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Full Length Research Paper

Decolourisation and degradation of textile dyes using a sulphate reducing bacteria (SRB) – biodigester microflora co-culture

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Successful decolourisation and degradation of textile dyes was achieved in a biosulphidogenic batch reactor using biodigester sludge from a local municipality waste treatment plant as a source of carbon and microflora that augmented a sulphate reducing bacteria (SRB) consortium. Orange II (O II) was decolourised by 95% within one day (24 h) producing 1-amino-2-naphthol and stoichiometric quantities of sulphanilic acid. The latter was degraded steadily (from ≈ 290 to 43 µM) over 20 days while 1-amino-2-naphthol disappeared from the reactor within two days. Other azo dyes, Reactive black 5 (RB 5), Reactive red 120 (RR 120), Remazol Brilliant violet 5R (RBV 5R), an anthraquinone dye Reactive blue 2 (RB 2) and an industrial azo dye mixture (Da Gama Textiles, King Williams Town, South Africa) were successfully degraded with the exception of Amido black 10B (AB 10B). The Orange II degrading cultures were freeze dried to investigate the feasibility of commercialising a powdered mixed starter culture for textile effluent bioremediation, but this decreased the dye degrading efficiency. Therefore bioremediation of textile effluent with sludge and SRB can concomitantly treat two wastes while providing a cheaper alternative of the carbon source. However, the fate of more aromatic amines needs to be investigated before full commercialisation of the process.

Key words: Bioremediation, textile effluent, 1-amino-2-naphthol, sulphanilic acid, sulphate reducing bacteria (SRB), azo dye, biosulphidogenic.

INTRODUCTION

Technological advance has seen an increase in diversity and complexity of synthesised textile dyes with the objective of product improvement through enhancement of dye properties such as resistance to fading, improved delivery of dyes to fabrics and increased variety of shades. This increase in diversity and complexity of dyes is coupled with higher resistance to environmental degradation leading to pollution problems by textile effluent. A larger proportion of these are azo dyes which can pass through normal water treatment procedures (Stolz, 2001; Pearce et al., 2003; Pandey et al., 2007) resulting in aesthetically unappealing water.

Adequate treatment of textile effluent requires more than one stage as there is need for both colour removal and degradation of aromatic compounds from the decolourisation process. Physico-chemical treatment methods are the least desirable owing to their high costs and generation of secondary pollutants. On the other hand, biological treatment methods are attractive due to their cost effectiveness, diverse metabolic pathways and versatility of microorganisms (Banat et al., 1996; Singh et al., 2004; Méndez-Paz et al., 2005; Van de Zee and Villaverde, 2005; Pandey et al., 2007).

In general, complete azo dye mineralisation requires both anaerobic and aerobic bacterial processes. Azo bonds are reduced under anaerobic conditions leading to generation of aromatic amines. Although laccases can be used for aerobic azo dye reduction, they polymerise the aromatic amines leading to secondary colour development and pollutant build-up (Zille et al., 2005). It is report-

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ed that aromatic amines are toxic, carcinogenic and recalcitrant to anaerobic degradation and usually require complete degradation under aerobic conditions (O'Neill et al., 2000; Pearce et al., 2003; Işik and Sponza, 2007). Use of co-cultures of facultative anaerobic and aerobic bacteria leads to mineralization of textile waste (Tan et al., 1999; O'Neill et al., 2000; Pandey et al., 2007). However, reactor supplementation with artificial media is costly (Pandey et al., 2007) and pure cultures have a narrow range of substrates (Tan et al., 2005), thus limiting such reactors to laboratory scale. Therefore there is a need for cheaper biological methods that can be readily adapted for textile effluent treatment.

To the best of our knowledge, little research has been done on the use of sulphate reducing bacteria (SRB) for textile effluent bioremediation (Yoo et al., 2001; Mutambanengwe et al., 2007) while the concept of single reactor for total mineralisation has been applied to facultative anaerobic and aerobic non-sulphate reducing bacteria (Van de Zee and Villaverde, 2005; Pandey et al., 2007). From our preliminary work, SRB grown in primary sludge was able to decolourise textile dyes (Mutambanengwe et al., 2007). However, decolourisation does not imply mineralisation of the aromatic amines. Therefore, the aim of this research was to confirm the ability of biodigester sludge and microflora-SRB co-culture to mineralise O II by monitoring the concentration of O II and its aromatic amines (sulphanilic acid and 1-amino-2-naphthol) in the reactors. Other dyes were used to evaluate the applicability of the co-culture for textile effluent. Use of municipal sewage sludge in textile waste treatment will minimise costs for artificial carbon sources while treating municipality organic waste, thus combining two negatives to come up with a positive outcome. Attempts were made to prepare a mixed starter culture by freeze drying the O II acclimatised samples.

MATERIALS AND METHODS

Orange II (O II) was used as a model (mono-) azo dye for fundamental experiments. Other commercial dyes were then used, these were: anthraquinone dye - Reactive blue 2 (RB 2); mono-azo dye - Remazol brilliant violet 5R (RBV 5R); di-azo - dyes Reactive black 5 (RB 5), Amido black 10B (AB 10B) and Reactive red 120 (RR 120), Da Gama Textiles (King Williams Town, South Africa) dye with silicates (ready for delivery to fabrics). A mixture of the above-mentioned commercial dyes (excluding Da Gama dye) was used with each dye at 100 mg/l in the reactor. All commercial dyes were supplied by Sigma (Steinheim, Germany), except AB 10B (Merck, Steinheim, Germany). The experiments were performed in triplicates with duplicate analyses for each sample. Results are reported as mean ± standard deviation (SD). Significant testing was performed at 5 % level ($P = 0.05$) using Microsoft Excel statistical analysis tool and significant results had $P < 0.05$.

Reactor set up

Sludge was obtained from a biodigester at a local waste water treatment plant (Grahamstown, South Africa) and sulphate reducing bacteria (SRB) from the Environmental Biotechnology Research Unit (EBRU) BioSURE® process (Rhodes University, South Africa). Sulphidogenic batch reactors were set up as described by Ngwenya and Whiteley (2006) and Mutambanengwe (2007). Modifications were made to the proportion of inoculum used, which was 10% (v/v) of the total reactor volume (1 000 ml). Sludge was added to give a final COD concentration of 2 000 mg/l in the reactor. Ratio of sulphate and COD was adjusted to 1:1 by adding sodium sulphate (Merck, Steinheim, Germany) prior to culturing. Nitrogen (99.99%) and hydrogen (99.98%) (Afrox, South Africa) were used to purge out oxygen before incubation and during sampling. In each case, controls that consisted of sterilised media (sludge + SRB) and dye in question were set up. The culture was acclimatised to O II degradation by successively transferring and incubating O II degrading cultures in a reactor with fresh O II (100 mg/l). The cultures were incubated in continuous stirring at ambient temperature (25 ± 2°C).

Sampling and analyses

Samples withdrawn from the reactors were centrifuged at 4 000 rpm (Eppendorf A-4-62 rotor, Merck, Steinheim, Germany) for 10 min at 4°C and filtered through 0.45 µm before UV/VIS scanning and HPLC. Sample analyses were performed immediately after sampling with minimised exposure to light and air. A Power wave$_x$ spectrophotometer with KC4 software (Analytic and Diagnostic Products, Cape Town, South Africa) was used to scan the samples (in UV transparent microtitre plate) from 200 to 800 nm at 5 - nm intervals and quantification of other commercial dyes. To confirm that colour removal was not due to adsorption to particulate matter, colour monitoring was performed both on the filtered supernatant and resuspended pellet.

HPLC analyses of O II and its reduction products (sulphanilic acid and 1-amino-2-naphthol) were performed using Beckman System Gold equipment (Beckman Coulter, Johannesburg, South Africa) with a Lichrocart® 250-4, Purospher® RP-18e (5 µM) column (Merck, Steinheim, Germany). Twenty microlitres of each sample were manually injected and the mobile phase, modified from Senan and Abraham, 2004; Zhao et al., 2005, was 60:40 v/v (acetonitrile :water) at a flow rate of 1 ml/min (pump module 126). Detection was performed at 248 and 483 nm using a diode array detector (module 168).

Freeze drying

Cultures acclimatised to O II degradation were freeze dried in their late log growth phase (after six days of culturing, Mutambanengwe, 2007) using a VisTis Bench Top SLC freeze drier (SP Industries, New York, USA) with minimal exposure to direct light and oxygen. The latter was achieved by keeping samples under a blanket of nitrogen before freezing. The solids per ml were calculated in order to use powder with weight that corresponded to 10% (v/v) of liquid inoculum per reactor to enable determination of effects of freeze drying on the samples. The powder was evaluated for decolourisation and degradation of O II, and decolourisation of all dyes that were used with liquid inoculum and sludge. However, yeast extract powder (2 g/l) was used as a carbon source since the powdered samples failed to decolourise dyes without a source of carbon whereas sludge would mask the effects of freeze drying by introducing fresh microflora. The reactors were set as described above.

RESULTS

In all cases, growth of the SRB was confirmed by a decrease in sulphate and formation of zinc sulphate pre-

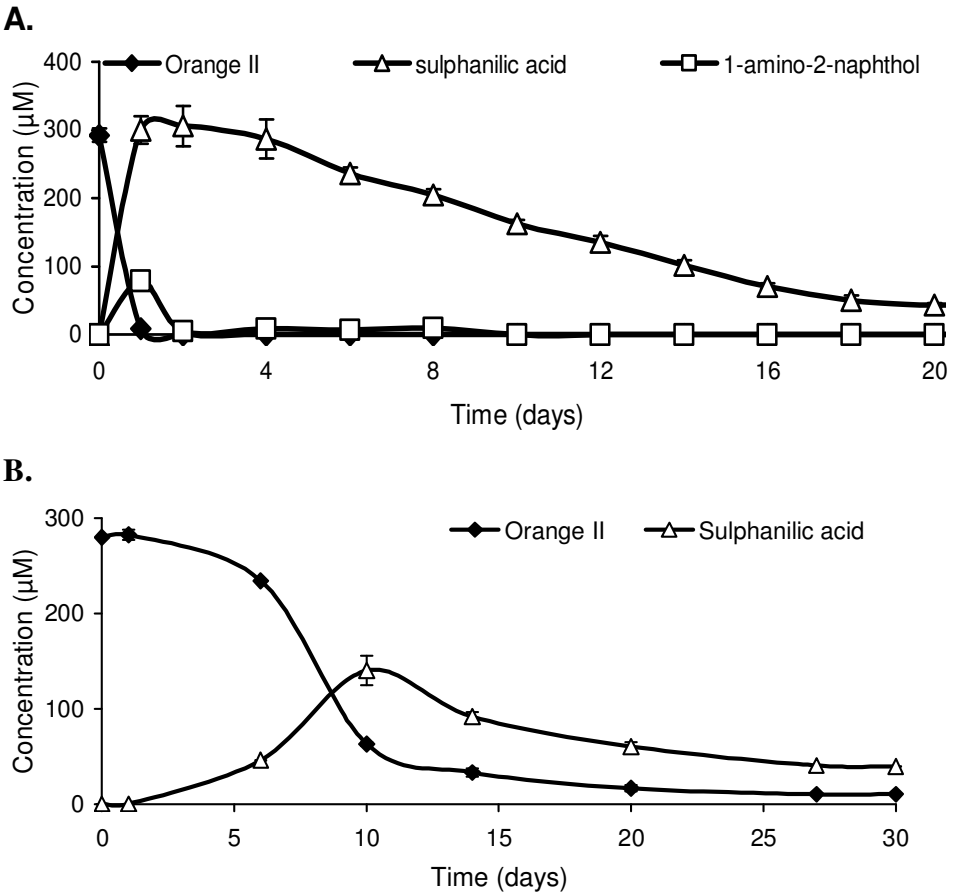

Figure 1. Degradation of O II and its immediate breakdown products (Sulphanilic acid and 1-amino-naphthol) in separate sulphidogenic reactors with liquid SRB and sludge (A), and freeze dried SRB-sludge O II degrading culture supplemented with yeast extract powder (B). All values represent the means ± SD (n = 3).

cipitate due to production of hydrogen sulphide. The decrease in COD supported the decolourisation and degradation of the dyes. Results from reactors with liquid SRB and sludge inocula (liquid inocula) and freeze dried (powder) inoculum are presented concurrently.

Orange II decolourisation and degradation

There was a rapid decrease (95.1%) in O II concentration within the first day of culturing and a concomitant stoichiometric increase in sulphanilic acid in reactors with liquid inocula (Figure 1A). However, 1-amino-2-naphthol concentration did not show a stoichiometric relationship with O II (Figure 1A). The absorption spectra (Figure not shown) of the O II cultures showed similar rapid decrease in O II absorbance peak and an initial increase in UV absorbance (around 250 nm). The UV absorbance decreased from day 1 as indicated for other dyes in subsequent sections. There was no colour development in the samples upon exposure to air and light. There was a lag phase in decolourisation and degradation of O II within the reactor with freeze dried inoculum (Figure 1B). Sulphanilic acid did not build up to the levels observed in the reactor with liquid inocula while 1-amino-2-naphthol was not detected (Figure 1B).

Commercial dyes – single dye reactors degradation studies

Reactors with the liquid inocula decolourised both azo and anthraquinone (RB 2) dyes more rapidly than the ones with freeze dried (powder) starter culture (Figure 2). Significant colour ($P < 0.05$) removal was observed during the first day of culturing for O II (95.1 ± 0.3 % colour removal), RB 2 (78.9 ± 2.9 %), and AB 10B (69.1 ± 2.1 %) while it took four days for 66.7 ± 3.1% colour removal in a reactor with RBV 5R (Figure 2). This was in contrast to decolourisation efficiencies in the reactors with powder inoculum where the shortest culturing period required for rapid dye decolourisation was six days with RB 5 (Figure 2). Insignificant colour changes were observed after the stage of rapid decolourisation in all

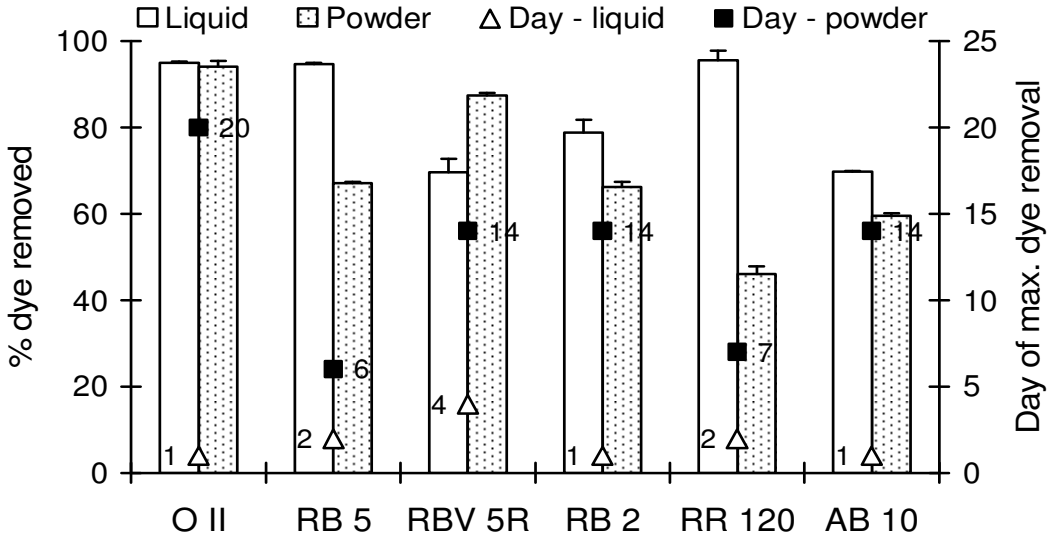

Figure 2. Decolourisation efficiencies of sulphidogenic reactors with different forms of inocula and the number of days taken to obtain maximum colour removal. All values represent the means ± SD (n = 3).

dyes as illustrated in Figure 3. All the samples from reactors with liquid inocula were visibly colourless after this phase except for AB 10B reactor samples that were purple throughout the culturing period. In addition to the similar observation for liquid inoculated AB 10B reactor, the powder inoculated reac-tors retained traces of the original dye colours. There was a shift in the absorption peak of the AB 10B after rapid decrease of the dye's maximum absorbance in both liquid inoculated (Figure 4) and powder inoculated reactors. In both sets of reactors, a progressive decrease in UV (220 – 285 nm) absorbance values for all dyes was observed (Figure 3 and 4). No specific decolourisation pattern was observed for all commercial dyes (Figures 2 and 5). There was no dye in the pellet fractions.

Mixed commercial dyes

The liquid inoculated reactors were more efficient than the powder inoculated ones in the decolourisation (Figure 5). However, liquid inoculated reactors were less efficient in decolourising individual dyes in a mixed dye reactor as opposed to the single dye reactor (Figures 2 and 5). For example, 89.7% O II was decolourised by the third day in a mixed dye reactor while 95.1% of the same dye concentration was decolourised within day 1 in a single dye reactor. In addition, the reactors with the powder inoculum had a shorter life span than the ones with the liquid inocula.

Da Gama textiles dye

Decolourisation of Da Gama Textiles was achieved in both sets of reactors (Figure 6) although reactors with liquid inocula were more efficient than those with powder inoculum. There were differences in the patterns and changes of the absorbance peaks for the reactors with liquid and powder inocula (Figure 6).

DISCUSSION

The HPLC results confirmed that decreases in O II absorbance in this and previous (Mutambanengwe et al., 2007) studies were due to reduction of the azo bond producing sulphanilic acid and 1-amino-2-naphthol. Stoichiometric ratio of sulphanilic acid to O II was expected because O II degradation proceeds in three ways, one of which is the symmetric cleavage of azo bond leading to formation of sulphanilic acid and 1-amino-2-naphthol (López et al., 2004) in stoichiometric quantities (Singh et al., 2007). However, the latter did not reach expected concentration (≈ 290 µM) either because of its instability (Coughlin et al., 1999; Singh et al., 2007) or rapid degradation within the reactor. The latter seems most likely since there was no pink colour associated with the auto-oxidation of 1-amino-2-naphthol after exposure to air and light (Pandey et al., 2007; Singh et al., 2007). Furthermore, neither the absorption spectra nor chromatograms showed any new peak to suggest new products from polymerisation or other transformation of the aromatic compounds. Therefore absence of detectable quantities of 1-amino-2-napththol in powder inoculated reactors can also be attributed to degradation.

Contrary to the reports of recalcitrance of sulphanilic acid under anaerobic conditions (Coughlin et al., 1999;

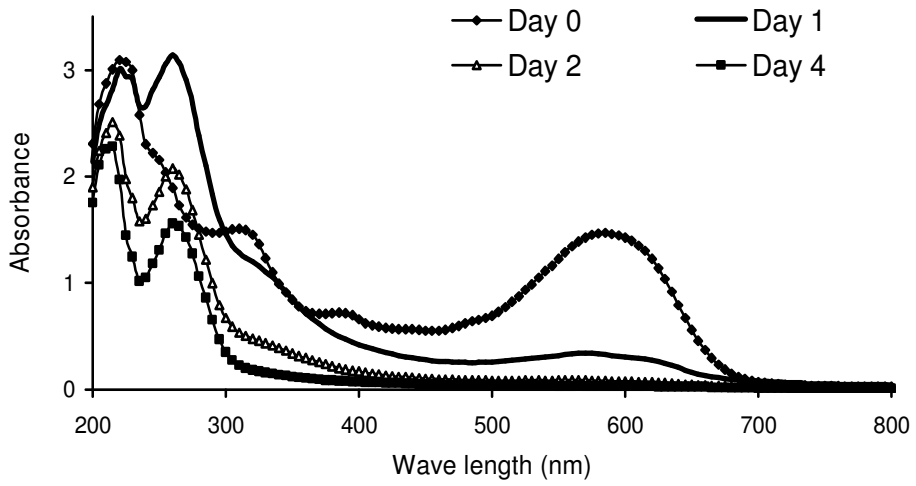

Figure 3. Decrease in absorbance due to colour removal (in the visible range) and decrease in aromatic amines (in the UV region) in a liquid inoculated sulphidogenic reactor with RB 5.

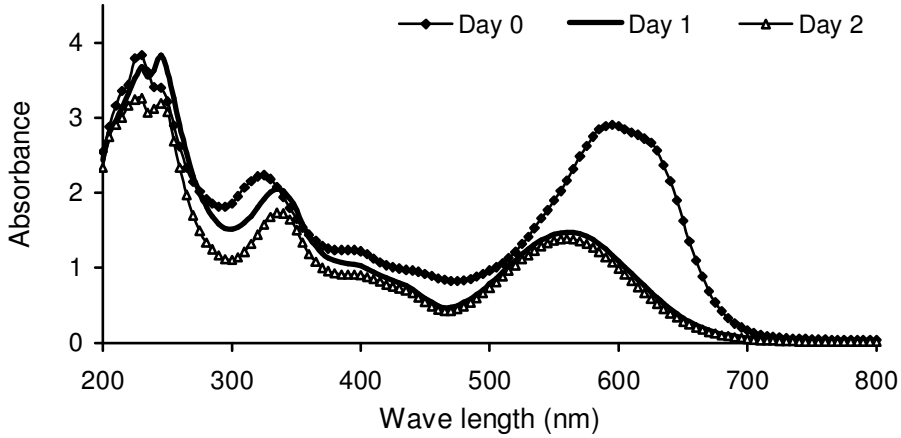

Figure 4. Typical shifting of absorbance peaks in a reactor with AB 10B with increase in culturing period (days).

Pearce et al., 2003; Tan et al., 2005; Van de Zee and Villaverde, 2005; Singh et al., 2007), the aromatic compound was degraded under biosulphidogenic conditions in this study. The degradation could be therefore a result of concerted effort of different organisms and factors, considering the microbial diversity and complexity nature of the reactors. Degradation of aromatic amines from other dyes is supported by the absence of sample recolourisation in single dye reactors, particularly that of RB 5 intermediates (Zille et al., 2005). It is therefore reasonable to assume that the observed decrease in visible and UV absorbance peaks signify degradation of dyes and their subsequent aromatic compounds. This is supported by absence of colour in the pellet fractions. The same applies for the Da Gama Textiles effluents in our previous studies (Mutambanengwe et al., 2007).

The spectral trend exhibited by AB 10B suggest either polymerisation of the aromatic amines after reduction of azo bonds or microbially-assisted changes in the functional groups leading to changes in the optical properties of the dye or a combination of the two. The trends were different from those observed in previous studies (Mutambanengwe et al., 2007), most probably because of differences in culture communities. Variations in culture compositions is supported by the observed rapid decolourisation of the anthraquinone RB 2 as opposed to 192 h in studies by Mutambanengwe et al. (2007) regardless of the point that anthraquinone dyes are the most recalcitrant due to the fused aromatic structure (Banat et al., 1996). Thus the use of non-defined environmental samples has a disadvantage of inconsistency and unpredictability of performance because of inherent variations

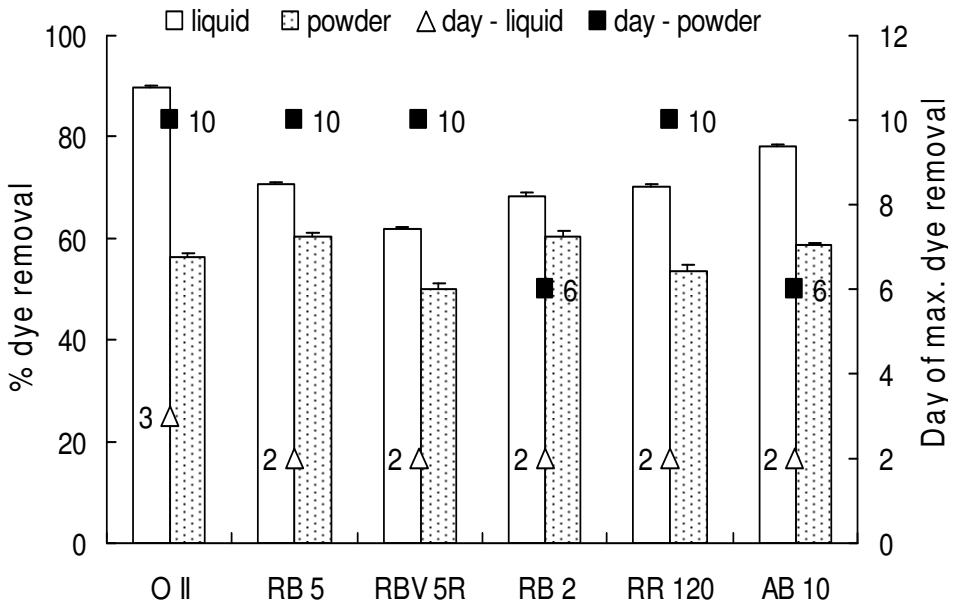

Figure 5. Percentage decolourisation of mixed commercial dyes in sulphidogenic reactors with different forms of inocula and the number of days taken to obtain maximum colour removal for each reactor set. The final concentration of each dye in the reactor was 100 mg/l. All values represent the means ± SD (n=3).

in microfloral and other factor compositions between samples.

Lower efficiencies in powder inoculated reactors could be due to structural changes in macromolecules during freeze drying. Changes in the microbial composition, after freeze drying, are supported by the differences in patterns of the absorption spectra of samples from the two sets of reactors with Da Gama Textiles dyes. Freeze drying of microorganisms is usually performed in the presence of cryoprotectants such as glycerol, saccharose and trehalose. Cryoprotectants were however not used in this study as it was assumed that they were inherent in the in situ cultures due to the complexity and diversity of components in the sludge and EBRU SRB culture. Although freeze drying is a convenient way of sample preservation, small volumes of the best performing SRB-sludge cultures were stored at 4°C for at least four months without significant loss in viability. Therefore, these small volumes can be used for back sloping when new reactors are set up without compromising degradation efficiency.

Absence of definite trends in the decolourisation of different dye structures is because of the non-specificity and extracellular nature of the decolourising processes (Russ et al., 2000; Stolz, 2001; Van de Zee and Villaverde 2005). In addition, sulphide and aromatic amines (1-amino-2-naphthol) reduce the azo bonds (Yoo et al., 2001; Méndez-Paz et al., 2005; Pandey et al., 2007). Consequently the size of azo dyes will not matter in the decolourisation.

Rate of dye degradation depends on the carbon source (Pandey et al., 2007). Therefore decreased efficiency in powder inoculated reactors can be attributed to this fact while dye toxicity and possible inhibition by non-sulphonated intermediates are the main reasons in mixed dye reactors. Failure of the freeze dried cultures, without a yeast extract powder; to degrade the dyes imply that the microorganisms could not use the dye as their sole carbon source unlike those reported by Singh et al. (2004).

Results from this study illustrated the synergistic effect of SRB and sludge microflora in dye degradation because BioSURE® SRB cultured in defined media cannot degrade aromatic amines (Mutambanengwe, 2007) while sulphonated aromatic amines are non-degradable under methanogenic conditions (Tan et al., 2005). Further support of the augmentation was observed by the improved decolourisation efficiency of 100 mg O II/l that was above 95% as opposed to 84% (Vijaykumar et al., 2007) within day 1. The SRB play a more significant role than the total sludge population in dye decolourisation (Yoo et al., 2001). This could be the case in this study considering that SRB produce sulphide that leads to azo dye reduction (Yoo et al., 2001; Méndez-Paz et al., 2005; Pandey et al., 2007).

More avenues of research and debate arise from this research. It maybe necessary to characterise the predominant bacterial species to enable control, consistency and predictability of the degradation processes. In consequence this will lead to standardisation of the effluent treatment process. From the industrial point of view it may be just effective to practise back sloping and bio-

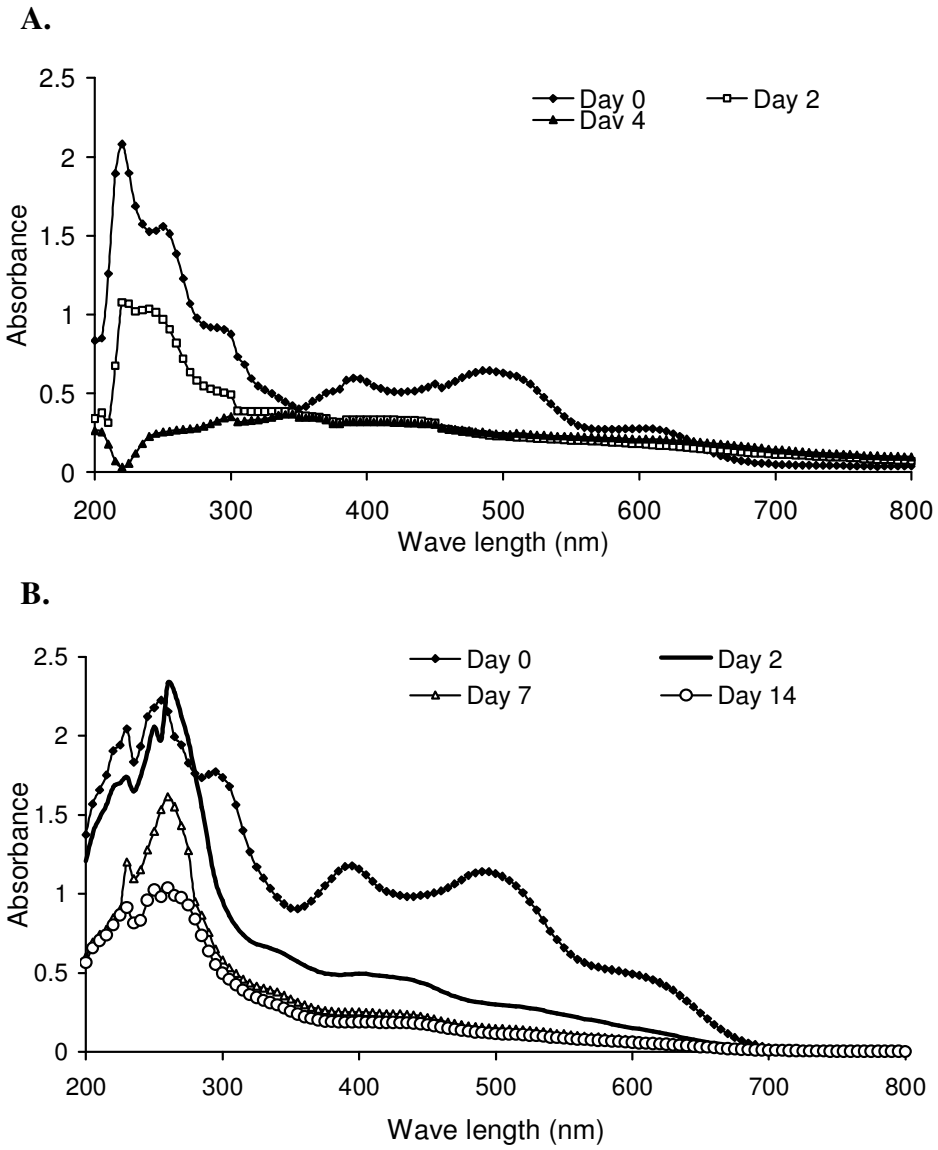

Figure 6. Absorption spectra illustrating decolourisation and degradation of a mixture of reactive dyes with silicates from Da Gama Textiles by the SRB-sludge liquid (A) and the powder (B) inoculated reactors.

remediate the textile effluent while minimising costs of specific species isolation and maintenance. Application of the study to more dyes and identification of end products of the dyes using mass spectrometry is required to confirm the fate of aromatic amines. Pathway description and mechanisms of aromatic amine cleavage need to be explored. Freeze drying may not be affecting the SRB population. Hence research is on going to determine this in order to try and use the powder as a source of SRB for inoculating the sludge.

Conclusions

The sulphonated aromatic amine and sulphanilic acid are degradable under biosulphidogenic conditions with a concerted effort of SRB and biodigester microflora and carbon. Use of BioSURE® SRB and biodigester sludge, doubling as a source of carbon and microflora, led to degradation of textile effluent, azo and anthraquinone dyes. While sludge is a cheap source of carbon, commercialisation of such a process requires determi-nation of the fate of the aromatic compounds to confirm safety of the resultant effluent. It is possible to use one waste material to treat another, positively contributing towards sustainable processes.

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