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FACTORS AFFECTING DEGRADATION AND DECOLOURISATION OF AN AZO DYE, METHYL RED, IN FIXED FILM BIOREACTORS

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ABSTRACT

The effects of texture, aeration, inorganic (PO_4-P and NO_3-N) and organic (milk whey) nutrients are reported here on degradation and decolourisation of methyl red. The effects have been quantified by comparing UV-visible spectra of untreated and treated 100ppm of the dye in two types of fixed film bioreactors that differed in composition of their solid matrix (one containing gravel only, gravel bed; while the other has gravel + coarse river sand, mixed bed). Methyl red degradation was better in gravel bed reactors in comparison to mixed bed reactors. Aeration improved its degradation in both the types of reactors. Nutrients favoured reduction in bandwidth of spectra in near UV region (hence degradation of benzenoid compounds) in outflows from gravel (non-aerated and aerated) and mixed bed (aerated) bioreactors, while an opposite trend was noted for their OD values in far UV and visible region responsible for the colour. Mixed bed (non-aerated) bioreactor was, however, the exception, where inorganic nutrients, especially NO_3-N improved reduction of bandwidth as well as OD values in the spectra of outflows.

INTRODUCTION

Today, bioremediation of xenobiotics has received considerable attention of scientists, since it mineralizes most of them eliminating health and ecological effects including future environmental liabilities. Besides, wherever applicable, it proves to be more economic than other technologies. During recent years microbial decolourisation and degradation of dyes have been detailed using mostly monoculture of bacterial and fungal species, whereas similar studies using either their consortia or mixed cultures of both fungi and bacteria are few (Sharma et al. 2004). In the present communication, we report optimization of degradation and decolourisation of an azo dye methyl red by a mixed culture of bacteria and fungi in the fixed film bioreactors.

MATERIALS AND METHODS

The gravel (length = 2-3 cm; width = 1.0-1.5 cm) and mixed bed {mixture (3:1) of gravel and coarse river sand} bioreactors were developed in 15 L plastic buckets, as described in detail elsewhere (Kumar et al. 2006). The interstitial volume of these bioreactors was kept constant (3 L). One bioreactor, each of gravel and mixed bed type, was aerated by inserting a tube through plastic pipe (Figs. 1a, b). Thus, four types of bioreactors, two each of gravel and mixed bed (aerated and non-aerated), were used during the present study.

The microbial community (comprising of two species of *Bacillus* and six of fungi *Aspergillus flavus, A. niger, A. fumigatus, Cladosporium cladosporioides, Trichoderma harzianum* and *Fusarium oxysporum*), isolated from the textile wastewater polluted habitats in Sanganer, Jaipur, was allowed to naturalize in the bioreactors for about 15 days in the greenhouse (temperature: maximum = 36.4± 3.3°C; minimum = 26.8±2°C; relative humidity: maximum = 65 ± 18%; minimum = 25 ± 11%).

During this period, bioreactors were fed with 3 L/day of 100ppm methyl red, prepared according to Kumar et al. (2006), at a constant rate for 12 h through the central pipe in the form of drips into the small bowl of bioreactor using a medical drip set. These bioreactors had vertical upflow hydraulics due to rising up of wastewater in the bucket during its loading (Figs.1a, b). The contact period between wastewater and biofilm in the bioreactor matrix (retention time) was 1day. Four type of inflows, used during optimization of methyl red degradation, were: (1) 100ppm methyl red (Treatment "A"); (2) 100ppm methyl red + 5ppm PO_4-P (Treatment "B"); (3) 100ppm methyl red + 5ppm PO_4-P + 50ppm NO_3-N (Treatment "C") and (4) 100ppm methyl red + 5ppm PO_4-P + 50ppm NO_3-N + 30 mL/d milk whey (Treatment "D"). Absorption spectra (UV-visible range) of both, inflow and centrifuged outflow of bioreactors, were scanned with the help of a UV-visible spectrophotometer (Model: Cintra 20- GBC). In comparison to inflow, percent reduction in the bandwidth and absorbance value (OD) of bioreactor outflows were calculated.

RESULTS AND DISCUSSION

The spectra of untreated methyl red (inflow) comprised of large number of intense peaks very close to each forming two distinct bands in UV region, hereafter referred to as Band-1 (B_1 200-295nm) and Band-2 (B_2 >295-340nm). Methyl red formed a distinct peak at 430nm (visible region). Another peak, noted in the visible region, was at 500nm (Fig. 2).

A comparison of spectra of four bioreactor outflows in 'A to D' treatments with that of inflow (untreated methyl red) revealed higher degradation of methyl red in the gravel bed reactors in comparison to mixed bed bioreactors, more particularly under aerobic conditions (Figs. 3-6). The bioreactor performance in 'A-D' treatments varied greatly with respect to percent reduction in bandwidth and OD values in comparison to inflow and, therefore, presented separately.

Reduction in Bandwidth

B1 band was conspicuous in the spectra of mixed bed bioreactor outflows while it was diffused in gravel bed bioreactor outflows indicating poor performance of the former. Percent reductions in B_1 bandwidth along with important findings are summarized below.

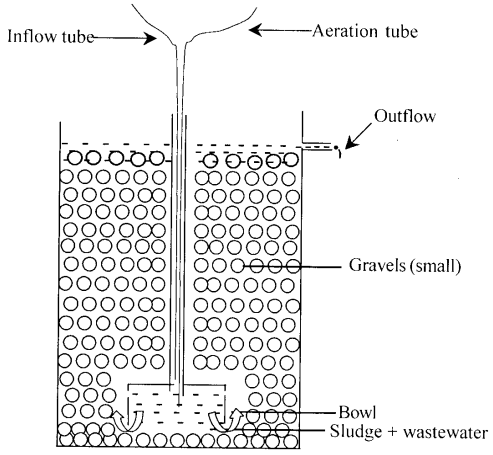

Fig. 1a: Gravel bed aerated bioreactor.

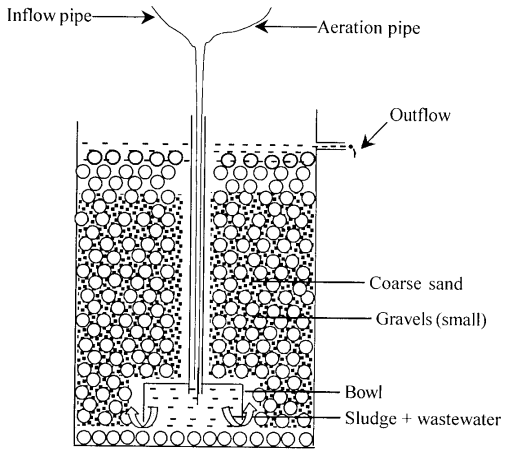

Fig. 1b: Mixed bed aerated bioreactor.

Fig. 2: UV-visible spectrum of 100 ppm methyl red (inflow).

1. Gravel bed (non-aerated): B = C ? A > D (39-53%)
2. Gravel bed (aerated): A > B > D ? C (20-50%)
3. Mixed bed (non-aerated): C > B > A > D (Nil-76%)
4. Mixed bed (aerated): B ? A ? D > C (4-47%)

- Supplementation of phosphorus improved performance of all the four bioreactors (Fig. 4).
- NO_3-N addition decreased efficiency of aerated reactors while it improved the same in non-aerated reactors (Fig. 5).
- Milk whey decreased adverse effect of NO_3-N only in aerated gravel bed reactor while it suppressed the efficiency of aerated mixed bed and non-aerated bioreactors (Fig. 6).

B_2 band was completely absent in the spectra of all the four bioreactors in treatments 'C' and 'D', whereas it was dull in treatment 'A' and 'B'(Figs. 3, 4, 5, 6). Thus, nutrients, especially nitrate and milk whey, increased microbial activities helping in degradation of compound/s responsible for this band. Percent reductions in B_2 bandwidth along with important findings are as below.

1. Gravel bed (non-aerated): C = D > A > B (40-100%)
2. Gravel bed (aerated): B = C = D > A (58-100%)
3. Mixed bed (non-aerated): C = D > B > A (38-100%)
4. Mixed bed (aerated): C = D > A = B (50-100%)

- Performance of gravel bed and mixed bed was almost similar.
- Addition of inorganic and organic nutrients improved performance of all the four bioreactors, being excellent in treatment 'C'.

Percent Reduction in Optical Density

No peak was found in bioreactor outflows at 430nm indicating complete decolourisation of methyl red. Four distinct peaks were, however, observed in bioreactor outflows; two each in UV (250 and 310nm) and visible region (410 and 500nm). Their OD values were compared with that of untreated methyl red (inflow) and important findings are given below.

1. At 250 nm

a. Gravel bed (non-aerated): A > B > C = D (30-51%)
b. Gravel bed (aerated): A > B > C ? D (25-42%)
c. Mixed bed (non-aerated): C > B > D > A (10-70%)
d. Mixed bed (aerated): A > B ? C > D (9-42%)

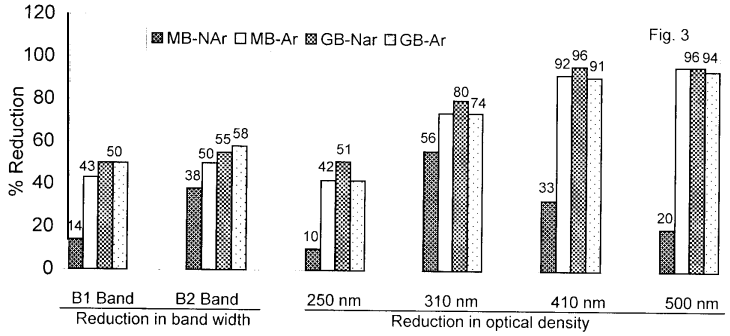

Fig. 3: Percent reduction in bandwidth and optical density in the spectra of bioreactor outflows in treatment 'A'.

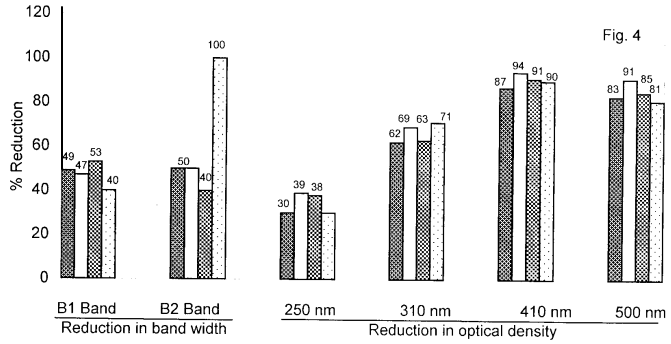

Fig. 4: Percent reduction in bandwidth and optical density in the spectra of bioreactor outflows in treatment 'B'.

2. At 310 nm
a. Gravel bed (non-aerated): A > C ? B ? D (59-80%)
b. Gravel bed (aerated): A ? B > C = D (60-74%)
c. Mixed bed (non-aerated): C > B > D ? A (56-87%)
d. Mixed bed (aerated): A > B ? C > D (55-74%)
3. At 410 nm
a. Gravel bed (non-aerated): A > B > D > C (73-96%)
b. Gravel bed (aerated): A > B > D > C (64-91%)
c. Mixed bed (non-aerated): C > B ? D > A (33-94%)
d. Mixed bed (aerated): B ? A > C > D (79-94%)
4. At 500 nm
a. Gravel bed (non-aerated): A > B > D > C (72-96%)
b. Gravel bed (aerated): A > D > C ? B (81-94%)
c. Mixed bed (non-aerated): C > B ? D > A (20-89%)
d. Mixed bed (aerated): A > B > D > C (76-96%)

In comparison to treatment 'A' (except non-aerated mixed bed bioreactor), percent reduction in OD values (UV region: 250 and 310 nm; visible region: 410 and 500 nm) decreased in treatments 'B, C and D' suggesting nutrient addition to inflow usually has unfavorable effects on other compounds present as impurities in methyl red (Figs. 3, 4, 5, 6).

Fig. 5: Percent reduction in bandwidth and optical density in the spectra of bioreactor outflows in treatment 'C'.

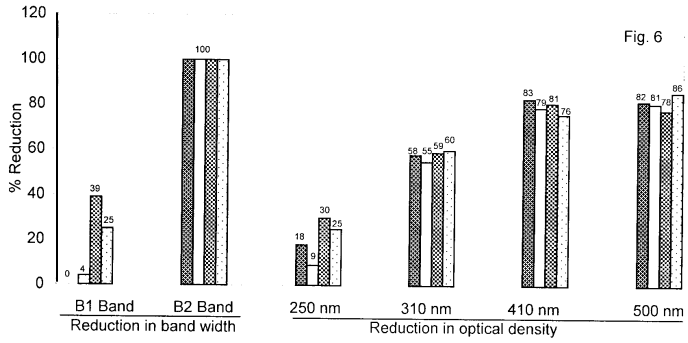

Fig. 6: Percent reduction in bandwidth and optical density in the spectra of bioreactor outflows in treatment 'D'.

The electrons in saturated organic compounds having single bonds absorb in vacuum UV region (<180 nm); whereas those present in unsaturated compounds, at the longer UV and visible region (390-760 nm). B_1 band (200-295 nm) is due to occurrence of compound/s having benzenoid ring. Whereas B_2 band (>295-340 nm) and peaks in the visible region of the spectra indicate presence of highly unsaturated compound/s having double and triple bonds, including conjugated compound/s. GC mass studies of microbially degraded methyl red has confirmed presence of benzoic acid and cyclotrisiloxane (unpublished data). Wong & Yuen (1996), however, reported microbial degradation (by *Acetobacter liquifaciens*) of methyl red into two colorless compounds namely 2-amino benzoic acid and N,N-dimethyl-p-phenylene diamine.

During the present study, percent reduction in band width (38-100%) and OD values (55-87%) was higher in far region (> 295-340nm - B2 band) as compared to near UV region (200-295nm - B1 band; bandwidth = Nil - 76%; OD values = 9-70%). Also OD values decreased greater in the visible region (20-96%) than UV region (9-87%). Thus, microbial degradation of relatively more unsaturated compounds was faster and compounds having aromatic ring degrade slowly.

Interestingly, as noted earlier, nutrients often favouring reduction in bandwidth, however, have adverse effects on reduction in OD values, except for mixed bed non-aerated bioreactor. Vijaya & Sandhya (2003) also reported an increase in the efficiency of mixed microbial culture to degrade and decolourise methyl red, when provided with glucose and PO_4-P. Perusal of literature has revealed

that nutrients favoring degradation of dyes by bacteria, however, discourage the same in case of fungi (Sharma et al. 2004). It seems that fungi played major role in methyl red degradation in the gravel bed (aerobic and anaerobic) and mixed bed (aerobic) bioreactors, whereas bacteria in the mixed bed (non-aerated) bioreactor. The bacterial species present in the bioreactors seem to be facultative anaerobes, as they degrade dye under both aerobic and anaerobic conditions, being rapid in the former. Other workers also reported faster degradation and decolourisation of methyl red aerobically (Wong & Yuen 1996, Moutaouakkil et al. 2004). The positive role of nitrate in the mixed bed anaerobic bioreactor is possibly due to increased availability of electron acceptors for bacteria. It, however, has adverse effects on decolourisation in gravel (aerated & non-aerated) as well as mixed bed (aerated) bioreactors, which improved a little after addition of milk whey containing lactose as soft carbon source. Wuhrmann et al. (1980) also reported inhibition of azo dyes reduction in the activated sludge, and hence, poor decolourisation, which was restored only after denitrification. It is likely that nitrate reacted with degradation products of methyl red forming more toxic compound/s that suppress methyl red degradation. The availability of lactose in milk whey perhaps favoured microbial growth that increased oxygen consumption, which perhaps resulted in anoxic pockets even in the aerated bioreactors. Under such conditions, toxic nitrate compound/s might have acted as electron acceptor forming colourless amine/s that improved decolourisation.

CONCLUSION

Present investigation has revealed better degradation as well as decolourisation of methyl red in the gravel bed bioreactors under aerobic conditions. Although nutrients adversely affected decolourisation, they favoured degradation of toxic benzenoid compound/s and also lower COD load in the bioreactor outflows (Kumar et al. 2006), hence improved overall performance of bioreactors.

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