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Degradation and Detoxification Analysis of Brilliant Green and Acid Blue 113 Dyes Using The Isolated Bacterial Strain From The Real Textile Waste in Free Cell and Upward Flow Packed Bed Reactor

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Abstract— **The present study aims to develop an environmental friendly and low-cost bioreactor having ability to mineralize the toxic, recalcitrant dye-containing wastewater. Bacterial species were isolated from the textile industry waste effluent and effective dye mineralizing species were selected based on their degradation performance. Brilliant Green and Acid Blue 113 dyes were selected as targeted pollutant for degradation study. Biofilm stability and active surface area available for microbial growth are important factors to be considered for immobilization and keeping this fact in the mind the column bioreactor was packed with solid residual waste foam and immobilized with the isolated bacterial consortium. Decolorization of selected dyes by the isolated microbial consortium was elucidated using a UV-Vis spectrophotometer, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) analysis. Bacterial free cell dye degradation analysis is effective for low concentration range and does not degrade a high concentration solution. Bacterial consortium immobilized within the Foam in Bioreactor has more mineralization potential. Phytotoxicity analysis revealed that the treated dye wastewater is less toxic in comparison to the untreated dye solution.**

Keywords— **Biodegradation, Immobilization, Microbial consortium, Phytotoxicity, Upflow packed bed reactor, Xenobiotic dyes.**

I. INTRODUCTION

Wastewater releases from the textile industry leads pandemonium to our natural water bodies. Dye containing wastewater dissemination in freshwater sources is very common worldwide particularly in developing countries due to unavailability of cost effective and environmental friendly treatment techniques that deteriorate the aquatic life tragically [1-6]. The annual production of dyestuff is 700,000 tons by using 100,000 commercial dyes. The low degree of fixation of dyes on the fibers leads to the mammoth discharge of dyes as waste [7]. Dye containing wastewater is carcinogenic, mutagenic and xenobiotic that can create various diseases such as ulceration of skin, skin irritation, neurosensory damage, bladder cancer, teratogenicity, and dermatitis, etc. [8]. The conventional physicochemical processes such as flocculation, coagulation, membrane filtration, irradiation, ozonation, etc. may be used for dye wastewater treatment but due to excess chemical utilization, high cost and extreme sludge generation refuted their use [12]. The biological techniques are gaining popularity nowadays for treatment of organic waste due to its advantage of no secondary sludge generation, low cost and ability to invade the targeted toxic waste completely. As dyes are synthetic compound, most of them are recalcitrant or very slow to natural and need specific isolated bacterial species for its effective degradation [9-11]. Bioremediation techniques seem valiant to supersede the dye-containing waste and have the potential to reinstate the natural habitat [13]. Bacterial species that are naturally occurring inflicted with contaminated sites have the utmost potential to degrade and detoxify the dye waste [14].

Immobilization of bacterial species on support materials such as foam facilitates the more effective degradation of targeted dye as compared to free cell system [11, 15]. The entrapment of microorganisms inside the pores support material or grow on the active surfaces acts as biocatalyst and significantly enhanced the degradation process [16]. The dye degradation process sometimes faces problems due to its high level of toxicity, complex nature and exiguous availability of nutrients. Effective dye dissolution endorsed by either aerobic or anaerobic mode; therefore the

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integrated degradation and detoxification study unblemished for the fate of dye biodegradation [17,18]. Mixed microbial consortiums are easy to maintain as comparison to a single species and different bacterial species degrade the dye molecule at different positions which is endorsed by nature of metabolites generated in the degradation process and more effective mineralization of dye [19]. Only Color removal does not signify the completion of mineralization of dye; the metabolites generated during the degradation may be more toxic than its parent dye molecule.

In the present study the biodegradation operation is conducted in fixed-film up-flow bioreactor due to its several advantages such as stable biofilm formation with ability to withstand hostile environmental conditions is which make it very effective and durable for degradation of dye [15]. Another important advantage of fixed-film up-flow bioreactor is its ability to be operated in both the aerobic and anaerobic modes [20]. Fixed film mineralization operation is proficient for xenobiotic compounds and prevailed over suspended growth reactors [21]. The main objective of this study is to evaluate the degradation potential of microbial consortium isolated from the textile waste contaminated sites using the sequential aerobic- anaerobic mode in the fixed-film up-flow bioreactor packed with foam under varying process conditions. The identification of stable metabolites and their toxicity potential was also evaluated. Brilliant Green and Acid Blue 113 dyes are selected for degradation study. Moreover, the physiochemical stringent parameters such as COD, TSS, and TOC are also ascertained for degradation efficiency.

II. Material and Methods

A. Dyes and Chemicals

The dye used in this study is Brilliant green (CAS number 633-03-4, $C_{27}H_{33}N_2HO_4S$, MW 482.64 g/mol) and Acid blue 113 (CAS number 3351-05-1, $C_{32}H_{21}N_5Na_2O_6S_2$, MW 681.65 g/mol), supplied by Sigma Aldrich (Fig. 1). All the chemicals and microbiological media used in the present study were analytical grade and of the highest purity.

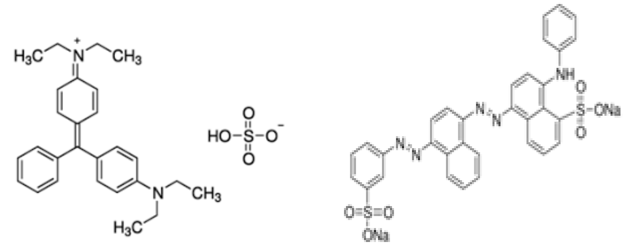

Fig.1 Brilliant Green Dye, and Acid Blue 113 Dye structure.

B. Microbial Consortium

Textile industries waste affected soil sample was gathered from the nearby contaminated sites of the Bhadohi carpet industry, Varanasi, U.P, India. Four bacterial strains capable of the dye degradation were isolated from the wastewater and contaminated soil sample by enriching with 50 mg/L of each dye namely Brilliant green and Acid Blue113 and stored on Nutrient broth at 4-8 ^{0}C for further use [22].

C. Batch Decolorization Study

Flask scale batch study was conducted for the manifestation of the isolated bacterial strain degradation potential. The stock solution of BG and AB 113 was prepared in deionized water and using stock solution simulated synthetic wastewater was prepared. The trace elements added as nutrient in the prepared synthetic wastewater were as follows (gm/L): K_2HPO_4- 1.73, KH_2PO_4- 0.63, $MgSO_4.7H_2O$- 0.1, NaCl- 4, $FeSO_4.7H_2O$- 0.03, NH_4NO_3- 1, $CaCl_2.2H_2O$- 0.02 and glucose-5 [12,13]. Entire batch analysis was performed under microaerophilic conditions. The inoculums size was optimized and the degradation was assessed by the reduction in optical density using UV- vis spectrophotometer. A control medium without dye was used as reference solution throughout the experiment. The entire experimental analysis was performed in a triplicate manner and results were expressed as their mean values along with deviation.

D. Laboratory Scale Anaerobic-Aerobic Reactor Setup, Design and Operating Conditions

Indigenously designed laboratory-scale up-flow fixed-film bioreactor made from borosilicate glass material with 1000 mm length, 50 mm diameter and 2.5 mm thickness was used for the experiments. The reactor was packed with foam and supported from both ends about 55 mm distance. The sampling port fixed at 500 mm distance from both ends. The reactor was operated at room temperature and adjusted pH of 7 ± 0.5. To eliminate the channeling effect and to enhance the retention time, the wastewater sample was introduced at the bottom. The average weight of the packed foam was 26.46 gm and the working volume of the reactor was 800 ml out of 1000 ml total volume. The isolated bacterial culture preserved in the nutrient broth was added to the reactor and then the reactor kept at room temperature for 15 days until the biofilm formation occurs. The contact angle value of foam is 64.32^0 with water and confirms its hydrophilic nature. The hydrophilic nature of foam governs the strong interaction with media (having bacterial consortium) and wastewater. The target dye wastewater was admitted at the bottom end of the reactor via the peristaltic pump (ELECTROLAB, Peristaltic Pump, Model- PP- 50V) at the flow rate of 120 ± 2 mL/h and the treated sample was withdrawn from the top corner at 800 mm distance.

To stimulate the degradation performance, 2.5 g/L peptone and 1g/L glucose were added with each new effluent to the reactor system. This addition was further repeated throughout each cycle (cycle indicates replenished the treated water with fresh effluent) of reactor operation. The treated effluent was collected, centrifuged at 5000 rpm (REMI CENTRIFUGE, RM- 12C BL) for 10 min, cell-free clear supernatant then examined for degradation performance.

E. Mineralization Analysis by Physicochemical Parameters

Dye wastewater degradation analysis was performed in both the batch and continuous operation. The extent of dye waste mineralized was assessed in terms of reduction of Chemical Oxygen Demand (COD), Total Suspended solids (TSS) and Total organic carbon (TOC). COD and TSS of the sample were determined by using APHA (1998) standard protocol. The total organic carbon (TOC) of the sample is determined by the TOC analyzer (Analytikjena, multi N/C 2100/2100S) [23, 24].

F. Phytotoxicity Assessment of Effluents

Toxicity analysis embedded with the treatment performance of the reactor. The analysis was conducted with *Vigna radiate* to assess the toxicity of the treated and untreated dye samples. The biomass free supernatants (2 mL) of the treated and untreated samples were added to the 10 seeds (in soil) that were incubated under controlled conditions (Temperature 30 ±5 ^{0}C, Relative humidity 55 to 65 %). 5 mL of distilled water was added after every 24 h. The supernatant was fungible with the addition of water in the case of a control sample at the same time. The experiment was performed with 50 mg/L of Acid Blue 113 and Brilliant Green dye. After the 7^{th} day of incubation toxicity assessment had articulated in terms of Germination (%) and length of plumule (shoot) and radical (root) [12,25].

G. Characterization

The degradation performance of bacterial consortium immobilized with the packing material (Foam) was disquisition by Fourier Transform Infrared Spectroscopy (FTIR) analysis and Screening electron microscopy (SEM). FTIR analysis was carried out for the confirmation of changes in the functional groups due to bioremediation. The spectra were recorded by the FTIR spectrophotometer (THERMO Electron Scientific Instruments LLC, Nicolet iS5) in the mid-infrared region of 400-4000 cm^{-1} with 32 scan speed and $4cm^{-1}$ resolution. The Foam was mixed with spectroscopically pure KBr in the ratio (5:95). Pellets were made by the 200 mg of KBr and 1 mg of each dried Foam sample and pressed at a pressure of 1MPa. The spectrum analysis was performed in a triplicate manner and necessary baseline correction was done to predict the change in the structure of BG and AB 113 dyes.26 SEM analysis was conducted (CARL ZEISS MICROSCOPY LTD, EVO - Scanning Electron Microscope MA15 / 18) with an accelerating voltage of 20 kV [27]. The standard curve of BG and AB 113 dye was withdrawn by using a UV-Visible spectrophotometer (ELICO SL 159) at room temperature. The λ_{max} of BG and AB 113 was 600 and 563 nm respectively. The dye degradation was calculated by using the following equation:

$$\text{Dye degradation (\%)} = (OD_1-OD_t)/OD_1 * 100$$

where OD_1 and OD_t refer to the initial absorbance before degradation and absorbance after degradation respectively.

III. RESULTS AND DISCUSSION

A. Batch Scale Biodegradation

The free cell bacterial degradation analysis of BG and AB 113 dye was elucidated for various concentration levels. The optimum inoculums volume of the bacterial consortium was found to be 2mL for BG and 4mL for AB 113. TSS and TOC analysis of the BG dye solution was analyzed for 5 days. COD and Dye's decolorization were analyzed for 8 days. 10 ppm BG dye solution shows a maximum of 89.97% decolorization and 20 ppm BG dye shows 61 % TOC and 55.6% TSS reduction. The batch scale COD analysis of BG dye was conducted for the concentration range from 50 ppm to 200 ppm.70.47%

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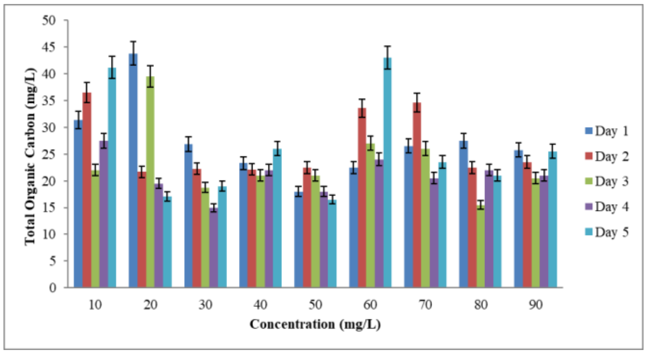
reduction in COD had predicted for 200 ppm dye solution as shown in Fig.2.



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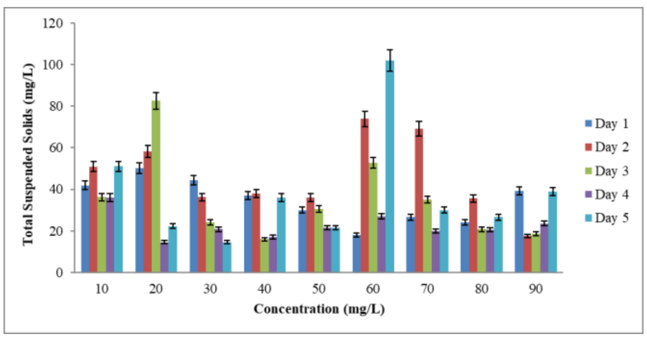

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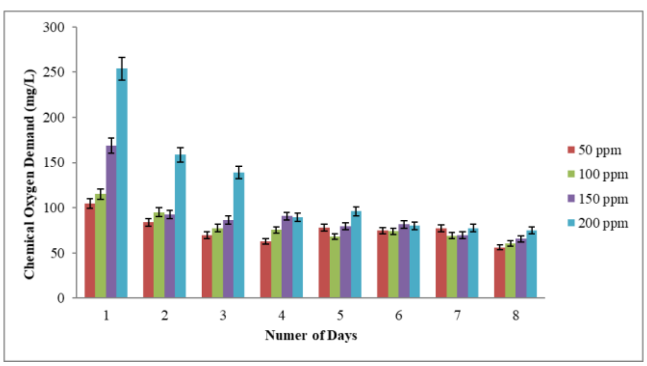

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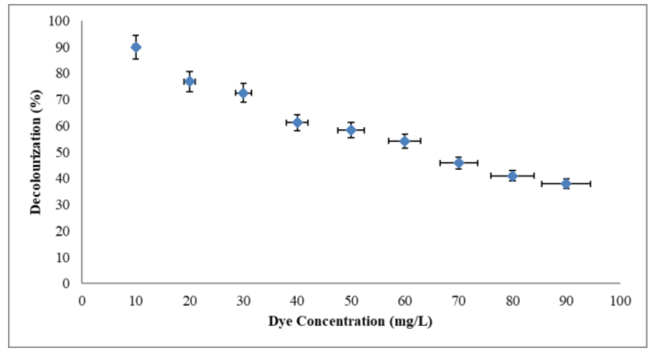
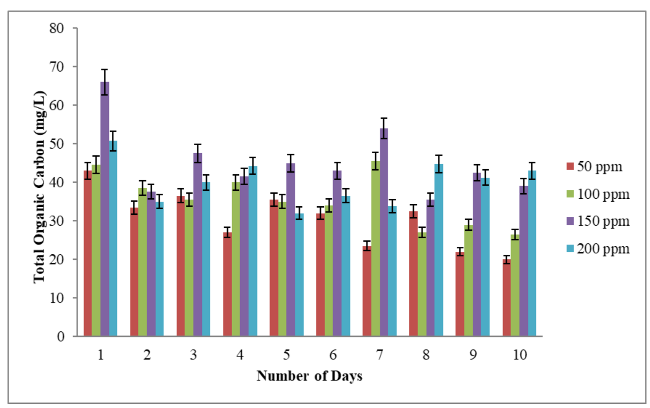


(d)

Fig.2. Batch scale (Free Cell) Bacterial Consortium degradation performance of Brilliant Green Dye (a) Total Organic Carbon, (b) Total Suspended Solids, (c) Chemical Oxygen Demand, and (d) Decolourization.

In the case of Acid Blue 113 COD, TSS and TOC were analyzed for dye concentration varying from 50 to 200 ppm while decolorization was analyzed from 20 to 200 ppm dye concentration. For a higher concentration of 200 ppm, AB 113 degradation was not accomplished with dexterity and a low level of dye concentration shows the varying amounts of decolorization. 20 ppm and 40 ppm dye concentration depict the major reduction in decolorization of 73.89 and 54.78% respectively. The observed reduction in COD, TSS, and TOC for 50 ppm AB 113 dye is 51.55, 93.59 and 53.49% respectively as shown in Fig.3.



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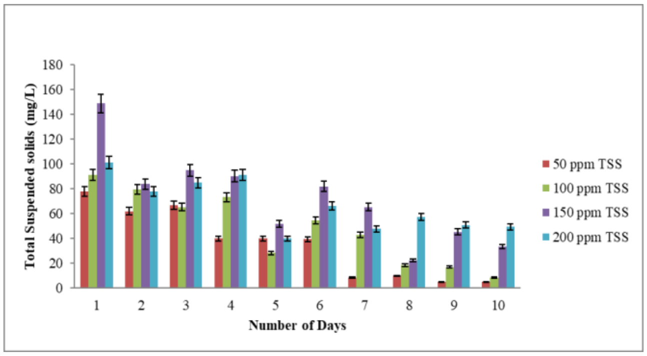

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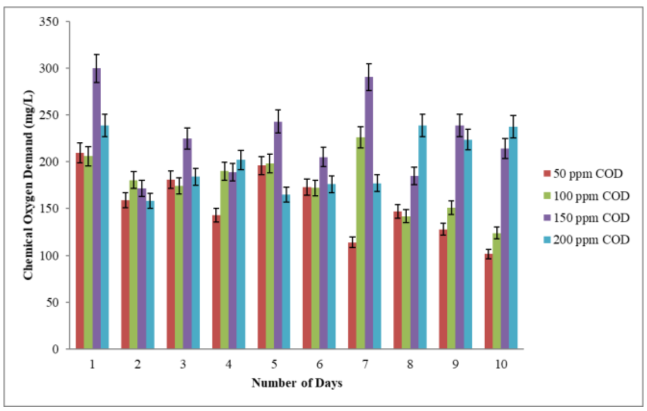

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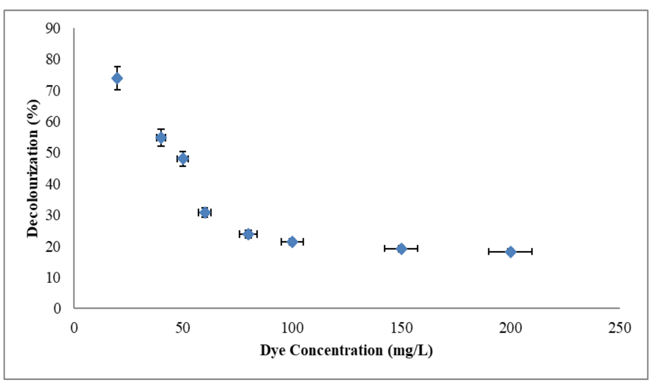

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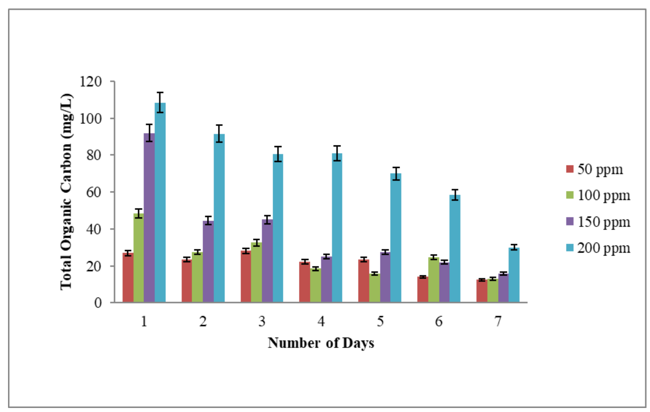
Fig.3. Batch scale (Free Cell) Bacterial Consortium degradation performance of Acid Blue 113 Dye (a) Total Organic Carbon, (b) Total Suspended Solids, (c) Chemical Oxygen Demand, and (d) Decolourization.

B. Degradation Analysis of Sequential Anaerobic-Aerobic Upflow Fixed-Film Bioreactor

The sequential aerobic-anaerobic and anaerobic-aerobic analysis was performed for BG and AB 113 dye respectively. The BG degradation analysis in an up-flow fixed bed reactor was conducted for the dye concentration range varying from 50 to 200 ppm and entire degradation analysis was observed for 7 days of operation. BG dye solution having a concentration of 200 ppm also shows 80.76% decolorization. 50 ppm dye solution shows maximum decolorization of 96.08%. The reduction in TSS, COD, and TOC is 73.81, 47.86, and 53.7% respectively for 50 ppm dye solution as shown in Fig.4. In the case of AB 113 dye, the degradation performance was also elucidated for the 7- day operation of the reactor. The maximum decolorization was found to be 86.14% in the case of a 50 ppm dye solution. The reduction in TSS, COD, and TOC is 80, 61.28, and 72.54% respectively as shown in Fig.5. The major advantage of this configuration is that dye mineralization of high concentration also occurs significantly.



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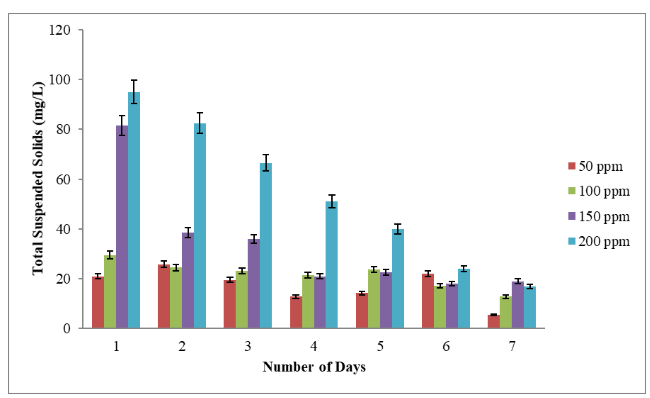

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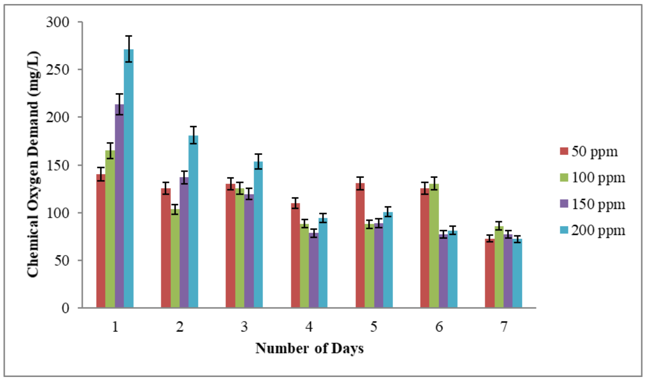

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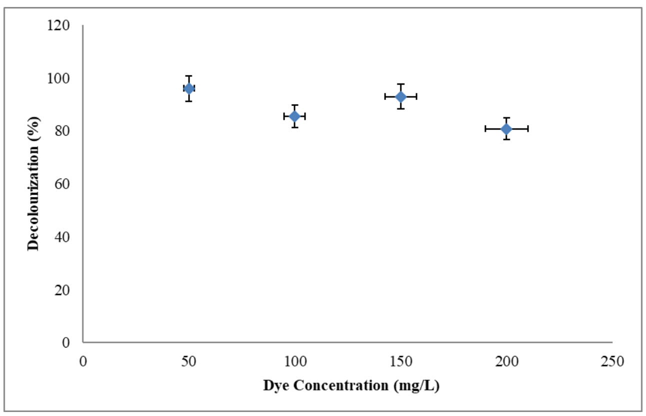

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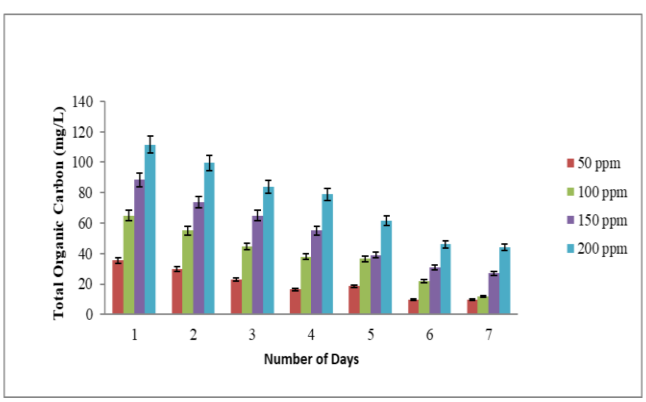
Fig.4. Fixed-film up-flow Packed Bed Tubular reactor degradation performance of Brilliant Green Dye (a) Total Organic Carbon, (b) Total Suspended Solids, (c) Chemical Oxygen Demand, and (d) Decolourization.



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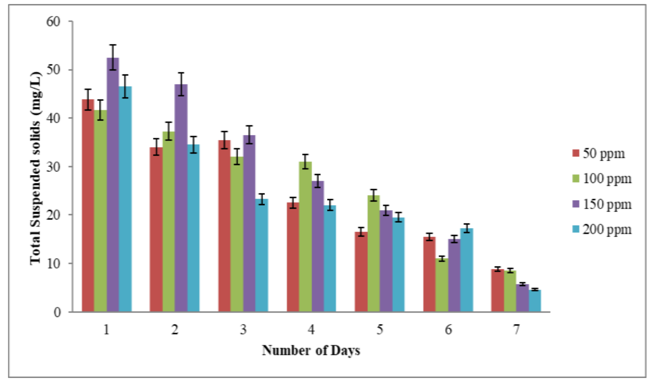

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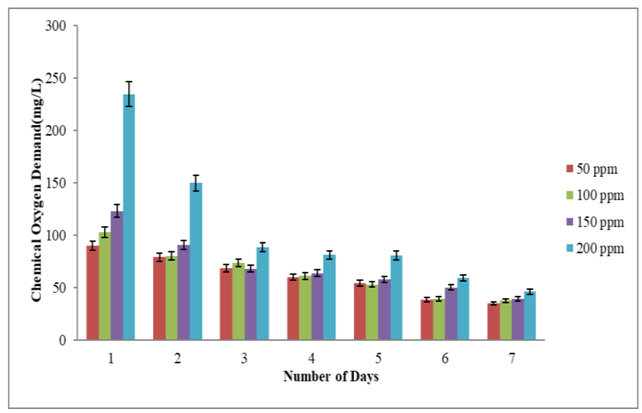

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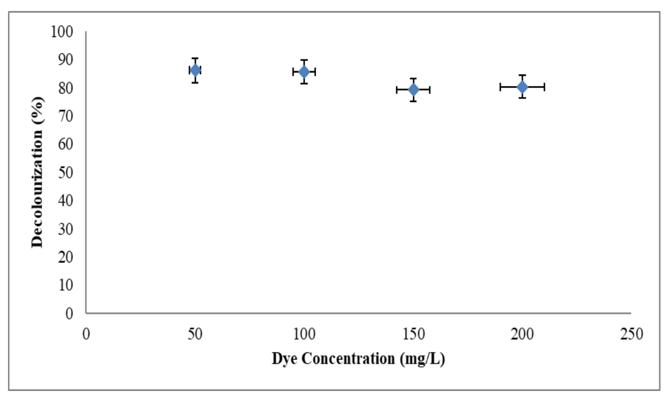

(d)

Fig.5. Fixed-film up-flow Packed Bed Tubular reactor degradation performance of Acid Blue 113 Dye (a) Total Organic Carbon, (b) Total Suspended Solids, (c) Chemical Oxygen Demand, and (d) Decolourization.

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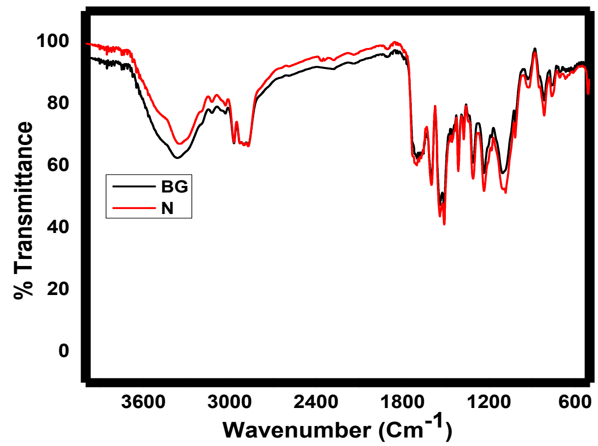
C. FTIR Characterization

FTIR spectra of the packing material foam were performed in its original form as well as with the treated dye solution of BG and AB 113. From Fig. 6(a), the original foam (N) depicts the peak at 3137.5 cm^{-1} is attributed to weak, broad O-H stretching intramolecular bonded. The peak at 3074.57 cm^{-1} is associated with strong, broad O-H stretching and belongs to the class of carboxylic acid. The absorption peaks at 2947.9 and 1925.65 cm^{-1} suggest strong, broad N-H stretching of amine salt and weak C-H bending of an aromatic compound. The peak at 1819.96, 1640.848, 1419.77, 1272.13, and 724.2969 cm^{-1} are due to weak C-H bending, medium C=N stretching imine/ oxime, strong C-O stretching of alkyl aryl ether and strong C=C bending of a disubstituted cis alkene.

BG dye FTIR spectra show the peak at 3727.29 cm^{-1} is attributed to medium, sharp O-H stretching of alcohol. The absorption spectra peak at 3158.48 and 3064.08 cm^{-1} are associated with strong, broad O-H stretching of carboxylic acid and alkyne strong, sharp C-H stretching. The peak around 2979.37, 2852.69, 1883.7, and 1704.58 cm^{-1} are medium C-H stretching of alkane, N-H stretching, weak C-H bending of an aromatic compound, and strong C=O stretching of a carboxylic acid dimer. The absorption spectra at the vicinity of 1577.91, 1504.49, and 1346.35cm^{-1} are linked with medium N-H bending of amine, strong N-O stretching of a nitro compound and strong S=O stretching of sulfonic acid. The presence of functional groups such as N-H stretching, N-H bending of amine, S=O stretching, and N-O stretching in the spectra govern the idea of Brilliant Green dye fragmentation.



(a)

In Fig. 6(b) FTIR spectra of Acid Blue 113 at 3353.73 cm^{-1} is attributed to strong, broad O-H stretching of intermolecular bonded alcohol. The peak at 2875.28 cm^{-1} is associated with strong, broad N-H stretching of amine salt. This is due to the cleavage of the N=N bond present in the dye. The absorption peak at 1600.51 cm^{-1} suggests the medium N-H bending of amine. Thus the formation of aromatic amine occurs after degradation. The spectral peaks at 1719.91, 1570.654, 1520.63, and 1410.9 cm^{-1} belong to the strong C=O stretching of α,β unsaturated ester, medium C=C stretching of cyclic alkene, strong N-O stretching of a nitro compound, and sulfate strong S=O stretching. The presence of a peak at 1251.15 cm^{-1} is due to the strong C-O stretching of an aromatic ester. The entire FTIR spectral analysis depicts the shift in major peak and confirms the cleavage of dye by bacterial consortium.

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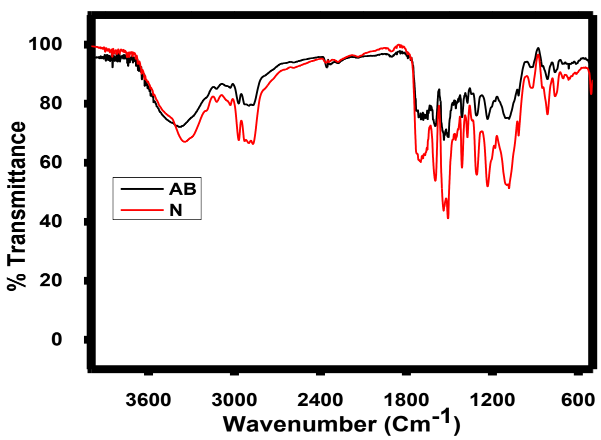

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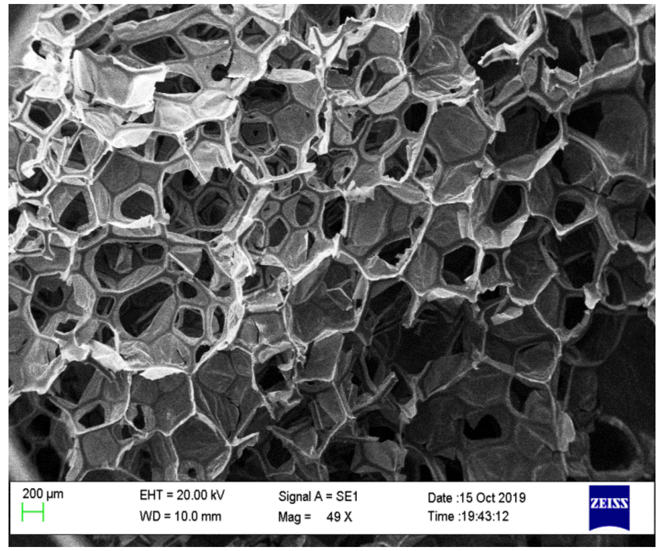
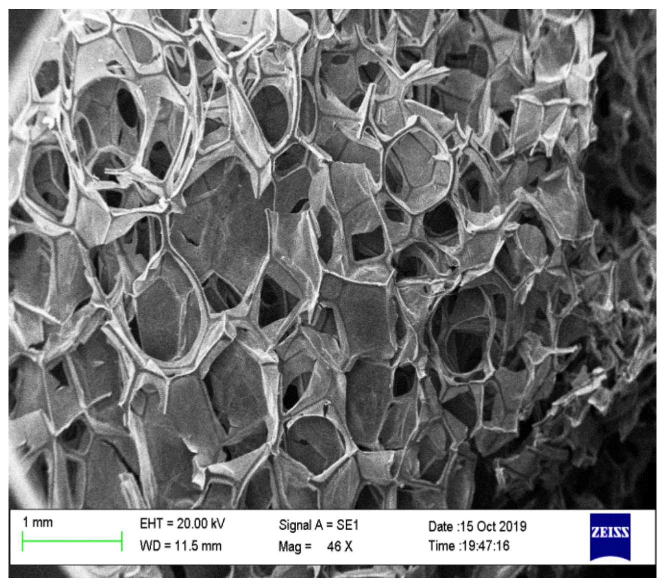
Fig.6. FTIR spectra analysis (a) Foam in original form (N) and Treated with Brilliant Green Dye (BG), (b) Foam treated with Acid Blue 113 (AB) and the original Foam (N).

D. SEM Analysis

Foam that was used as an immobilization and biofilm formation were morphologically analyzed. SEM images at the same magnification of original foam as well as the treated with BG and AB 113 dyes are shown in Fig.7. The SEM analysis confirms that the packing foam is porous and has the distribution of pore opening of various sizes. This inner morphological analysis confirms that the Foam was suitable for immobilization and the stability of biofilm. The images confirm that the microbial biofilm strongly adhered with foam. O. Anjaneya et al. (2013) used Sheep bones for immobilization of microbial species and performed the treatment of Amaranth dye. A similar analysis was also conducted by Alejandro et al. (2009) that used Luffa fruit material for microbial growth and treats domestic wastewater. Sharma et al. (2004) also suggest the naturally occurring sea shells as another good packing material for the decolorization of Acid Violet dye.



(a) Brilliant Green



(b) Acid Blue 113

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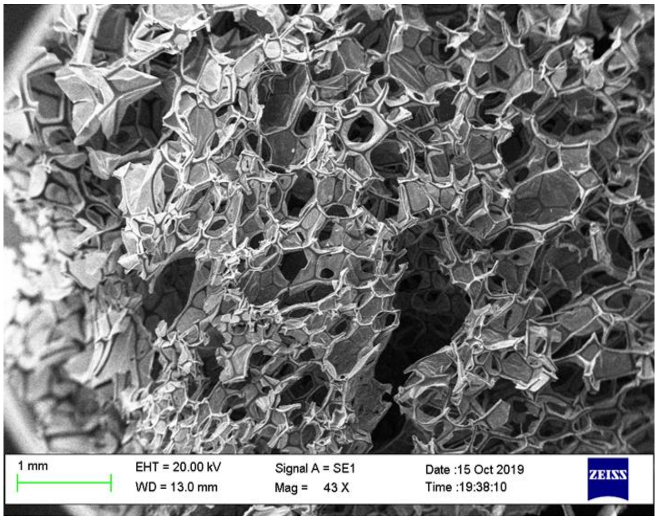

(c) Original Foam

Fig.7. SEM analysis of the Foam treated with (a)Brilliant Green, (b) Acid Blue 113 and (c) Original Foam.

E. Phytotoxicity Analysis

Bioremediation of BG and AB 113 with the help of bacterial consortium in the bioreactor system leads almost complete mineralization. The toxicity assessment is necessary for end products so that the treated water can further be utilized for irrigation and other uses. The root and shoot length analysis of *Vigna radiata* after exposure to the environment of treated and untreated dye illustrates that the plant growth under-treated dye furfurals while the untreated dye diminishes the growth. The mean length of root and shoot for both untreated dyes and treated dyes and their corresponding germination % are shown in Table 1. Phytotoxicity analysis of both dyes revealed that the treated end products were significantly less toxic than the untreated dye solutions. Similar observations have been reported in previous studies. Buntić et al. [6] performed seed germination analysis of Crystal Violet and Safranin T dyes using *Triticum aestivum* seeds and confirmed the non-toxic nature of the degradation products. Shanmugam et al. [1] also reported detoxification of various azo dyes using *P. mungo* seeds after biological treatment.

TABLE I

PHYTOTOXICITY STUDIES OF BRILLIANT GREEN AND ACID BLUE 113 DYES UNDER CONTROLLED CONDITIONS

Parameters	**Control**	**Brilliant Green Untreated**	**Brilliant Green Treated**	**Acid Blue 113 Untreated**	**Acid Blue 113 Treated**
Germination (%)	100	44	78	41	81
Root Length (cm)	7 ± 1	5.4 ± 0.2	8.4 ± 0.5	6.7 ± 0.7	8.5 ± 1.1
Shoot Length (cm)	15 ± 0.6	8.2 ± 1.1	10.1 ± 0.6	9.1 ± 0.5	12.3 ± 0.4

IV. CONCLUSIONS

Brilliant Green and Acid Blue 113 dyes are recalcitrant and their improper discharge to the environment leads to lancination effects on the environment. The bacterial consortiums isolated from the real textile waste have significant potential for dye mineralization. The immobilization technique further resolves the degradation problem. The novel sequential anaerobic-aerobic up-flow fixed-film bioreactor system shows significant potential towards the degradation of a higher concentration of both BG and AB 113 dyes within 7 days of continuous operation. FTIR and SEM analysis unveiled the dye molecule degradation in the bioreactor system. The residual solid waste foam seems efficacious for the growth of bacterial consortium and sustains its biofilm stability. Phytotoxicity study confirms the nontoxic nature of final treated products.

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