Summary and Conclusion
A well-flourished textile industry development has originated increasing use of a wide variety of synthetic dyes. It is estimated that annually 280,000 tonnes of textile dyes are discharged in such industrial effluent. Azo dyes are about a half of all known dyestuffs in the world, making them the largest group of synthetic colorants released into the environment. Azo dyes account for the majority because of an ease and cost effectiveness of their synthesis, their superior fastness to the applied fabric, high photolytic stability, recalcitrant to microbial degradation and the variety of colors available compared to natural dyes. They are extensively used in the textile, paper, food, leather, cosmetics and pharmaceutical industries. Inefficiency of the textile processing results a large amount of dyestuff being directly lost in the wastewater, which ultimately finds way into the environment. It is estimated that 5-10% of the dyes is lost in the effluent during the dyeing process, while in the case of reactive dyes, as much as 50% of the initial dye load is present in the dye bath effluent. Thus pollution by wastewater contaminated with dyestuff is becoming increasingly alarming worldwide. The release of colored effluents into the environment is undesirable, not only because of their color, but also because of many dyes, dye precursors, and their biotransformation products such as aromatic amines have been found to be toxic, mutagenic, carcinogenic in nature, and having the potential of bioaccumulating in the food chain. In addition, it also affects water transparency, water quality and gas solubility in water bodies, and depicts acute toxic effects on aquatic flora and fauna. Where as on the land it inhibits the germination rate of several plants which having important ecological function and decrease soil fertility. Therefore, the treatment of industrial effluents containing azo dyes and their metabolites becomes necessary prior to their final discharge in to the environment. The implementation of existing physical/chemical methods have inherent drawbacks of being economically unfeasible (more energy consumption and chemicals uses), unable to completely remove the recalcitrant azo dyes and their organic metabolites, generating a significant amount of sludge that may cause secondary pollution problems, and involving complicated procedures.
In recent years, microbial or enzymatic decolorization and degradation of azo dyes has received much attention due to their eco-friendly, inexpensive nature, do not produce large quantities of sludge. Microorganisms such as aerobic and anaerobic bacteria, fungi, actinomycetes and algae have been found efficient not only for color removal but also for the complete mineralization of the dyes. In order to develop a practical bioprocess for the treatment of dye wastewater, it is necessary to identify the capable microorganisms in the degradation of azo dyes. To make this concern, we have studied yeast and bacteria for the decolorization and degradation of various azo dyes.

Bacterial cells represent an inexpensive and promising tool for the removal of various azo dyes from the textile dye effluents. Bacteria have many advantages as compared to filamentous fungi such as, faster growth rate, higher hydraulic retention time and could be efficient in treating high-strength organic wastewaters. Individual bacterial strain usually cannot degrade azo dyes completely and the intermediate products are often carcinogenic aromatic amines, which needs further decomposition. The advantage of mixed culture is apparent as some strains can collectively carry out effective biodegradation tasks that no individual pure strain can achieve due to synergistic metabolic activities of microbial community. Keeping these points in the mind, we have developed a microbial consortium-GR, consisting of *Proteus vulgaris* NCIM-2027 (PV) and *Micrococcus glutamicus* NCIM-2168 (MG). As both the bacteria are found to be efficient in the dye degradation, we have used this consortium for the decolorization of an industrial diazo dye Scarlet RR as well as mixture of various industrial dyes under static anoxic condition. The consortium-GR significantly decolorized Scarlet RR with an average decolorization rate of 16666 µg h\(^{-1}\); which is much faster than that of the pure cultures (PV, 3571 µg h\(^{-1}\); MG, 2500 µg h\(^{-1}\)). We have systematically investigated various physicochemical conditions Viz. agitation rate, temperature, pH and initial dye concentration of individual (PV and MG) and developed consortium-GR to achieve faster decolorization and degradation of Scarlet RR. Addition of carbon/nitrogen sources appeared to enhance decolorization activity of
consortium-GR in the presence of peptone and beef extract and also in the extracts of agricultural waste (namely, rice husk and rice straw). The use of agricultural waste for the decolorization makes the process more economically feasible and applicable. Consortium-GR showed best decolorization performance with nearly complete mineralization of Scarlet RR (over 90% TOC and COD reduction) within 3 h, much shorter relative to the individual strains. The ability of pure culture (PV and MG) and consortium-GR to decolorize repeated additions of Scarlet RR dye aliquots (50 mg l⁻¹) under static conditions were tested. Individual strains have ability to decolorize up to third dye aliquot addition sequentially increasing incubation period (48 h). In contrast, consortium-GR can decolorize up to seventh cycle within 48 h and further dye aliquot addition decreases decolorization performance with the increase in the incubation period. The eventual cessation of decolorization is likely to be due to nutrient depletion.

The difference in the fate of metabolism of Scarlet RR by an individual strain and with the consortium-GR is demonstrated using enzymatic status and analysis of the degraded products. A significant induction in consortium-GR culture for riboflavin reductase was 1897 and 730% relative to that obtained in pure culture of *P. vulgaris* and *M. glutamicus*, respectively. Likewise, the activity of NADH-DCIP reductase in consortium-GR culture was also 145 and 226% when compared to an individual strains. In contrast, there was slight induction in the lignin peroxidase in consortium-GR (105%) compared to *P. vulgaris*, whereas moderate induction in lignin peroxidase (285%) and laccase (183%) relative to *M. glutamicus*. Induction in the riboflavin reductase and NADH-DCIP reductase was observed in the consortium, suggesting the involvement of these enzymes during the fast decolorization process. The final product, 1,4-benzenediamine was characterized by GC-MS analysis. Phytotoxicity studies revealed non toxic nature of the biodegraded products of Scarlet RR by consortium-GR. In addition, consortium-GR applied for the mixture of twelve industrial dyes (at concentration, 30 mg l⁻¹ each) showed 88%
Decolorization under static condition with significant reduction in TOC (62%) and COD (68%) within 72 h.

Enhanced decolorization of various reactive dyes was observed by using developed consortium-GR. Among which, higher decolorization and degradation of toxic, sulfonated reactive dye Green HE4BD (50 mg l\(^{-1}\)) was observed by consortium-GR, with an average decolorization rate (2083 µg h\(^{-1}\)), which is much faster than that of the pure cultures (PV, 694 µg h\(^{-1}\); MG, 1190 µg h\(^{-1}\)). Effect of different physicochemical parameters (agitation rate, temperature, pH etc.) to achieve maximum dye degradation and decolorization by an individual strain and consortium-GR were systematically investigated. The consortium-GR has ability to decolorize repeated additions of Green HE4BD dye aliquots (50 mg l\(^{-1}\)) up to fifth dye aliquot addition under static conditions. Extent of mineralization was determined with TOC and COD measurement, shows nearly complete mineralization of Green HE4BD by consortium-GR (up to 90% TOC and COD reduction) within 24 h, relatively higher to the individual strains. A significant induction in the extracellular lignin peroxidase (219%), riboflavin reductase (465%), azoreductase (439%) and intracellular lignin peroxidase (487%) was observed in the consortium-GR relative to the pure culture of \textit{P. vulgaris} and \textit{M. glutamicus}, respectively. Likewise, there was moderate induction in oxidative; laccase (139 and 177%) and reductive; NADH-DCIP reductase (156 and 151%) in the consortium-GR compared to individual strains. In contrast, slight induction in intracellular lignin peroxidase (139%) and moderate induction was observed in riboflavin reductase (198%), and azoreductase (181%) enzyme activity in the consortium-GR relative to \textit{M. glutamicus}. UV-Visible, TLC and HPLC analysis of extracted products confirmed the biodegradation of Green HE4BD by consortium-GR. Phytotoxicity and microbial toxicity studies demonstrated no toxicity of the biodegraded products of Green HE4BD by consortium-GR. The composition of textile effluent consists of a mixture of many synthetic dyes and the effluent characteristic in terms of pH, dissolved oxygen, organic, and inorganic chemical content etc. depends upon the textile processing. Thus, the microbial population
to be used as inoculum in the treatment process for removing color from these effluents must have the capability to decolorize different dyes. For that purpose, we have applied consortium-GR to mixture of four and eight toxic reactive dyes (at concentration, 50 mg l\(^{-1}\) each) showing up to 80% decolorization (in terms of decrease in ADMI value), with significant reduction in TOC and COD within 72 h. Thus the concerted activities of the consortium-GR consisting of \textit{P. vulgaris} and \textit{M. glutamicus} were able to decolorize and degrade Scarlet RR and sulfonated toxic Green HE4BD and also have ability to decolorize mixture of dyes with significant decolorization rate and required much less incubation time relative to individual strains under static condition. The foregoing results suggest the potential application of this developed consortium in the bioremediation of dye-containing wastewater via appropriate bioreactor operations.

The study of decolorization of Green HE4BD by an individual strain \textit{Micrococcus glutamicus} NCIM-2168 was performed. \textit{M. glutamicus} exhibited complete decolorization and degradation of Green HE4BD (an initial concentration of 50 mg l\(^{-1}\)) within 42 h at temperature 37 °C and pH 8, under static condition. Decolorization performance of Green HE4BD was 100 and 30% under static and shaking condition, respectively. Addition of carbon/nitrogen sources appeared to enhance the decolorization activity of \textit{M. glutamicus}, while extracts of agricultural waste (namely, rice husk and rice straw), instead of peptone and beef extract were found to be more economically feasible supplements to enhance the decolorization of Green HE4BD by \textit{M. glutamicus}. Extent of mineralization was determined with total organic carbon (TOC) and chemical oxygen demand (COD) measurement, showing a satisfactory reduction of TOC (72%) and COD (66%) within 42 h. A significant induction in the enzyme activity of riboflavin reductase (1692%), azoreductase (1117%), NADH-DCIP reductase (523%) was observed over the period of Green HE4BD decolorization by \textit{M. glutamicus} as compared to lignin peroxidase (278%) and laccase (112%) after complete decolorization (42 h) Enzyme studies show an involvement of the oxidoreductive enzymes in the
Analytical studies of the extracted metabolites confirmed the significant degradation of Green HE4BD into various metabolites and the final product naphthalene was confirmed by GC-MS analysis. The microbial toxicity and phytotoxicity assay revealed nontoxic nature of Green HE4BD metabolites. In addition, the *M. glutamicus* strain was applied to decolorize a mixture of ten reactive dyes (at concentration 30 mg l\(^{-1}\)), that showed 63% decolorization (in terms of decrease in ADMI value) within 72 h, along with 48 and 42% reduction in TOC and COD under static condition. To our knowledge, this could be the first report on the biodegradation of sulfonated Green HE4BD and mixture of ten recalcitrant reactive dyes by *M. glutamicus*.

We have studied the decolorization performance of Navy Blue HER using five different microorganisms Viz. *Trichosporon beigelii* (100%); *Yarrowia lipolytica* (42%); *Bacillus megatarium* (38%); *Cellulomonas biazotea* (40%); *Acinetobacter sp.* (38%). Among which *T. beigelii* gave better performance after 24 h incubation. Decolorization performance of Navy Blue HER was 100% under static condition and 30% under shaking condition. The growth of *T. beigelii* was observed to be more at static (9.2 g l\(^{-1}\)) as compared to shaking condition (4.2 g l\(^{-1}\)). The optimum condition for the decolorization of Navy Blue HER by *T. beigelii* was observed at temperature at 37 °C and at pH 7. The complete decolorization performance of Navy Blue HER in 100 ml batch culture of *T. beigelii* in nutrient broth at different cell concentrations (wet weight) 6, 12, 18, and 24 g l\(^{-1}\) was observed after 42, 24, 16 and 11 h, respectively. The results indicate that the rate of decolorization increases with an increase in the cell concentration and sequentially reduced the time required for complete decolorization of Navy Blue HER by *T. beigelii*. The effect of different concentrations of Navy Blue HER on the decolorization was observed by taking 30, 50, 70, 80, and 100 mg l\(^{-1}\), the required time was 12, 24, 28, 38 and 48 h, respectively. The percentage of decolorization decreased beyond 100 mg l\(^{-1}\) dye concentration. Only 40 and 20% decolorization was observed after 48 h at 150 and 200 mg l\(^{-1}\) dye concentration, respectively. Decrease in the decolorization rates in *T. beigelii* may result due to the toxicity of the dye to
yeast cells and/or inadequate biomass concentration for the uptake of higher concentrations of dye. The percent of decolorization was observed maximum with yeast extract (100%), while less decolorization with other supplements of carbon and nitrogen source within 24 h. Decolorization of repeated addition of dye aliquots showed the effective dye decolorization up to 2 cycles. Sequentially, complete decolorization and decrease in TOC (95%) of Navy Blue HER by *T. beigelii* showed complete mineralization of the dye. A significant increase in the enzyme activity of NADH-DCIP reductase (689%) and azoreductase (706%) was observed over period of Navy Blue HER decolorization as compared to laccase (123%) and tyrosinase (130%) after complete decolorization (24 h) which presumably indicates an enzymatic reduction mechanism. UV-Visible, TLC, HPLC and FTIR analysis of extracted products confirmed the biodegradation of Navy Blue HER. Phytotoxicity study demonstrated no toxicity of the biodegraded products with respect to plants viz. *Phaseolus mungo* and *Sorghum vulgare*. In addition to Navy Blue HER, *T. beigelii* has ability to decolorize various industrial dyes (50 mg l⁻¹) Viz. Malachite Green, Crystal Violet, Methyl Violet, Orange HE2R, Red HE7B, Golden Yellow 4BD, Scarlet RR and Green HE4BD in the nutrient broth (pH 6.6) at 37 °C under static condition.

Cell immobilization by an entrapment within natural or synthetic matrices is particularly suitable for the bacterial decolorization of azo dyes since it creates a local anaerobic environment favorable to oxygen-sensitive decolorization. Immobilized-cell systems increase the biomass concentration; enhance the stability, mechanical strength, and reusability of the immobilized cell beads. The immobilized cell treatment system also allows an easier solid-liquid separation in a settling tank and eliminates the problems associated with bulking occurrence. Moreover, the dye can absorb to the gel matrix to enhance the decolorization efficiency. Therefore, immobilization of desirable specific bacteria (azo-dye-degrading bacteria) to remove refractory pollutants from the textile wastewater becomes feasible.
Decolorization performance of Reactive Blue 172 (50 mg l\(^{-1}\)) were studied using six different microorganisms viz. *Proteus vulgaris* NCIM-2027 (100%), *Trichosporon beigelii* NCIM-3326 (24%), *Bacillus megatarium* (38%), *Micrococcus glutamicus* NCIM-2168 (65%) *Pseudomonas desmolyticum* NCIM-2112 (40%) and *Acinetobacter sp.* (38%). Among them, *P. vulgaris* gave better decolorization performance (10,000 μg h\(^{-1}\)) and completely decolorized Reactive Blue 172 (50 mg l\(^{-1}\)) within 5 h incubation. In addition *P. vulgaris* has ability to decolorize various industrial dyes (50 mg l\(^{-1}\)) Viz. Reactive Red 120, Direct Orange 34, Reactive Red 2, Reactive Blue 25, Mordant Black 11, Methyl Orange, Reactive Orange 16, Reactive Blue 172, Reactive orange 4, Reactive Blue 203, Reactive Green 19A, Reactive Blue 171, Reactive Yellow 84 and Reactive Blue 59 in the nutrient broth (pH 6.6) at 37 °C under static condition. The potentiality and wide spectrum of decolorization ability of *P. vulgaris* induced us to perform the immobilization study using this strain. In this study, cells of *P. vulgaris* were immobilized in natural calcium alginate and K-carrageenan (CA and K-CGN) and synthetic polymer matrix polyacrylamide and polyvinyl alcohol (PAA and PVA). The decolorization rate was very low in case of PAA, PVA, and CGN-immobilized cells when compared to CA immobilized beads. The decolorization capacity of the CA immobilized cells was studied systematically investigating the effects of agitation rate, temperature, pH, dye concentration, immobilized bead concentration and using different pure carbon and nitrogen sources and 5 ml extract of agricultural by-products (1%) using synthetic media and pure distilled water to make the process applicable and economically feasible. Repeated-batch operations were performed to examine the reusability of the immobilized cells in azo dye decolorization. In free cells after five cycles, the decolorization rate dropped below 60%, while the complete decolorization was observed up to seventh cycle in CA immobilized cells. After seventh cycle there is a marginal reduction in the decolorization may be attributed to a significant decrease in the mechanical strength of CA immobilized cells. Reduction in TOC (84%) and COD (84%) results suggests nearly complete mineralization of this dye with nontoxic
residual metabolites evaluated by microbial toxicity tests. When mixture of various six reactive dyes (each at concentration; 50 mg l\(^{-1}\)) applied to CA immobilized cells of *P. vulgaris* in batch culture at 37 °C under static anoxic condition achieved higher color removal (in terms of ADMI value) with significant reduction of TOC (70%) and COD (69%) after 24 h incubation. Significant induction in the enzyme activity of azoreductase (608%), riboflavin reductase (540%), and NADH-DCIP reductase (385%) was observed over the period of Reactive Blue 172 decolorization by *P. vulgaris* as compared to lignin peroxidase (326%) and laccase (120%) after complete decolorization (5 h). The enzymatic profile presumably indicates involvement of oxidoreductive enzymes for the degradation of Reactive Blue 172 into simple metabolites by *P. vulgaris*. SEM analysis indicated the presence of *P. vulgaris* cells (rod shaped bacteria) on the surface (rough) as well as at the internal (multilayered) cross-sectional view. The potential of CA immobilized cells of *P. vulgaris* in concern with diversity of dyes and their nonspecificity could be useful for the treatment of the textile effluents containing a mixture of dyes.

However, in case of immobilized cells with the variety of entrapment matrices for the decolorization and degradation of dyes many problems have been experienced including diffusion restrictions, reduced enzyme activity, lack of open spaces for the growing cells, rupture due to insufficient mechanical strength and toxicity caused by synthetic polymers. To overcome these problems, we report the decolorizing ability of *P. vulgaris* immobilized on agricultural waste product *L. cylindrica* (biological inert material) for Reactive Blue 172 and various types of reactive dyes having large scale of industrial applications in India using immobilized *P. vulgaris* cells on *Luffa cylindrica*. The ability of 48 h grown *P. vulgaris* cells immobilized on *L. cylindrica* to decolorize various nineteen industrial dyes (each at concentration; 50 mg l\(^{-1}\)) were tested in the nutrient broth (pH 6.6) at 37 °C under static condition. The effect of various physicochemical conditions (agitation, temperature, pH, and dye concentration) on the decolorization performance of Reactive Blue 172 by using *P. vulgaris* cells immobilized on *L. cylindrica* was studied in detail.
Extent of mineralization was determined with total organic carbon (TOC) and chemical oxygen demand (COD) measurement, showing a satisfactory reduction of TOC (85%) and COD (89%) within 5 h incubation. In addition, we also evaluated the decolorization ability of mixture of various nineteen reactive dyes (each at concentration; 50 mg l\(^{-1}\)) by using \(P. \ vulgaris\) cells immobilized on \(L. \ cylindrica\) in batch culture at 37 °C under static anoxic condition. The results suggesting that \(P. \ vulgaris\) cells immobilized on \(L. \ cylindrica\) could achieve higher color removal in terms of ADMI value and sequentially moderate reduction in TOC (58%) and COD (55%) in the culture within 48 h incubation, indicates the mineralization of dye mixture. The SEM analysis shows highly fibrous structure and with magnification the \(P. \ vulgaris\) cells (rod shaped bacteria) were observed at surface as well as at the internal cross-sectional view. We supposed that due to high surface and multilayered structure possess higher biomass for the interaction with the dye.

It is crucial to select appropriate types of biocatalyst (suspended or immobilized cells) and bioreactor operation (e.g., CSTR, batch, fed-batch cultures) for a more viable application of the bacterial decolorization system. The effective diffusion coefficient of a substrate and the intrinsic kinetic parameters of immobilized cells are required for the sophisticated design of a biochemical reactor that can maximize the advantage of immobilized cells. In the present study, fixed-bed reactor containing CA immobilized \(P. \ vulgaris\) and \(P. \ vulgaris\) cells immobilized on \(L. \ cylindrica\) were used for the continuous decolorization. The fixed-bed decolorizers were conducted under different feeding conditions (e.g. volumetric flow rate, dye loading concentration, etc.). At a constant feeding dye concentration of 50 mg l\(^{-1}\), the optimal volumetric decolorization rate was achieved at a volumetric feeding rate of 20 ml h\(^{-1}\) and 30 ml h\(^{-1}\), for fixed beds containing CA- and \(L. \ cylindrica\) immobilized cells, respectively. A higher volumetric feeding rate led to a higher dye-loading rate, but would lower the hydraulic retention time (HRT). The results seem to imply that the effect of mass transfer on the decolorization in CA-immobilized cells was more significant than in \(L. \ cylindrica\)-immobilized cells. The rate of
mineralization (on the basis of reduction of TOC and COD) of dye in case of CA immobilized cells was higher at lower volumetric feeding rate, whereas, it sharply declines at higher feeding rate, might be due to mass transfer effect and less removal of degraded metabolites from immobilized beads. In contrast *L. cylindrica* immobilized cells also showed good removal of TOC and COD compared to CA immobilized beads. In *L. cylindrica* immobilized cells due to highly porous and multilayered structure bacteria adhere very well, thus acts as membrane due to which dye passes from one side to other easily, becomes less mass transfer effective and also helpful in the removal of degraded metabolites from immobilized beads. In addition, we have used both immobilized matrices for the decolorization of mixture of thirteen reactive dyes; (each at concentration; 50 mg l\(^{-1}\)), at different volumetric feeding rate and dye concentration. *L. cylindrica* immobilized cells effectively decolorized the mixture of reactive dyes in terms of ADMI values with significant reduction in TOC and COD at higher volumetric feeding rate, whereas, in CA immobilized cells showed less decolorization efficiency due to mass transfer effect. It appears that the beds packed with agricultural waste *L. cylindrica* immobilized cells may be more technically favorable, environmentally benign, and inexpensive, and could be useful for the large scale applications to remove the synthetic dye from dye-laden wastewaters. Moreover, the rapidly expanding information from genomics and molecular genetics combined with an improved genetic engineering technologies offer a wide range of possibilities for enhancing the performance of bacterial decolorization and mineralization of azo dyes.