CHAPTER 2

Literature Review

2.1 Sustainable approaches of dye treatment

The treatment of industrial effluents has employed a variety of physical, chemical, physicochemical, biological, enzymatic, and combination procedures, depending on the type of pollutant to be eliminated and the quality of the water to be discharged. Adsorption, ion exchange, coagulation-flocculation, oxidation, membrane filtration (including RO), nanofiltration, and electrodialysis are some of the physicochemical approaches used to treat textile effluents. The main disadvantages of physical approaches are that they are only successful when there is a small amount of effluent, and they merely transfer dye molecules to another phase rather than decomposing contaminants. One of the biggest downsides of membrane approaches is membrane fouling, which happens quickly and necessitates membrane replacement, which is costly. Chemical methods have been widely utilized to remove colors from wastewater; nevertheless, there are various problems connected with their use. The key disadvantages include high costs, sludge accumulation, disposal issues, and the production of secondary sludge because of excessive chemical use.

Biological methods appear to be economically feasible and produce environmentally friendly sludge compared with other techniques. The bioremediation of textile dyes can be achieved using microorganisms and biocatalyst. Different strains of microorganisms can secrete an enzyme that can remove hazardous pollutants from contaminated sites.

2.2 Biodegradation of dyes

Biodegradation of dyes is an intriguing approach to treating textile effluents as it is an eco-friendly option for complete mineralization with very less sludge generation (Ihsanullah et al., 2020; Varjani et al., 2020a). In recent years, there have been significant advancements in the development of biological systems for the efficient and cost-effective removal of dyes from wastewater. For dye degradation and decolorization, microorganisms such as bacteria, fungi, yeast, and algae were used. The degradation of dyes is affected by differences in growth conditions and metabolic mechanisms of microorganisms. The main factors affecting the biodegradation of textile dyes via various microorganisms are discussed in the following subsections.

2.3 Environmental factors affecting the biodegradation process

Several environmental factors such as pH, substrate concentration, temperature, inoculum dose, and dissolved oxygen could affect the biodegradation rate. Optimization of these process parameters will result in enhanced biodegradation and will also be helpful in designing industrial-scale bioreactors.

2.3.1 Effect of pH

pH is a key parameter in bacterial growth and is also required for effluent treatment. Depending on the dyes and salts used, the pH can be acidic, alkaline, or neutral. The rate of degradation of dyes in dye-containing effluent can vary depending on its pH (Varjani et al., 2020a). The optimum pH range for microbial culture generally varies from 6.0 – 9.0, while fungi can grow even in a low pH range 4.0 – 5.0 (Shi et al., 2021). Similarly, ligninolytic enzymes produced by fungi are most active at low pH. The optimal pH promotes azo dye molecule movement across the cell membrane and results in the highest color removal rate (Kapoor et al., 2021). The issue can be resolved by either (a) adjusting effluent pH to encourage the growth of bacteria that degrade dyes or (b) choosing a microbial species that can flourish at effluent pH.

2.3.2 Effect of temperature

Temperature is a key process variable and has an impact on microbial activity and the process of microbial dye degradation. It also affects metabolic activities, microbial growth, reaction mechanism, and bioavailability (Angelova et al., 2008). When bacteria are present in wastewater, extreme temperatures can either kill the bacteria or affect their growth. The optimal temperature for bacterial culture, which is typically reported as 30–40 °C for the majority of bacteria, can be used to accelerate the rate of dye degradation (Varjani et al., 2020b). Therefore for optimum performance, the temperature level should be maintained within a certain limit. Liu et al., 2021 performed a dye degradation experiment using *Bacillus amyloliquefaciens* W36 and reported maximum degradation (95.42%) at 30℃. Ponraj et al., 2011 tested the effect of temperatures ranging from 4℃-37℃ for the degradation of orange 3R dye and reported max dye degradation at 27℃.

2.3.3 Effect of substrate concentration

Substrate concentration is also an important process variable in the case of biodegradation of organic pollutants. Various studies have suggested that the growth of microorganisms enhanced under optimum loading conditions, which was inhibited after increasing the substrate concentration (Abu Talha et al., 2018). Swain et al., 2021 has performed a biodegradation study for the degradation of acid orange (AO7) dye by a bacterial consortium and reported substrate inhibition after 631.97 mg/L. Sonwani et al., 2019 studied the biodegradation of naphthalene in an integrated aerobic treatment plant and reported substrate inhibition beyond 33.2 mg/L. Kureel et al., 2017 performed biodegradation of benzene in a packed bed bioreactor by *Bacillus sp*.-M3 and reported substrate inhibition after 435.84 mg/L.

2.4 Biodegradation of dyes via bacterial strain

Bioremediation of textile dyes can be accomplished using microorganisms and biocatalysts. Different strains of microorganisms are competent in releasing enzymes that can remove hazardous pollutants from contaminated sites (Xiang et al., 2016). Bacterial degradation is faster than fungal degradation for the decolorization and mineralization of azo dyes (Ahmad et al., 2015a; Shinkafi et al., 2016).

Previously, numerous bacterial species namely *Enterobacter sp.* EC3 (Wang et al., 2009), *Staphylococcus hominis* RMLRT03 (Singh et al., 2014), *Bacillus cohnii* RAPT1 (Padmanaban et al., 2016), *Brevibacillus parabrevis* (Abu Talha et al., 2018), *Aeromonas hydrophila* MTCC 1739 and *Lysinibacillus sphaericus* MTCC 9523 (Srinivasan and Sadasivam, 2018a), *Alcaligenes faecalis* (Bharti et al., 2019), etc. have been employed for degradation of various dyes.

The basic principle of biodegradation is microbial metabolism, which degrades pollutants in wastewater. Biodegradation is one of the most common and widely used methods for decomposing textile effluents (Chaturvedi et al., 2022b). Microorganism play most vital role in biodegradation and each microorganism has a distinct degradation efficiency and operating environment. Several process parameters, however, including pH, temperature, dye concentrations, and aerobic/anaerobic conditions, have a significant impact on the biodegradation of various synthetic dyes (Srivastava et al., 2022).

The mechanism of bacterial degradation of azo dye involves the cleavage of azo bonds (-N=N-) under anaerobic condition which results in the formation of colorless solution that contain potential hazardous aromatic amines, which further degraded aerobically. Bacterial degradation is faster in comparison to fungal degradation with regards to decolorization and mineralization of azo dyes (Saratale et al., 2011). It has been observed that microbial consortia is more useful than pure bacterial culture in terms of capability to degrade azo dyes (Ahmad et al., 2015a) . Corso and Maganha De Almeida, 2009, used *Aspergillus oryzae* to biosorb reactive dye Procion Red HE7B and Procion Violet H3R at different pH Values (2.5, 4.5 and 6.5). In another study consortia of *Pseudomonas species SUK1* and *Pseudomonas rettgeri strain HSL1* were used to degrade different type of azo dye (Reactive black 5, Direct red 81, Disperse red 78, Reactive orange 16) (Lade et al., 2015). McMullan et al., 2001 shows the ability of actinomycetes to decolorize textile dye. These are *Sterptomyces* species, are known to produce extracellular peroxidase that have key role in biodegradation of lignin.

2.5 Biodegradation of dyes via fungal strain

White-rot fungi are the most efficient microorganisms at breaking down synthetic dyes. White-rot fungi are a type of microorganism that produces enzymes to degrade dyes under aerobic conditions. They generate a variety of oxidoreductases that break down lignin and other aromatic compounds (Ali, 2010). Acoording to Forss and Welander, 2009 wood-rotting microorganisms may be suitable for the biodegradation of synthetic dyes. This is due to the fact that materials containing lignocellulose sometimes contain complex molecules with structures resembling those of textile dyes, which may enable microorganisms to become adapted to refractory organic compounds when growing on these materials. Various researchers have utilized white-rot fungi such as, *Pleurotus florida*, *Phanerochaete chrysosporium*, and *Agaricus bisporus* for dye degradation (Kumari and Naraian, 2016). Apart from white-rot fungus strain, some other strains were also utilized for the dye treatment such as *Rhizopus arrhizus*, *Aspergillus niger*, and *Rhizopus oryzae* (Sen et al., 2016).

Venkatesh Prabhu et al., 2016 successfully achieved 89% decolorization and 72% COD reduction for real textile wastewater after treating with white-rot fungi *Pleurotus ostreatus* in batch condition. This degradation and COD reduction were further enhanced to 92% and 76% when treated in an inverse fluidized bed bioreactor (IFBBR). In another study Kumari and Naraian, 2016 utilized two lignocellulolytic fungus strains *Pleurotus florida* and *Rhizoctonia solani* to degrade brilliant green dye. The results show that the co-culture of these strains degraded the maximum amount of dye (98.54%) than the monoculture (81.12% and 68.89% for PF and RS respectively). In another study, Krishnamoorthy et al., 2018 degraded three azo dyes namely congo red, methyl red, and reactive blue by the mixed fungal culture of *Dichotomomyces cejpii* MRCH 1-2 and *Phoma tropica* MRCH 1-3. They have reported 97.4%, 87.14%, and 90.6% degradation of congo red, methyl red, and reactive blue respectively. They have also proposed a degradation pathway for congo red dye degradation via mixed fungal cultures. Salem et al., 2019 have reported that *Aspergillus niger* D2-1 has degraded 98.62% reactive yellow dye and 92.42% reactive red dye. According to Singh and Dwivedi, 2020, a novel fungal strain *Aspergillus terreus* GS28 successfully decolorized direct blue dye after 168 h of incubation, and maximum decolorization efficiency of 98.4% was reported. Sosa-Martínez et al., 2020 have tested the potential of the enzyme extract of *Phanerochaete chrysosporium* CDBB 686 for the degradation of seven different azo dyes. Critical variables influencing the *in-vitro* decolorization process were investigated further, and the results were compared to those of an *in-vivo* decolorization system. In *in-vitro* condition they are successfully able to decolorize 41.84% congo red dye, 56.86% poly R-478, and 69.79% methyl green dye.

Gao et al., 2020 have tested a novel fungus strain *Bjerkandera adusta* SWUSI4 for decolorization and detoxification of triphenylmethane dyes. The degradation study shows effective degradation of triphenylmethane dyes within 14 days. Ameen et al., 2021 have tested the potential of different *Aspergillus strains* to degrade acid blue 29, congo red, and disperse red 1 dyes and found more than 86% degradation for all the dyes.

Due to their large surface area and ease of solid-liquid separation, fungi provide an efficient system. Fungi have a variety of mechanisms for degrading both organic and inorganic contaminants. However, the majority of research on pollutant removal has used pure cultures and single-pollutant exposures.

2.6 Biodegradation of dyes via Algae

Algal bioremediation of textile wastewater has utilized both viable and nonviable algae. Algae decolorizes azo dye via three distinct mechanisms including chromophore assimilation for algal biomass production, CO_2 and H_2O production during decolorization, and adsorption of chromophores by algal biomass (Singh and Singh, 2017). Various algal strain have been utilized for dye degradation such as *Chlorella vulgaris*, *Lyngbya lagerlerimi*, *Nostoc lincki*, *Oscillatoria rubescens*, *Elkatothrix viridis* and *Volvox aureus* (El-Sheekh et al., 2009).

Aravindhan et al., 2007, have reported biosorption of basic yellow dye by *Caulerpa scalpelliformis* algal strain, and they found 94% dye removal at 10g/L adsorbent dose. Daneshvar et al., 2007 have investigated the potential of *Cosmarium sp*. as a viable biomaterial for malachite green dye degradation. They have reported 87.1% dye degradation at 4.5×10^6 cells/mL algal biomass concentration.

In another study, Kousha et al., 2012 have examined the potential of the chemically modified algal strain *Stoechospermum marginatum* for acid orange II dye adsorption. Adsorption kinetics was well explained by pseudo-second-order model. El-Sheekh et al., 2021 have utilized algal consortium to degrade variety of azo dye. The maximum decolorization of 98.54% was observed for 20 ppm Reactive Orange 122 dye with *Oscillatoria sp*. combines with *S. obliquus*. Chaieb et al., 2023 used the *Shewanella algae* B29 algal strain to degrade textile dyes. They have reported 91.04% degradation of reactive red 66 dye under optimum conditions.

2.7 Detailed review on biodegradation of dyes via bacteria

Padmanaban et al., 2016 have studied the degradation of Reactive red 120 dye in a packed bed bioreactor using *Bacillus cohnii RAPT1* immobilized polyurethane foam as a packing material. The process parameters such as pH, dye concentration, time of immobilization, inoculum dose, and temperature were optimized and the optimum value was found to be 8.0, 200 mg/L, 36 h, and $300*10^6$ respectively. Under these optimum conditions, complete degradation was reported within 4 h of study. According to the results, the rate of degradation of RR120 in the immobilized batch was double that of the batch with free cells.

In another study, Krishnan et al., 2017, assessed the biodegradation of three azo dye mixtures (reactive brilliant red X-3B, direct black-19, and direct blue 6) in batch mode. The effect of process parameters such as pH, inoculum dose, and dye concentration was assessed in aerobic conditions. The process parameters were optimized using the central composite design (CCD) of response surface methodology (RSM). The maximum removal efficiency was found to be 31.2%, 71.5%, and 87.6% for RBRX-3B, DB-6, and DB-19 dye respectively. Biodegradation kinetics was evaluated using Monod and Michaelis – Menten equation.

Srinivasan and Sadasivam, 2018b have utilized adopted (*Aeromonas hydrophila* MTCC 1739) and non-adopted (*Lysinibacillus sphaericus* MTCC 9523) to degrade 100 mg/L of Drimaren Red CL-5B in a nutrient bath at pH 8.0 and temperature 37°C. The molecular interactions between laccase and azoreductase of both bacteria with dye were calculated using docking software, which also helped to identify active site residues. The assessment of biodegraded product was carried out using UV-Vis spectrophotometer, FTIR, GC-MS, and HPLC analysis. *A. hydrophila* MTCC 1739 and *L. sphaericus* MTCC 9523 respectively achieved decolorization scores of 91.96 and 88.35% while docking scores for laccase and azoreductase were -31.9921, -18.1289, and -27.2792, -2.5185 kJ/mol. Docking can help with extensive bacterial strain screening to find the best decolorizer.

Bharti et al., 2019 have studied the biodegradation of Methylene blue (MB) dye via *Alcaligenes faecalis* immobilized on the *Casuarina* seed-biochar. The process parameters were optimized and under these conditions, maximum 96% MB removal was obtained. The maximum %degradation was assessed for both free cell and immobilized cells in packed bed bioreactor and found 81.5% and 89.1% respectively. The pseudo-second order kinetics fits well ($R^2 > 0.978$) to describe biochar absorption in batch mode for the removal of MB dye. A regeneration study shows that after 5 cycles of treatment, the absorption capacity of biochar only reduces up to 10% from 88.0% to 79.9%. The economic assessment shows the viability of the bioreactor process in industrial-scale applications.

Sonwani et al., 2020a used *Bacillus sp.* MH587030.1 species to degrade Congo red (CR) dye in moving bed bioreactor (MBBR). The process conditions (pH, dye concentration, and media filling ratio) were optimized using a central composite design of response surface methodology (RSM). At the optimum condition, an MBBR was continuously operated for 564 h at various flow rates and the maximum removal efficiency obtained was 95.7%. The modified Stover – Kincannon model was used to evaluate the biodegradation kinetics. The rate control mechanism suggests that at a low inlet loading rate, the substrate diffusion is slow leading to the rate of mass transfer control. Degraded samples were analyzed by FTIR analysis.

Srinivasan and Sadasivam, 2021 have studied the biodegradation of decolorization and degradation of three azo dyes by non-adapted and adapted *Aeromonas hydrophila* bacteria under optimized physicochemical conditions. The decolorization study was performed for culture in nutrient media at pH 7.0, temp 35°C, time 16 h, at shaking conditions. In dye medium (100 mg/L) at pH 8.0, temp 37°C, time 72h, at static condition. The %degradation of Remazol Yellow RR, Reactive Yellow F3R, and Joyfix Red RB dye was found to be 94.0%, 89.02%, and 90.32% respectively for non-adapted bacteria. For adapted bacteria, the value was found to be 90.0%, 90.4%, and 88.75% respectively. The final degraded metabolites were analyzed by FTIR, HPLC, and GC-MS analysis. Degradation pathways were proposed for the degradation of these three dyes by adapted and non-adapted bacteria.

Kishor et al., 2021b have successfully isolated a novel bacteria *Bacillus albus* MW407057 for the degradation of Methylene Blue dye. The bacterial sample decolorizes 99.7% MB dye and removed 83.87% COD within 6h when operated at optimized conditions (pH 7.0, temperature 30°C, dye concentration 100 mg/L). The degraded metabolites were analyzed by FTIR and LC-MS analysis and a degradation pathway was proposed. The effect of carbon and nitrogen source was also assessed. The toxicity test reveals that the treated sample shows 90% seed germination. In another study, Barathi et al., 2022a successfully degraded Reactive Red 170 dye by bacterial consortium isolated from the dye-contaminated site. In this study, a bacterial consortium of three different bacteria (*Bacillus subtilis*, *Brevibacillus borstelensis*, and *Bacillus firmus*) have been utilized to degrade the dye. Biofilm growth and extracellular polymeric substance (EPS) were evaluated at various dye concentrations. The effect of dye concentration on EPS composition was assessed by evaluating protein and polysaccharide levels. The degradation was confirmed by UV-Vis spectroscopy, FTIR, 2D-FTIR, and HPLC analysis. The degraded product was tested for genotoxicity and cytotoxicity using *Allium cepa* root cells. Together, the examined bacterial consortium degraded the RR 170 dye and produced metabolites that are harmless to living cells.

Saravanan et al., 2022 studied the biodegradation of Rhodamine-B dye by a novel bacterial strain *Brevundimonas diminuta.* This strain showed 90 – 95% degradation at optimum conditions (pH 7.0, temperature 30℃, and dye concentration 10 mg/L). The degradation efficiency was tested for various process conditions such as dye concentration being varied from 10 – 50 mg/L, pH of the solution being varied from 3 – 9, the temperature being varied from 30 – 90℃, and carbon and nitrogen sources. Degraded metabolites were analyzed using GC-MS and FTIR. In another study Al-Ansari et al., 2022 studied the decolorization of Acid orange (AO) dye by a novel bacteria *Enterobacter aerogenes* ES014 isolated from dye effluent. The degradation study was performed in a batch reactor at optimum conditions (pH 7.0, temperature 35℃, and NaCl 6%) and the maximum degradation efficiency obtained was 100% for 100 mg/L of AO dye and it was in the range of > 80% for 100 – 300 mg/L AO dye concentration. The effect of carbon and nitrogen sources on the degradation of AO dye has been studied. An enzymatic assay was performed to assess the laccase activity. Phytotoxicity assessments was carried out using *A. hypogaea* seed and treated water shows 94.3% seed germination.

A summary of bacteria biodegradation of dyes is reported in **Table 2.1.**

Table 2.1 A summary of biodegradation of various dyes by bacterial strains.

S. No.	Bacterial Strain	Dye	Process condition				Degradation (%), Reaction mechanism	References
			Conc. (mg/L)	pH	Temperature (°C)	Time (h)		
1	*Enterobacter sp.* EC3	Reactive Black 5	200	7.0	37.0	108	92.56, Anaerobic	Wang et al., 2009
2	*Staphylococcus hominis* RMLRT03	Acid Orange	100	7.0	35	60	92.32, NADH azo reductase	Singh et al., 2014
3	*Brevibacillus parabrevis*	Congo red	150	7.0	30	144	95.71, Aerobic	Abu Talha et al., 2018
4	*Serratia liquefaciens*	Azure-B	100	7.0	30	48	90, Lignin peroxidase (LiP) enzyme	Haq et al., 2018
5	*Bacillus sp. MH587030.1*	Congo red	50	7.0	35	564	95.7, Aerobic	Sonwani et al., 2020a

6	*Bacillus albus* MW407057	Methylene blue	100	7.0	30	6	99.27, LiP enzyme	Kishor et al., 2021b
7	*Lysinibacillus fusiformis*	Congo red	60	7.0	35	72	87.33, Aerobic	Maurya et al., 2021
8	*Aeromonas hydrophila* MTCC 1739 *Aeromonas hydrophila* SK16	Remazol Yellow RR Joyfix Red RB Reactive Yellow F3R	100	7.0	37	96	89.02, 90.32, 94.0 90.4, 88.75, 90.0 aerobic	Srinivasan and Sadasivam, 2021
9	Consortium *(Bacillus subtilis, Brevibacillus borstelensis and Bacillus firmus*)	Reactive red 170	40	7.0	35	48	85.0, Aerobic	Barathi et al., 2022a
10	*Brevundimonas diminuta*	Rhodamine-B	10	7.0	30	24	90 – 95, NADH azo reductase	Saravanan et al., 2022
11	*Enterobacter aerogenes* ES014	Acid orange Methyl orange, Congo red	100	7.5	35	48	82.3 78.2 81.5, Laccase	Al-Ansari et al., 2022

12	*Alcaligenes faecalis*	Methylene blue	150	7.0	30	120	96.2, enzymatically catalyzed	Bharti et al., 2019
13	*Dietzia sp.* PD1	Congo red	177.63	5.56	30	42.64	99.97	Das et al., 2016
		Indigo carmine	135.62	5.85		43.29	99.95, Aerobic	

2.8 Ozone based treatment system for dyes degradation

The principle of advanced oxidation processes (AOPs) is to produce strong oxidizing radicals, specifically the hydroxyl radical ($OH^{\bullet}$), which have high oxidation potential ($E^{\circ} \cong 2.8\,V$) and is capable of oxidizing highly recalcitrant contaminants (Asghar et al., 2015; Ledakowicz and Pázdzior, 2021). Hydroxyl radical ($OH^{\bullet}$) can be generated by individual processes which includes ozone (O_3), UV fenton, hydrogen peroxide (H_2O_2), UV/H_2O_2, $O_3/UV/H_2O_2$, and $O_3/TiO_2/H_2O_2$ system (Bilińska and Gmurek, 2021). Among all these existing advanced treatment processes, ozonation is preferred as a viable alternative for textile wastewater treatment owning to its high selectivity towards the chromophoric group of an azo dye, less sludge formation, and adaptability across a wide pH range (Chaturvedi and Jaiswal, 2022; Fanchiang and Tseng, 2009).

In recent decades, the ozonation process has been successfully used to treat industrial wastewater containing toxic and recalcitrant organic contaminants. Initially, ozone is used for detoxification, disinfection, and sludge reduction. Whereas micropollutant removal, COD reduction, and decolorization are the most common applications of ozone. In fact, several studies have confirmed the effectiveness of the ozone process as a pre-treatment option for the textile wastewater treatment (Abidin and Ridwan, 2011; Malik et al., 2020; Somensi et al., 2010).

Wang et al., 2003 have assessed the effect of ozonation on the degradation of Remazol black 5 dye. The efficacy of the ozonation process was analyzed on the basis of color removal, COD removal, TOC removal, and improved biodegradability index (BOD_5/COD). They found that 6 h of ozonation results in complete decolorization, 40% reduction in COD, and a 25% reduction in TOC for 2000 mg/L of RB5 dye. Zhang et al., 2006 have performed ozonation coupled with ultrasonic irradiation for the degradation of methyl orange dye. The results show that more than

95% of degradation was achieved within 20 min of operation for 400 mg/L of MO dye. Tapalad et al., 2008 have performed a degradation experiment for Congo red dye via catalytic and non-catalytic ozonation. They found that catalytic ozonation with $(CuNO_3)_2$ has resulted in 90% color removal, and 60% COD reduction within 48 min of the experiment.

Fanchiang and Tseng, 2009 have degraded reactive blue 19 dye via ozonation. They have found complete decolorization and 60% COD reduction after 10 min of ozonation. They have also reported that ozonation primarily results in the formation of intermediate metabolites on an acidic nature. Khadhraoui et al., 2009 have reported 54% COD removal and 32% TOC removal for ozonation of congo red dye and also found that the kinetics of degradation is of pseudo-first order. Somensi et al., 2010 have performed ozonation for raw textile wastewater in both basic (pH 9.0) and acidic (pH 3.0) conditions. They reported that 4h of ozonation results in better degradation and mineralization in basic conditions than acidic conditions. In basic conditions the ozone reacts via $OH^{\bullet}$ mechanism which has very high oxidation potential and results in better mineralization and degradation. Ozonation results in an improved biodegradability ratio and less toxic metabolites than raw wastewater.

Turhan et al., 2012 have performed decolorization experiments on methylene blue dye by ozonation. They have reported 64.96% COD reduction under basic conditions (pH 12) for 12 min of ozonation. They have also reported that degradation kinetics for direct ozonation was of pseudo-first order. Venkatesh et al., 2015 have assessed the efficacy of the ozonation process for Congo red dye degradation. The experiments were performed in a batch reactor with constant ozone flowrate of 5 g/hr and the efficacy was assessed on the basis of color and COD removal. Ozonation was carried out for 25 min and 90% color and 50% COD removal was reported.

Zhang et al., 2015 have performed ozonation experiments in a semi-batch reactor for the degradation of Reactive red 195 dye. They have reported enhanced degradation in the acidic medium than the basic medium. Ozonation was carried out for 30 min for 400 mg/L of RR 195 dye and more than 99% color removal was achieved. Hassaan et al., 2021 have compared the efficacy of ozonation and ultraviolet-assisted ozonation for the degradation of acid yellow 11 dye. They have reported better degradation in the case of sole ozonation than UV/O_3, and achieved up to 99% degradation within 20 min of the ozonation process. Mohan and Oke, 2022 aimed to test ozonation as a pre-treatment option for biodegradation to treat real textile wastewater. At optimum conditions, solely ozonation treatment of real textile effluent resulted in 94.6% colour removal and 67.4% COD removal. Ozonation enhanced the biodegradability of the sample by 81.8% and the oxygen uptake rate by 126%. Chaturvedi and Jaiswal, 2022 have utilized ozonation to treat reactive blue dye in bubble column reactor. They have reported up to 90% removal after 120 min of ozonation. Average Specific Electricity Consumption (SEC_{av}) was defined as a parameter to compare the cost of ozonation under various process conditions. It was discovered that if the dyeing water was treated at high initial dye concentrations, the specific power consumption during ozonation could be reduced significantly (by 25-30%).

Some researchers have also employed catalytic ozonation process for the treatment of dye wastewater. Babar et al., 2022 have used ozonation catalyzed by Fe-loaded biochar for methylene blue dye treatment. They have compared single ozonation (SOP) with catalytic ozonation process (HCOP) and found only 76% removal in the case of SOP while it increases to 95% for HCOP. Javed et al., 2023 have utilized Fe and Co loaded borosilicate glass for catalytic ozonation of methylene blue dye. They have reported that Co-BSG/O_3 resulted in better degradation of MB dye and found 92% degradation in 8 min.

2.9 Integration of ozonation with biodegradation for dye treatment

Industrial dye wastewater is typically resistant to biological treatment; therefore, an integration of chemical and biological processes for these effluent treatment processes is of interest. The ozonation process uses a lot of energy and creates toxic byproducts. Combining ozonation and biodegradation can result in a cost-effective dye wastewater treatment because biological processes are advantageous to the environment and affordable (Goswami et al., 2020; Punzi et al., 2015).

The majority of recent research on ozonation has been on its application as a pre-treatment when combined with biological processes. The typical reason for this is that industrial effluents frequently contain substances that are toxic to the microorganisms used in biological treatment. The pre-treatment process therefore aims to enhance the biodegradability of the effluent samples. Gökçen and Özbelge, 2006 have utilized ozonation as a pre-treatment to the biological degradation (activated sludge process) for acid red-151 dye treatment. They have found 47% dye degradation in the case of coupled ozonation and biodegradation processes, whereas only 25% degradation was reported in the case of a solely activated sludge process. van Leeuwen et al., 2009 have studied the use of pre-ozonation prior to the activated sludge process for orange II dye degradation. The observations show that ozonated sample results in 96% color removal, while the non-ozonated sample results only in 67% removal. Orange II ozonolysis products were identified as primarily biodegradable. In the integrated process, these products were supposed to be removed through biodegradation. Lu et al., 2009 aimed to treat wastewater containing reactive brilliant red X-3B via sequential ozonation and biodegradation (Upflow biological filter). They have reported complete degradation after 120 min of ozonation and biodegradability also increased from 0.102 to .406, which makes the sample more biodegradable. The average color and COD removal efficiency under optimal conditions was 97% and less than 30%, respectively. Following ozone pre-oxidation, the UBAF process can significantly reduce the COD of the wastewater. The UBAF process had a COD removal efficiency of more than 85% on average.

In another study de Souza et al., 2010 studied the use of a combined treatment approach (ozonation and biodegradation) for the successful removal of color and COD of hydrolyzed textile dye. Ozonation results in partial oxidation and complete decolorization of Remazol black B dye for even a very high concentration (500 mg/L) of dye samples. Degradation kinetics was pseudo-first order in terms of dye concentration. The toxicity assessments show ozonation increases the toxicity of the sample which was reduced after subjecting it to biological treatment. Abidin and Ridwan, 2011 studied the color and COD removal of 4 different azo dyes for the integrated ozonation and biological degradation process. At lower ozone doses, ozonation and biological treatment mechanisms both contributed to COD removal. However, direct oxidation at higher ozone doses played a significant role.

Punzi et al., 2015 have utilized combined ozonation and biological to treat both simulated and real textile wastewater. They have used ozonation as a post-treatment to anaerobic biological treatment process. For coupled process, 99% color removal and 85-90% COD removal were reported for simulated textile wastewater containing 100 – 1000 mg/L dye, while for real wastewater only 70% COD removal was reported (HRT for ozonation 3min, Biodegradation 3 days). Abidin et al., 2015 aimed to treat azo dye (reactive red 120) solution via integrated ozonation and biodegradation (semi-batch bubble reactor). They have reported 100% color removal, 60% COD removal, and 37% TOC removal for 40 min of ozonation. In another study, Castro et al., 2017 aimed to degrade reactive orange 16 dye via coupling ozonation and biodegradation in the moving bed bioreactor process. The results showed that after 5 minutes of ozone exposure, more than 97% of the color was removed from the dye solutions with various RO16 ranging from 25 – 100 mg/L. Whereas TOC removal was only 48% indicating partial mineralization. However, in the MBBR experiment, they reported 93% COD removal and 96% ammonium removal.

Gong, 2016 studied the degradation of real textile wastewater via a combination of anaerobic biodegradation – ozonation – aerobic biodegradation in a moving bed bioreactor. Under optimum conditions, they reported 94.3% COD removal, 97.8% suspended solid removal, 85.3% ammonia removal, and 96.3% color removal. The anaerobic MBBR process improved raw wastewater biodegradability, whereas the two aerobic MBBRs were critical in removing COD and ammonia. Venkatesh et al., 2017 aimed to develop cost-effective technology for dye wastewater treatment via coupling of ozonation and biodegradation (anaerobic Upflow sludge blanket). The integrated system shows 90% COD reduction and 94% dye removal and color removal of 10 on the Pt-Co scale. Variation in the pH of the solution with the ozonation process indicated that metabolites formed after ozonation is of acidic nature. Paździor et al., 2017 studied the degradation of azo dye via both coupling and sole physical and chemical processes and compared the parameter on the basis of acute toxicity. As a chemical process, ozonation was tested in two reactors stirred cell and bubble column, while biodegradation was carried out in two different systems Sequence Batch Reactors and Horizontal Continuous Flow Bioreactor. Coupling of SBR with ozonation results in 96% toxicity removal and HCFB followed by ozonation resulted in 98% toxicity removal.

Dias et al., 2019 studied degradation of reactive red 239 via integrated system of ozonation and biodegradation in MBBR. A 20 min of ozonation results in complete color removal, 60% COD removal, and 30% TOC removal which was completely mineralized when subjected to the biological treatment. Toxicity assessment of the ozonated sample was carried out using a bioluminescence bacteria *Aliivibrio fischeri* and they reported that 4 min of ozonation remove complete toxicity of the sample. Goswami et al., 2020 developed a hybrid system (ozonation and biodegradation) for the treatment of congo red dye. The biodegradation study was carried out in PBBR packed with immobilized Arjuna seed biochar and ozonation was used as a post-treatment strategy. A biodegradation experiment was performed for 248 h at 30°C resulting in 92% color removal which was completely decolorized by subsequent ozonation.

Based on the above review it can be suggested that an integrated AOP-biodegradation methodology, in which an AOP is used for recalcitrant waste and the biodegradation route is used for biodegradable components, could be significantly less expensive than a pure AOP technology at large-scale textile-water treatment.

Table 2.2 Brief summary of integration of Ozonation with biodegradation for dye wastewater treatment.

S.No.	**Type of dye**	**Objective**	**Integrated process**	**Result**	**References**
1.	Real textile wastewater	Influence of integration of ozonation and biological treatment for real textile effluents.	Biodegradation (SBR) in conjunction with ozonation. Biodegradation (HCFB) in conjunction with ozonation.	96% of toxicity removal. 98 % of toxicity removal	Paździor et al., 2017
2.	Raw textile wastewater	Ozonation was used as a pre-treatment for the assessment of the efficacy of	Pre-ozonation and sequential biological oxidation	After ozonation color removal was 67.5% and COD reduction was 25.5%. Biodegradability was	Somensi et al., 2010

		pilot-scale system.		enhanced up to 6.8 fold.	
3.	Remazol Black B	Integrated system of ozonation and biofilm mediated biodegradation.	Ozonation in conjunction with biodegradation.	For 500 ppm of dye solution 96% color removal was achieved. Ozone kinetics evaluation revels pseudo-first-order reaction with respect to the dye concentration.	de Souza et al., 2010
4.	Reactive Red 239	Decolorization and organic matter removal in batch assay.	Ozonation followed by biodegradation in MBBR	Toxicity was completely removed after 4 min of ozone. Ozonated dye shows reduced nitrification	Dias et al., 2019

				efficiency when fed to the bioreactor.	
5.	Congo red (CR) dye	Removal of Congo red dye in PBBR where packing material for the reactor was biochar of Arjuna seeds immobilized with bacterial cells.	Biodegradation integrated with ozonation	Complete decolorization in hybrid treatment. Economic analysis suggested, energy required to degrade 1 Kg of CR dye is about 3.5 kW.	Goswami et al., 2020
6.	Reactive Black 5	A new technology comprise of ozonation and biodegradation	Integration of ozonation with anaerobic biodegradation in Upflow anaerobic	90% of COD reduction and 94% color removal was achieved.	Venkatesh et al., 2017

		has been developed to control the dye wastewater treatment cost.	sludge blanket (UASB) reactor.	The new integrated system has shown enhanced color removal on platinum cobalt (Pt–Co) scale.	
7.	Reactive Orange 16 (RO16)	Degradation of RO 16 by hybrid treatment. Comparison of two reactor was done on the basis of Color removal , COD removal, and ammonium removal.	Ozonation followed by biodegradation in MBBR	MBBR removes 93% COD and 97% ammonium removal for both ozonolysis dye and non-ozonated dye. For non-ozonated dye color removal was not effective.	Castro et al., 2017

8.	Remazol Red (RR)	In order to treat the real textile wastewater, a new setup comprising of an anaerobic biofilm reactor integrated with ozonation was developed.	Anaerobic biofilm reactor integrated with ozonation	Ozonation removes the complete toxicity from the samples. Even though biologically treated sample shows some mutagenic effect .After 1 min of ozonation the mutagenicity further increased.	Punzi et al., 2015
9.	Reactive Blue 19 (RB-19)	Degradation of dye wastewater using ozonation in a semi-batch reactor and propose a	Ozonation in semi batch reactor	Ozonation has enhanced the biodegradability. After 10 min of ozonation the biodegradability	Fanchiang and Tseng, 2009

	degradation pathways.	(BOD/COD) increases from 0.15 to 0.33.

2.10 Summary of the literature review and research gap

Textile and dyeing industries are considered the most polluting industries based on both the amount and the toxic content of effluents. Dye-contaminated effluents discharged from industries are considered one of the major concerns among environmentalists. The remediation and abatement of these toxic dyes from the environment is a serious concern since these pose adverse impacts on humans as well as on the aquatic ecosystem.

Several treatment techniques comprised of chemical, physical, and biological have been used over the past few decades for the degradation of dyes. These are adsorption, coagulation, membrane filtration, ozonation, etc. However, these techniques are associated with demerits such as high sludge production and expensive cost. The biological method has several merits such as low-cost, eco-friendly, and less harmful sludge production over conventional physicochemical processes. Various researchers adopted the free-cell technique for dye degradation, which involves the addition of free-cell microorganisms directly to the bioreactor. However, free cell processes often have limited application under a high inlet loading rate. To rectify the above problem, the immobilized cell technique has received major attention. In this technique, the microorganisms grow on a carrier surface, and subsequently, immobilized carriers are used as packing material in the bioreactor.

Among the attached growth bioreactors, the packed bed bioreactor (PBBR) has been widely utilized for the biodegradation of various organic pollutants. Various packing materials such as sodium alginate, polyurethane foam (PUF), biochar, and polymeric support were used for the immobilization of microorganisms. In order to scale up these lab-scale reactors to industrial scale accurately for biodegradation, the role of mass transfer correlations is an important aspect of the effective operation of bioreactor.

However, several studies have shown the existence of non–biodegradable or low-biodegradable compounds in textile effluent. The existence of these non–biodegradable compounds causes a lower biodegradability index (BOD_5 : COD < 0.2) and makes biological treatment ineffective for the majority of industrial wastewater, including textile industry. The biological methods are typically effective for wastewater having BOD_5 : COD ratios greater than 0.4. In this context, new technologies for the treatment of textile wastewater have been developed. The principle of advanced oxidation processes (AOPs) is to produce strong oxidizing radicals, specifically the hydroxyl radical ($OH^{\bullet}$), which is having high oxidation potential ($E^{\circ} \cong 2.8\ V$) and is capable of oxidizing highly recalcitrant contaminants. Hydroxyl radical ($OH^{\bullet}$) can be generated by individual processes which include ozone (O_3), UV fenton, hydrogen peroxide (H_2O_2), UV/H_2O_2, $O_3/UV/H_2O_2$, and $O_3/TiO_2/H_2O_2$ system. Among all these existing advanced treatment processes, ozonation is preferred as a viable alternative for textile wastewater treatment owing to its high selectivity towards the chromophoric group of an azo dye, less sludge formation, and adaptability across a wide pH range.

In connection with the optimization and process designing of the bioreactor, RSM is one of the useful statistical experimental designs and provides information about the combined effect of various parameters. However, the major issue in the treatment of dye-containing wastewater by the biological process is the nonlinear nature of the process, modeling of such a complex process cannot be successfully done by RSM. To rectify these limitations, ANN can be used to approximate the functions.

ANN is a computational-based model which is influenced by biological neural processing, which can be used to model highly nonlinear processes. ANN could be advantageous over RSM as it does not require preparatory specification of fitting function.

Based on the above literature survey the research gaps are summarized below:

1. Limited work reported on the Mass Transfer aspect in the biodegradation of dye effluents.
2. Parameter optimization using advanced mathematical techniques such as; Artificial Neural Networks is an under–explored area.
3. Limited studies are available on the role of hybrid processes (Advanced oxidation + Bioremediation) for dye treatment.
4. Which process should be used in the first stage (pre – treatment) is also interesting and less explored.
5. Economic viability of the process is an important concern from real scale application point of view.

2.11 Objective of my Research work

The overall objective of the present research work is to develop an effective and economically viable treatment strategy for textile wastewater treatment. Based on the above research gaps these objectives were formulated:

1. Acclimatization, isolation, and identification of potential bacteria obtained from dye-contaminated sites.
2. Mass transfer assessment in the Packed Bed Bioreactor (PBBR) for dye degradation also evaluation of kinetics using substrate growth and inhibition models.
3. Application AOP (Ozonation) in combination with the bioreactor for dye degradation.

a. Comparison of the efficacy of the standalone bioreactor and hybrid process for dye removal.

b. Optimization of process parameters (pH, Ozone dose, Ozonation time).

4. Toxicity analysis of degraded samples via phytotoxicity and bacterial toxicity tests.

5. Techno-economic assessment of coupling process (Ozonation and Biodegradation) for dye treatment.