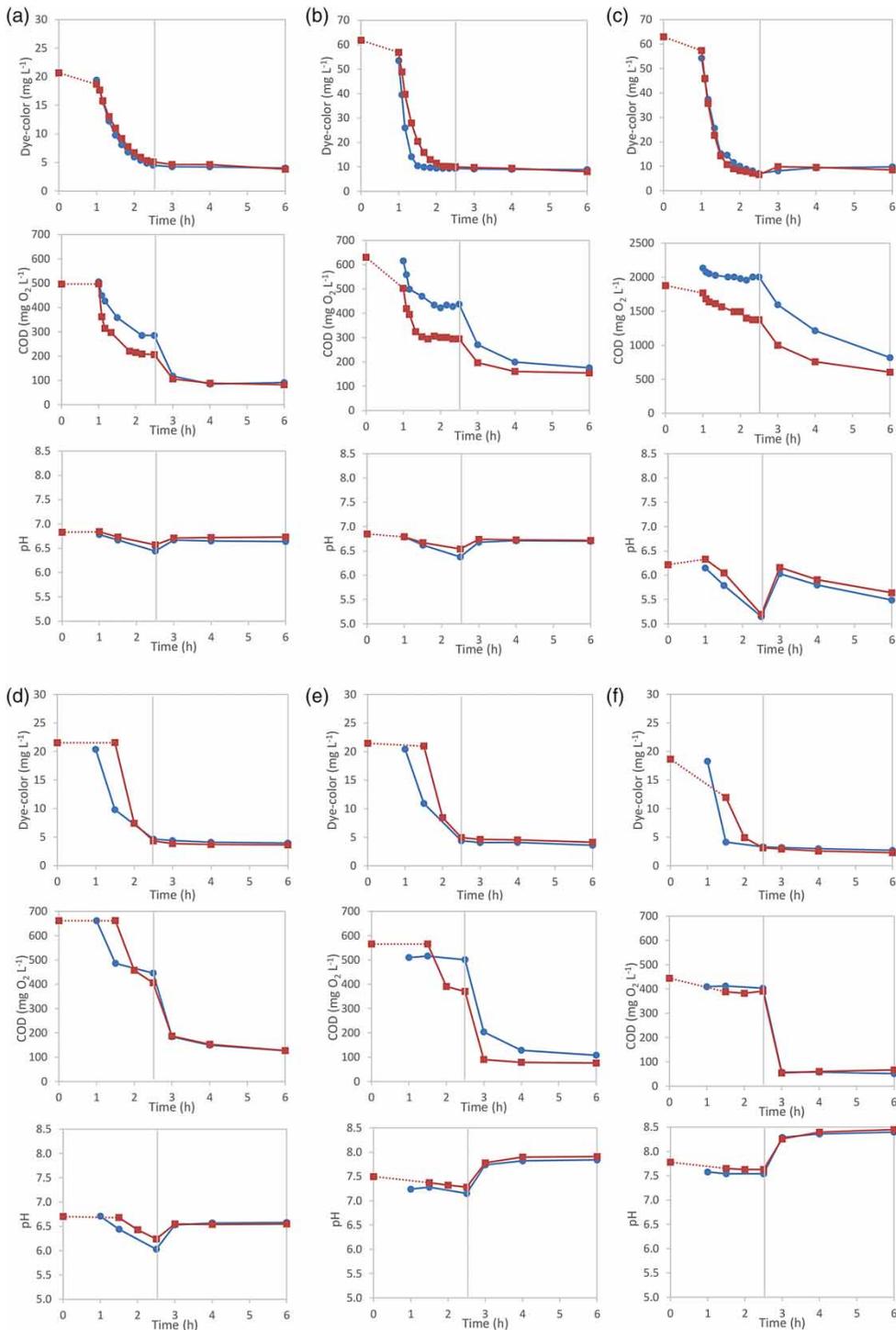


Figure 3 | Color, COD and pH profiles along the reaction phase in SBR1 (●) and SBR2 (■) cycles representative of periods A–F (Table 1). The dotted line depicts the plug-flow feeding period.

transfer conditions were provided. Owing to a lower microbial growth rate in the aerobic phase, complete COD removal under anaerobic conditions has been shown to increase AG density and structural stability (Pronk *et al.* 2015a). In fact, despite the negligible COD removal during

the plug-flow feeding phase of SBR2, the subsequent stirred anaerobic phase led to faster anaerobic COD removal than in SBR1. This might explain the lower average SVI values in SBR2, from period D on (Table 2). Yet, complete anaerobic COD removal was never achieved in the SBRs, and this

situation worsened when the carbon source was changed from hydrolyzed hydroxypropyl starch (fermentable substrate) to acetate (VFA).

In spite of the limited acetate uptake during the anaerobic phase (Figure 3(f)), results from Nile Blue staining of biomass samples taken along one treatment cycle, during period F, suggest that AGS in both SBRs accumulated intracellular lipid aggregates during the anaerobic phase (Figure 4). These storage compounds were apparently partly consumed during the aerobic period in SBR1 (Figure 4). Therefore, AGS in SBR1 was possibly under carbon starvation at the end of the reaction phase, since the biodegradable COD was exhausted from the mixed liquor 30 min after the start of aeration (Figure 3(f)). This was not observed in SBR2.

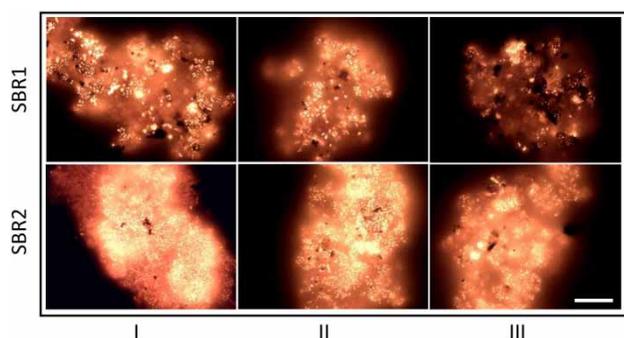


Figure 4 | Fluorescence micrographs (magnification 1,000, excitation wavelength 540 nm) of Nile Blue-stained biomass samples harvested from SBR1 and SBR2 on day 313 (period F; 100% acetate) at the end of the anaerobic phase (I), 30 min after the aeration onset (II) and at the end of the aerobic stage (III). Scale bar = 20 μm .

Azo dye biodegradation and metabolite formation

HPLC analysis confirmed that the azo dye AR14 was anaerobically reduced (Figure 5(a)). Two aromatic amines result: 4-amino-naphthalene-1-sulfonic acid (4A1NS) and 1-naphthol-2-amino-4-sulfonic acid (1N2A4S). Only the former was detected by HPLC, as 1N2A4S probably undergoes autoxidation reactions (Kudlich *et al.* 1999). The 4A1NS profiles show that this amine was not aerobically biodegraded (Figure 5), which might be a consequence of the low SRT (Table 2). In fact, 4A1NS has been noted as recalcitrant and the few studies reporting its biodegradation (Koupaie *et al.* 2013; Franca *et al.* 2015) used strong biomass retention strategies, i.e. biofilms and AGS SBRs under high SRT values, producing more diverse microbial communities.

CONCLUSIONS

The effects of the feeding strategy and feed composition on the stability of AGS in the treatment of textile wastewater were assessed in two non-tubular SBRs, a statically fed, anaerobic–aerobic SBR (SBR1), and an anaerobic plug-flow fed, anaerobic–aerobic SBR (SBR2), the latter allowing the biomass to contact with the feed during the fill stage. Overall, high color and COD removal levels were attained in both SBRs. However, dye and COD shock loads had a negative effect on AG integrity and further aerobic bioconversion of the amine metabolite was not observed. COD uptake was

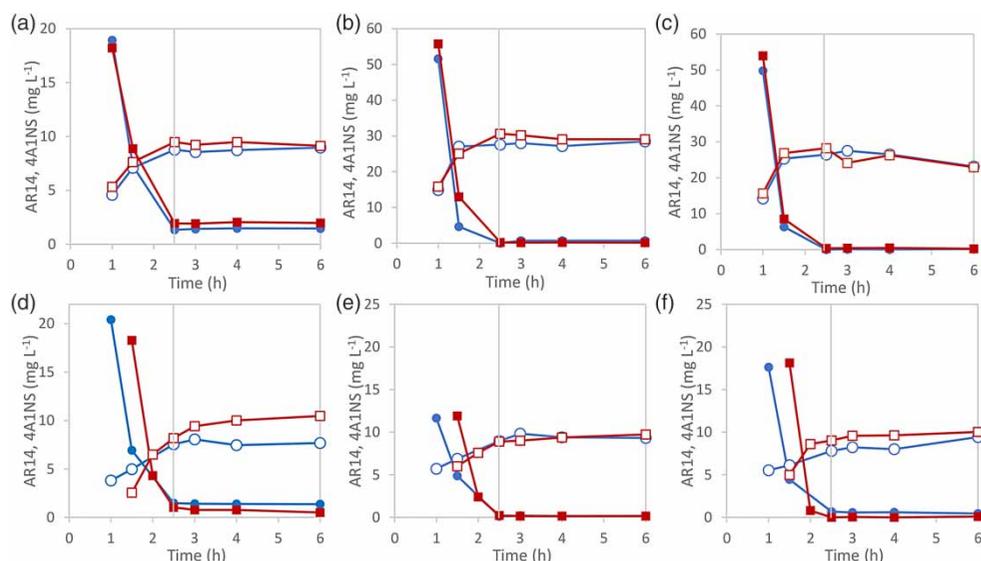


Figure 5 | Concentration profiles of AR14 (filled markers) and 4A1NS (open markers) along the reaction phase in SBR1 (● and ○) and in SBR2 (■ and □), registered in periods A–F (Table 1).

minimal in the plug-flow feeding stage of SBR2, but the fraction of COD removed anaerobically was generally higher than in SBR1. The change from the hydrolyzed starch-based to the acetate-based feed resulted in AGS settleability deterioration in both SBRs. After adaptation, lower SVIs were attained in SBR2 and decolorization yields during its plug-flow feeding period improved, revealing a higher capacity of SBR2 to deal with substrate-related variations.

ACKNOWLEDGEMENTS

This work was financed by Fundação para a Ciência e a Tecnologia (FCT, Portugal), projects PTDC/EBB-EBI/120624/2010, PTDC/AAG-TEC/4501/2014 and UID/BIO/04565/2013. Funding from Programa Operacional Regional de Lisboa 2020 (Project No. 007317) is also acknowledged. R. D. G. Franca and N. D. Lourenço acknowledge FCT for doctoral (SFRH/BD/95415/2013) and post-doctoral (SFRH/BPD/88095/2012) research grants, respectively.

REFERENCES

- APHA – American Public Health Association 1995 *Standard Methods for the Examination of Water and Wastewater*. Eaton A. D., Clesceri L. S. & Greenberg A. E. (eds), 19th edn. American Public Health Association, American Water Works, Water Environment Federation, Washington, DC, USA.
- Beun, J. J., Hendriks, A., Van Loosdrecht, M. C. M., Morgenroth, E., Wilderer, P. A. & Heijnen, J. J. 1999 [Aerobic granulation in a sequencing batch reactor](#). *Water Res.* **33** (10), 2283.
- de Kreuk, M. K. & van Loosdrecht, M. C. M. 2004 Selection of slow growing organisms as a means for improving aerobic granular sludge stability. *Water Sci. Technol.* **49**, 9.
- de Kreuk, M. K., Kishida, N., Tsuneda, S. & van Loosdrecht, M. C. M. 2010 [Behavior of polymeric substrates in an aerobic granular sludge system](#). *Water Res.* **44**, 5929.
- Delée, W., O'Neill, C., Hawkes, F. R. & Pinheiro, H. M. 1998 [Anaerobic treatment of textile effluents: a review](#). *J. Chem. Technol. Biotechnol.* **73** (4), 323.
- dos Santos, A. B., Cervantes, F. J. & van Lier, J. B. 2007 [Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology](#). *Bioresour. Technol.* **98**, 2369.
- Franca, R. D. G., Vieira, A., Mata, A. M. T., Carvalho, G. S., Pinheiro, H. M. & Lourenço, N. D. 2015 [Effect of an azo dye on the performance of an aerobic granular sludge sequencing batch reactor treating a simulated textile wastewater](#). *Water Res.* **85**, 327.
- Koupaie, E. H., Moghaddam, M. R. A. & Hashemi, S. H. 2013 [Evaluation of integrated anaerobic/aerobic fixed-bed sequencing batch biofilm reactor for decolorization and biodegradation of azo dye Acid Red 18: comparison of using two types of packing media](#). *Bioresour. Technol.* **127**, 415.
- Kudlich, M., Hetheridge, M. J., Knackmuss, H. J. & Stolz, A. 1999 [Autoxidation reactions of different aromatic o-aminohydroxynaphthalenes that are formed during the anaerobic reduction of sulfonated azo dyes](#). *Environ. Sci. Technol.* **33**, 896.
- Lee, D.-J., Chen, Y.-Y., Show, K.-Y., Whiteley, C. G. & Tay, J.-H. 2010 [Advances in aerobic granule formation and granule stability in the course of storage and reactor operation](#). *Biotechnol. Adv.* **28**, 919.
- Lourenço, N. D., Novais, J. M. & Pinheiro, H. M. 2000 [Reactive textile dye color removal in a sequencing batch reactor](#). *Water Sci. Technol.* **42** (5–6), 321.
- Muda, K., Aris, A., Salim, M. R., Ibrahim, Z., Yahya, A., Van Loosdrecht, M. C., Ahmad, A. & Nawahwi, M. Z. 2010 [Development of granular sludge for textile wastewater treatment](#). *Water Res.* **44**, 4341.
- O'Neill, C., Hawkes, F. R., Hawkes, D. L., Lourenço, N. D., Pinheiro, H. M. & Delée, W. 1999 [Colour in textile effluents – sources, measurement, discharge consents and simulation: a review](#). *J. Chem. Technol. Biotechnol.* **74**, 1009.
- Ostle, A. G. & Holt, J. G. 1982 Nile blue A as a fluorescent stain for poly-beta-hydroxybutyrate. *Appl. Environ. Microbiol.* **44**, 238.
- Pronk, M., Abbas, B., Al-zuhairy, S. H. K., Kraan, R., Kleerebezem, R. & van Loosdrecht, M. C. M. 2015a [Effect and behaviour of different substrates in relation to the formation of aerobic granular sludge](#). *Appl. Microbiol. Biotechnol.* **99**, 5257.
- Pronk, M., de Kreuk, M. K., de Bruin, B., Kamminga, P., Kleerebezem, R. & van Loosdrecht, M. C. M. 2015b [Full scale performance of the aerobic granular sludge process for sewage treatment](#). *Water Res.* **84**, 207.
- Rocktäschel, T., Klarmann, C., Helmreich, B., Ochoa, J., Boisson, P., Sørensen, K. H. & Horn, H. 2013 [Comparison of two different anaerobic feeding strategies to establish a stable aerobic granulated sludge bed](#). *Water Res.* **47**, 6423.
- Sarayu, K. & Sandhya, S. 2012 [Current technologies for biological treatment of textile wastewater – a review](#). *Appl. Biochem. Biotechnol.* **167** (3), 645.
- Van der Zee, F. P. & Villaverde, S. 2005 [Combined anaerobic-aerobic treatment of azo dyes – A short review of bioreactor studies](#). *Water Res.* **39**, 1425.
- Zhang, Q., Hu, J. & Lee, D.-J. 2016 [Aerobic granular processes: current research trends](#). *Bioresour. Technol.* **210**, 1.

First received 10 February 2017; accepted in revised form 5 May 2017. Available online 20 May 2017