

# 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 *Trichoderma 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 waste water 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% clorox, 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: C<sub>23</sub>H<sub>25</sub>ClN<sub>2</sub> and Nigrosin disodium; 4-amino-3-[(4-nitrophenyl) diazenyl] - 5-oxo-6- (phenylhydrazinylidene) naphthalene-2, 7-disulfonate, Molecular formula: (C<sub>22</sub>H<sub>14</sub>N<sub>6</sub>Na<sub>2</sub>O<sub>9</sub>S<sub>2</sub>), 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 Nigrosine 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;

Percentage of decolorization = {Initial Absorbance - Final absorbance/Initial Absorbance} X100

### 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 contained 100ml of Potato Dextrose Broth (PDB) and Nutrient Broth (NB) mixture added with filter sterilized 0.5g/L of Malachite green and Nigrosine disodium dyes separately. The inoculated media were incubated on rotary shaker in 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 Minitab 16 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 GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-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'TACGGCTACCTTGTTACGACTT3') with 1  $\mu$ L 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

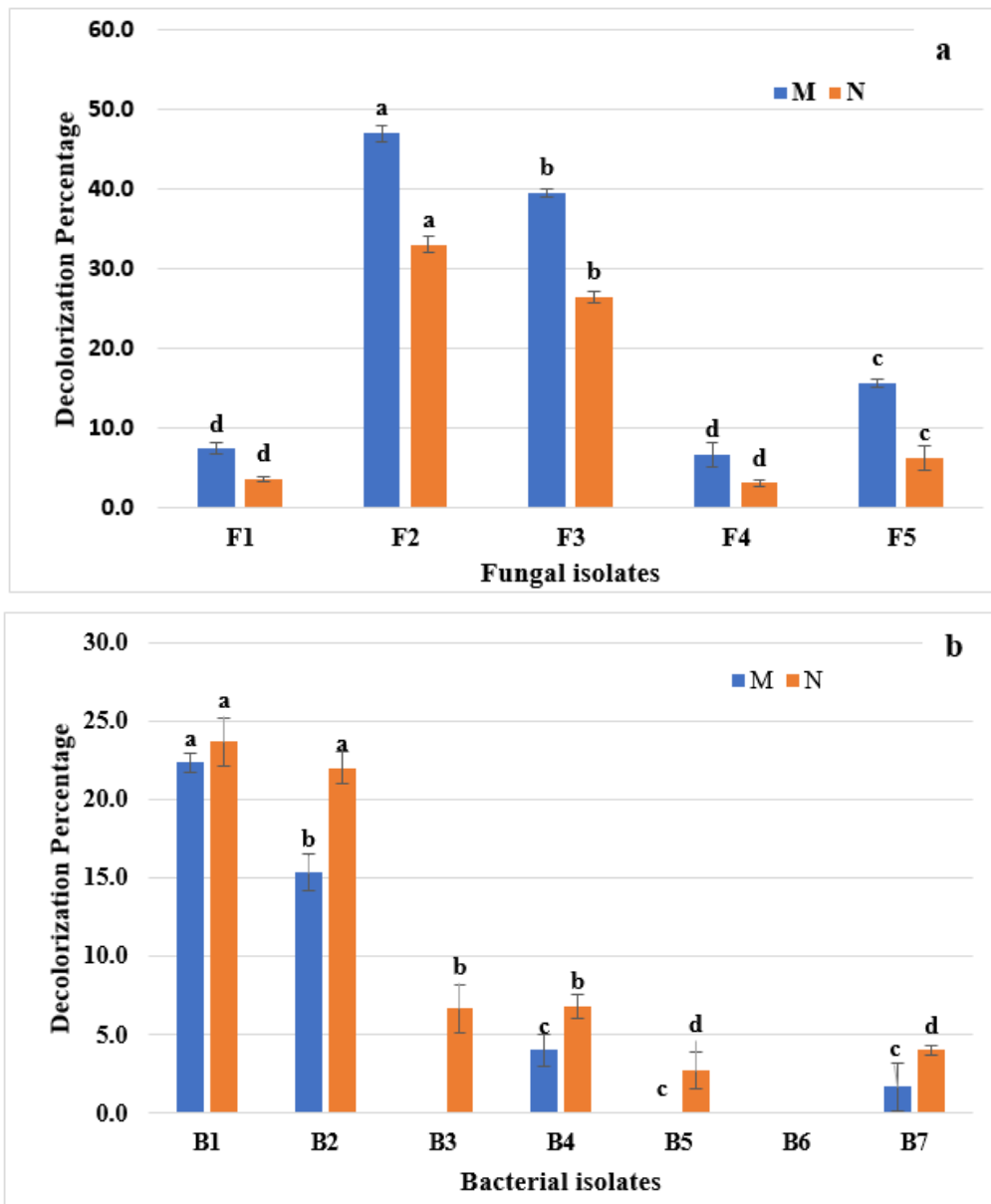


Fig. 1- a decolorization ability of different fungal isolates on malachite green and nigrosin disodium dyes. b. decolorization ability of different bacterial isolates on malachite green and nigrosin disodium dyes. M- malachite green and N- nigrosin disodium. columns with the same letter are not significantly different at 5% probability level. vertical bars show standard deviations.

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 ( $47 \pm 0.762$ ) and Nigrosin disodium ( $33 \pm 0.845$ ) 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 ( $22.3 \pm 0.435$ ) and the highest decolorization percentage for Nigrosin disodium ( $23.7 \pm 0.612$ ) 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

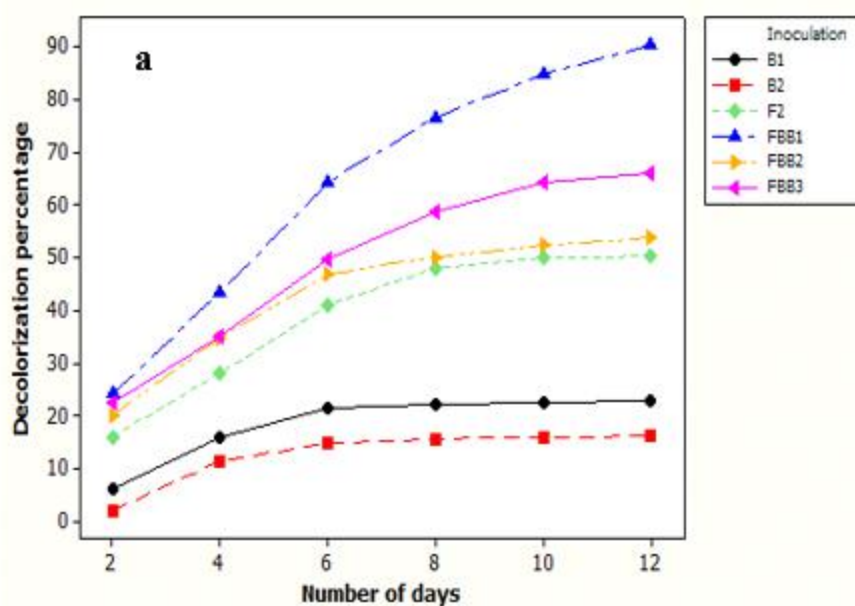
Microscopical observation revealed that except the microbial combinations FBB6, all other combinations showed a biofilm formation and the strength of the attachment varied depending on the microbial composition. Out of all combinations, the highest strength of attachment was observed in FBB1. Biofilm 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

Microbial combination	Strength of the attachment
F2B1 (FBB1)	*****
F2B2 (FBB2)	*****
F2B2B1 (FBB3)	****
F3B1 (FBB4)	*
F3B2 (FBB5)	**
F3B1B2 (FBB6)	-

### 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.



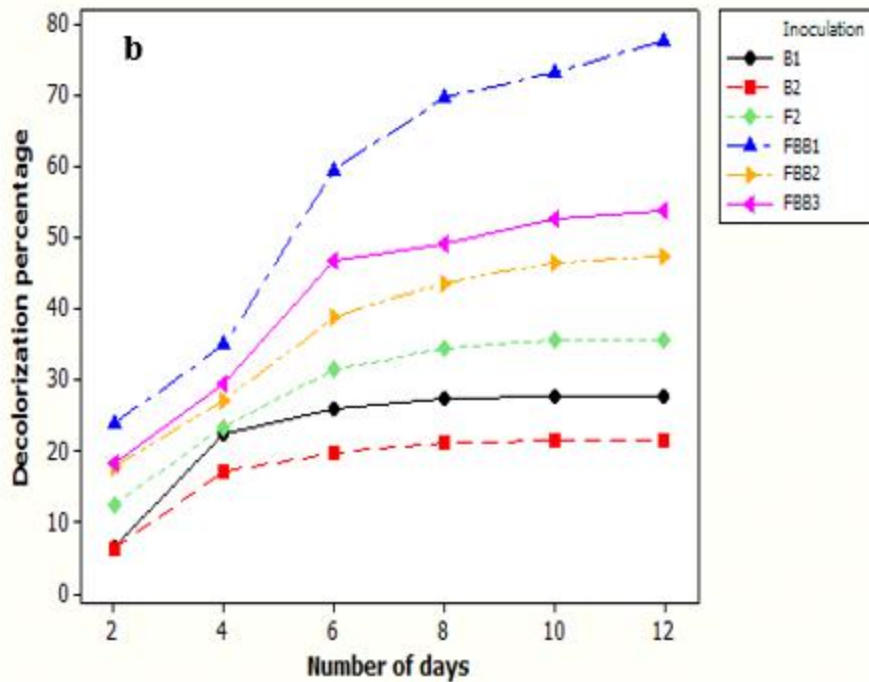


Fig. 2- a Decolorization ability of different biofilm combinations and their monocultures on malachite green dye with the time. b. Decolorization ability of different biofilm combinations and their monocultures on malachite green dye with the time

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 *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens*.

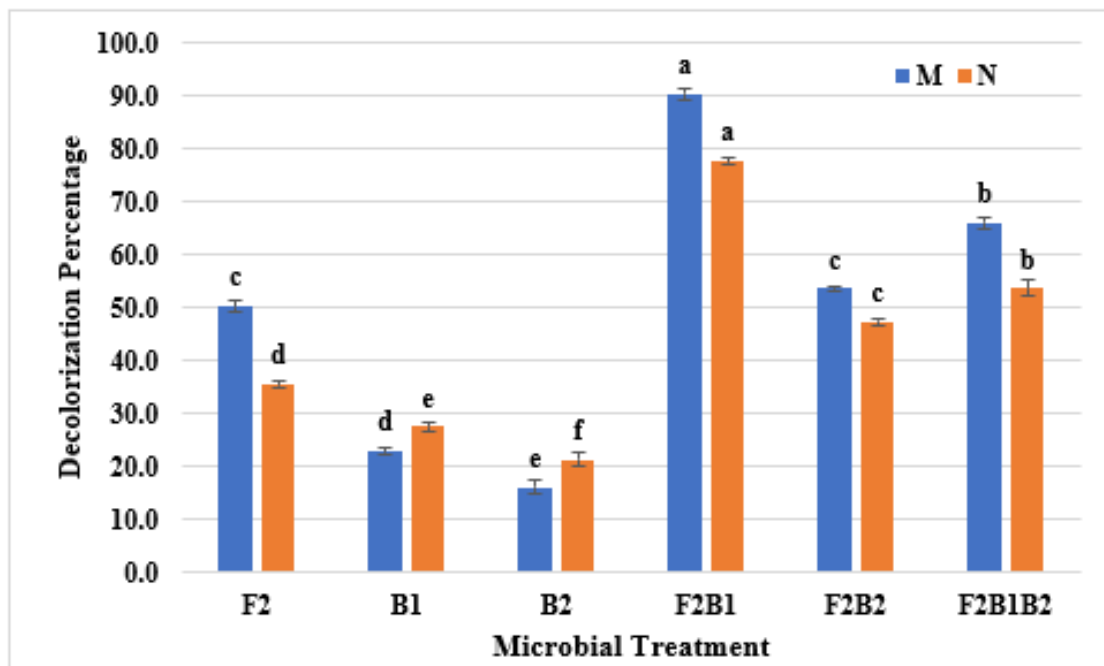


Fig.3- Decolorization ability of different biofilm combinations and their monocultures on malachite green and nigrosin disodium dyes. Columns with the same letter are not significantly different at 5% probability level. Vertical bars show standard deviations.

#### 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 (Casieri *et al.*, 2008). Fungi produce various extracellular enzymes, resulting in enhanced bioremediation rates for most of the pollutants (Kaushik 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 sclerdermeus* 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 *Trichoderma 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.

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#### REFERENCES

- [1] Ali, L., Alhassani, H., Karuvantevida, N., Rauf, M.A. and Ashraf, S.S. (2014). Efficient aerobic degradation of various azo dyes by a *Sphingomonas* sp. isolated from petroleum sludge. *Journal of Bioremediation and Biodegradation* 5 (3): 1-10.
- [2] Allam, N.G. (2017). Bioremediation Efficiency of Heavy Metals and Azo Dyes by Individual or Consortium Bacterial Species Either as Free or Immobilized Cells: A Comparative Study. *Egyptian Journal of Botany*, 57 (3):555 – 564.
- [3] Azbar, N., Yonar, T., Kestioglu, K. (2004). Comparison of various advanced oxidation processes and chemical treatment methods for COD and color removal from a polyester and acetate fibre dyeing effluent. *Chemosphere*. 55:35-43.
- [4] Banat, I.M., Nigam, P., Singh, D., Marchant, R. (1996). Microbial decolorization of textile-dye containing effluents: A review. *Bioresource Technology* 58:217-227.
- [5] Casieri, L., Varese, G.C., Anastasi, A., Prigione, V., Svobodova, K., Marchisio, V.F., Novotny, C. (2008). Decolorization and detoxification of reactive industrial dyes by immobilized fungi *Tramet*

- spubescens* and *Pleurotoso streatus*. *Folia Microbiologica* 53(1):44-52.
- [6] Cha, C.J., Doerge, D.R., Cerniglia C.E. (2001). Biotransformation of Malachite Green by the Fungus *Cunninghamella elegans*. *Applied and Environmental Microbiology* 67(9): 4358–4360.
- [7] Chang-jun, C.H.A., Daniel, R., Doerge, Carl, E. (2001). Biotransformation of Malachite Green by the Fungus *Cunninghamella elegans*. *Applied and Environmental Microbiology*. 67(9): 4358-4360.
- [8] Cheriaa, J., Khaireddine, M., Roubhia, M., Bakhrouf, A. (2012). Removal of triphenylmethane dyes by bacterial consortium. *Scientific World Journal* doi:10.1100/2012/5112454
- [9] Culp, S. J., and Beland, F. A. (1996). Malachite green: a toxicological review. *Journal of the American College of Toxicology*. 15:219–238
- [10] Dos Santos, A.B., Cervantes, F.J., Van Lier, J.B. (2007). Review paper on current technologies for decolourisation of textile wastewaters: perspective for anaerobic biotechnology. *Bioresource Technology*. 98: 2369-2385.
- [11] Drumond Chequer, F.M., de Oliveira, G.A.R., Ferraz, E.R.A., Cardoso, J.C., Zaroni, M.V.B., de Oliveira, D.P. (2013). Textile Dyes: Dyeing Process and Environmental Impact. *INTECH*, 151-176.
- [12] Elisangela, F., Andrea, Z., Fabio, D.G., Cristiano, R.M., Regina, D.L., Artur, C.P. (2009). Biodegradation of textile azo dyes by a facultative *Staphylococcus arlettae* strain VN-11 using a sequential microaerophilic/aerobic process. *International Biodeterioration and Biodegradation* 63:280-288.
- [13] Ezeronye, O. U., Okerentugba, P.O. (1999). Performance and efficiency of a yeast biofilter for the treatment of a Nigerian fertilizer plant effluent. *World Journal of Microbiology and Biotechnology* 15:515-516.
- [14] Fu, Y., Viraraghavan, T. (2002). Removal of Congo red from an aqueous solution by fungus *Aspergillus niger*. *Advances in Environmental Research* 7:239-247.
- [15] Galindo, C., Kalt, T. (1999). UV/H<sub>2</sub>O oxidation of azo dyes in aqueous media: evidence of a structure - degradability relationship. *Dyes and Pigments* 42:199-207.
- [16] Gieg, L.M., Fowler, S.J., Berdugo-Clavijo, C. (2014). Syntrophic biodegradation of hydrocarbon contaminants. *Current Opinion in Biotechnology* 27: 21–29.
- [17] Kaushik, P., Malik, A. (2009). Fungal dye decolorization: recent advances and future potential. *Environment International* 35:127-141.
- [18] Knapp, J.S., Newby, P.S. (1995). The microbiological decolorization of an industrial effluent containing a diazo-linked chromophore. *Water Research*. 29(7):1807-1809
- [19] Liu, S., Ren, H., Gao, Q., Roach, D.J., Loder Jr., R.T., Armstrong, T.M., Mao, Q., Blaga, I., Barker, D.L., and Jovanovich, S.B. (2000). Automated parallel DNA sequencing on multiple channel microchips. *Proceedings of the National Academy of Science of the United States of America*. 97: 5369-5374.
- [20] Mahmood, R., Sharif, F., Ali, S. and Umar Hayyat, M. (2015). Enhancing the decolorizing and degradation ability of bacterial consortium isolated from textile effluent affected area and its application on seed germination. *The Scientific World Journal* 62: 81-95.
- [21] McMullan, G., et al. (2007). Microbial decolourisation and degradation of textile dyes. *Applied Microbiology and Biotechnology*. 56:81-87.
- [22] Mitra, A. and Mukhopadhyay, S. (2015). Biofilm mediated decontamination of pollutants from the environment. *AIMS Bioengineering*, 3(1): 44-59.
- [23] Moorthi, P.S., Selvam, S., Sasikalaveni, A., Murugesan, K., Kalaichelvan P.T (2007). “Decolorization of textile dyes and their effluent using white rot fungi”. *African journal of Biotechnology*. 6(4): 424429
- [24] Padmesh T.V.N., Vijayaraghavan, K., Sekaran, G., Velan, M. (2005). “Batch and column studies on biosorption of acid dyes on fresh water macro alga *Azolla filiculoides*.” *Journal of Hazardous Materials*. 125(3):121–129.
- [25] Papinutti, L., Mouso N., Forchiassin, F. (2006). Removal and degradation of the fungicide dye malachite green from aqueous solution using the system wheat bran- *Fomes sclerodermeus*. *Enzyme and Microbial Technology*. 39:848-853.
- [26] Phugare, S. S., Kalyani, D.C., Surwase, S. N., Jadhav, J. P. (2011). Ecofriendly degradation, decolorization and detoxification of textile effluent by a developed bacterial consortium. *Ecotoxicology and Environmental Safety* 74: 1288–96.
- [27] Robinson, T., McMullan, G., Marchant, R., Nigam, P. (2001). Remediation of dyes in Textile effluent: A critical review on current treatment technologies with a proposed alternative. *Bioresource Technology* 77:247-255.
- [28] Shahid, A., Singh, J., Bisht, S., Teotia, P., Kumar, V. (2013). Biodegradation of textile dyes by fungi isolated from North Indian field soil. *Environment Asia* 6(2):51-57.

- [29] Sriram, N., Reetha, D., Saranraj, P. (2013). Biological Degradation of Reactive Dyes by Using Bacteria Isolated from Dye Effluent Contaminated Soil. *Middle-East Journal of Scientific Research* 17 (12): 1695–1700.
- [30] Stolz, A. (2001). Basic and applied aspects in the microbial degradation of azo dyes. *Applied Microbiology and Biotechnology*. 56(1-2):69-80.
- [31] Sudha, M., Saranya, A., Selvakumar, G. and Sivakumar, N. (2014). Microbial degradation of azo dyes: a review. *International Journal of Current Microbiology and Applied Science* 3(2): 670-690.
- [32] Sweetey, Vats, S., Kumar, M., Sharma, S., Kumar, V., Garg, S. (2017). Mycoremediation of Textile Dyes: Application of Novel Autochthonous Fungal Isolates. *Environment Asia*. 10 (2), 147-161.
- [33] Wang, H., Su J.Q., Zheng, X.W., Tian, Y., Xiong, X.J., Zheng, T. L. (2009). Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by *Citrobacter* sp. CK3. *International Biodeterioration and Biodegradation* 63:395-399.
- [34] Yang, X.Q., Zhao, X.X., Liu, C.Y., Zheng, Y., Qian, S.J. (2009). Decolorization of azo, triphenylmethane and anthraquinone dyes by a newly isolated *Trametes* sp. SQ01 and its laccase. *Process Biochemistry* 4:1185-1189.
- [35] Zhang, G., Guo, G., Hu, X. (2010). Deep RNA sequencing at single base- pair resolution reveals high complexity of the rice transcriptome. *Genome Research* 20: 646-654.
- [36] Zhang, H., Rogiers, P., Preiser, J. C., Spapen, H., Manikis, P., Metz, G., Vincent J. L. (1995). "Effects of methylene blue on oxygen availability and regional blood flow during endotoxic shock," *Critical Care Medicine*, 10: 1711-1721.