African Journal of Biotechnology Vol. 10 (4), pp. 584-588, 24 January, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.1683
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Decolourisation and degradation of reactive blue 2 by sulphate reducing bacteria (SRB) and zero valent iron in a biosulphidogenic reactor

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Accepted 11 February, 2010

This work was performed to determine the influence of heat treatment on sewage sludge and addition of zero valent iron (ZVI) on the degradation and decolourisation of an anthraquinone dye, reactive blue 2 (RB 2). A consortium of sulphate reducing bacteria (SRB) in a biosulphidogenic batch reactor with biodigester sludge was used. The latter supplied carbon and augmenting microorganisms. Reactors with heat treated sludge were outperformed by those with unheated sludge for the larger part of the reactor life span. A 75% decolourisation efficiency was achieved within 24 h of inoculation when 4 g ZVI/l were added in an SRB reactor with unheated sludge as opposed to 59% colour removal after four days in the same reactor without ZVI. However, decolourisation was also noted in the presence of ZVI alone, indicating existence of chemical reaction between ZVI and RB 2.

Key words: Decolourisation, degradation, reactive blue 2, zero valent iron, sulphate reducing bacteria, sulphidogenic.

INTRODUCTION

Textile industrial effluent accounts for the largest proportion of dye effluent pollution worldwide because the dye delivery process to the fabrics is not efficient with between 10 and 40% of the dye lost in effluent (Pearce et al., 2003). The coloured effluent in water has a number of undesirable effects such as the reduc-tion of aesthetic properties, decreased photosynthesis and gas solubility (Asad et al., 2007). Furthermore, when the dyes are transformed they can give rise to toxic and carcinogenic compounds (Pearce et al., 2003; Kandelbauer and Guebitz, 2005).

Continual dye improvement to develop shades that withstand harsh environmental conditions and to satisfy the ever-growing market has exacerbated the pollution problem by dye effluents. This is because of the need for matched continual improvements in treatment methods for such effluents. There exists a variety of treatment methods for dye effluent that are broadly classified as chemical, physical and biological. Chemical and physical methods generally lead to production of secondary pollution and are costly. Biological treatment methods are more desirable as they are environmentally friendly, do not produce secondary pollutants and have a higher possibility of wider application (Forgacs et al., 2004; Georgiou et al., 2004; Moosvi et al., 2005; Asgher et al., 2007; Togo et al., 2008). However, viable biological treatment using microorganisms requires cheap carbon sources.

Most of the bioremediation studies concentrated on azo dyes while the anthraquinone dyes have been lagging. Anthraquinone dyes are not readily removed from water and constitute the second largest proportion (approximately 15%) of dyes used in the textile industry (Banat et al., 1996; Lee et al., 2005; Lee et al., 2006; Iqbal, 2008). Current research is moving towards

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Abbreviations: SRB, sulphate reducing bacteria; **ZVI,** zero valent iron; **RB 2,** reactive blue 2 (anthraquinone dye).

applications of nano- and micro-sized particles, such as zero valent iron, in azo dye bioremediation (Lin et al., 2008; Fan et al., 2009). Zero valent iron particles are preferred because they can occur naturally in the environment, are environmentally friendly, cheap and reduce azo dyes and other pollutants (Bigg and Judd, 2000; Deng et al., 2000; Tratnyek and Johnson, 2006; Fan et al., 2009). Potential for the application of methanogenic sludge-driven sulphidogenic reactors in the remediation of a wide variety of dyes was demonstrated by Mutambanengwe et al. (2007) and Togo et al. (2008). Therefore the purpose of this work was to ascertain the applicability of the sulphidogenic reactor and ZVI to remediation of anthraquinone dye reactive blue 2 (RB 2).

MATERIALS AND METHODS

Reactor design and 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). Bioreactors were set up as described by Ngwenya and Whiteley (2006) and Togo et al. (2008). The experimental bioreactors consisted of untreated sludge, RB 2 (100 mg/l) and 10% (v/v) SRB. Two control reactors were set up: (i) heat treated (autoclaved, 121oC for 15 min) sludge, dye and no SRB, and (ii) heat treated sludge, dye and SRB. The former was mainly used to assess adsorption of the dye while the latter was to help in assessing the role played by the microflora from the sludge. The cultures were incubated at room temperature (25 ± 2°C) with gentle stirring.

Sampling and analyses

Samples were withdrawn from the bioreactors at 24 h intervals and analysed as described by Togo et al. (2008). The analyses entailed absorbance wavelength scans between 200 and 800 nm, at 5 nm intervals using a PowerWavex spectrophotometer with KC4 software (Analytical Diagnostics, Cape Town, South Africa). Absorbance at 590 nm was used to determine the concentration of RB 2 while that at 280 nm was used for aromatic compounds in the samples.

Effects of dye concentration and ZVI on RB 2 decolourisation and degradation

Different concentrations of RB 2 (50, 100, 125 and 150 mg/l) were added to untreated sludge, heat treated sludge and controls, incubated, sampled and analysed as described above to determine the best dye concentration for subsequent experiments.

Zero valent iron ranging from 2 to 8 g/l were added to different sulphidogenic reactor set ups to determine their effects on the decolourisation of RB 2 (100 mg/l). In addition to the previously described controls, one with double distilled water, ZVI and 100 mg RB 2/l was prepared.

RESULTS

Effects of heat treatment and dye concentration

Figure 1 shows typical changes in absorbance peaks for the sulphidogenic degradation and decolourisation of RB 2 (100 mg/l) by SRB grown in untreated sludge. There was a significant ($P < 0.05$) decrease in the absorbance peaks in the UV range and at 590 nm throughout the reactor operation period. A 59% decrease in colour intensity was observed between inoculation (Figure 1, day 0) and day 4 that continued until day 12 (95%; Figure 2), after which no further significant decrease was observed (results not shown). Thus subsequent experiments were terminated on the 12th day. There was slow decolourisation in the reactor with autoclaved sludge (Figure 2) with the non autoclaved one exhibiting significantly higher performance between days 1 and 9, exclusive. No decolourisation of RB 2 was observed in the control flask without inoculum using heat treated sludge.

Decolourisation efficiency was directly proportional to dye concentration from 50 to 100 mg RB 2/l, after which it dropped significantly ($P < 0.05$) at 200 mg RB 2/l (Figure 3).

Effect of ZVI

Decolourisation efficiency increased with ZVI concentration up to 4 g/l, which resulted in 75% colour removal within 24 h, as opposed to 59% in four days (Figure 4). Interestingly, a 78% colour intensity removal was observed after two days in a reactor with heat treated sludge and SRB and there was a degree of colour removal (44%) in the control that had dye, ZVI and distilled water.

DISCUSSION

Differences in initial performances between heat treated and untreated reactors highlighted the importance of microbial consortium supplied by sludge in the degradation of the dye. This becomes an advantage as the pre-treatment will not be a necessary step for best reactor performance. Although the heat treated sludge alone could not remove the dye, it is reasonable to say that the autoclaving may have destroyed the useful microorganisms for dye degradation but may not have necessarily rendered the sludge sterile. This is because the time and temperature used may not be sufficient to get rid of all the microbial life due to the viscosity and complex composition of the sludge. Unautoclaved sludge alone has been noted to degrade the dyes but to a less extend when compared to that with SRB (Togo et al., 2008).

The type and concentrations of dyes, sludge source and microbial concentration have been suggested to affect decolourisation (Dos Santos et al., 2007). Failure by the cultures to decolourise dye below 50 mg/l imply that there is a threshold level at which the microbial degrading mechanisms are stimulated and above which (e.g. 200 mg RB2/l) are inhibited. Mutambanengwe et al. (2007)

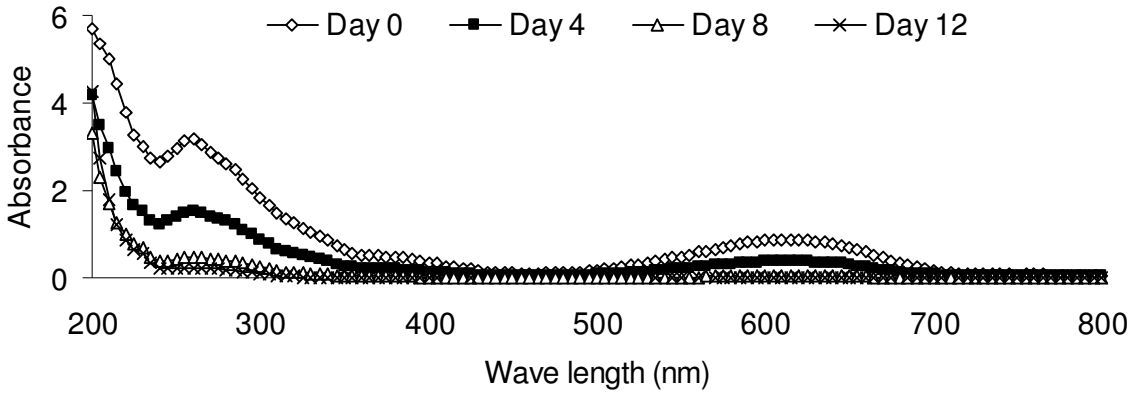

Figure 1. Typical changes in absorbance peaks monitored in a bioreactor for the anaerobic degradation and decolourisation of Reactive Blue 2 (100 mg/l) by sulphate reducing bacteria in untreated sewage sludge.

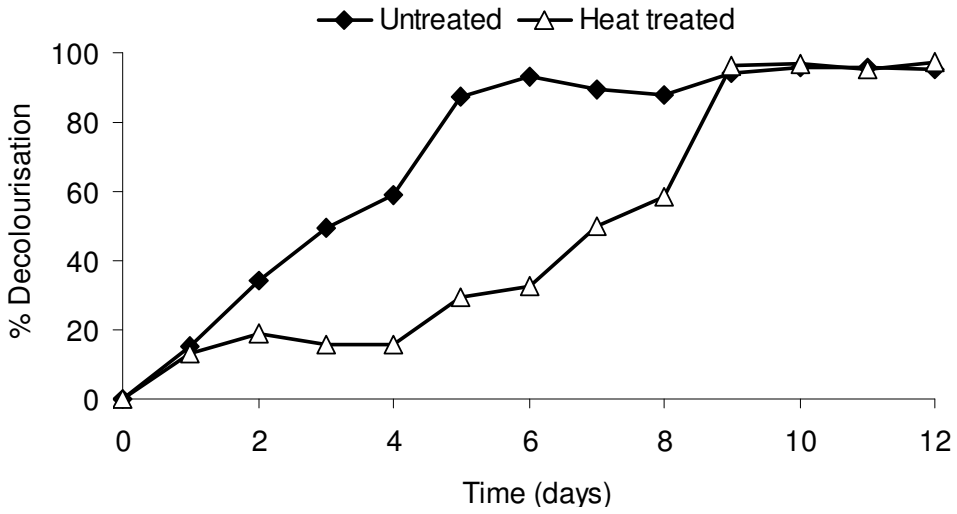

Figure 2. Percent decolourisation of Reactive Blue 2 (100 mg/l) dye by sulphate reducing bacteria grown in untreated sludge and heat treated sludge over 12 days.

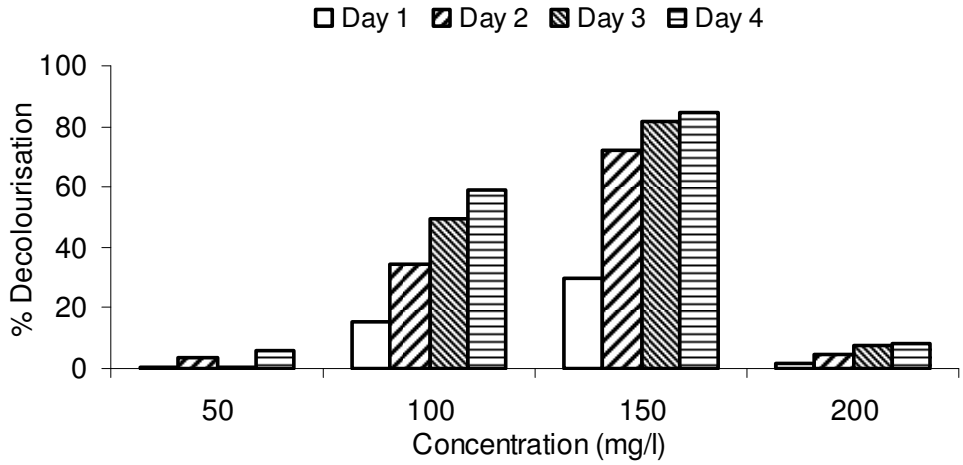

Figure 3. Effects of different concentration of Reactive Blue 2 on decolourisation by sulphate reducing bacteria in untreated sludge.

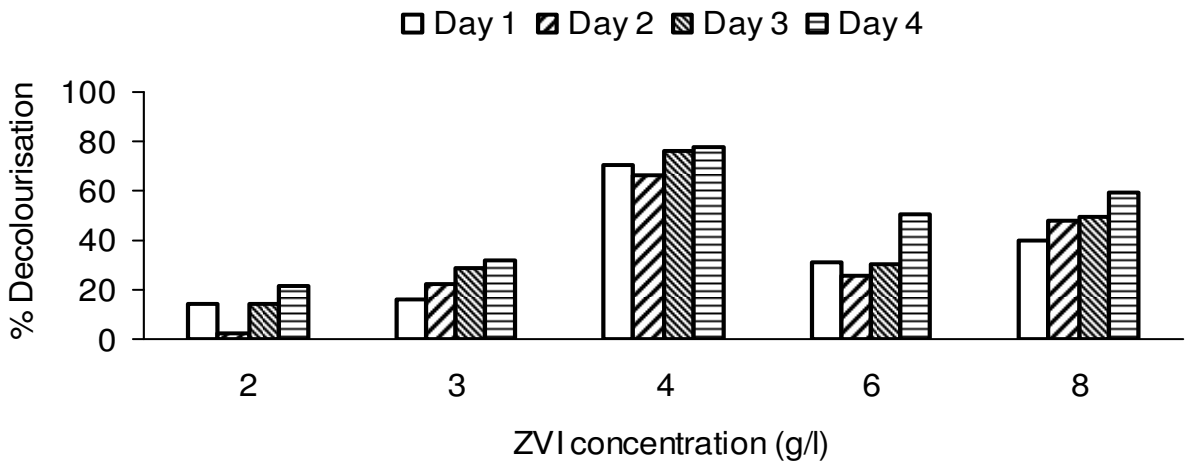

Figure 4. Effect of zero valent iron (ZVI) concentration on decolourisation of Reactive Blue 2 (100 mg/l) with SRB's in untreated sludge.

noted an induction of azo dye decolourisation in the SRB while Ramya et al. (2007) observed toxicity at elevated concentration of RB2 (200 mg/l) on the Aspergillus sp. These stimulating and toxic levels have an important bearing on reactor performance when treating the industrial effluent. Consequently, there will be a need for the determination of initial dye concentrations in the effluent and necessary adjustments before the bioremediation processes.

The ZVI enhanced the dye decolourisation and degradation by the microbial culture. While the ZVI have been described to reduce the azo bond in azo dye decolourisation (Lin et al., 2008; Fan et al., 2009), the mechanisms of decolourisation on anthraquinone dye are supposedly different. Other mechanisms of ZVI action on dyes include microelectrolysis, and adsorption and flocculation (Lin et al., 2008; Fan et al., 2009). Microelectrolysis is more likely, in this work, since there were no flocculants observed in the control with the distilled water. Furthermore, the results suggest that the proposed microelectrolysis mechanism is enhanced by factors (biotic or abiotic) in the reactors with sludge and SRB. This opens avenues for further research to ascertain these processes and mechanisms. A decrease in decolourisation at above 4 mg ZVI/l suggests toxicity or inhibition of microbial activities.

While the bioremediation of textile dyes and subsequent application of the ZVI have been largely focused on azo dyes, this study provides hope for application of these processes to other recalcitrant non azo dyes. Thus further research can enhance understanding, control and application of this process.

ACKNOWLEDGEMENT

The authors wish to extend their gratitude to Rhodes University, South Africa, for funding.

REFERENCES

Asad S, Amoozegar MA, Pourbabaee AA, Sarbolouki MN, Dastgheib SMM (2007). Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. Bioresour. Technol. 98: 2082-2088.

Asgher M, Bhatti HN, Shah SAH, Asad EMJ, Legge RL (2007). Decolorization potential of mixed microbial consortia for reactive and disperse textile dyestuffs. Biodegradation, 18: 311-316.

Banat IM, Nigam P, Singh D, Marchant R (1996). Microbial decolorization of textile-dye-containing effluents: a review. Bioresour. Technol. 58: 217-227.

Bigg T, Judd SJ (2000). Zero-valent iron for water treatment. Environ. Technol. 21: 661-670.

Deng N, Luo F, Wu F, Xiao M, Wu X (2000). Decolorization of aqueous reactive dye solutions in the UV/Feo system. Water Res. 34: 2408-2411.

Dos Santos AB, Cervantes FJ, Van Lier JB (2007). Review paper on current technologies for decolourisation of textile wastewaters: Perspectives for anaerobic biotechnology. Bioresour. Technol. 98: 2369-2385.

Fan J, Guo Y, Wang J, Fan M (2009). Rapid decolorization of azo dye methyl orange in aqueous solution by nanoscale zerovalent iron particles. J. Hazard. Mater. 166: 904-910.

Forgacs E, Cserháti T, Oros G (2004). Removal of synthetic dyes from wastewaters: a review. Environ. Int. 30: 953-971.

Georgiou D, Metallinou C, Aivasidis A, Voudrias E, Gimouhopoulos K (2004). Decolorization of azo-reactive dyes and cotton-textile wastewater using anaerobic digestion and acetate-consuming bacteria. Biochem. Eng. J. 19: 5-79.

Iqbal M (2008). Textile Dyes. Rahaber publishers, Karachi, Pakstan.

Kandelbauer A, Guebitz GM (2005). Bioremediation for the decolorization of textile dyes - a review. In: Lichtfouse E, Schwarzbauer J, Robert D (eds). Environmental Chemistry: Green Chemistry and Pollutants in Ecosystems. Springer-Verlag, Berlin, pp. 269-288.

Lee YH, Matthews RD, Pavlostathis SG (2005). Anaerobic biodecolorization of textile reactive anthraquinone and phthalocyanine dyebaths under hypersaline conditions. Water Sci. Technol. 52: 377-383.

Lee YH, Matthews RD, Pavlostathis SG (2006). Biological decolorization of reactive anthraquinone and phthalocyanine dyes under various oxidation-reduction conditions. Water Environ. Res. 78: 156-169.

Lin YT, Weng CH, Chen FY (2008). Effective removal of AB24 dye by nano/micro-size zero-valent iron. Sep. Purif. Technol. 64: 26-30.

Moosvi S, Keharia H, Madamwar D (2005). Decolorization of textile dye

Reactive Violet 5 by a newly isolated consortium RVM 11. 1. World J. Microbiol. Biotechnol. 21: 667-672.
Mutabanengwe CCZ, Togo CA, Whiteley CG (2007). Decolourisation and degradation of textile dyes with biosulphidogenic hydrogenases. Biotechnol. Prog. 23: 1095-1100.
Ngwenya N, Whiteley CG, (2006). Recovery of Rhodium (III) from solutions and industrisl wastewaters by a sulphate-reducing bacteria consortium. Biotechnol. Prog. 22: 1604-1611.
Pearce CI, Lloyd JR, Guthrie JT (2003). The removal of colour from textile wastewater using whole bacterial cells: a review. Dyes Pigments. 58: 179-196.
Ramya M, Anusha B, Kalavathy S, Devilaksmi S (2007). Biodecolorization and biodegradation of Reactive Blue by *Aspergillus* sp. Afr. J. Biotechnol. 6: 1441-1445.
Togo CA, Mutabanengwe CCZ, Whiteley CG (2008). Decolourisation and degradation of textile dyes using a sulphate reducing bacteria (SRB)- biodigester microflora co-culture. Afr. J. Biotechnol. 7: 114-121.
Tratnyek PG, Johnson RL (2006). Nanotechnologies for environmental cleanup. Nano. Today, 1: 44-48.