



Sequential anaerobic-aerobic decolourization of a real textile wastewater in a two-phase partitioning bioreactor



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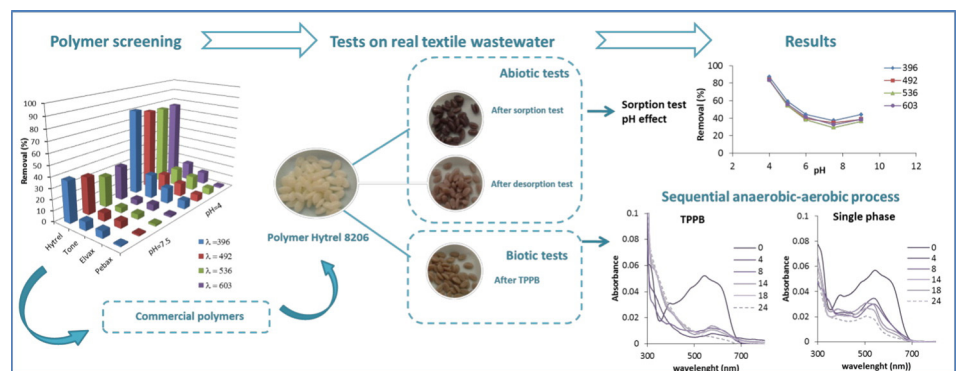
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HIGHLIGHTS

- A two-phase partitioning bioreactor is applied to treat real textile wastewater.
- DuPont polymer Hytrel 8206 is effective in colour absorption reaching removal of 84%.
- Low pHs favour the sorption process with equilibrium times ≤ 24 h.
- The polymer improves biological colour removal in the two-phase system.

GRAPHICAL ABSTRACT



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ABSTRACT

This work describes the application of a solid-liquid two-phase partitioning bioreactor (TPPB) for the removal of colour from a real textile wastewater containing reactive azo-dyes. Four polymers were tested over the pH range of 4–9 to select the most effective absorbant to be used as the partitioning phase in the TPPB. The best results were obtained with Hytrel 8206 at pH 4 achieving ~70% colour removal, based on the dominant wavelength, in the first 5 h of contact time, and 84% after 24 h. Wastewater treatment was undertaken in a solid-liquid TPPB operated with Hytrel 8206 in sequential anaerobic-aerobic configuration. The reaction time of 23 h was equally distributed between the anaerobic and aerobic phases and, to favour colour uptake, the pH was controlled at 4.5 in the first 4 h of the anaerobic phase, and then increased to 7.5. Colour removal (for the dominant wavelength, 536 nm) increased from 70 to 85% by modifying the bioreactor operation from single-phase to TPPB mode. Based on COD measurements nearly complete biodegradation of the intermediates produced in the anaerobic phase was obtained, both in the single-phase and two-phase mode, with better performance of the TPPB system reaching 75% COD_{Dye} removal.

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1. Introduction

The textile industry is one of the largest producers of aqueous industrial emissions since dyeing, and associated finishing operations,

generate among the largest volumes and pollution levels of discharged water (Sen and Demirel, 2003). Because dyes are designed to provide a high degree of chemical and photolytic stability to resist breakdown over time, microbial attack, and the action of water and soap, they are recalcitrant and resistant to biodegradation (Solis et al., 2012). Azo dyes are the most common synthetic dyes used in industry, and thus the most commonly released into the environment and are used in

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the textile industry, paper printing (Chang et al., 2004) and in plastics, leather, cosmetics and food industries (Telke et al., 2008). Release of untreated or poorly treated effluents containing azo dyes is harmful to the aquatic environment, causing inhibition of photosynthesis and growth of aquatic biota, reduction of dissolved oxygen, potential toxicity to humans, flora and fauna (Saratale et al., 2011), and also for aesthetic reasons as colour in wastewater can be detected even at very low levels (<1 mg/L) (Pereira and Alves, 2012).

Conventional wastewater treatment, such as activated sludge, can be ineffective for decontaminating dye effluents because of the physical-chemical stability and poor biodegradability of these pollutants, as noted above (Forgacs et al., 2004), but, it is worth noting that relative to chemical and/or physical processes, biological methods have the advantages of being cost-competitive and more environmentally friendly. Moreover, if operated with effective technologies, are often able to achieve complete mineralization of the pollutants at ambient conditions. Several research and review articles have focused on the biological decolorization of textile wastewater, especially on synthetic azo dye solutions summarizing the use of different microorganisms, reaction environments and technologies (Sponza and Isik, 2002; Pandey et al., 2007; Saratale et al., 2011; Solis et al., 2012).

The biodegradation of reactive azo dyes is difficult since their complex structure and synthetic nature often require a variety of bacteria, both aerobic and anaerobic, able to achieve the reductive cleavage of the —N=N— bond, resulting in the formation of generally colourless, but potentially hazardous, aromatic amine by-products. These aromatic intermediates could in principle be degraded aerobically or anaerobically (Joshi et al., 2004), however these metabolites can be more toxic than the parent dyes (Solis et al., 2012), and their biodegradation is generally more effective under aerobic conditions (van der Zee and Villaverde, 2005). This has led to the use of sequential anaerobic-aerobic processes aimed at more effective removal of azo dyes from wastewater, recently reviewed by Popli and Patel (2015), who have considered biological anaerobic-aerobic decolorization of azo dyes for various types of reactors, under different operating conditions. They concluded that the anaerobic stage of the sequential process can be negatively affected by the high initial concentration and complex structure of dyes, and confirmed the possibility of achieving mineralization of the intermediates under aerobic conditions.

Here, we propose the application of two-phase partitioning bioreactors (TPPBs), which have been demonstrated to be advantageous for xenobiotic removal (Tomei et al., 2011b). Employment of a TPPB in this case can be effective in reducing the exposure of biomass to toxic substrate concentrations, with the additional feature, in contrast to the use of activated carbon proposed by Kuai et al. (1998) for the same purpose, of providing gradual substrate release and subsequent biodegradation in the aqueous phase. The uptake of toxic substrates by amorphous polymers in TPPB systems occurs via absorption rather than adsorption: i.e. when biodegradation occurs in the cell-containing aqueous phase the thermodynamic equilibrium of the system is restored by the release of the absorbed compound driven by the cellular metabolic demand. This feature maintains sub-inhibitory concentration levels in the liquid phase to allow the biomass to achieve high biodegradation rates of the compound, which is consumed, and not simply adsorbed. The mechanism of uptake-release also provides for polymer bioregeneration, and the consequent restoring of its sorption capacity, which is not exhausted. As a final distinction between absorptive amorphous polymers and adsorptive activated carbon, rational selection of such polymers allows the use of materials, which are specifically targeted to the substrate in question, unlike activated carbon which is generally non-selective to organic molecules.

To date, most studies and applications of this powerful technology were related to synthetic solutions, and in this study we have investigated for the first time the application of TPPB systems to a real wastewater. The main objective of this study was to evaluate colour removal for a sequential anaerobic-aerobic process operated in a conventional

single-phase mode and in a TPPB system for the treatment of an industrial effluent containing Remazol Black 5, Remazol Yellow RR and Remazol Brilliant Red 21. In addition, COD levels during the treatment steps were followed to have a cumulative estimate of the fate of the intermediates during the biodegradation process. Finally, according to Muda et al. (2011) and Ricco et al. (2004), on line measurement of the specific oxygen uptake rate (SOUR) was used to assess the toxicity of dyes/by-products on the aerobic biomass in the different contacting configurations.

2. Materials and methods

2.1. Textile wastewater

Textile wastewater was obtained from the dyeing bath of a factory located in the textile district of the Como area, in the North of Italy. The contaminant load is mainly comprised of a mixture of mono and di-azo reactive dyes (commercially named Remazol Black 5, Remazol Yellow RR and Remazol Brilliant Red 21), accounting for ~90% of the pollution load, and chemical additives used for industrial processing. Wastewater characterization is reported in Table 1.

2.2. Polymers

A first selection of the polymers has been performed on the basis of their previous applications in TPPBs and on their predicted affinity for the dyes under investigation (Bacon et al., 2014).

Four commercial polymers were tested in this study, and their source, properties and composition are shown in Table 2. Before use, the polymers were pre-treated in order to remove any impurities arising from the manufacturing process by adding a known mass of polymer (~100 g) to an equal volume of methanol (Fluka, Italy) in a flask and mixing it vigorously for at least 20 min. The polymers were then repeatedly washed with distilled water until a clean (i.e. non-turbid) wash water was obtained. Each step lasted 20 min and, generally, five washing steps were sufficient, at the end of which the polymers were dried by air exposure for several days.

2.3. Sorption and desorption tests

Sorption tests of the wastewater colour by polymers were performed in batch mode in flasks containing a fixed volume (in the range of 100–200 mL) of wastewater diluted 1:10 with tap water. Magnetic stirrers provided the mixing, and the temperature was controlled at 25 ± 0.5 °C. The duration of the tests varied in the range of 24–72 h, i.e. a time estimated to be sufficient to reach equilibrium. Liquid samples were taken from the flasks at time intervals of 1 h, during the first 8 h, then after 24 h and then at subsequent intervals of 24 h. The pH was regularly monitored and adjusted by acid/base addition in order to maintain the desired pH values, which were pH 4 and 7.5 at a fixed polymer/solution ratio of 5% (v/v).

Table 1
Textile wastewater characteristics: average values and standard deviation (SD).

| Parameter | Unit | Average value | SD |
|-------------------|------|---------------|-------|
| pH | – | 9 | 0.50 |
| COD | mg/L | 1117 | 58.00 |
| TSS | g/L | 0.54 | 0.10 |
| VSS | g/L | 0.23 | 0.05 |
| TC | mg/L | 471.1 | 35.2 |
| TOC | mg/L | 158.0 | 9.80 |
| N-NH ₄ | mg/L | 40.0 | 4.50 |
| Chlorides | g/L | 38.6 | 3.10 |
| Nitrate | g/L | 3.8 | 0.35 |
| Phosphates | g/L | 3.2 | 0.33 |
| Sulphates | g/L | 4.5 | 0.38 |

Table 2
Suppliers, properties and compositions of commercial polymers used in this study.

| Commercial name | Grade | Supplier | Density (g/cm ³) | Size (mm) | Tg ^a (°C) | Composition |
|-----------------|-------|----------|------------------------------|----------------------|----------------------|--|
| Hytrel | 8206 | DuPont | 1.170 | 1.5–5 ^b | -59 | poly(ether- <i>block</i> -butylene terephthalate) |
| Tone | P787 | Dow | 1.145 | 4 ^c | -69 | poly(caprolactone) |
| Elvax | 770 | DuPont | 0.928 | 3.5–5.5 ^b | -100 | poly(ethylene- <i>co</i> -vinyl acetate) |
| Pebax | 2533 | Arkema | 1.010 | 3–5 ^b | -65 | poly(tetramethylene ether- <i>block</i> -amide 12) |

^a Glass Transition Temperature.^b Minimum – maximum dimension.^c Diameter.

Hytrel showed the best results in the sorption tests so it was selected as the partitioning phase for the TPPB and its uptake-release capacity was more thoroughly investigated through sorption and desorption tests under different operating conditions according to the test plan shown in Table 3.

In the desorption tests the colour-loaded polymer, resulting from the sorption tests, was put in contact with fresh tap water under mixed conditions. The same experimental apparatus, sampling and analytical procedures of the sorption tests were utilized. Desorption tests were performed at pH = 7.5, which is the typical value of the biological processes.

Parallel control tests for sorption and desorption were performed under the same operating conditions with tap water and polymers, but without wastewater, to take into account the possible presence of released substances from the polymers, which could affect the measurements. The absorbance readings of the control samples were subtracted from the corresponding test sample readings.

Sorption tests have been also executed to verify that Hytrel does not absorb acetate. Initial concentration was 100 mg/L and applied modalities are the same above reported. The tests were conducted for 24 h in two operating conditions: at pH 7.5 and with the pH pattern applied in the bioreactor, i.e. pH 4.5 in the first 4 h and pH 7.5 for the subsequent 20 h.

2.4. Bioreactors: type and operation modes

Experiments were conducted in lab scale sequencing batch reactors (SBRs) (volume 0.5 and 1.2 L) operating at 27 ± 1 °C, mixed with a magnetic stirrer, and equipped with peristaltic pumps (Cellai) for feeding and discharge of the effluent. Additional information about timing and control devices and management are given elsewhere (Tomei et al., 2004).

Each SBR working cycle, consisting of fill (20 min), reaction (1360 min), settle (40 min) and draw (20 min) periods, lasted one day, with the reaction time being equally distributed between the anaerobic and aerobic periods during each 24 h cycle. The time distribution of the SBR was established on the basis of previous studies on xenobiotic removal in SBRs (Tomei and Daugulis, 2013; Mosca Angelucci and Tomei, 2015). Furthermore, no wastage of biomass was utilized during the entire experiment because due to the high reaction

Table 3
Experimental plan and operating conditions for sorption and desorption tests with Hytrel 8206.

| Test | Polymer/water ratio % (v/v) | Sorption | | Desorption | |
|------|-----------------------------|----------|----------|------------|----------|
| | | pH | time (h) | pH | time (h) |
| H1 | 2.5 | 7.5 | 72 | 7.5 | 96 |
| H2 | 5 | 7.5 | 72 | 7.5 | 96 |
| H3 | 5 | 4 | 72 | 7.5 | 96 |
| H4 | 10 | 7.5 | 72 | 7.5 | 96 |
| H5 | 10 | 4 | 24 | 7.5 | 96 |
| H6 | 10 | 5 | 24 | 7.5 | 96 |
| H7 | 10 | 6 | 24 | 7.5 | 96 |
| H8 | 10 | 9 | 72 | 7.5 | 96 |

cycle duration a minimal biomass increase was expected, as reported in Tomei et al. (2016), and the biomass wastage associated with the effluent discharge balanced the biomass growth. The reactor exchange ratio (added volume/total volume) was controlled at 0.3 in all tests in order to achieve data suitable for comparison. The feed contained the real textile wastewater and sodium acetate with a dye-acetate ratio of 1:20, in terms of COD, diluted with tap water to reach the desired load, and supplemented with a mineral medium (Williams and Unz, 1989) to ensure an adequate supply of nutrients for microbial growth. The sequential anaerobic-aerobic process was applied in three series of tests and a microbial consortium, previously acclimated to the same textile wastewater, was employed as inoculum (Tomei et al., 2016). In the first series (I) the bioreactor was operated in conventional (single-phase) mode, with the first part of the experiment dedicated to microbial acclimatization achieved by progressively increasing the dye loading (in the range of 0.005–0.075 kg_{COD}/(m³ d), followed by reactor operation for 45 days at a fixed feed of 0.01 kg_{COD}/(m³ d). The same load was applied in the second series (II), while in the third one (III) the dye load was increased to 0.013 kg_{COD}/(m³ d).

The three series of tests were performed at different pH conditions. The first one (I) was conducted at pH 7.5, while in the second (II) and the third (III) ones, the pH was controlled at 4.5 during the first 4 h of the anaerobic period, then increased to 7.5 to restore suitable conditions for microbial growth during the residual anaerobic phase (440 min) and the following aerobic period.

For the TPPB operation mode the polymer Hytrel 8206 was added to the bioreactor at a polymer/aqueous phase ratio of 10% (v/v) at the beginning of experimental phase III and kept in the system until the end of the experiment. This value has been chosen on the basis of previous studies on TPPBs demonstrating their effective performance with polymer/water ratio ≤ 10% (Amsden et al., 2003; Tomei et al., 2009; Tomei et al., 2011b).

Treatment performance was evaluated by daily analysis of the colour content and COD in the influent and the effluent, and kinetic tests were carried out in the three different operating modes described above. Table 4 shows an overview of the operating conditions for the three series of tests.

2.5. Kinetic tests

Colour removal in the three experimental series of tests was characterized by kinetic tests carried out in the bioreactors under the operating conditions reported in Table 4. Kinetic tests allow to follow the colour pattern during the subsequent biological phases. Tests were performed during the reaction phase of the SBR working cycles, by taking samples from the bioreactors at time intervals of 1–2 h. Samples were centrifuged (10–20 min, 13,000 rpm), and spectrophotometric analysis was performed on the supernatant. COD and VSS concentrations were also monitored at the start (after feeding the bioreactor) and at the end of the test. Additionally, during the aerobic period, the dissolved oxygen (DO) concentration was monitored and controlled (in the range of 3–4 mg/L) through an on-off aeration strategy, and DO data were employed to calculate the specific oxygen uptake rate of the biomass (SOUR) according to the procedure reported in Tomei et al. (2004).

Table 4
Operating conditions in the three series of tests.

| Test Series | Operation mode | Working volume (L) | Acclimation | | Stable operation | |
|-------------|---|--------------------|--------------|--|------------------|--|
| | | | Duration (d) | Dye load $\text{kg}_{\text{COD}}/(\text{m}^3 \text{ d})$ | Duration (d) | Dye load $\text{kg}_{\text{COD}}/(\text{m}^3 \text{ d})$ |
| I | Conventional | 1 | 22 | 0.005–0.0075 | 45 | 0.01 |
| II | Conventional pH modified ^a | 0.3 | 7 | 0.0075 | 21 | 0.01 |
| III | TPPB ^b pH modified ^a | 0.3 | 7 | 0.01 | 21 | 0.01–0.013 |

^a pH controlled at 4.5 in the first 4 h of the anaerobic phase.

^b Polymer to water ratio 10% (v/v).

In order to verify data reproducibility, all kinetic tests were conducted in two replicates under the same operating conditions.

2.6. Analytical methods

COD Cell Tests (MERCK-referring to EPA 410.4 method) based on potassium dichromate oxidation and spectrophotometric determination (Spectroquant Nova30) were employed to measure the COD concentration. Samples were appropriately diluted to eliminate the effects of chloride interferences on the measurements.

TOC, employed for wastewater characterization, was analyzed with a total organic carbon analyser (TOC-V CSN SHIMADZU), which determines the TOC concentration by the difference between the amount of total and inorganic carbon in the sample.

Ion concentrations were measured with an ionic chromatograph (DIONEX, DX 100) equipped with a Dionex AS-14 column.

Biomass concentration in the reactor was determined as VSS concentration.

VSS concentrations and pH were determined according to standard procedures (APHA, 1998).

Acetate was analyzed by using a PerkinElmer Auto System gas chromatograph equipped with $2 \text{ m} \times 2 \text{ mm}$ stainless steel column packed with 60/80 mesh Carboxen B-DAX 80–120 4% CW 20 M Supelco. Other operating conditions were: N_2 carrier gas 20 mL/min, oven temperature 120 °C, injector temperature 200 °C, flame ionization detector (FID) at temperature 200 °C.

Colour content was determined according to Standard Methods (APHA, 1998) from the UV–visible absorbance scans recorded with a

spectrophotometer (PerkinElmer, Lambda 25), in the 200–900 nm spectral band. From a spectrum analysis three wavelengths, 396 nm, 492 nm and 603 nm, were identified as being representative of the different intervals of colour distribution in the sample on the basis of the positions of the peaks. The dominant wavelength was 536 nm and can be considered to be the most representative of the entire spectrum. Decolourization performance was evaluated as percent colour removal from the absorbance readings at the three representative wavelengths and at the dominant wavelength. The absorbance of the liquid in the bioreactor before feeding was accounted for the measurement and the evaluation of colour removal.

3. Results

3.1. Polymer screening: sorption and desorption tests

The first step to evaluate the potential applicability of a two-phase bioreactor is polymer screening to determine the best absorptive material in terms of uptake-release. Previous studies have generally been performed on single target dyes, however in this case a real wastewater comprised of a mixture of dyes was investigated, making it more difficult to find a polymer suitable for all the components in the mixture.

Four polymers were selected for testing based on their previous use for solute uptake, and an overview of the screening results is reported in Fig. 1, expressed as percent colour removal at the different wavelengths for 24 h of contact time. A noticeable pH effect is evident with the best performance given by Hytrel 8206 at pH 4 for all wavelengths, reaching colour removal in the range of 83–87%.

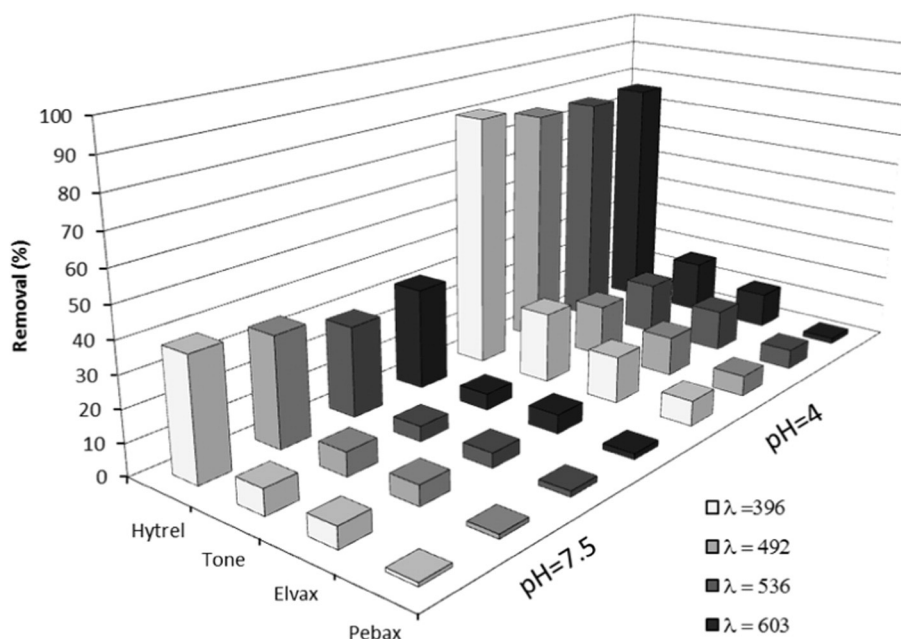


Fig. 1. Sorption tests: % colour removal at different pH values for the different wavelengths (nm).

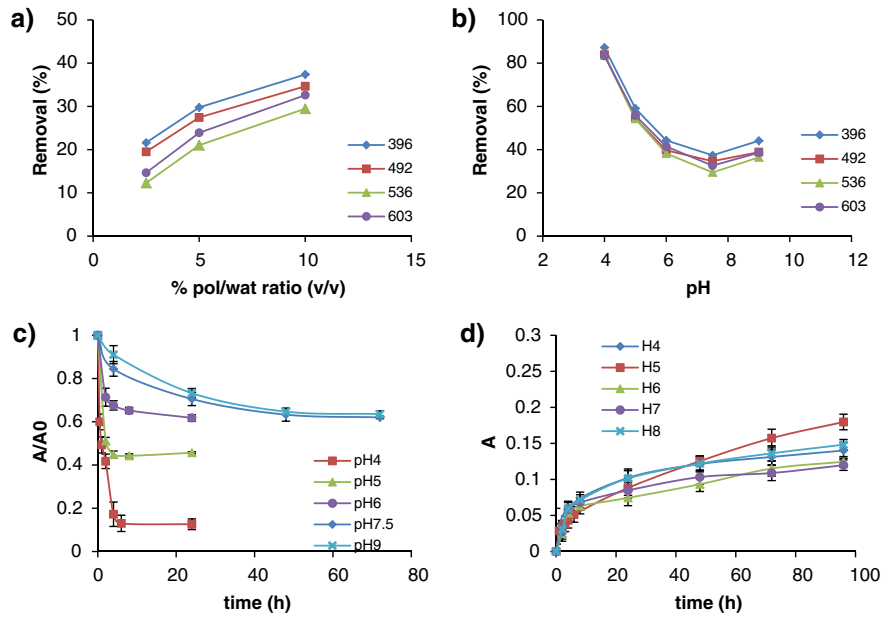


Fig. 2. Overview of the sorption tests results, polymer Hytrel. a) Sorption colour removal vs. polymer/water ratio for the different wavelengths (nm); contact time 72 h, pH = 7.5 (Test H1, H2, H4); b) Sorption colour removal vs. pH for the different wavelengths (nm); polymer/water ratio 10%, contact time 24 h (Test H5, H6, H7) and 72 h (Test H4, H8); c) Sorption kinetics (H4–8) at different pH: absorbance profile vs. time for the dominant wavelength i.e. 536 nm, polymer/water ratio 10%; A = absorbance at time t; A0 = absorbance at time 0; d) Desorption kinetics (H4–8): absorbance profile vs. time for the dominant wavelength i.e. 536 nm, polymer/water ratio 10%; pH = 7.5. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

On the basis of the obtained results, Hytrel was chosen as the partitioning phase in the TPPB and investigated more thoroughly for sorption and desorption.

Fig. 2a displays the % colour removal vs. the polymer/water ratio for Hytrel and demonstrates enhanced colour uptake with increasing polymer fraction utilized. The test, conducted at pH = 7.5, reached colour uptake in the range of 30–37% even at the highest polymer fraction (10%) and 72 h of contact time, so the test was repeated with a fixed polymer/water ratio of 10% and pH in the range of 4–9 with the results reported in Fig. 2b. Increased colour removal can be seen for decreasing pH with the maximum value occurring at pH 4. Lower pH values were not explored due to their likely negative effect on biomass activity. No appreciable improvement was observed for pH \geq 7.5.

The sorption and desorption kinetic results are reported in Figs. 2c and d respectively and provide an estimation of the time required for the physical processes of absorption/desorption to occur, which is one of the important parameters to be considered in practice. The absorbance profile displayed in Fig. 2c shows that at lower pH values (4–5), ~6 h of contact time are sufficient to reach maximum dye removal. Desorption data reported in Fig. 2d do not show significant differences in the desorbed amounts reached after contact times \leq 24 h, which is the duration for the work cycle of the bioreactor.

3.2. Biotic tests: colour removal

Table 5 shows a summary of the removal efficiencies achieved in the biotic tests. Colour removal is expressed as average values \pm (SD) for the different wavelengths in the three series (i.e. calculated by taking into account all work cycles performed in the series) in order to provide an exhaustive representation of the removal efficiencies achieved during the different experimental campaigns. For each series, the total percent removal (i.e. for the complete sequential anaerobic-aerobic process) and the removal only in the anaerobic phase are reported.

The results of a typical kinetic test performed during a work cycle of the first series are shown in Fig. 3. Fig. 3a shows the absorbance profile at the dominant wavelength, which is the most representative of colour removal during the test, while Fig. 3b shows the absorbance spectra vs. time (in the range 300–800 nm), which gives an overview of the colour

components during the treatment process. Finally, Fig. 3c shows the percent colour removal for the sequential process at the same selected wavelengths, and the total colour removal (TCR) calculated as the difference between the area of the two surfaces bounded by the spectrum curves at the start and at the end of the anaerobic and aerobic phases. The colour appears to be mainly removed during the anaerobic phase (~50%) with additional removal in the aerobic phase of ~18%. The percent removal increases with the wavelength, as also reported in Table 5. The first series is the reference condition for comparison of the subsequent operation modifications applied in the 2nd and 3rd series of tests.

Since colour uptake by Hytrel is greater at low pH values (see Fig. 2b), which can be detrimental to biomass activity, the second series of tests examined the possibility of operating at low pHs for a reduced portion of the biological reaction time. The results of a typical kinetic test performed during a work cycle of series II are shown in Fig. 4. No significant differences are observed in comparison with the first series of tests (see Fig. 3): removal efficiencies at $\lambda = 536$ nm are 52% and 19% for the anaerobic and aerobic phases, respectively. For the other wavelengths removal efficiencies are comparable and even higher with a slight decrease only for the lowest wavelength, $\lambda = 396$ nm, which contributes minimally to the colour content. The positive results of this second series confirmed the possibility of operating at lower pH in the TPPB.

In the third series of tests the bioreactor was operated in TPPB mode at increasing dye loading rates, and the results of the kinetic tests are

Table 5

Removal efficiencies in the different experimental series: for each series, the first line gives the average percent removal (\pm SD) referred to the sequential anaerobic-aerobic process while in the second line the removal in the anaerobic phase is reported. Bold characters refer to the dominant wavelength.

| Series | Dye removal (%) | | | |
|-------------|--------------------|--------------------|--------------------------------------|--------------------|
| | $\lambda = 396$ nm | $\lambda = 492$ nm | $\lambda = 536$ nm | $\lambda = 603$ nm |
| I - Total | 53.7 \pm 11.4 | 61.3 \pm 10.4 | 68.2 \pm 8.1 | 90.5 \pm 7.4 |
| Anaerobic | 38.2 \pm 6.8 | 47.5 \pm 9.8 | 49.9 \pm 3.9 | 66.0 \pm 11.1 |
| II - Total | 48.0 \pm 14.4 | 63.8 \pm 14.0 | 67.1 \pm 12.9 | 83.7 \pm 12.7 |
| Anaerobic | 35.2 \pm 5.6 | 54.5 \pm 10.4 | 55.5 \pm 4.5 | 69.4 \pm 4.9 |
| III - Total | 64.4 \pm 16.9 | 79.9 \pm 11.7 | 84.5 \pm 8.9 | 92.0 \pm 4.9 |
| Anaerobic | 42.4 \pm 6.1 | 79.4 \pm 7.6 | 82.0 \pm 5.9 | 84.3 \pm 4.4 |

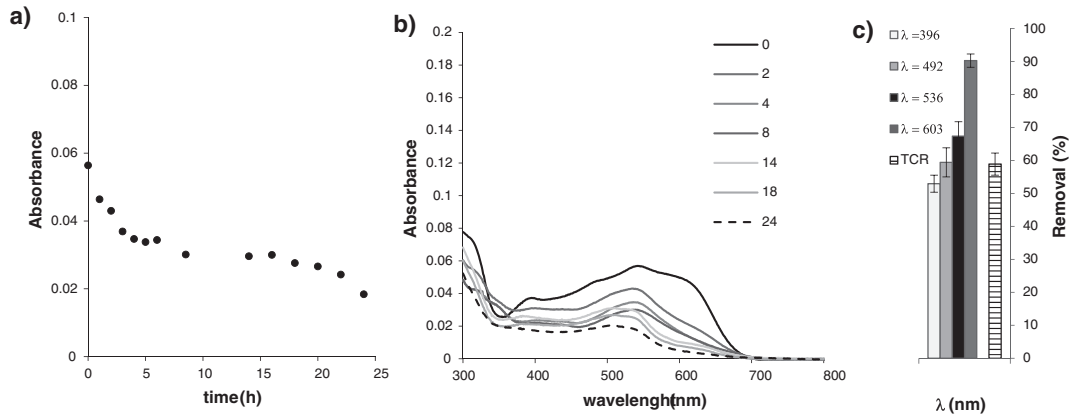


Fig. 3. Results of the kinetic tests: series I: a) Absorbance pattern for the dominant wavelength; b) Evolution of the colour spectrum vs. time; c) Colour removal at the different wavelengths (evaluated on the test replicates).

shown in Figs. 5 and 6 for work cycles at the two loading conditions. In Fig. 5a, corresponding to the same operating conditions of the two previous series, an improvement in colour removal for the dominant wavelength is observed with a value of 84%. There is also a different evolution of the colour spectrum (Fig. 5b), in comparison to the previous tests of series I and II. In the conventional biological systems, even with the modified pH, a progressive and regular lowering of the spectrum vs. time is observed, while in the TPPB, a distinct decrease of the spectrum is observed after 4 h followed by a slight increase, more obvious for the higher loading. It is also observed that good performance of the TPPB system is maintained for a colour load increase of 30%, with colour removal at $\lambda = 536 \text{ nm} > 80\%$ (see Fig. 6 and Table 5).

3.3. COD and SOUR pattern

COD is a parameter commonly used in wastewater treatment plants to evaluate the process efficiency and the effluent quality when specific characterization of a wastewater component is not required, or is difficult. COD concentrations in the feed and at the end of the anaerobic and aerobic phases and the related colour removal for the kinetic tests performed at $0.01 \text{ kgCOD}/(\text{m}^3 \text{ d})$ load in the three series, are reported in Table 6. The two COD components, i.e. biogenic (acetate) and dye mixture, have been differentiated in the mass balance. In the last two columns, the TCR (evaluated from the absorbance spectra) is reported for comparison. Concerning the acetate fraction, the control tests excluded its absorption on the polymer in all the investigated conditions (variation coefficient $\leq 3\%$ for both neutral and acid-neutral conditions). Acetate is a readily biodegradable substrate both under anaerobic and aerobic conditions, thus, if it not absorbed, it can be reasonably assumed

that its biodegradation takes place in a short time in the very first part of the anaerobic phase, and the residual COD at the end of the anaerobic and aerobic phase is attributable to the colour component (COD_{Dye}). Total COD removal efficiencies of $\geq 96\%$ and $\geq 98\%$ after the anaerobic and aerobic phase respectively, have been achieved in the three series, while a different trend is observed for COD_{Dye} : a low anaerobic removal for COD_{Dye} (11–14%) is observed in Series I and II, which increases up to 41% in Series III. At end of the aerobic phase COD_{Dye} percent removal of ~ 59 and 75% are achieved in Series I – II, and III, respectively.

A first quantification of the toxicity effect on the biomass may be evaluated by SOUR data continuously recorded during the kinetic tests. In Fig. 7 the SOUR profiles of kinetic tests of Series I and III show a distinct difference between the maximum detected values, i.e. 8 and $28 \text{ mgO}_2/(\text{g}_{\text{VSS}} \text{ L})$, respectively. The higher specific oxygen consumption rates reached in Series III successfully demonstrated the increased activity of the biomass operating in TPPB mode, as already pointed out with xenobiotic compounds such as 2,4-dichlorophenol (Tomei et al., 2014).

4. Discussion

The results of the abiotic sorption tests showing the best performance for Hytrel, can be explained considering that the dye molecules are very polar and have a strong affinity for water. As such, polar polymers, such as Hytrel can potentially work well. As for the effect of pH, the positive effect of low pH could be justified considering that a lower pH provides conditions that would protonate the sulphate groups and allow for enhanced absorption into the polymer. Sorption characteristic times ($\sim 6 \text{ h}$) observed for Hytrel at lower pH values (4–5) are similar in magnitude to the kinetics of many biological processes, thus

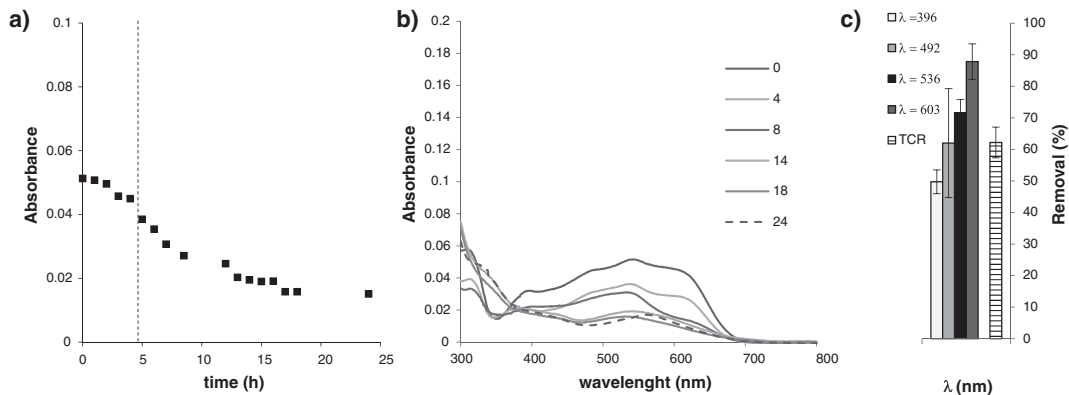


Fig. 4. Results of the kinetic tests: series II: a) Absorbance pattern for the dominant wavelength; b) Evolution of the colour spectrum vs. time; c) Colour removal at the different wavelengths (evaluated on the test replicates).

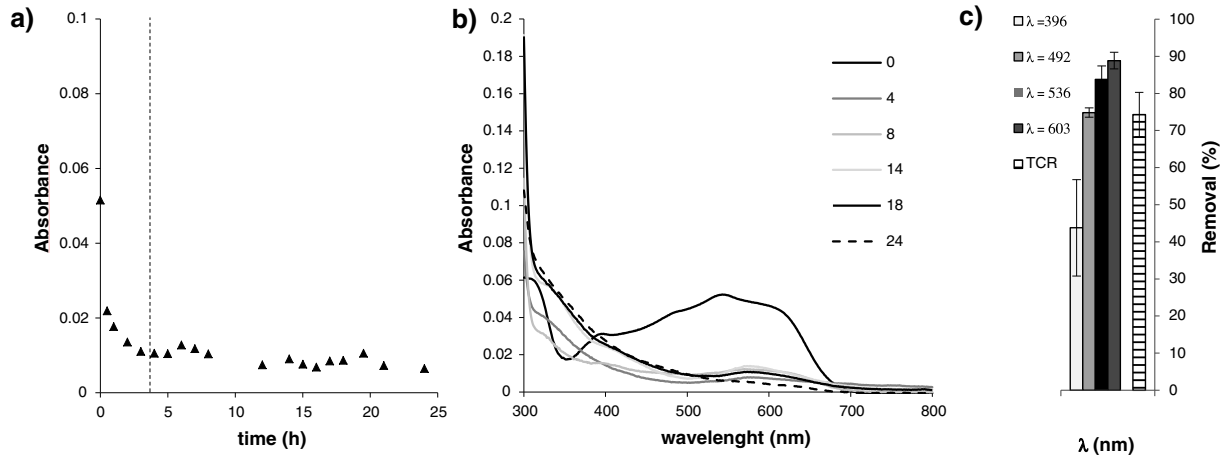


Fig. 5. Results of the kinetic tests: series III; Dye load = $0.01 \text{ kg}_{\text{COD}}/(\text{m}^3 \text{ d})$: a) Absorbance pattern for the dominant wavelength; b) Evolution of the colour spectrum vs. time; c) Colour removal at the different wavelengths (evaluated on the test replicates).

suitable for TPPB application. Higher pH requires longer contact times, which could be limiting when coupled with a biological process, and this is why low pH values have been applied in TPPB operation here (Series II and III).

Biotic tests were performed in sequential anaerobic-aerobic mode, because, as already mentioned, the two reaction environments act in a synergic mode with the aerobic stage completing the biodegradation process of compounds and intermediates not biodegraded in the anaerobic period. This approach has been successfully applied for treating synthetic textile wastewater (Kapdan et al., 2003; Supaka et al., 2004; Lourenco et al., 2006), and in other biological systems, i.e. anaerobic followed by post-aerobic treatment of urban wastewater (Chan et al., 2009), and a sequential anaerobic-aerobic process for sludge stabilization (Tomei et al., 2011a).

In Series I, i.e. the conventional single-phase system, greater decolorization with increasing wavelengths is observed. A possible explanation of this finding is given by the different biodegradability of the single dyes in the mixture. According to Shah et al. (2014) and Sanmuga Priya et al. (2015), Remazol Red (peak 580–590 nm) is characterized by higher biodegradability, followed by Remazol Yellow (peak 398–480 nm), while Remazol Black (peak 595 nm) is the most difficult to biodegrade. Remazol Red and Remazol Black are characterized by absorbance peaks at $\lambda \geq 580 \text{ nm}$ that is in the higher wavelength region. Thus, considering the higher biodegradability of Remazol Red (with respect to the Black) it may be plausible to assume a higher contribution of Remazol Red to the colour removal.

The good performance of the biological system in Series II, in spite of the low pH applied in the first part of the anaerobic period, can be explained considering that the bacterial strains catalysing the first steps of the anaerobic reaction chain (i.e. the Acidogens and the Acetogens), in contrast to the Methanogens, are not affected by lower pH (Kim et al., 2004; van Lier et al., 2008; Chen et al., 2008). In this study, the objective was not to maximize methane production but to have colour removal so, in principle, it is not detrimental to operate a portion of the anaerobic reaction time at low pH, thus providing conditions for enhancing colour uptake by the polymer. Moreover, in two-phase anaerobic digestion (Acidogenic phase followed by the Methanogenic one) applied pH values are in the range 4.3–6 (Demirel and Yenigün, 2002), and in specific applications, such as in the production of hydrogen, the pH is controlled around 4 (Chen et al., 2008; Fang and Liu, 2002). According to Firmino et al. (2010), due to the competition of dye reduction with methanogenesis for the electrons generated upon electron donor oxidation, the two-stage anaerobic system could be an interesting option to enhance colour removal. In our case, the sequential system can be considered as a two-stage anaerobic system where the two phases are undertaken in the same unit on a time basis rather than in two separate units, thus the low pH in the first part of the reaction is compatible with the acidogenic step. In addition, the high pH tolerance of decolorizing bacteria reported in Saratale et al. (2011) can also explain why pH change had no effect on the performance of the bioreactor in series II with respect to Series I. In any case, the inhibitory effect on methanogenesis can also be mitigated by appropriate technologies:

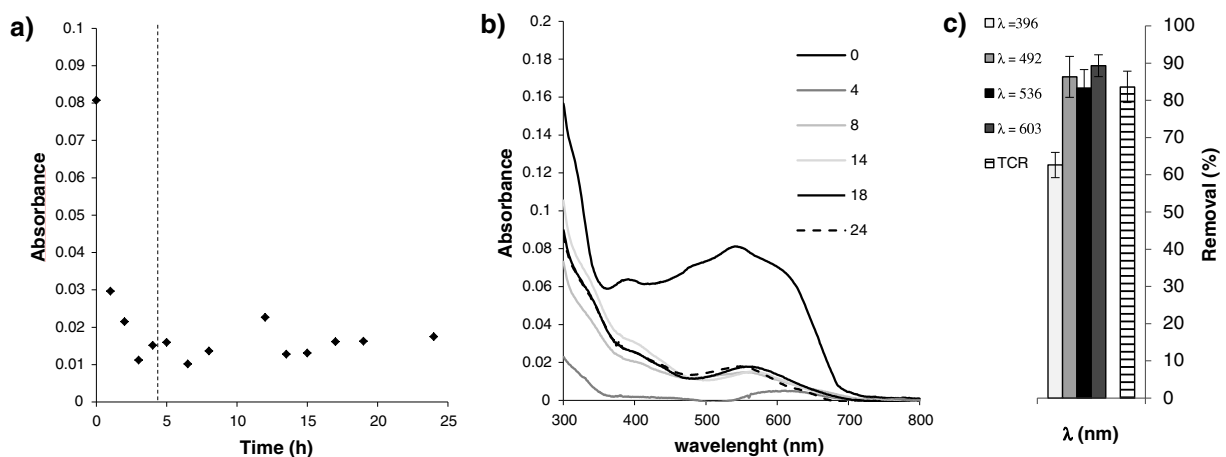


Fig. 6. Results of the kinetic tests: series III; Dye load = $0.013 \text{ kg}_{\text{COD}}/(\text{m}^3 \text{ d})$: a) Absorbance pattern for the dominant wavelength; b) Evolution of the colour spectrum vs. time; c) Colour removal at the different wavelengths (evaluated on the test replicates).

Table 6

COD data and % removal. AN = at the end of the anaerobic phase; AE = at the end of the aerobic phase (average values of the two replicates of the kinetic tests in the three Series).

| Series | COD (mg/L) | | | | Removal (%) | | | | | |
|--------|------------|--------------------|------|-----|--------------------|------|--------------------|------|------|------|
| | Feed | | AN | AE | COD _{Tot} | | COD _{Dye} | | TCR | |
| | Tot | (dye) ^a | | | AN | AE | AN | AE | AN | AE |
| I | 425 | 21.2 | 18.2 | 8.8 | 95.7 | 97.9 | 14.4 | 58.6 | 50.3 | 58.9 |
| II | 443 | 22.2 | 19.6 | 9 | 95.6 | 98.0 | 11.5 | 59.4 | 55.7 | 62.2 |
| III | 485 | 24.3 | 14.4 | 6 | 97.0 | 98.8 | 40.6 | 75.3 | 70.6 | 74.9 |

^a Estimated taking into account that the total COD of feed solution is constituted by a 1:20 acetate:dye ratio (on COD basis).

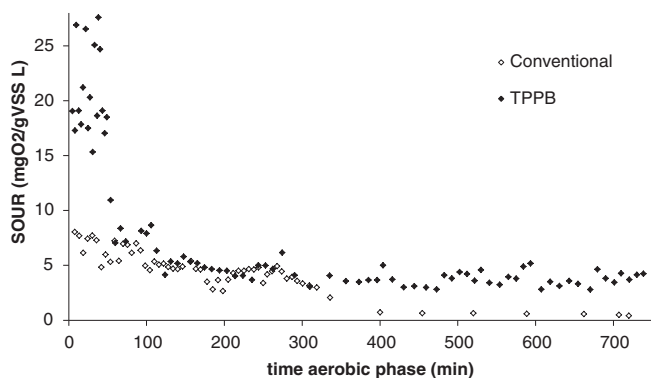


Fig. 7. SOUR time profiles during the first and the third experimental series: Dye load = $0.01 \text{ kg}_{\text{COD}}/(\text{m}^3 \text{ d})$; Biomass concentration = 2760 and 2560 $\text{mg}_{\text{VSS}}/\text{L}$ for 1st and 3rd series, respectively.

Patel and Madamwar (2000) reported high COD removal (95%) and efficient biomethanation for a fixed-film anaerobic bioreactor applied to the treatment of petrochemical acidic wastewater (pH 2.5).

In the third Series, a clear beneficial effect of the polymer on colour removal is observed, with efficiencies reaching values up to 90%. Moreover, the different evolution of the spectrum, with respect to Series I and II, may be due to colour removal attributable to polymer uptake in the

first part of the anaerobic phase followed by dye release. In other words, the maximum decolourization is achieved just after the first 4–5 h due to sorption into the polymer, but this does not imply actual degradation of dyes, which is achieved only after their release into the aqueous phase. The mechanism of uptake–release in the TPPBs is highlighted by the stable low colour level observed during the aerobic period: this pattern can be explained with the near-complete removal of the colour released from the polymer. Performance of TPPB system was followed for more than 20 days: it is worth noting that no saturation of the polymer and/or loss in the overall efficiency of the process were observed during this period, in spite of the increased load. This confirms the robustness of the uptake/release mechanism of amorphous polymer and the added advantage of sub-inhibitory and optimal conditions for the biomass.

The COD trend in the three series gives indirect indications on the fate of intermediates and on the role of the polymer. The low anaerobic removal for COD_{Dye} (11–14%) in Series I and II, if compared to the corresponding high anaerobic removal of TCR (50–56%), highlights the formation of intermediates (presumably aromatic amines) not biodegraded in the anaerobic step. According to the better aerobic biodegradability of aromatic amines under aerobic conditions, the removal of COD_{Dye} reaches 59% for both series and is comparable with the final TCR removal of 59–62%.

The beneficial effect of the polymer addition in the third series is evidenced by higher anaerobic COD_{Dye} removal (41%) reaching 75% after the aerobic period. Also in this case the difference between the COD_{Dye} and TCR removal in the anaerobic step may be attributable to the intermediates not removed under anaerobic conditions, which are biodegraded in the aerobic phase as demonstrated by same removal efficiency (~75%) for COD_{Dye} and TCR.

In series III, the positive effect of the added polymer is reflected in the reduction of the toxicity on the biomass after the sequential treatment, as highlighted by the SOUR profiles reported in Fig. 7. This finding highlights the increased biomass activity, attributable to the reduced dye concentration in the liquid phase to which the biomass is exposed.

Finally, a visualization of the polymer effectiveness in the abiotic and biotic tests is given in Fig. 8, which shows very dark colour after absorption, and light colouration of the beads after three weeks of use in the TPPB.

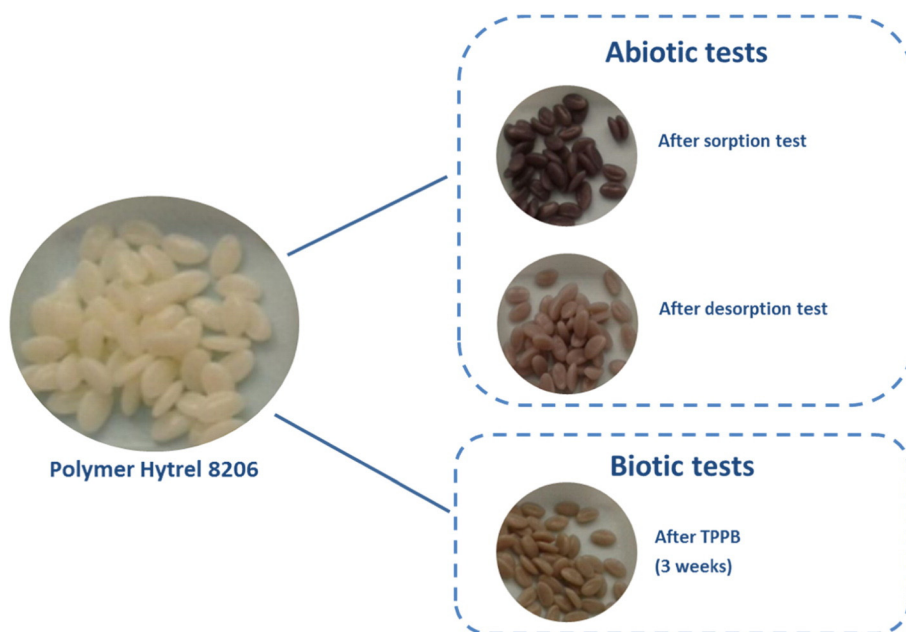


Fig. 8. Colour changes in polymer beads, new beads and beads utilized in abiotic and biotic experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5. Conclusions

TPPB bioreactor improved the performance of the sequential anaerobic-aerobic process applied to the removal of azo dyes from a real dye bath wastewater, reaching 84% removal for the characteristic wavelength, and 75% for TCR in comparison to 68% and 59% observed in the conventional sequential process.

The COD mass balance demonstrated that for all investigated operating conditions, the aerobic step achieved an almost complete removal of the intermediates produced in the first anaerobic step.

The study is a first demonstration of the applicability of TPPB bioreactors to industrial wastewater treatment, in this specific case, given the homogeneity of the fed substrate (i.e. the same group of dyes) in the segregated wastewater stream, one polymer has been effective for enhancing colour removal and reducing the toxicity to the biomass. For more complex industrial wastewater, characterized by different groups of substrates, the flexibility of the proposed sequential TPPB system can be exploited by operating with a mixture of different polymers tailored to the different substrate components, and by varying the operating conditions, i.e. duration and reaction environments to optimize the performance of the biological process.

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