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Biodecolorization and Bioremediation of Denim Industrial Wastewater by Adapted Bacterial Consortium Immobilized on Inert Polyurethane Foam (PUF) Matrix: A First Approach with Biobarrier Model

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Abstract

The present experiments were studied on bioremediation of denim industry wastewater by using polyurethane foam (PU foam) immobilized bacterial cells. About 30 indigenous adapted bacterial strains were isolated from denim textile effluent out of which only four isolates were found to be efficient against crude indigo carmine degradation using broth decolorization method. The selected bacterial strains were identified as *Actinomyces* sp., (PK07), *Pseudomonas* sp., (PK18), *Stenotrophomonas* sp., (PK23) and S*taphylococcus* sp., (PK28) based on microscopic and biochemical characteristics. The bacterial immobilized cells have the highest number of viable cells (PK07, PK18, PK23 and PK28 appeared to be 1×10^8, 1×10^9, 1×10^6 and 1×10^7 CFU/ml respectively) and maximum attachment efficiency of 92% on PU foam. The complete degradation using a consortium of PU foam immobilized cells was achieved at pH 6, 27°C, 100% of substrate concentration and allowed to develop biofilm for one day (1.5% W/V). In SEM analysis, it was found that immobilization of bacterial cells using PUF stably maintained the production of various extracellular enzymes at levels higher than achieved with suspended forms. Finally, isatin and anthranilic acid were found to be degradation products by NMR and TLC. The decolorized dye was not toxic to monkey kidney cell (HBL 100) at a concentration of 50 μl and 95% of cell viability was retained. A mathematical model that describes bacterial transport with biodegradation involves a set of coupled reaction equations with non-standard numerical approach based on the time step scheme.

Key words: biobarrier model, bioflim, bioremediation, cytotoxicity test, polyurethane foam, secondary metabolites

Introduction

The textile industry involves a wide range of raw materials, machineries and processes to engineer the required shape and properties of the final products. Indigo dyes are used by a wide number of industries and textile mills predominantly use them. Today denim is the basis of the Indian fashion industry in many cases. It is a challenging dye to use because of its insoluble nature in water; to be dissolved, it must undergo a chemical change under high alkaline condition. When a submerged fabric is removed from the dyebath, the indigo quickly combines with oxygen in the air and reverts to its insoluble form producing color on the fabric (Ciardelli *et al*., 2001; Bes-Pia *et al*., 2004). This textile chain begins with the production of raw fiber continues with pretreatment, dyeing, finishing, printing, coating, and other processes. Among these processes, dyeing and finishing are major water consuming processes that generate highly polluted effluents.

The dyeing step in the textile production has the largest risk on the environment due to high concentrations of organic dyes, additives and salts used. Therefore, among the processes applied in the textile industry, dyeing process wastewater should be dealt with seriously. The districts Coimbatore, Tirupur and Karur are largely polluted (Ranganathan *et al*., 2007) with discharged toxic wastes from dyeing and bleaching units. The problem of disposal of textile wastes is likely to become serious in the days ahead and there is possibility of affecting the groundwater in the study area. Most of the time, this process constitutes the major part

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of the water consumption and generates wastewaters distinguished by high chemical oxygen demand (COD), high dissolved and suspended solids, and high color contents (Azbar and Tonar, 2004; Chan *et al.*, 2008). Many of the conventional and even advanced treatment technologies suffer the limitation of not being able to treat highly colored wastewaters from textile manufacturing units (Vandevivere *et al.*, 1998). One of the main drawbacks of these treatments is their high energy costs and low efficiency in degrading the dye stuffs. In some studies on membrane treatment of denim textile mill effluents for reuse, the wastewater is directly filtered after a pretreatment to remove only coarse particles to reduce membrane fouling and this problem has not yet been solved (Chatcharee, 2011). An alternative method of sludge management involves use of microorganisms for in situ degradation in the soil. It has been successfully applied for cleanup of soil, surface water, groundwater, sediments and ecosystem restoration (Prasad, 2011). It has been unequivocally demonstrated that a number of xenobiotics can be cleaned up through bioremediation.

Biofilms can be composed of either single or multiple species. It provides the benefit of a stable environment for the enclosed microbes, which makes them such a prolific and widespread phenomenon in nature. The aim of the present study was the screening of potent adapted bacterial strains from the denim effluent sample. These strains were allowed to form a biofilm on an inert substrate like the polyurethane foam and retain their ability to efficiently degrade H-chromophore of Indigo dye in several optimum conditions and to check their efficiency on an industrial scale.

Experimental

Materials and Methods

Sample collection. The textile effluent sample contaminated with crude indigo dye was collected from the outlet of denim industry dyeing unit (indigo dye) in Perundurai KG Fabrics, Coimbatore. The sample was brought to laboratory and stored at 7°C and used for further process.

Isolation, screening and identification of adapted bacterial strains from denim textile effluent. The dilution plates after incubation showed zone of clearance around number of colonies. Among this only 30 predominant cultures were isolated. The zone of clearance was due to the dye degrading ability of the isolated organisms. A zone of clearance was observed after 24 hrs in the nutrient agar plate (0.5% peptone, 0.3% yeast extract, 1.5% agar and 0.5% NaCl) containing 0.1% dye. The plates were incubated at 37°C as for 24 hours. The cultures which showed a zone of clearance around their colonies were isolated and used for further screening on nutrient broth (0.5% peptone, 0.3% yeast extract and 0.5% NaCl) (Rajendran *et al.*, 2011) amended with 0.1% of indigo carmine separately for the quantitative estimation of decolorization. The decolorization pattern was calculated by using formula *i.e.* (Initial OD-Final OD)/ Initial OD X 100. After incubation the tubes were centrifuged and the dye decolorization was measured spectrophotometrically at 610 nm. The selected bacterial strains (PK07, PK18, PK23 and PK28) were examined for their shape, gram staining and motility. The biochemical tests were performed according to Bergey's manual of systemic bacteriology (Holt *et al.*, 1994). The selected bacterial stains were identified though 16S rRNA which was carried out at Chromous Biotech Pvt Ltd, Bangalore, India.

Characterization of untreated denim industrial effluent. The raw effluent was characterized by measuring the values of 11 different physico-chemical parameters. These parameters (Total Dissolved Solids (TDS) (mg/l), Total Solids (TS) (mg/l), Chemical Oxygen Demand (COD) (mg/l), pH, color, turbidity (NTU), hardness (mg/l), conductivity (mS), resistivity (Ω) and alkalinity (mg/l)) were chosen in accordance with the regulations of Tamil Nadu Pollution Control Board. All the above mentioned physico-chemical analyses were done immediately after the effluent sample was collected (APHA, 1992).

Toxicity Test for Polyurethane foam (PUF): About 0.1 ml of 3-day old liquid nutrient broth cultures were individually spread over on nutrient agar plate (Jerabkova *et al.*, 1997). The PUF of each density was cut into slices of about 1 × 1 cm and the plates without PUF were used as a control. The plates were incubated at 37°C for 2 days. The area around and in contact with the PUF cubes were observed for bacterial growth.

Enumeration of viable cells immobilized on polyurethane foam: Polyurethane foam cubes were weighed and placed into a 100 ml of conical flask containing 20 ml of nutrient broth and autoclaved at 121°C for 15 min, 2 ml of 3 day old cultures were then inoculated separately into each flask. The cultures were incubated at 30°C with orbital shaking (120 rpm) for ten days. Enumeration of viable cells was carried out 2, 4, 6, 8 and 10 days after incubation. To ensure that only the immobilized cells were further quantified, the PUF was first rinsed with sterile nutrient broth. It was torn into pieces using sterile forceps and then suspended in nutrient broth and vortexed to dislodge the immobilized cells. About 0.1 ml aliquots were spread onto nutrient agar plate and incubated at 37°C until colonies appeared. The experiment was repeated four to five times. The attachment efficiency (AE) was calculated as the fraction of the total viable cells that was immobilized (Jerabkova *et al.*, 1997).

Comparison of treatment trails on Bioremediation of DENIM industry wastewater by pure broth culture, alginate immobilized cells and PU foam immobilized cells. Using broth cultures: To 95 ml of the effluent sample, 5 ml of individual strains and consortium (combination of four bacterial strains) were inoculated. After inoculation the samples were incubated in a metabolic shaker for 24 hours at room temperature for a period of 5 days. Samples were retrieved from the flasks after 5 days of incubation and the bioremediation efficiency of the individual cultures as well as that of the consortium were studied as described above.

Using Ca-alginate immobilized cells: The Ca-alginate entrapment of bacterial cells was performed as per the method described by Kierstan and Coughlan (1985). The three gram of beads was measured and it was added to 97 ml of denim textile effluent. This was incubated at room temperature in a metabolic shaker at 120 rpm for 5 days. Sample without inoculation was also used as a control. The treated effluent samples were retrieved and studied as described above.

Using PU foam cells: The polyurethane foam was sterilized using water at 100°C for 20 minutes and the excess water was drained off after sterilization. The pure bacterial broth cultures were added in such a way that the foam was immersed in the broth. Cultures were incubated for 24 hours at room temperature in a metabolic shaker for the biofilm to develop in the matrix of the polyurethane foam. After incubation the excess of nutrient broth was drained off and added 100 ml of denim industry wastewater. The samples were incubated in a metabolic shaker for 24 hours at room temperature for a period of 5 days. The treated effluent samples were retrieved and studied as described above.

Optimization of cultural conditions for maximum biodegradation ability (APHA, 1992). The efficient adapted bacterial consortium (four) immobilized on polyurethane foam was selected and optimized under different parameters such as retention time (on 3rd day), pH (5, 6, 7, 8 and 9), temperature (7°C, 17°C, 27°C and 37°C), Initial organic load concentration (20%, 40%, 60%, 80%, and 100%), inert substrate concentration (0.5%, 1%, 1.5%, 2% and 2.5% W/V) were used for the biofilm formation and its efficiency in biodegradation and also the decolorization.

Treatment trails of denim effluent under optimized cultural conditions: About 1.5% W/V of the foam was taken and allowed to develop biofilm for one day. A substrate concentration of 40% was added and incubated at 27°C for 3 days. The pH of the effluent was adjusted to pH 6.

Characterization of Indigo Dye Degrading Compounds Through GCMS. GC-MS is particularly useful for identification of products from disperse vat dyes. Treated and untreated effluents were centrifuged. Equal volumes of the supernatant collected were mixed with diethyl ether separately in order to retrieve the organic content of the treated and untreated effluent samples. The organic layer was then collected and allowed to evaporate at room temperature. The residue that remains was then suspended in 5 ml 100% methanol. The methanol solution was then used for GC-MS analysis for both the treated and untreated effluent samples separately. GC-MS was performed using a THERMO GC-TRACE ULTRA VER: 5.0, THERMO MS DSQ II (version 1.10 beta, Shimadzu) (Adosinda *et al.*, 2003).

Biodecolorization and degradation of Indigo dye using various analytical methods. In order to uncover the possible mechanism of indigo dye decolorization and to identify the metabolites generated from indigo dye after bacterial treatment, various analytical techniques were used. The thin layer chromatography (TLC) (Campos *et al.*, 2001) and nuclear magnetic resonance spectroscopy (NMR) (Ramya *et al.*, 2008) have been proved to be ideal methods with the quantification of secondary metabolites from treated indigo dye. Biofilm developed on polyurethane foam was taken and air dried and SEM analysis was done. HBL 100 cells (28-homo-brassinolide/Breast cancer cell line) were purchased from National Centre for Cell Science (Pune, India) and maintained in DMEM and McCoys medium, supplemented with non-essential amino acids. Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2 in a CO_2 incubator. Cells were cultured and $\sim 1 \times 10^4$ cells/wells were seeded into 96 well tissue culture plates and incubated for 48 h. HBL 100 cells were treated with 50 μL concentration of processed indigo dye and crude indigo dye.

$$\text{Percentage of Cytotoxicity} = \frac{\text{Mean OD value of experimental sample}}{\text{Mean OD value of experimental control (untreated)}} \times 100$$

Note: OD – Optical Density values of treated and untreated HBL cells

Adsorption isotherm kinetics. Adsorption isotherms were studied with bacterial consortium fixed inert PUF to evaluate the dye adsorption capacity of denim industrial wastewater. The constant in the *Langmuir isotherm* was determined by plotting Ce/(x/m) versus Ce and making use of Eq. 1 written as,

$$Ce/(x/m) = 1/ab + 1/a(Ce) \quad (1)$$

x/m = mass of g adsorbate (PUF), a, b = empirical constant, Ce = equilibrium concentration of adsorbate in solution after adsorption (mgl^{-1}). In *Freundlich isotherm* was determined by plotting Ce/(x/m) versus Ce and making use of Eq. 2 written as,

$$Log(x/m) = Log\ Kf + (1/n) \log Ce \quad (2)$$

Lagergren (Pseudo first order)

$$\text{Log}\,(q_e - q) = \log q_e - K_{ad} / 2.303 \times t \quad (3)$$

Whereas, q and q_e are amounts of adsorbate adsorbed (ml l^{-1}) at time, t (h) and at equilibrium respectively. K_{ad}, is the adsorption rate constant (1 per h).

Pseudo-second order

$$t/Q_t = (1/K_1 Q_e^2) + (1/Q_e)\, t \quad (4)$$

Where, Q_e, is the amount of adsorbate adsorbed at equilibrium (ml l^{-1}). Q_t, is the amount of adsorbate adsorbed at time *t* (h); K_1, is the second order equilibrium rate constant (ml per h).

Mathematical modeling on bioremediation efficiency of multiple species used as biobarrier model. The model of water flow, the transport of nutrients and degradation of indigo dye as well as the growth of biofilm forming bacteria are done. Considering the limiting nutrients in the simulations further nutrients are added. In this section it is discussed about qualitative results of some dual species biobarrier simulations and the multispecies biofilm experiments that could lead to the design of more effective bioremediation techniques.

In order to model multi-species biofilm interactions in porous media a three phase mixture consisting of a liquid phase, a solid PU foam phase and a biofilm phase is taken. The fundamental equation for saturated transient ground water flow of constant density in horizontal direction (E-1),

$$S_s \frac{\partial h}{\partial t} - \frac{\partial}{\partial x}(K \frac{\partial h}{\partial x} = 4) = f \quad \text{(E-1)}$$

Statistical analysis. The statistical analysis was done by comparing various physico-chemical parameters with bioremediation of denim textile wastewater. All analysis was conducted in triplicate and results presented are the mean of triplicate ± standard deviations (SD).

Results and Discussion

Isolation, screening and identification of adapted bacterial strains. The indigo carmine dye containing plates after incubation showed zone of clearance around number of colonies. Among these only 30 bacterial colonies which showed a zone of clearance only 4 bacterial isolates were effective in the degradation of indigo carmine by showing a reduction pattern of more than 60%. These microorganisms (PK07, PK18, PK23 and PK28) were capable of degrading the pie bond which in turn reduced the H – chromophore of the indigo carmine dye without any biosorption characters. A majority of the bacterial strains which were primarily selected from the solid media showed biosorption characteristics in broth assay.

The UV-visible spectrophotometer showed different absorbance values for the media incubated with the 30 bacterial isolates. This variation for each culture depends on the metabolites produced by them and the ability of those metabolites to react with the dye compound and converting them into their secondary derivatives. The absorbency was measured at 610 nm (Rajendran *et al.*, 2011). From the morphological and biochemical results, all the selected adapted bacterial isolates were again identified on the basis of 16S rRNA gene sequence. Based on the sequence alignment on BLAST revealed the isolate belongs to *Actinomycetes* sp. (PK07), *Pseudomonas aeruginosa* (PK18), *Stenotrophomonas rhizophila* (PK23) and *Staphylococcus pasteuri* (PK28) with 77%, 92%, 99% and 86% sequence similarities respectively. Similar work (Balan and Monteiro, 2001) was proved among many indigo carmine degrading microorganisms and the predominant cultures.

Comparison of treatment trails on bioremediation of denim industry wastewater by pure broth culture, alginate immobilized cells and PU foam immobilized cells. The treated effluent samples of broth cultures were checked for their bioremediation ability after 5 days of incubation. A maximum percentage of reduction was seen in consortia and their reduction percentages were COD – 61.7%, Colour – 75.38%, TS – 60.10%, TSS – 79%, TDS – 73.2%,Turbidity – 42.3%, Hardness – 63.04%, Conductivity – 14%, Resistivity – 29.3%, Salinity – 11.9%, pH – 12.2%. Mixed bacterial cultures from a wide variety of habitats have shown to decolorize the diazolinked chromophore of dye molecules in 15 days (Knapp and Newby, 1995). A specific bacterium *Pseudomonas putida* was chosen to utilize the aromatic structures.

A maximum reduction percentage was seen in bacterial consortia (Ca-alginate) and their reduction pattern were, COD – 77.3%, Colour – 78.1%, TS – 47.5%, TSS – 47.02%, TDS – 34.9%, Turbidity – 69.5%, Hardness – 54.3%, Conductivity – 5.14%, Resistivity – 45.63%, Salinity – 12.9%, pH – 11.08%. On comparing, other two treatment trails (PUF immobilized cells and free cells) were suitable for the bacterial biomass production as well as increasing the bioremediation efficiency. Therefore, it can be concluded that the entrapment technique is not suitable for the immobilization of mixed bacterial consortia because of surrounding environmental conditions (Trevors *et al.*, 1992).

A maximum percentage of reduction was seen in consortia and their reduction percentages were COD – 88.3%, Colour – 93.1%, TS – 84.5%, TSS – 50.8%, TDS – 60.9%,Turbidity – 66.7%, Hardness – 76.9%, Conductivity – 5.1%, Resistivity – 29.3%, Salinity – 11.9%, pH – 12.2%. The storage stability and microbial activity of

encapsulated cells in PUF are better than those of cells encapsulated in Ca-alginate beads as well as pure broth culture. Similar works (Kim *et al.*, 2002; Forgas *et al.*, 2004) have explained the efficiency of decolorization and biodegradation enhanced by the absorption of the dye on the biomass.

Optimization of cultural conditions for maximum biodegradation ability.

Effect of retention time. A reduction was observed for the selected bacterial combination immobilized on polyurethane foam from the first day onwards till 7 days. A significant reduction was seen in third day of incubation. The reduction percentages were shown in Table I. This was similar to the work (Kim *et al.*, 2002) has explained, a decrease of COD removal efficiency by 20% was observed in fourth day due to the sloughing of biofilm in the fixed film bioreactor.

Effect of inert substrate concentration (PUF). A maximum reduction was seen in the foam quantity of 1.5%W/V. The maximum percentage of reduction for all the parameters was shown in Table IIa. Zaiat *et al.* (2000) developed a new reactor configuration, the horizontal-flow anaerobic immobilized-biomass (HAIB) reactor utilizing polyurethane foam matrices for biomass immobilization.

Effect of initial pH. A percentage of reductions of all the parameters are shown in Table IIb. The pH 7 was considered as optimum range because only in the neutral pH organisms survive better (Rump and Krist, 1992). Increase in pH affects various other parameters present in polluted water. Highly alkaline or highly acidic pH is not suitable for the indigenous organisms present in the effluent.

Effect of incubation temperature. An efficient reduction was observed at 27°C (Table IIIa). The optimal temperature for bacteria ranges from 20°C to 37°C. The metabolic activities will be higher only at this range. Thus maximum degradation was seen in 27°C. In the similar experiments (Khlifi *et al.*, 2010) were conducted at different temperatures, 20°C, 25°C, 30°C, 35°C. The maximum degradation of waste water was found at 30°C.

Effect of initial substrate concentration. When different concentration of effluent samples were supplemented for the consortium, an efficient reduction was observed at 40% of the effluent sample (Table IIIb). From the results, a significant reduction occurred in diluted organic load rather than direct effluent. This was because at very low concentrations the quantity of organic content will be low. Similar results were observed in the work of Khlifi *et al.* (2010) where optimal decolorization occurred with 20% effluent at pH 5.

Effect of incubation time for biofilm development. When biofilm was allowed to develop over increased period of incubation time (1 day up to 5 days), biofilm developed on first day of incubation was found to be more efficient in the bioremediation of effluent (Table IV). When cultures were grown as biofilm the growth rate will be faster due to matrix formation and maintain a stable log phase.

Treatment trails of denim effluent under optimized cultural conditions. The maximum degradation under all the above optimized conditions were found to be TS – 41%, TSS – 75%, Turbidity – 42%, Color – 86%, COD – 89%, pH – 21%, Conductivity – 38%, TDS – 64%, Salinity – 30%, Hardness – 54% and Resistivity – 38%.

GC-MS analysis. The compounds present for the respective peaks were 1,2-benzene dicarboxylic acid, bis (2-ethylexyl) ester, Hexadecane-2-methyl, Hexadecane-2,6,10,14-tetramethyl, octacosone. The compounds

Table I
Effect of retention time on bioremediation of denim industrial effluent (Mean and Standard deviation, n = 3).

	Effect of retention time (days)						
	1	2	3	4	5	6	7
Total Solids	39.45 ± 0.92	40.51 ± 0.54	45.10 ± 0.60	54.06 ± 0.73	66.47 ± 0.72	77.52 ± 0.65	76.40 ± 1.20
TSS	40.89 ± 0.50	45.75 ± 0.45	49.84 ± 0.58	58.30 ± 0.85	61.41 ± 0.89	61.26 ± 1.10	60.21 ± 1.01
Turbidity	17.93 ± 0.40	21.45 ± 0.89	22.94 ± 0.58	26.40 ± 0.89	31.53 ± 0.87	29.44 ± 0.96	29.38 ± 1.28
Color	45.13 ± 0.51	53.86 ± 0.34	60.65 ± 0.63	63.58 ± 0.93	74.55 ± 0.91	72.32 ± 0.81	71.31 ± 1.10
COD	50.58 ± 0.65	55.42 ± 0.88	61.86 ± 0.40	68.45 ± 0.88	79.41 ± 0.69	79.35 ± 0.91	79.17 ± 0.77
pH	5.11 ± 0.24	5.63 ± 0.65	5.67 ± 0.54	5.86 ± 0.91	6.69 ± 0.97	6.39 ± 0.79	6.41 ± 1.30
Conductivity	41.25 ± 1.00	43.71 ± 0.56	48.66 ± 0.61	60.26 ± 0.90	70.40 ± 1.27	70.33 ± 0.94	70.38 ± 1.07
TDS	16.60 ± 0.61	30.40 ± 1.10	35.82 ± 0.50	49.50 ± 1.13	59.82 ± 0.99	58.54 ± 0.94	57.28 ± 0.98
Salinity	15.92 ± 0.30	18.69 ± 0.57	24.84 ± 0.48	34.66 ± 0.85	43.60 ± 1.06	42.69 ± 0.61	42.48 ± 1.25
Hardness	38.05 ± 0.50	49.07 ± 0.52	56.01 ± 0.65	68.32 ± 0.89	75.53 ± 1.17	74.88 ± 0.91	74.42 ± 1.29
Resistivity	22.93 ± 0.40	28.65 ± 0.60	34.89 ± 0.40	48.65 ± 1.39	56.71 ± 0.65	52.32 ± 0.97	49.42 ± 1.28

Note: TSS – Total Suspended Solids; COD – Chemical Oxygen Demand; TDS – Total Dissolved Solids

Table II
Effect of inert PUF concentration (a) and initial pH (b) on bioremediation of denim industrial effluent (Mean and Standard deviation, n = 3).

	(a) Effect of inert PUF concentration (g/ml)					(b) Effect of initial pH				
	0.5	1	1.5	2	2.5	5	6	7	8	9
Total Solids	35.38 ± 0.88	39.07 ± 0.63	55.45 ± 0.87	49.23 ± 0.90	41.55 ± 0.63	49.62 ± 0.68	64.61 ± 0.58	56.41 ± 0.98	51.18 ± 1.03	45.47 ± 0.92
TSS	38.61 ± 0.68	45.29 ± 0.65	72.02 ± 1.47	61.49 ± 1.31	36.54 ± 1.48	45.82 ± 0.91	59.31 ± 0.97	52.34 ± 0.93	49.25 ± 0.86	46.67 ± 1.17
Turbidity	46.13 ± 0.19	50.85 ± 0.82	64.26 ± 0.78	48.72 ± 1.45	44.60 ± 0.73	23.70 ± 0.88	40.13 ± 1.02	31.45 ± 0.93	29.85 ± 0.48	25.48 ± 1.15
Color	42.99 ± 0.75	61.22 ± 0.94	71.25 ± 1.10	54.87 ± 0.94	48.51 ± 0.87	49.45 ± 0.88	69.15 ± 0.77	55.34 ± 0.95	57.74 ± 0.91	50.12 ± 1.04
COD	43.01 ± 0.56	45.82 ± 0.41	74.67 ± 1.06	51.31 ± 0.89	45.27 ± 0.87	40.19 ± 1.03	66.17 ± 0.99	55.44 ± 1.21	49.65 ± 0.51	47.18 ± 0.91
pH	5.91 ± 0.88	6.55 ± 0.62	14.56 ± 0.79	6.95 ± 0.43	8.53 ± 0.69	7.02 ± 0.81	6.61 ± 0.54	6.42 ± 0.78	6.16 ± 0.61	5.51 ± 0.69
Conductivity	49.56 ± 0.62	61.12 ± 0.78	78.18 ± 0.87	71.25 ± 1.00	66.02 ± 0.89	60.19 ± 0.93	72.61 ± 3.41	65.99 ± 0.79	59.56 ± 0.62	55.47 ± 0.96
TDS	36.88 ± 0.32	45.58 ± 0.57	59.41 ± 0.77	47.49 ± 0.93	40.59 ± 1.11	33.86 ± 0.98	54.76 ± 1.11	46.30 ± 0.76	47.55 ± 1.09	46.05 ± 0.71
Salinity	33.98 ± 1.23	45.42 ± 1.48	45.45 ± 0.46	31.15 ± 1.40	30.83 ± 1.43	55.21 ± 0.90	64.28 ± 0.96	61.45 ± 0.84	60.16 ± 1.03	45.39 ± 0.84
Hardness	35.86 ± 0.84	39.54 ± 0.64	68.42 ± 0.68	43.52 ± 0.98	31.42 ± 1.16	41.25 ± 1.00	58.03 ± 0.72	47.80 ± 0.85	48.11 ± 0.92	45.23 ± 1.07
Resistivity	52.06 ± 0.90	54.02 ± 0.19	72.84 ± 0.65	64.34 ± 0.87	62.25 ± 1.65	60.37 ± 1.07	70.54 ± 0.38	68.40 ± 0.91	66.51 ± 0.48	59.37 ± 0.90

Note: TSS – Total Suspended Solids; COD – Chemical Oxygen Demand; TDS – Total Dissolved Solids

Table III
Effect of temperature (a) and initial substrate concentration (b) on bioremediation of denim industrial effluent (Mean and Standard deviation, n = 3).

	(a) Effect of temperature (°C)				(b) Effect of initial substrate concentration (g)				
	7	17	27	37	0.2	0.4	0.6	0.8	1
TS	53.2 ± 0.64	59.18 ± 0.63	75 ± 0.35	68.03 ± 0.49	40.66 ± 0.54	46.95 ± 0.49	53.51 ± 0.90	65.33 ± 0.81	76.87 ± 0.46
TSS	30.2 ± 0.64	43.82 ± 0.37	54.25 ± 0.58	45.16 ± 0.49	39.94 ± 0.57	45.05 ± 0.65	48.98 ± 0.50	57.97 ± 0.44	61.10 ± 0.63
Turbidity	14.59 ± 1.20	25.66 ± 0.56	38.7 ± 0.64	20.77 ± 0.52	15.95 ± 0.50	18.08 ± 0.20	26.00 ± 0.75	27.88 ± 0.44	31.51 ± 0.87
Color	43.85 ± 0.60	48.72 ± 0.56	65.91 ± 0.79	55.14 ± 0.43	40.09 ± 0.66	53.91 ± 0.34	60.23 ± 0.99	64.00 ± 0.24	76.93 ± 0.51
COD	40.39 ± 1.10	45.16 ± 0.33	51.23 ± 1.03	40.96 ± 0.72	48.41 ± 0.50	54.85 ± 0.48	62.10 ± 0.32	68.07 ± 0.55	79.60 ± 0.64
pH	7.03 ± 0.20	6.83 ± 0.68	7.13 ± 0.13	7.88 ± 0.35	5.08 ± 0.24	5.00 ± 0.27	4.81 ± 0.37	5.43 ± 0.34	6.50 ± 0.63
Conductivity	54.71 ± 0.49	60.34 ± 0.91	75.04 ± 0.42	47.99 ± 0.42	39.92 ± 0.58	43.75 ± 0.56	48.47 ± 0.65	60.55 ± 0.62	69.70 ± 0.54
TDS	49.58 ± 0.68	58.51 ± 0.92	63.96 ± 0.67	53.72 ± 0.63	36.82 ± 0.50	38.95 ± 0.78	43.17 ± 0.76	49.67 ± 0.49	59.80 ± 0.45
Salinity	32.40 ± 0.73	40.91 ± 0.37	54.79 ± 0.68	40.10 ± 0.51	14.89 ± 0.71	18.01 ± 0.45	24.27 ± 1.04	34.57 ± 0.72	43.79 ± 0.40
Hardness	33.88 ± 0.52	44.21 ± 0.55	50.85 ± 0.35	41.49 ± 0.99	21.68 ± 1.15	27.96 ± 0.38	34.93 ± 0.36	48.00 ± 0.46	56.83 ± 0.45
Resistivity	65.03 ± 0.49	68.38 ± 0.87	73.78 ± 0.59	54.01 ± 0.49	40.65 ± 0.43	51.43 ± 0.43	65.13 ± 0.63	71.28 ± 0.84	75.20 ± 0.24

Note: TSS – Total Suspended Solids; COD – Chemical Oxygen Demand; TDS – Total Dissolved Solids

Table IV
Effect of biofilm optimization on bioremediation of denim industrial effluent
(Mean and Standard deviation, n = 3).

	Effect of biofilm optimization (days)				
	1	2	3	4	5
TS	34.38 ± 0.407	22.84 ± 1.50	21.10 ± 1.39	19.42 ± 0.89	18.30 ± 0.65
TSS	23.06 ± 0.12	20.07 ± 1.88	13.53 ± 1.13	13.54 ± 0.50	15.20 ± 0.64
Turbidity	34.79 ± 0.20	28.92 ± 1.54	31.42 ± 1.09	24.43 ± 0.81	23.06 ± 0.89
Color	65.37 ± 0.23	62.17 ± 0.81	56.19 ± 0.70	51.32 ± 0.92	49.74 ± 1.39
COD	43.27 ± 0.28	25.48 ± 0.91	35.58 ± 0.89	30.46 ± 0.74	31.74 ± 1.25
pH	12.23 ± 0.27	12.49 ± 0.73	24.31 ± 0.91	19.26 ± 0.79	16.33 ± 1.30
Conductivity	14.45 ± 0.17	22.00 ± 1.39	13.33 ± 0.84	26.25 ± 0.86	24.79 ± 1.48
TDS	46.53 ± 0.33	44.54 ± 0.88	38.33 ± 0.85	29.46 ± 0.76	31.34 ± 1.36
Salinity	50.22 ± 0.08	50.48 ± 1.03	44.31 ± 0.81	37.45 ± 2.07	35.26 ± 1.01
Hardness	35.28 ± 0.24	25.58 ± 0.85	26.73 ± 1.15	25.33 ± 0.52	24.51 ± 0.64
Resistivity	49.19 ± 0.15	36.07 ± 1.48	41.43 ± 1.00	33.48 ± 0.74	32.97 ± 0.05

Note: TSS – Total Suspended Solids; COD – Chemical Oxygen Demand; TDS – Total Dissolved Solid

present in the treated sample were found to be octadecane, S-phenyl-1-butylethene-2-(monothio) carboxylate which showed peak ranges 23.14, 19.51 and 11.88 respectively which were nontoxic. Utkarsha and Jyoti (2011) reported the degradation product of the textile industry effluent by *Penicillium* was studies by GCMS analysis.

Biodecolorization and degradation of Indigo dye using various analytical methods.

ʻH-NMR analysis. The ʻH-NMR spectrum of the Indigo dye showed two singlets in the downfield and aromatic proton at 7.6 δ and corresponding to NH and two doublets at 6.9 δ and 6.8 δ, which accounts for the presence of two adjacent protons on the aromatic ring. The overall spectrum clearly depicts the structure of Indigo dye. On the other hand, the Indigo dye which has undergone effective degradation was recorded by spectrum. The end product isatin exhibited three singlets at 7.5 δ, 6.9 δ and 6.8 δ which reveal the presence of the proton of SO_3H group an aromatic proton (Ramya *et al*., 2008).

Morphological studies of immobilized bacterial cells on PUF by scanning electron microscope (SEM). The PU foam substrates used were having highly flexible and porous surface providing large surface area for immobilizing adapted bacterial cells for biofilm formation. The bacterial biofilm consisted of heterogeneous population of short and long rods, cocci and filaments. Rile *et al.* (1999) has observed the majority of F92 cells were immobilized on the outer surfaces of PUF in SEM.

Thin layer chromatography. The indigo dye is used as a unique source of carbon and nitrogen available to adapted bacterial consortium in this assay, it indicates the possibility that indigo undergoes a biodegradation process. The degradation was demonstrated by thin layer chromatography of treated indigo dye extract, it is observed that the control dye has slight mobility and one known metabolite *i.e.* Rf = 0.2 which indicates the presence of isatin which has been detected in the extracts where indigo was present. Very few reports are available on the biodegradation products of indigo dyes. The degradation of indigo by laccases produces isatin (indole-2,3-dione), it is further degraded to anthranilic acid (2-aminobenzoic acid) and is observed through HPL analysis (Campos *et al*., 2001).

Cytotoxicity test. The adapted mixed bacterial secondary metabolites which were released into the treated medium during biodegradation were not cytotoxic and the cells died as a result of the toxicity of indigo dye. However the decolorized indigo dye was nontoxic to the cells at a concentration of 50 μl in 48 hours while the crude indigo dye killed over 98% of the cells. It is evident from that the percent viability of HBL 100 cells was not drastically affected when the tissue was stored at 37°C up to 3 days. Labib *et al.* (2012) reported a case of fatal poisoning in a 3-year-old child after administration of indigo for therapeutic purposes (diarrhea, vomiting and fever).

Adsorption isotherms. In Langmuir plots for adsorption, the linear plots of C_e / q_e vs C_e confirm that the adsorption follows the Langmuir isotherm model. Langmuir constants, Q_o and b were determined from the slope and intercept of the respective plots. From the Q_o and b values it could be depicted that PU foam immobilized bacterial cells was efficient in adsorbing the dyes. The Q_o and b values of indigo dye containing denim wastewater adsorption by PU foam immobilized cells were 20.4 and 140.74 mg/g, respectively. There was a wide difference in adsorption capacities between

bacterial biomasses studied, in particular with indigo dye adsorption. The Freundlich equation is used for heterogenous surface energies in which the energy term, Q_o, in the Langmuir equation varies as a function of the surface coverage, q_e, strictly due to variation in the heat of adsorption.

'n' gives an indication of favorability and Kf [mg/g (mg/l-1)n], the capacity of the adsorbent. This Freundlich adsorption isotherm was applied for the adsorption of indigo dye containing denim wastewater by PU foam immobilized cells, free cells and Ca-alginate immobilized cells in agitated mode (McKay *et al*., 1985). In the present study, the n values were found to be in the range of 5.865 and K_f values at 85.176 for adsorption of dyes studied onto PU foam immobilized cells in agitated mode. On the other hand, the Free (0.405) and Ca-alginate immobilized cells (0.625) were noted for 'n' values ranging at less than one.

Adsorption kinetics. To study the adsorption kinetics, two kinetic models were used that include Lagergren (Pseudo-first order) and Pseudo-second order models. The linearized form of Lagergren (Lagergren, 1898) and Pseudo-second order models can be studied in below. The straight line plots of log (q_e-q) vs t (time) indicates the applicability of the Lagergren equation. The K_{ad} values were calculated from the slope of the linear plots and were observed to be in the range at $\times 10^{-2}$ 1 per min at 27°C, respectively for adsorption of indigo dye by PU foam immobilized cells in agitated mode. The adsorbent rate was about 1.4×10^{-2} 1 per min (PU foam immobilized cells) at 100 mg/l^{-1} dye concentration, respectively. Khattri and Singh (2000) reported that the temperature did not have any significant effect on the rate constant of crystal violet adsorption on neem sawdust. The K_{ad} values obtained in the present study are comparable with these observations, only PU foam immobilized cells were effective for removal of indigo dye than other treatment trails. Initially, the validity of the two models was checked by studying the kinetics under different initial dye concentrations. For Lagergren plot (free and Ca-alginate immobilized cells), correlation coefficients were found to be 0.82 to 1, but the calculated Q_e varied widely to experimental Q_e, suggesting the sufficiency of the model to fit the kinetic data for the initial concentrations examined. The reason for these differences in the Q_e values is that there is a time lag, possibly due to a boundary layer or external resistance controlling at the beginning of the sorption process. Whereas, the PU foam immobilized cells of correlation coefficient was found to be 0.99, hence from these results, we concluded that this model was highly sufficient for removal of indigo dye. The equilibrium rate constant of second order kinetics model, K_1, was calculated from the slope of linear plots. The values of K_1, ranged at 0.032 g^{-1} mg^{-1} min $\times 10^{-2}$ for the dyes studied in PU foam immobilized cells. The data obtained in the present study showed that dye adsorption by PU foam immobilized cell biomass fits well with the pseudo second order model than Lagergren model. Correlation coefficients were mostly greater than 0.99, and the lowest correlation coefficient (0.94) was better than the first order model correlation coefficients.

Mathematical modeling on bioremediation efficiency of multiple species used as biobarrier model. A multispecies biofilm interaction in porous media was considered with a three phase mixture consisting of a liquid phase, a solid PU foam phase and a biofilm phase. The biofilm can be considered to be part of the solid phase, it is simpler to take it as a separate phase. The four bacterial species present in the porous medium develops strong biofilm forming microbes with the nutrients. The fundamental equation for saturated transient ground water flow of constant density, in horizontal direction, can be written in the form (Allen *et al*., 1988),

$$S_s \frac{\partial h}{\partial t} - \frac{\partial}{\partial x}(K \frac{\partial h}{\partial x} = 4) = f \quad (1.1)$$

The single fluid flow equation (1.1) arises from the mass balance law

$$S_s \frac{\partial h}{\partial t} + \frac{\partial v}{\partial x} = f \quad (1.2)$$

Substitute for the specific discharge vector υ using Darcy's law

$$v = -K \frac{\partial h}{\partial x} \quad (1.3)$$

Here h (x, t) denotes the hydrolic head, S_s is the specific storage, K is the saturates hydraulic conductivity and f (x, t) represents source. The specific discharge vector υ (x, t) called Darcy velocity represents the speed of the water. We assume there are no sources for the fluid, therefore = 0 in equation (1.1). Invoking the above simplifying assumptions to equations (1.1),

$$-\frac{\partial}{\partial x}\left(K \frac{\partial h}{\partial x}\right) = 0 \quad (1.4)$$

The transport and reaction of nutrients and contaminants, and the growth of the two microbial species are governed by a system of partial differential equations. Finally we can work only with the liquid and biofilm phases,

$$\frac{\partial}{\partial t}\left(\phi^L \rho C\right) + \frac{\partial}{\partial x}\left(\upsilon \rho C\right) - \frac{\partial}{\partial x}\left(D \frac{\partial \rho T}{\partial x}\right) = rC\left(\rho B, \rho K, \rho C, \rho T\right) \quad (1.5)$$

Here ρi, i = B, K, C, T, represents the intrinsic mass density of the contaminant degrading microbes, the strong biofilm forming microbes and nutrients respec-

tively. It follows that the small initial biobarrier-forming microbial concentrations are given by,

$$\phi\left(\tilde{X}_B, \tilde{X}_K\right) = \phi_0\left(1-\tilde{X}_B-\tilde{X}_K\right)$$
$$K\left(\tilde{X}_B, \tilde{X}_K\right) = K_0\left(1-\tilde{X}_B-\tilde{X}_K\right)^{nk} \quad (1.6)$$

Incorporating the above simplifying assumptions into equations (1.5) and using normalized concentrations as the unknowns yields the following governing system of deferential equation,

$$\frac{\partial S_C}{\partial t}+\nu\frac{\partial S_C}{\partial x}-\frac{\partial}{\partial x}\left(D\frac{\partial S_C}{\partial x}\right)=-\frac{1}{Y_K}\mu^K\left(S_C\right)X_K\,G\left(X_B+X_K\right) \quad (1.7)$$

with γ typically small. One common assumption in Monod's growth rate for bacteria is that there is only one nutrient that limits the growth. That is, there is an excess of the other nutrients. In this case the rate of substrate utilization has been usually described by Bailey and Ollis (1986).

$$\mu^j\left(S_i\right)=\mu^j_{\max}\frac{S_i}{K^j_{S_i}+S_i^{'}}$$

j = B, K, where Si is the concentration of the limiting nutrient.

For the biobarrier model;

$$\mu\left(S_1, S_2, ..., S_m\right)=\mu^j_{\max}\prod_{i=1}^{m}\frac{S_i}{K^j_{S_i}+S_i^{'}} \quad (1.8)$$

j = B,K, is used to describe the kinetics of microbial transformations of nutrient and contaminant. In System the Monod expressions are given by,

$$\mu\left(S_C\right)=\frac{\mu^K_{\max}S_C}{K^K_{S_C}+S_C^{'}} \quad (1.9)$$

Simulations. To determine the solution of an ordinary deferential equation (1.4) we use a standard finite deference method to calculate h. Then we were using numerically differentiate Darcy's law (1.3) to get the velocity field v. The temporal differentiation in the microbial species equations (1.5) uses a forward Euler time integration. The nutrients and bacterial transport equations are solved using a Ghill shooting method (Kojouharov and Chen, 2000).

The first dual-species biobarrier model (1.7) with standard Monod kinetics (1.9) has been validated in with the porous media experiments done by Cunningham *et al.* (1991) for a 1 × 1 cm long PU foam cube with 0.70 mm, in diameter. In our first simulation, we consider initial and boundary conditions for S_C that correspond to a high nutrient concentration experiment, *i.e.* S_C (x, 0) = S_C (0, t) = 175 μg/ml. This is clearly the case, since the thresholds are smaller than the steady state values for the nutrient. In this scenario, the steady-state biofilm forming bacterial population density was able to degrade the contaminant Indigo dye containing denim industrial wastewater.

Conclusions. Operational stability and longevity of cells encapsulated in PU foam are significantly better than free and alginate cells. Biodegradation of industrial effluents rich in organic pollutants with immobilized bacterial strains in PU foam is an extremely versatile approach that can be used in detoxification of denim waste for longer periods, provided the process is made economical and convenient for use on a large scale.

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