7. CONCLUSION:

The current norms for industries which are generating toxic effluent is to maintain zero discharge. It necessitates the complete removal of contaminates so that this can be recycled back into various processes in the industries. In case of textile industries especially carpet, color is the major deterrent in the use of recycled water. The present investigation aimed at removal of azo dyes which are most frequently used in carpet industries.

Various treatment techniques have been investigated by researchers; most of these techniques are either expensive or generating secondary pollutants which are also harmful to environment and living organisms. The review of biological methods reveals that this method hold promises if a suitable organism/consortia can be utilized with better designed process. To address this problem isolation of various bacterial strains were done from the effluent/contaminated soil. The efficacy of pure and mixed culture was investigated for degradation of color in batch condition. A sequential batch reactor was also designed and fabricated to test the efficiency of degradation of the most efficient mixed consortia. The following conclusions ware derived:

- The *Staphylococcus saprophytic* BHUSS X3, *Staphylococcus saprophytic* BHUSS X5, *Stenotrophomonas sp*. BHUSSp. X2, *Arthrobacter* sp. BHUAS X16, *Micrococcus* sp. BHUMC X14, *Planococcus* sp. BHUP X11, *Staphylococcus arlettae* strain BHUSA X17, *Microbacterium* sp. BHUMSp X4, *Pseudomonas putida* strain BHUPP X10 and *Exiguobacterium* sp. IITES X12, bacterial strains

were isolated from contaminated soil collected from Sant Ravidas Nagar (Bhadohi), Uttar Pradesh, India and 16S rRNA gene sequence of bacterial strains were deposited in NCBI for Accession Number.

- The most promising of these bacterial strains for biological degradation as pure culture are *Staphylococcus saprophytic* BHUSS X3 and *Stenotrophomonas sp*. BHUSSp. X2 under static condition.
- Mixed consortia consisting of *Arthrobacter* sp. BHUAS X16 (KM199789), *Micrococcus* sp. BHUMC X14 (KM199787), *Planococcus* sp. BHUP X11, (KM199788), *Exiguobacterium* sp. IITES X12 (KM199786) and *Pseudomonas putida* strain BHUPP X10 (KJ40223) have rendered best degradation in batch culture under aerobic conditions.
- The batch studies were utilized for optimization of operating parameters such as pH, temperature, agitation speed, initial dye concentration and nutrient concentrations which are tabulated below for pure and mixed consortia.

S.No.	Name of Bacterial strain	Accession Numbers	Phylum	Operating parameter	Dye	% of dye degradation
1.	*Staphyolococcus saprophytic* BHUSS X3	KJ439576	Firmicutes	Static condition, pH 8.0 Temperature 35 °C, initial dye 100 mg/l and 4% inoculum	Navy N5RL1	95.5% in 4h

2.	*Staphyolococcus saprophytic* BHUSS X3	KJ439576	Firmicutes	Shaking condition, pH 8.0 Temperature 35 °C, initial dye 100 mg/l and 4% inoculum	Navy N5RL1	25% in 16h
3.	*Stenotrophomonas sp*. BHUSSp X2	KJ740220	Proteobacteria	Static condition, pH 8.0 Temperature 35 °C, initial dye 100 mg/l and bacterium $=7\times10^7$cells/ml.	ACID RED G (C.I. Acid Red 1)	97% in 6h
4.	*Stenotrophomonas sp*. BHUSSp X2	KJ740220	Proteobacteria	Shaking condition, pH 8.0 Temperature 35 °C, initial dye 100 mg/l and bacterium = 7×10^7cells/ml	ACID RED G (C.I. Acid Red 1)	42% in 24h
5.	*Arthrobacter* sp. BHUASX16 , *Planococcus*sp. BHUP X11, *Micrococcus* sp. BHUMCX14, *Pseudomonas putida* strain BHUPP X10, *Exiguobacterium* sp. IITES X12 **:(BC1 mixed consortia)**	KM199789, KM199788, KM199787, KJ740223	Actinobacteria Firmicutes, Actinobacteria, Proteobacteria	Shaking condition 120 rpm, pH 8.0 Temperature 35 °C, initial dye 50 mg/l and and bacterium=1.5×10^6 cells/ml.	ACID RED G (C.I. Acid Red 1)	99% in 14
6.	*Arthrobacter* sp. BHUASX16 , *Planococcus*sp. BHUP X11, *Micrococcus* sp. BHUMCX14,	KM199789, KM199788, KM199787,	Actinobacteria Firmicutes, Actinobacteria, Proteobacteria	Static condition 120 rpm, pH 8.0 Temperature 35 °C, initial dye 50 mg/l and and bacterium=1.5×10^6	ACID RED G (C.I. Acid Red 1)	44% in 14h

	Pseudomonas putida strain BHUPP X10, *Exiguobacterium* sp. IITES X12 **:(BC1 mixed consortia)**	KJ740223		cells/ml.		
7.	*Arthrobacter* sp. BHUASX16 , *Planococcus*sp. BHUP X11, *Micrococcus* sp. BHUMCX14, *Pseudomonas putida* strain BHUPP X10, *Exiguobacterium* sp. IITES X12 **:(BC1 mixed consortia)**	KM199789, KM199788, KM199787, KJ740223	Actinobacteria Firmicutes, Actinobacteria, Proteobacteria	Shaking condition 120 rpm, pH 8.0 Temperature 35 °C, initial dye 50 mg/l and and bacterium=1.5×10^6 cells/ml.	Scarlet 4BS (C. I. Direct RED23)	90% in 20h
8.	*Arthrobacter* sp. BHUASX16 , *Planococcus*sp. BHUP X11, *Micrococcus* sp. BHUMCX14, *Pseudomonas putida* strain BHUPP X10 *Exiguobacterium* sp. IITES X12 **:(BC1 mixed consortia)**	KM199789, KM199788, KM199787, KJ740223	Actinobacteria Firmicutes, Actinobacteria, Proteobacteria	Shaking condition 120 rpm, pH 8.0 Temperature 35 °C, initial dye 50 mg/l and and bacterium=1.5×10^6 cells/ml.	Scarlet 4BS (C. I. Direct RED23)	52% in 20h

- Various enzymes identified in azo dye degradation during present investigation are laccase, lignin peroxidase, tyrosinase, azoreductase and NADH reductase. This suggests induction of extracellular and intracellular enzyme activities in the presence of dye.
- The Azoreductase seems to be the most prominent enzyme for decolorization using *Stenotrophomonas* sp. BHUSSp X2 followed by laccase and NADH reductase as significant increase in their amount has been observed as compared to control.
- The agricultural residues such as wheat bran and bagassa have shown potential to be used as sole carbon sources as degradation efficiency for glucose, wheat bran and baggasa were 82%, 85%, and 72% respectively even in absence of nitrogen source. Yeast used as a sole nitrogen source in absence of any carbon source has shown reasonable degradation efficiency (80%).
- The maximum decolrization efficiency achieved by *Stenotrophomonas* sp. BHUSSp X2 was found to be 97%.
- The analytical investigation using UV-Vis spectrophotometer, FT-IR and HPLC revealed that degradation by pure culture resulted into intermediate products such as aromatic amines which are harmful to the aquatic and human life, whereas mixed consortia shows complete degradation and mineralization.
- The analytical investigation using UV-Vis spectrophotometer, HPLC, and FT-IR confirmed dye degradation through absence or shifting of respective peaks whereas GC-MS helped in identification of intermediates products by microbial

degradation. The spectral analysis shows complete breakdown of azo bond into aromatic amines and finally aromatic amines broken into other compounds under aerobic condition by bacterial consortia BC1.

- The kinetic studies revealed that degradation follows first order kinetics with low activation energy indicating it to be a faster process.
- Efficacy of mixed consortia for the treatment of dyes contaminated was reconfirmed through phytotoxicity investigations which made it a potential candidate to be used in the reactor.
- The mixed consortia used in indigenously designed and fabricated bioreactor having klinkers as packing material resulted into 72% decolorization efficiency.
- To achieve complete degradation adsorption was integrated with biological degradation. The biologically treated wastewater was sent for adsorption into cylindrical column packed with TiO_2 coated klinkers for further treatment.
- Decolorization upto 96% could be achieved with this integrated approach.
- Phytotoxicity studies revealed that only 26% germination was observed in without treatment of dye bearing wastewater and 66% of germination in biologically treated wastewater and 100% germination took place after complete treatment of dye wastewater.