Bioremediation of Textile dyes by Fungal-Bacterial Biofilms

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Abstract—Textile waste pollutants are the most polluting waste water and their treatment is greatly challenging for their safe discard. Microbial communities have potential ability to decolorize synthetic commercial dyes used for textile dyeing. Therefore, this study was aimed to develop potential dye degrading microbial biofilms from endophytic fungi and soil bacteria. Endophytic fungi were isolated from the leaves of Eleusine indica (Linn) and bacteria were isolated from soil samples obtained near textile effluent dumping site in Biyagama Industrial zone, Sri Lanka. Biofilms were developed after screening the fungal and bacterial isolates with Malachite green and Nigrosin disodium dyes separately and the decolorization assay was performed for biofilms along with monocultures to evaluate their ability for dye decolorization. The highest significant (P< 0.05) decolorization percentages were observed by Tricoderma harzianum (F2) and Bacillus subtilis (B1) for both dyes. All the biofilm combinations showed higher decolorization percentage than that of the monocultures. Thus, it can be concluded that the biofilms can be used as an efficient biological tool for textile effluent treatment.

Keywords—Biofilms, Textile dyes, Bioremediation, Decolorization.

I. INTRODUCTION

Environmental pollution has been recognized as one of the major hazards of the modern world. Due to the rapid industrialization and urbanization, various types of chemicals manufactured and applied in day to day life (Moorthi et al., 2007). Among industrial effluents, wastewater from textile industries is one of the most difficult to be treated since the dyes used are usually synthetic and contain complex aromatic molecular structures (Padmesh et al., 2005). During manufacturing and processing, approximately 10-15% of the dye is lost and released directly as wastewater that accumulates in the environment (Elisangela et al., 2009). Unfortunately, most of these dyes persist in the environment due to their high stability to physical factors like light and temperature (Drumond Chequer et al., 2013). The disposal of such reactive dyes into the environment even at very low concentrations causes considerable damage as they intensely affect the photosynthetic activity of aquatic organisms by limiting the light penetration and their breakdown products may be toxic to them (Wang et al., 2009). Nigrosin disodium and Malachite green are good examples which are used in many fields and also have a wide variety of toxicological effects (Zhang et al., 1995). Both dyes have been reported for their negative impact on living cells and organisms (Culp and Beland, 1996). Because of the toxicity to major microorganisms, its presence in wastewaters makes difficult the biodegradation.

Commonly applied treatment methods for the removal of colored effluents consist of integrated processes involving various combinations of biological, physical and chemical decolorization methods (Galindo and Kalt, 1999; Azbar et al., 2004). However, chemical and physical methods for the treatment of dye wastewater are not widely applied to textile industries because of the costs and disposal problems (Yang et al., 2009). The available conventional wastewater treatment systems are unable to completely remove the recalcitrant dyes and other organic residues from such effluents. Therefore, there is an immense effort to develop a cost-effective and ecofriendly alternative to conventional waste treatment methods.

Among all the technologies, bioremediation of textile dye containing effluents using microorganisms which are capable of degrading dye, is still seen as an attractive alternative solution (Shahid et al., 2013). Although, bacterial, fungal and algal species have the ability to adsorb and/or degrade dyes (Stolz, 2001; Don Santoz, 2007), the moderate decolorization rate and complexity of textile effluents limit the performance of microorganisms in bioremediation (Banat et al., 1996; Elisangela et al., 2009; Wang et al., 2009). Further, it has been observed that the biological treatment along with the current conventional microbiological treatment processes have not been not proved satisfactory for color elimination (Robison et al., 2001). Therefore, bioremediation through microbial biofilms is emerging as one of the promising approaches due to its power of degrading various environmental pollutants, high tolerance towards harsh environment, low-cost and environmentally friendliness (Mitra and Mukhopadhyay, 2015). Thus, present study focused on investigating the potential of fungal bacterial biofilms to decolorize the selected textile dye effluent.
II.  METHOD

2.1 Isolation of endophytic fungi and soil bacteria

Endophytic fungi were isolated from the leaves of *Eleusine indica* (Linn.), a plant grown near a dumping site of textile effluents in Biyagama Industrial zone, Sri Lanka. The leaves of the *Eleusine indica* (Linn.) were cut into smaller pieces and surface sterilized with 70% ethanol, 1% chlorox, and rinsed with sterilized water. The PDA plates inoculated with the surface sterilized leaf parts were incubated at 25°C and observed for growth. The pure fungal cultures were transferred periodically onto fresh Potato Dextrose Agar (PDA) plates. Bacteria were isolated from soil samples obtained from the same location near the textile effluent dumping site. The pure bacterial cultures were transferred periodically onto fresh Nutrient Agar (NA) plates.

2.2 Screening of microbial isolates on dye decolorization

Malachite green (4-[4-(dimethylaminophenyl) phenyl-methyl]-N, N-dimethylaniline) Molecular formula: C23H25CIN2 and Nigrosin disodium; 4-amino-3-[4-nitrophenyl) diazenyl]- 5-oxo-6-(phenylhydrazinylidene) naphthalene-2, 7-disulfonate, Molecular formula: (C22H14N6Na2O9S2), dyes in textile industries were used to screen the fungal and bacterial isolates for their decolorization ability. Fungal and bacterial isolates were inoculated into Erlenmeyer flasks containing 100 ml of nutrient broth and potato dextrose broth supplemented with filter sterilized 0.5g/L of Malachite green and Nigrosin disodium dyes separately. Dyes containing uninoculated medium served as control and all assays were performed in triplicate. Inoculated medium and control were incubated at room temperature (25°C) for five days on rotary shaker at 150 rpm. Growth and dye decolorization were noted every day. After incubation, samples were withdrawn, centrifuged at 5000 rpm at 4°C for 10 minutes using cooling centrifuge (REMI C-24BL). Absorbance reading of the cell free supernatant was measured using Spectrophotometer at 620 nm for Malachite green and at 600 nm for Nigrosin disodium dyes. The decolorizing activity was expressed in terms of percent decolorization which was determined by using the following formula;

\[
\text{Percentage of decolorization} = \left( \frac{\text{Initial Absorbance - Final absorbance}}{\text{Initial Absorbance}} \right) \times 100
\]

2.3 Formation of the biofilms

The screened fungal (F1 and F2) and bacterial (B2, B3 and B6) isolates having the dye decolorization ability were inoculated into Potato dextrose and Nutrient broths separately and incubated at 29±1 °C for 10 days and at 25± 1°C for 3 days respectively. Fungal- bacterial biofilms were developed by combining the fungal and bacterial isolates grown in potato dextrose and nutrient broths and incubated at room temperature with continuous shaking for five days. The strength of the attachment of the biofilm was continuously observed under optical microscope model BX43F by staining with Methylene blue. Biofilms which had the best attachments (F2B3, F2B2B3, F2B2) and their mono cultures were selected to analyze their efficiency of heavy metal absorption in the liquid media.

2.4 Dye decolorization assay

Ten milliliters of five-day old biofilms and mono cultures were inoculated into series of 250ml Erlenmeyer’s flask containing 100ml of Potato Dextrose Broth (PDB) and Nutrient Broth (NB) mixture added with filter sterilized 0.5g/L of Malachite green and Nigrosin disodium dyes separately. The inoculated media were incubated on rotary shaker at 150 rpm speed at room temperature 27°C for 10 days with control contained medium having dye without biofilm and monoculture inoculation and all assays were performed in triplicate. During the incubation, 5 ml of samples were withdrawn at two days’ time intervals and centrifuged at 5000 rpm at 4°C for 10 minutes using cooling centrifuge (REMI C-24BL). Absorbance reading of the cell free supernatant was measured using Spectrophotometer at 620 nm for Malachite green and at 600 nm for Nigrosin disodium dyes and the decolorizing activity was expressed in terms of percent decolorization using the formula mentioned above.

2.5 Statistical analysis

Values reported in this paper are the means ± Standard Deviation. The decolorization capacity of each isolate was studied by one-way ANOVA followed by post-Hoc multiple comparisons by Duncan’s method using Minitab16 software package.

2.6 Molecular identification of microbial components in the responsive biofilm for the dye decolorization

Genomic DNA of the fungal component of the biofilm was extracted from 5-day old fungi cultures grown on plates using thermolysis method (Zhang et al., 2010). Fungal DNA was amplified using universal primers of fungal DNA ITS1 (5’–TCC GTA GGT AAG CTC GCG G-3’) and ITS4 (5’–TCC OCT TAT TGA TAT OC-3’) (White et al., 1990). PCR products were purified and were sent for sequencing and obtained sequences were compared with the other related sequences using BLAST search in GenBank (NCBI) (Liu et al., 2000). Identification of the bacterial component in the responsive biofilm was done through 16S rDNA sequence analysis. The genomic DNA of each biofilm-forming isolate grown in TSB for 24 to 48 hours was extracted using ZR Fungal/Bacterial DNA Kit™ (Zymo Research California USA) according to the manufacturer’s protocol. Polymerase Chain Reaction (PCR) procedure
was done using primers 11F (5’GTTTGATCMTGGCTCAG 3’) and 1492R (5’TACGGACTCACCTTGTTACGACTT3’) with 1 µL of undiluted genomic DNA extract as template. Agarose gel electrophoresis was employed to detect amplification of the 16S rDNA of the biofilm-forming isolates. The amplified products were sequenced at the Macrogen Sequencing facility in Korea and sequences were compared with those stored in the Genbank databases of the National Center for Biotechnology Information available on-line using pairwise alignment or BLAST algorithm.

III. RESULTS

3.1 Screening of fungal and bacterial isolates on dye decolorization

All the fungal isolates showed decolorization of both Malachite green and Nigrosin disodium dyes (Fig.1a). The results revealed that fungal isolate F2 and F3 showed higher decolorization percentage for both dyes than that of the other fungal isolates. Out of all fungal isolates, the highest significant (P< 0.05) decolorization percentage for both Malachite green (47± 0.762) and Nigrosin disodium (33 ± 0.845) was observed from F2 fungal isolate. All the bacterial isolates showed decolorization of both dyes except B5 and B6 (Fig.1b). Further, bacterial
isolates B1 and B2 showed higher decolorization percentage for both dyes than that of the other bacterial isolates. The highest significant (P< 0.05) decolorization percentage for Malachite green (22.3± 0.435) and the highest decolorization percentage for Nigrosin disodium (23.7 ± 0.612) was observed from B1 bacterial isolate. Decolorization was not recorded from B6 isolate for both dyes. It was observed that all the fungal isolates decolorized more Malachite green dye than Nigrosine disodium dye whereas all the bacterial isolates decolorized more Nigrosine disodium dye than Malachite green dye.

3.2 Biofilm formation

Microscopical observation revealed that except the microbial combinations FBB6, all other combinations showed a biofilm formation and the strength of the attachment varied depending on the microbial composition. Out of all combinations, the highest strength of attachment was observed in FBB1. Biofilm combinations FBB2 and FBB3 also showed higher attachment compared to other biofilm combinations. Therefore, the biofilm combinations (FBB1-FBB3) showed the higher attachments under microscope, were considered for the decolorization assay.

Table 1 - Strength of attachment of different monocultures during the formation biofilms

<table>
<thead>
<tr>
<th>Microbial combination</th>
<th>Strength of the attachment</th>
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<tbody>
<tr>
<td>F2B1 (FBB1)</td>
<td>*****</td>
</tr>
<tr>
<td>F2B2 (FBB2)</td>
<td>****</td>
</tr>
<tr>
<td>F2B2B1 (FBB3)</td>
<td>****</td>
</tr>
<tr>
<td>F3B1 (FBB4)</td>
<td>*</td>
</tr>
<tr>
<td>F3B2 (FBB5)</td>
<td>**</td>
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<tr>
<td>F3B1B2 (FBB6)</td>
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3.3 Dye decolorization assay

All the biofilm combinations showed higher decolorization percentage than that of the monocultures at every time intervals from day 2 to day 12 for both dyes (Fig.2). Although, a sharp increment was observed in the dye decolorization percentage up to day 6 by the fungal isolate F2 and biofilm combinations FBB2 and FBB3 for both dyes, the rate of increment was gradually decreased with the time until day 12. After day 6, it was clear that the dye decolorization rate by the bacterial isolates became constant. However, this pattern was not observed from the biofilm combination FBB1 where the rate showed continuous increment even after day 6 until day 12 and the increment rate showed high compared to other biofilms. Out of all the biofilms, FBB1 showed the highest decolorization percentage values for both dyes at every time intervals until 12. Further, all the bacterial isolates (B1 and B2) showed the least dye decolorization percentage values at every time intervals from day 2 to day 12.
It was observed that the biofilm combinations showed higher dye decolorization percentage than that of the monocultures for both dyes (Fig. 3). Out of the two dyes all the biofilm combinations and the fungal isolate, F2 decolorized more Malachite green dye than Nigrosin disodium whereas bacterial isolates decolorized more Nigrosine disodium dye than Malachite green dye. Out of all the microbial treatments, the highest significant (P<0.05) dye decolorization percentage was observed by the biofilm combination FBB1 for both dyes. Further, molecular identification confirmed the composition of the highest responsive biofilm FBB1 as Tricoderma harzianum, Bacillus subtilis and Pseudomonas fluorescens.

Fig. 2- a Decolorization ability of different biofilm combinations and their monocultures on malachite green dye with the time. b. Decolorization ability of different biofilm combinations and their monocultures on malachite green dye with the time.

Fig. 3- Decolorization ability of different biofilm combinations and their monocultures on malachite green and nigrosin disodium dyes. Columns with the same letter are not significantly different at 5% probability level. Vertical bars show standard deviations.
IV. DISCUSSION

On the basis of the observations of the current study, it was found that the endophytic fungal isolates performed better in decolorizing targeted dyes than bacterial isolates. A previous study reported that fungi have more tolerant to high concentrations of polluting chemicals than bacteria (Casiier et al., 2008). Fungi produce various extracellular enzymes, resulting in enhanced bioremediation rates for most of the pollutes (Kausik and Malik, 2009). Further, fungi have a greater physical contact with the environment due to the presence of increased cell-to-surface ratio. Therefore, fungal systems appear to be most appropriate in the treatment of textile dyes (Ezeronye and Okerentugba, 1999) and also, they had shown better dye reduction potential over the bacteria (Fu and Viraraghavan, 2002). It has been reported that the white rot fungus Phanerochaete chrysosporium, grown under ligninolytic conditions, had shown to metabolize crystal violet by sequential N demethylation of the parent compound, which had catalyzed by lignin peroxidase (Cha et al., 2001). In a previous report it was demonstrated that supernatants from Fomes sclerodermeus with laccase activity were able to degrade malachite green dye (Papinutti et al., 2006).

Decolorization studies with Trichoderma virens showed high decolorization ability (99.6%) for brilliant blue dye (Sweety et al., 2017). The current study also showed the ability of the reduction of both dyes by the bacterial isolates. The reason for effective and faster decolorization of the textile dye by bacteria might be associated with the metabolic activities and interactions of the strains (McMullan et al., 2001; Phugare et al., 2011). It has been reported in a previous study that the significant ability of dye reduction by bacterial species, Bacillus sp. and Pseudomonas sp. isolated from textile dye effluent contaminated soil (Sriram et al., 2013). Further, the efficacy of Bacillus subtilis in decolorization of azo dyes have been previously reported (Cheria et al., 2012; Ali et al., 2014).

The current study clearly showed that biofilms has high decolorization ability than their monocultures. It has been reported that different microbial species present in consortia of biofilms each with different metabolic degradation pathway are capable of degrading several pollutants including dyes and heavy metals either individually or collectively (Gieg et al., 2014; Mitra and Mukhopadhyay, 2015). Decolorization of synthetic dyes using consortia offers advantages over the use of single microbial strains (Sudha et al., 2014) due to higher degrees of biodegradation resulted from synergistic metabolic activities of the microbial community (Allam, 2017). It has been reported that mixed bacterial cultures from different habitats showed high decolorization of dye molecules in 15 days (Knapp & Newby, 1995). Similar results have been reported by another study that immobilized bacterial consortium of the three bacterial species (Sphingomonas paucimobilis, Rhizobium radiobacter, and Bacillus subtilis) had the ability to decolorize azo dyes more efficient than free bacterial cells of single culture (Allam, 2017). Mahmood et al. (2015) found that, the consortium of 6 bacterial isolates was able to decolorize 84% of 200 ppm of red, green, black, yellow, and mixed dyes within 24 hours while individual strain required 72 hours.

V. CONCLUSION

Malachite green and Nigrosin disodium dyes are highly degradable with fungal bacterial biofilms in comparison with their mono cultures. Biofilm combination Tricoderma harzianum, Bacillus subtilis and Pseudomonas fluorescens showed the highest dye reduction ability for both Malachite green and Nigrosin disodium dyes. Therefore, it can be concluded that the biofilms can be used as an efficient biological tool for textile effluent treatment.

VI. ACKNOWLEDGEMENT

University Grants Commission is acknowledged for funding the research (Grant No. UGC/VC/DRIC/PG/2013SP/UWA/01).

REFERENCES


