Effect of Sequencing Batch Cycle Strategy on the Treatment of a Simulated Textile Wastewater with Aerobic Granular Sludge

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Abstract

Aerobic granular sludge was successfully developed on a simulated textile effluent with an added azo dye, on a non-tubular sequential batch reactor (SBR), with better granulation results than in a dye-free control SBR fed with the same base medium. The overall performance of the treatment was very good, with high color and chemical oxygen demand (COD) removal yields (up to 85% and 80%, respectively). Operation under two sequencing batch cycle strategies, i.e., with a single anaerobic/aerobic reaction phase or under intermittent aeration, showed that the latter generally improved the treatment performance. Under intermittent aeration, biomass accumulation was induced, with faster settling aggregates, leading to a higher COD removal yield. Although a lower decolorization rate was observed in the first 30 minutes of anaerobic reaction, as compared to the single phase strategy, the overall color removal remained high. This work demonstrates the applicability of the new aerobic granular sludge technology for the treatment of textile effluents.

Keywords

Wastewater Treatment; Biodegradation; Aggregation; Bioreactors; Sequential Batch Reactor; Azo Dye

List of Abbreviations

2A1N4S - 2-Amino-1-naphthol-4-sulphonate

4A1NS - 4-Amino-1-naphthalene sulphonate

AR14 – Azo dye Acid Red 14

COD - Chemical oxygen demand

$E_{Ag/AgCl}$ - Oxidation-reduction potential value against the silver/silver chloride standard

H/D - Height-to-diameter ratio of the working bioreactor volume

HPLC - High performance liquid chromatography

MLVSS - Mixed liquor volatile suspended solids

ORP - Oxidation-reduction potential

OUR - Oxygen uptake rate

RSD - Relative standard deviation

SBR - Sequential batch reactor

SBR1 - Sequential batch reactor operated without dye

SBR2 - Sequential batch reactor operated with dye

SOUR - Specific oxygen uptake rate

SVI - Sludge volume index; measured after a 5-min (SVI_5) and a 30-min (SVI_{30}) settling time

TSS - Total suspended solids

VSS - Volatile suspended solids

1. Introduction

The modern textile industry has been swift to respond to the increased demand for textile products worldwide, and its wastewater load has been increasing proportionally [1]. This situation has sometimes led to the degradation of the quality of receiving water bodies, limiting their use for several purposes [2]. This industrial sector generates large quantities of wastewater, e.g., in Europe, a water consumption of 50-240 m³ per ton of textile product has been estimated [3]. These effluents are usually heavily loaded with organic carbon and color compounds, the latter being azo dyes in an estimated 70% of the cases [4]. The treatment of textile effluents poses a major challenge in environmental protection, namely in what regards cost and quality of the final effluent. Physicochemical treatments are available but costly, and in spite of notable achievements lately reported on the application of bio-treatments to textile wastewaters [1], the latter are not yet efficient enough notably in what regards the elimination of synthetic colorants.

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4 Biological systems combining anaerobic and aerobic phases have been proposed for the treatment
5 of textile effluents, with dye reduction occurring in the anaerobic phase, resulting in
6 decolorization, and further organic load removal being obtained in the subsequent aerobic phase,
7 with possible oxidation of dye reduction metabolites [5-7]. Sequential batch reactors (SBR) have
8 been often reported as good options for this purpose [5, 7] due to their operational flexibility and
9 simplicity, and compact layout [5]. The SBR configuration in activated sludge treatment systems
10 is characterized by cyclic, fill-an-draw operation of a single tank, dispensing the use of dedicated
11 settlers. The standard cycle comprises five steps: fill (influent feed, static or with agitation and/or
12 aeration), react (with agitation and/or aeration), settle (sedimentation of the biomass/clarification
13 of the treated liquor), draw (clarified effluent discharge) and idle [8]. Excess biomass can be
14 withdrawn during the react or draw phases. This system allows the manipulation of reaction and
15 settling times in each cycle, in response to variations in input organic loads and biomass
16 settleability. This feature is particularly useful for wastewaters coming from batchwise industrial
17 processes, such as textile dyeing, which often exhibit wide variations in flow rate and
18 composition [5, 6]. SBR operation also easily allows the implementation of the two-step
19 anaerobic-aerobic reaction sequence required for reductive decolorization and organic load
20 oxidation in dye-containing textile wastewaters [6, 7].

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35 Aerobic granular sludge is a new and promising technology for the treatment of industrial
36 wastewaters [9]. Aerobic granules are self-aggregated microorganism clusters with outstanding
37 settling properties, when compared with conventional activated sludge flocs. In the present state-
38 of-the-art they are obtained and maintained only in SBR [10, 11] reducing to a few minutes the
39 settling time in these single-tank systems [12]. Very high biomass concentrations can be achieved
40 in the bioreactor, resulting in high organic load treatment capacities and very compact systems
41 [12]. Aerobic and anoxic/anaerobic zones can coexist inside the same granule, in response to the
42 adjustment of aerated and non-aerated periods in the SBR cycle. This provides a unique system
43 for the removal of organic load and nutrients since the major biological reactions can occur
44 simultaneously even throughout the aerated phase, namely, the accumulation of polyphosphates
45 and nitrification in the outer layers and denitrification in the inner granule [10, 13, 14]. Other
46 advantages of aerobic granules are their robustness in the presence of toxic compounds [15] and
47 the rapid recovery of biological activity and maintenance of granule integrity even after extended
48 storage periods [16-19]. These are especially important in industrial wastewater treatment

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4 systems, since toxic compounds can be present and wastewater production often faces
5 interruptions for several days or weeks, according to the manufacturing schedules. Due to these
6 exceptional characteristics, the application of the aerobic granular sludge technology to the
7 treatment of industrial effluents has recently raised attention among researchers [15, 20].
8 Nevertheless, studies on the application of this technology to the treatment of textile wastewaters
9 are still incipient [21].

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15 In most of the reported studies, the tubular configuration has been used in laboratory SBR for
16 aerobic granulation. This configuration is characterized by high height-to-diameter ratios (H/D),
17 in the range of 14 to 30 [13, 22-25], but this geometry is likely to pose implementation
18 difficulties in full-size systems.

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22 In the present study, a non-tubular SBR, with a H/D value of 2.5, was tested. To the authors' best
23 knowledge this is the lowest H/D value reported for aerobic granulation in laboratory conditions,
24 and it was chosen with the purpose of easing a subsequent scale up to industrial system sizes.
25 Thus, in this work, the possibility of aerobic granule development was assessed, using a
26 flocculent activated sludge inoculum fed with a simulated textile wastewater. Treatment
27 performance was evaluated under two cycle strategies, namely, a two-phase anaerobic-aerobic
28 cycle and an intermittent aeration regimen throughout the cycle.

29 30 31 32 33 34 35 36 37 38 **2. Materials and Methods**

39 40 41 *2.1. Simulated textile effluent feed solutions*

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45 A carbon source stock solution was prepared with a starch-derived sizing agent used in the textile
46 industry, Emsize E1 (hydroxypropyl-starch, Emsland-Starke GmbH, Germany) as the carbon
47 source. This substrate was pre-hydrolyzed in alkaline conditions (NaOH) as described previously
48 [5], followed by pH adjustment to 7.0 (HCl) and dilution with deionised water to 100 g.L⁻¹.

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52 A base feed solution was prepared in deionized water using the carbon source stock solution
53 diluted to a chemical oxygen demand (COD) content of 1000 mg O₂.L⁻¹, and supplemented with
54 pH buffering phosphates and nutrients to the following concentrations: KH₂PO₄ (760 mg.L⁻¹),
55 Na₂HPO₄.12H₂O (2310 mg.L⁻¹), NH₄Cl (140 mg.L⁻¹), MgSO₄.7H₂O (23 mg.L⁻¹), CaCl₂ (28
56 mg.L⁻¹), FeCl₃.6H₂O (250 µg.L⁻¹), MnSO₄.4H₂O (40 µg.L⁻¹), H₃BO₃ (57 µg.L⁻¹), ZnSO₄.7H₂O
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(43 $\mu\text{g.L}^{-1}$), and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}.4\text{H}_2\text{O}$ (35 $\mu\text{g.L}^{-1}$). All salts were analytical grade. This solution exhibits a COD:N:P mass ratio of 100:3.7:30. This was the only feed to bioreactor SBR1, which was used as a dye-free control.

An azo dye stock solution was also prepared. The tested azo dye was Acid Red 14 (AR14, Chromotrope FB, Sigma-Aldrich, 50% dye content), used without further purification. It was dissolved in deionised water to a concentration of 3.0 g.L^{-1} .

The synthetic textile effluent (dye-containing) feed was obtained by adding 10 mL of the dye stock solution after 750 mL of the base feed solution, at each cycle, corresponding to an initial, added dye concentration of 20 mg.L^{-1} in the bioreactor. The dye stock solution was added from the top directly into bioreactor SBR2, at the end of the fill phase of the operational cycle.

2.2. Non tubular sequencing batch reactor (SBR) configuration

The experiments were conducted in two non tubular SBR, SBR1 and SBR2, each with a working volume of 1.5 L, operated with a volumetric exchange ratio of 50%, the settled effluent being withdrawn at half-height of the reactor's content, and at a hydraulic retention time value of 12 h. Each reactor had a working height of 25 cm and an inner diameter of 10 cm, the height-to-diameter ratio (H/D) value being 2.5. For aeration an air compressor model SPP 15 (Highblow, Japan) was used with a porous membrane diffuser placed at the bottom of each reactor. The air flow rate was approximately 3.0 L.min^{-1} for each reactor, giving an aeration rate of 2 v.v.m. The SBR were operated under mechanical stirring during the entire reaction periods, both aerated and non-aerated. The magnetic stirrer used was a bioMIXdrive 1 model with the bioMIXcontrol MS4 control unit run at 200 r.p.m. (2mag, Germany). The base feed solution was inserted at the bottom of the reactor, at the level of the air diffuser, using a separate peristaltic pump for each reactor (Mini-S 660, Ismatec, Switzerland), and the rapid discharge of the settled supernatant was achieved using a single gear pump for the two reactors (Reglo-Z, Ismatec, Switzerland). A separate peristaltic pump was used to feed the dye stock solution to SBR2. The pumping, aeration and agitation functions were automatically controlled, via an interface, by a dedicated program installed on a personal computer.

2.3. Experimental strategy

The two SBR were operated under the same operational conditions, except for the presence of dye in the feed. In this way, the effect of the dye could be assessed with two independent experiments in all conditions imposed throughout the study. The duration of the operational cycle was kept at 6 hours, totaling 4 cycles per day. Each cycle comprised the following phases (Table 1): fill, during which fresh feed was pumped into the reactor, over the settled biomass leftover from the previous cycle, without mechanical mixing; reaction, during which mechanical mixing was applied, both without and with aeration; settling, without mechanical mixing for biomass sedimentation; discharge, during which half of the operational volume was rapidly removed from the clarified supernatant; and idle, a quiescent period to complete the cycle time. Thus, the reactors' liquid volume was renewed twice each day (cycle exchange ratio: 50%). The organic loading rate fed to the reactors was kept at $2.0 \text{ kgCOD.m}^{-3}.\text{d}^{-1}$ in all periods.

The experimental strategy included two objectives: 1) following the process of converting the biomass' initial floc morphology to granule morphology (granulation), in the presence and absence of the dye, using an operational cycle which included a prolonged anaerobic phase; 2) testing the system's performance and biomass morphology stability when subjected to changes in the operational cycle.

Within the first objective, all parameters were kept constant except for a progressive reduction in the time allowed for biomass settling before the onset of the supernatant discharge phase (period Ia). At the start of this period the two reactors were seeded with activated sludge flocs (2.7 gTSS.L^{-1}) harvested at a full-scale wastewater treatment plant (Chelas, Lisbon) and operated during the first 4 days with a settling time of 40 min, for adaptation of the biomass to the synthetic feed. Subsequently, the settling time was shortened to 10 min (days 5-12), 5 min (days 13-28), and 4 min (days 29-33) to induce granulation [10, 22, 26-29]. Except for the first, all step reductions of the settling time were introduced as soon as the total suspended solids (TSS) level in the effluent was reduced to 100 mg.L^{-1} or less.

Within the second objective, the duration of the anaerobic phase was first extended (days 34-52), to induce further carbon source storage by the biomass possibly favoring the stability of the granule morphology (period Ib). After this, the biomass was collected and used to re-inoculate the reactors under the final operational conditions of period Ia. After a few cycles, an alternative

operational strategy was imposed (period II), replacing the reaction phase composed by a single anaerobic followed by a single aerobic stage with an intermittent aeration regime, starting with an anaerobic stage, totaling the same 3-to-2 h anaerobic-to-aerobic duration ratio of period Ia. At the same time, the settling time was further reduced from 4 to 3 minutes. This final regimen was operated for 23 days.

The operational conditions employed in each experimental period are summarized in Table 1. Throughout the two periods, the reactors were monitored with respect to biomass inventory, morphology and activity (oxygen uptake rate), pollutant conversion (dye and COD), and chemical parameters (oxidation-reduction potential, pH) indirectly related to biological activity. Each imposed operational conditions were kept for at least 4 days, completing at least 8 liquid renovations in the bioreactors.

Table 1. Operational cycle phase durations for the two SBR during the successive experimental periods.

SBR cycle phase duration	Period I		Period II
	Ia	Ib	
Operational time (days)	1 to 33	34 to 52	1 to 23
Fill (no mixing)	15 min	15 min	15 min
Anaerobic phase (with mixing)	3 h	3.5 h	Intermittent aeration: 6 × (30 min anaerobic + 20 min aerated)
Aerobic phase (with mixing and aeration)	2 h	2 h	
Settling	40 to 4 min	4 min	3 min
Discharge	1 min	1 min	1 min
Idle	4 to 40 min	10 min	41 min
Reaction time / Total cycle time	5 h / 6 h	5.5 h / 6 h	5 h / 6 h

2.4 Analytical Methods

Bioreactor parameters measured in situ were oxidation-reduction potential (ORP), dissolved oxygen and temperature. All other parameter values were measured on samples collected from the reactors' liquid contents. For COD, dye concentration, pH and high performance liquid chromatography (HPLC) samples were centrifuged for 10 min at 4000 rpm, and measurements

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4 were performed on the supernatant. Soluble COD was determined following a standard procedure
5 (APHA, 1998). Each sample was measured in duplicate and mean values are presented (relative
6 standard deviation, RSD: average 1.4%, maximum 6.0%). The contribution of the azo dye to the
7 COD level was measured in a deionized water solution at 20 mg/L and found to be negligible,
8 below the detection limit of the method ($\leq 10 \text{ mgO}_2\text{.L}^{-1}$).
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13 Total suspended solids (TSS) and volatile suspended solids (VSS) were measured on samples
14 from the mixed liquor and from the settled effluent, using standard methods (APHA, 1998). Each
15 sample was measured in duplicate and mean values are presented (RSD: average 2.5%, maximum
16 21%).
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21 Sludge volume index (SVI) was determined by measuring the volume occupied by the sludge
22 settled from 1 L of mixed liquor, after 5 (SVI₅) and 30 (SVI₃₀) minutes of settling, and dividing it
23 by the mixed liquor TSS value. This measurement was carried out offline in an Imhoff cone, and
24 the mixed liquor sample was promptly returned to the reactor.
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29 The concentration of azo dye was determined spectrophotometrically, by reading the absorbance
30 of samples at 515 nm (the wavelength of maximum absorbance in the visible range) using a
31 Specord 200 spectrophotometer (Analytik Jena, Germany). Each sample was measured in
32 duplicate and mean values are presented (RSD: average 0,2%, maximum 2,4%).
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37 HPLC analyses were performed using a Merck-Hitachi (Germany) system comprising a L-6200A
38 Intelligent Pump, a L-4250 UV-Vis Detector and a L-7200 Autosampler with a D7000 Interface
39 for computer control, fitted with a reversed phase RP-18, LiChroCART 250-4 column (Merck,
40 Germany), with spectrophotometric detection at 220 nm. The mobile phase, fed at 0.7 mL/min,
41 was composed of sodium phosphate buffer (25 mM, pH=5.5) and acetonitrile, run on a 30-min
42 linear gradient from 100:0 to 50:50 (v/v), followed by a 5-min linear gradient up to 15:85 (v/v)
43 and ending with a step back to 100:0, kept for a further 5-min period. Standard solutions of the
44 dye Acid Red 14 and of the amine 4-amino-1-naphthalene sulphonate (Sigma, USA) in distilled
45 water were run for their respective peak identification and concentration calibration against peak
46 area. Each sample was measured in duplicate and mean values are presented (RSD: average
47 0.1%, maximum 0.9%)
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56 Biomass samples from both reactors were observed at magnifications of 40× and 100×, under a
57 transmission light microscope (BA200, Motic, China) fitted with a digital camera and respective
58 software (Moticam 1000, Motic, China).
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The value of pH was measured on centrifuged samples using a Metrohm 6.0202.100 glass electrode connected to a Metrohm 691 potentiometer (Switzerland).

Oxidation-Reduction Potential (ORP) was measured in situ, using a SenTix ORP electrode (platinum with silver/silver chloride as reference electrode, 3M KCl) (WTW, Germany) connected to a digital pH meter (pH 538, WTW, Germany). Measured values are presented as read. Conversion to values relative to the standard hydrogen electrode (E_h) can be done by adding +207 mV to the readings.

Dissolved oxygen was measured in situ, using a LDO101 Standard Luminescent Dissolved Oxygen Probe (Hach, USA) connected to a multi parameter meter HQ40d (Hach, USA) fitted with a temperature probe. Oxygen uptake rate (OUR) measurements were taken during the aerated reaction phase, by briefly (up to 5 min) interrupting aeration and registering the evolution of dissolved oxygen values in time. For this, the probe was immersed in the mixed liquor, at its surface, to a depth of approximately 3 cm.

3. Results and Discussion

3.1 Biomass concentration, settling properties and morphology

Figure 1 shows the evolution of the TSS values in the mixed liquor. During the first part of period Ia biomass concentration levels were maintained in SBR1 (no dye) and increased in SBR2 (with dye) in spite of the consecutive shortening of the settling period with consequent biomass washout. This strategy of settling time reduction to induce granulation was successful, with SVI_{30} in SBR2 (with dye) dropping sharply from an initial value of 325 to 67 $mL.g^{-1}$ on day 27 (Fig.2, Ia). This result indicated an increase in aggregate cohesion [30] with a correspondent alteration in biomass morphology. Microscopic examination (Fig. 3) showed that granules could be observed from day 21 on, in both reactors, with well defined, round or oval shapes, reaching 0.5 mm in diameter on day 30 in SBR2. Granules showed much less defined outlines in SBR1, where they became enveloped in a diffuse coating of exopolymeric material (filamentous bacteria were apparently not involved) towards the end of period Ia. This observation in SBR1 followed the reduction of the settling time from 5 to 4 min which also resulted in a sharp increase in SVI still in period Ia. This granule deterioration trend was aggravated in period Ib with the extension of

the anaerobic phase as seen in figures 2 and 3. Mass balances to the TSS in the reactors gave average sludge age values of 5 days for SBR1 and 12 days for SBR2 in period I, reinforcing that granulation proceeded better in SBR2. In period Ib granules kept their size and shape definition in SBR2, as opposed to those of SBR1, and their abundance even increased. Some granules were observed which were of a much lighter color than the rest, which probably were composed essentially of exopolymeric material.

The period taken for granulation to be achieved in this study is similar to the ranges reported in the literature [14, 31] for anaerobic/aerobic granular systems treating urban wastewaters and synthetic effluents in tubular SBR. However, the granule size obtained, up to 0.5 mm, is lower than most of the literature values. This could have been partly due to the non-tubular geometry of the reactors, leading to a lower selective pressure for granule development, since granules obtained in a sequential batch air-lift bioreactor with a H/D value of 3 showed also a small size of 0.78 mm [32]. The use of mechanical stirring could have also contributed to the granules' reduced size through shear stress [13]. It was previously reported that granules obtained in a SBR with a H/D value of 5.5, operated under the same stirring rate value of the present study (200 rpm), though initially larger in size evolved to a uniform size range of 0.6 to 0.8 mm [33].

In period II, the intermittent aeration regimen apparently reduced the accumulation of the diffuse exopolymeric material in SBR1, resulting in denser clusters (Fig. 3, II), but at same time some filamentous growth was revealed. Nevertheless, the SVI_{30} values improved, becoming similar to those obtained in period Ia before day 30. The average sludge age value was 6 days. In this period, SBR2 also performed better, the intermittent aeration leading to biomass accumulation above 5.0 gTSS.L^{-1} , and faster biomass settling with SVI_{30} values reaching their best (55 mL.g^{-1}). Still, full granulation could not be achieved, since the SVI_5 and SVI_{30} did not attain comparable values, a distinctive characteristic of granular biomass [34]. Dense aggregates were observed (Fig.3, II) of a size similar to that of granules in period I, but with a different, less regular shape. The average sludge age rose in SBR2 up to 15 days, denoting an improvement in system stability. In both reactors, the settled effluent TSS levels were generally around 200 mg.L^{-1} .

In both reactors, the overall better performance in period II, as compared to period Ib, could have been due to the intermittent exposure of the granules to increased shear from the air flow, as opposed to a 3.5-hour long initial period without this effect (the anaerobic reaction phase). Shear has been reported to favor aerobic granulation [10, 11, 27, 35, 36]. The biomass concentration

levels and SVI_{30} values are in the same range of those obtained by other authors treating a textile wastewater with aerobic granules in a tubular SBR with a H/D value of 12.5 [21]. This result is remarkable, and proves the feasibility of a non-tubular SBR for aerobic granulation on a synthetic dye containing feed.

3.2 Carbon source transformation, oxygen uptake and pH changes

Representative examples of the profile of residual COD along the SBR cycle reaction time are shown in figure 4. During period I the sharpest drop in COD levels consistently occurred following the onset of aeration. COD removal yields in the anaerobic phase were consistently higher in SBR2 (up to 30%) than in SBR1 (less than 20%), with overall removal yields around 70%. A high removal yield of organic substrate during the anaerobic phase, preferably 50% or more [37], has been shown to be determinant for efficient granulation. This effect possibly contributed to a better granulation process in SBR2. In period II, the cumulative COD removal yield during the anaerobic periods improved slightly, to 23%, in SBR1 and reached 30% in SBR2, with the overall COD removal yield also increasing in both SBR, reaching 80%. These overall COD removal levels were comparable to those observed for the same carbon source in a two-phase, 24-hour cycle SBR with flocculent activated sludge [38]. In Figure 4 it can be seen that the residual COD reaches the low concentration levels much sooner in period II than in period I, for both SBR. Several authors consider that exposure of the biomass to a feast/famine regimen promotes aerobic granulation [26, 35, 37, 39-42]. A prolonged famine stage, in particular, is important for granule stability [24] and it should take up about 2/3 of the cycle time [36]. This effect is also a possible explanation for the better granulation obtained in period II, with the apparent consumption of the **diffuse** exopolymeric material, the lowest SVI values of the experiment and the highest biomass accumulation.

Oxygen uptake rate (OUR) was measured at the start and end of the aeration phases during period I, and at the start of the 1st and end of the 6th aeration phases during period II. Average values of the specific OUR readings (SOUR), the OUR divided by the mixed liquor VSS value (MLVSS), are presented in Table 2. Initial SOUR values for period Ia (68-69 $\text{mgO}_2\cdot\text{gMLVSS}^{-1}\cdot\text{h}^{-1}$) matched the values reported by other authors for aerobic granular sludge, namely values of 69 and 56 $\text{mgO}_2\cdot\text{gMLVSS}^{-1}\cdot\text{h}^{-1}$ for glucose and acetate feeds, respectively [28], and of 51 $\text{mgO}_2\cdot\text{gMLVSS}^{-1}\cdot\text{h}^{-1}$

$^1.h^{-1}$ in an aerobic granular system treating a textile effluent [21]. In period Ib significant differences were noted in SBR1, with much higher SOUR values, but not in SBR2, which showed values similar to those from previous period Ia. Indeed, in period Ib SBR1 had a consistently different behavior both at the start and end of aeration, and this could have been related to the presence of the exopolymeric substances in the liquid. In Period II, under intermittent aeration, both reactors showed the same trend of lower SOUR values, roughly 60% of those measured in Period Ia at the start of aeration. The SOUR was also lower at the end of the reaction cycle for SBR2, roughly half of that of Period I, but this was not observed in SBR1, which maintained the SOUR final levels of period Ia.

Table 2. Representative values of the specific oxygen uptake rate (SOUR) measured in SBR1 (no dye) and SBR2 (with dye), along the experimental periods. Average values of 2 or 3 measurements are given (except for period Ib), with standard deviation values in brackets.

Operational periods		SOUR (mgO ₂ .gMLVSS ⁻¹ .h ⁻¹)			
		SBR1 (no dye)		SBR2 (with dye)	
		Aeration start	Aeration end	Aeration start	Aeration end
Period I	Ia	68 (10)	16 (1)	69 (5)	15 (3)
	Ib	116 (-)	26 (-)	73 (-)	15 (-)
Period II		40 (1)	16 (2)	45 (6)	8 (1)

As exemplified in Figure 5, a consistent acidification profile was detected along the anaerobic phase, which was, also consistently, reversed very quickly upon the onset of aeration. This happened in spite of the buffering effect of the phosphate salts in the medium. Differences between SBR1 and SBR2 were not very significant, typically as shown in Figure 5. This result is probably due to the production and subsequent consumption of free volatile fatty acids, indicating the ready fermentation of the carbohydrate carbon source by the biomass. Thus, reducing equivalents required for dye decolorization are apparently readily available.

3.3 Dye decolorization

Color removal yields measured spectrophotometrically in SBR2 for the overall cycle remained stable in the 80-85% range along the entire experimental run, with a good yield even on the first day of operation, and best by a small margin in period II under intermittent aeration, as it can be seen in Figure 6. These anaerobic biodecolorization yields are in accordance with those of previous reports for the same dye, Acid Red 14 [43-45].

Also, color removal occurred almost solely in the anaerobic reaction phase. During period II it was clear that the onset of aeration after the first 30 min of anaerobic reaction interrupts decolorization, the latter being resumed only in the following anaerobic phase (Figs 6 and 7a). Moreover, in period I most of the final color removal yield was achieved during the first 30 min of anaerobic reaction, but in the intermittent aeration regime (period II), color removal yields in the first 30-min anaerobic phase dropped to about half, the yield values of period I being attained only after the second anaerobic phase.

Nevertheless, these results together with those discussed in sections 3.1 and 3.2, indicate that in the two phase regimen of period I, the anaerobic phase could best be much shortened. Decolorization yields could probably be maintaining in the same range, prolonging the aerobic phase, which would increase both the exposure to shear stress and the famine period, possibly improving the granulation and COD removal performance of both reactors.

It should be noted that color readings using spectrophotometry at 515 nm do not entirely reflect residual dye concentration, since some color development (brownish) was noted after the initial reduction. This is probably due to reactions involving the dye reduction metabolites, namely the unstable *ortho* 2-amino-1-naphthol-4-sulphonate (2A1N4S). Aromatic amines have been shown to undergo auto-oxidation in the presence of oxygen with the resulting products forming colored oligomeric structures [46-48]. Examples of HPLC chromatograms and analytical results are presented in Figure 7 and indicate that less than 1% of the initial dye content was present at the end of the 3-hour anaerobic phase in period I, and at the end of the overall reaction phase in period II, in contrast with the circa 80% color removal yields measured spectrophotometrically on aliquots from the same samples. HPLC results (Fig. 7a) confirm the azo bond cleavage stoichiometry, with the dye reduction forming the corresponding amount of the stable amine 4-amino-1-naphthalene sulphonate (4A1NS), also indicating that this metabolite was not

biologically degraded in the aerobic phase. Several authors have reported the difficulty of aerobic biodegradation of sulphonated aromatic amines [6, 49, 50] . In more specific studies, the aerobic removal of the amine 4A1NS was not observed [51], but it was found to be neither toxic [51] nor mutagenic [52, 53].

The results obtained indicate that the SBR system with aerobic granular sludge achieves comparable or better performances, in what regards dye decolorization and COD removal, when compared with flocculent activated sludge SBR systems reported previously [5, 7]. The present option furthermore has the advantage of achieving these performances in short cycles of merely 6 hours (3-h anaerobic/2-h aerobic), as opposed to the 24-h SBR cycles operated with flocculent sludge (12- to 20-h anaerobic/4- to 12-h aerobic) [5, 7]. The present system is therefore preliminarily validated for textile wastewater treatment, raising the possibility for exploitation of the full potential of aerobic granular sludge features. These include different, simultaneous biological reactions in the inner and outer layers of larger granules, high biomass concentrations, and operation at high sludge age values. The latter allow the development of slow-growing populations enlarging the biodegradation capabilities and reduce the production of waste biosludge [13].

3.4 Oxidation-reduction potential profiles

Typical ORP monitoring cycles in the two SBR are presented in Figure 8. Despite the consistent differences observed in the ORP profiles from the two SBR, the measurements of dissolved oxygen concentration in the mixed liquors gave similar results, i.e., consistently under 0.05 mg O₂.L⁻¹ during the anaerobic phase and around 7 mg O₂.L⁻¹ during the aerated phase. Interesting to note is the shoulder in the ORP profile after the onset of aeration, more pronounced in SBR2, suggesting the presence of accumulated reduced substances produced in anaerobic phase being readily oxidized. The subsequent depletion of these reduced substances would account for the second rise in ORP (end of the shoulder). This shoulder pattern is also found in the results of Muda et al. [21] for dissolved oxygen concentration profiles in an anaerobic/aerobic granular sludge SBR treating a synthetic wastewater containing a defined dye mixture. The absence of this shoulder, in the profiles of both reactors during the 1st aeration stage of period II could possibly explain the lower SOUR values measured.

SBR1 displayed a slower ORP reduction during the anaerobic phase than SBR2, the latter starting this phase with ORP readings already around -300 mV (coming from the static fill phase). On the other hand, SBR2 readings tended to be stable in the -300 to -350 mV range in the anaerobic phase regardless of the operational regimen (Ia, Ib or II), while ORP values in SBR1 kept decreasing throughout the anaerobic phase in period I, and drifted upwards along the anaerobic stages of period II (Fig. 8). This result indicates that the reduced decolorization yield in the first anaerobic stage of period II (half of that obtained in period I after 30 min of anaerobiosis) cannot be explained by different (higher) ORP values. Thus, it is probably related to a biological response of the active biomass to the different environmental conditions created by the intermittent aeration regimen. Still regarding ORP, it is interesting to refer that a supernatant sample (without biomass) collected from SBR2 after the anaerobic reaction of one of the cycles in period I, gave similar ORP readings while still in the absence of aeration (-300mV) in a separate vessel, but when subjected to aeration increased its ORP value to $E_{Ag/AgCl} = 0$ mV. This is much higher than the value attained by the rest of the mixed liquor in the presence of biomass in the subsequent aerated phase and is close to the values attained in SBR1, indicating that the redox equilibrium in the presence of oxygen and dye reduction metabolites is biologically mediated. The involvement of the metabolites from the reduction of AR14 in reversible redox processes was also reported by Liu et al. [43]. It is conceivable that the better granulation obtained in SBR2, with higher COD removal yields in the anaerobic phase, is related to the dye acting as an electron acceptor and allowing for increased metabolic energy yields, even if the redox equivalents involved are in small amounts. The reversible redox action of the produced metabolites could enhance this process, possibility through the shuttling of electrons with the consequent increase in reaction rates.

4. Conclusions

Aerobic granular sludge was successfully developed on a simulated textile effluent in the presence of an azo dye, with better overall granulation results than in its absence, as observed through the operation of the dye-free control SBR1 on the same base feed. The overall performance of the treatment was very good, with high color and COD removal yields.

In the first experimental period, an operational strategy was tested using a single reaction phase per batch cycle, composed of an extended anaerobic phase followed by an aerated phase. Results indicate that this strategy can probably be improved by using a shorter anaerobic phase, keeping color removal at high yields and possibly promoting COD removal and biomass granulation.

The intermittent aeration cycle strategy subsequently tested led to marked performance improvements in the two SBR, inducing the formation of dense, faster settling aggregates. The biomass from SBR1, which had accumulated diffuse exopolymeric materials during the first experimental period, deteriorating its settling properties, was able to recover from this condition. In SBR2, fed with dye, a significant biomass accumulation was achieved. The intermittent aeration regime gave also very interesting treatment performance results, improving the overall removal of COD and maintaining or slightly increasing the color removal yields, in spite of a lower decolorization rate during the first 30 minutes of anaerobic reaction. The reasons for the latter result were not fully understood, but were apparently not related to either higher ORP or dissolved oxygen values.

This work demonstrates the applicability of the new aerobic granular sludge technology to the treatment of textile effluents in a laboratory scale SBR with a non-tubular configuration, which would possibly be easier to scale up for industrial scale operation.

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FIGURE 1

Fig. 1. – Total suspended solids measurements in the mixed liquor of (□) SBR1 (no dye) and (○) SBR2 (with dye) and in the discharged effluent of (■) SBR1 (no dye) and (●) SBR2 (with dye), along the experimental periods.

FIGURE 2

Fig. 2. – Sludge volume index values measured along the experimental periods I and II, namely, SVI₃₀ in (□) SBR1 (no dye) and (○) SBR2 (with dye), and SVI₅ in (■) SBR1 (no dye) and (●) SBR2 (with dye).

FIGURE 3

Fig. 3. Microscopy images from biomass samples taken from the activated sludge flocculent inoculum, and from the mixed liquor of the two SBR during period I (Ia - day 21, Ib - day 40) and period II (day 22).

FIGURE 4

Fig. 4. Representative mixed liquor dissolved COD profiles along the reaction phase of the operational cycle in SBR1 (no dye) and SBR2 (with dye), for each of the experimental periods, I and II. Period I(a): (■) day 1, (Δ) day 26; period I(b): (●, dashed line) day 42; Period II: day 9.

FIGURE 5

Fig. 5. Representative pH profiles along the reaction phase of the operational cycle in (●, ○) SBR1 (no dye) and (■, □) SBR2 (with dye), along experimental periods I and II. In period I: (□, ○) I(a); (■, ●) I(b).

FIGURE 6

Fig. 6. Representative azo dye concentration profiles (by spectrophotometry) along the reaction phase of the operational cycle in SBR2, for each of the experimental periods, I and II. Period I(a): (□) day 1, (△) day 26; period I(b): (●, dashed line) day 49; Period II: day 15.

FIGURE 7

Fig. 7. a) Representative concentration profiles (by HPLC) of (■, ●) azo dye AR14 and (□, ○) amine 4A1NS, along the reaction phase of SBR2 for each of the experimental periods: (■, □) period I; (●, ○) Period II. **b)** Examples of HPLC chromatograms of samples taken during the reaction phase of one of the cycles in period Ia (same scale on both chromatograms).

FIGURE 8

Fig. 8. Representative ORP profiles along the reaction phase of the operational cycle in SBR1 (no dye) and SBR2 (with dye), for each of the experimental periods, I and II. Period I: I(a), straight line; I(b), dashed line.