Chapter 2

2. 1. Literature Review

2.1.1 Biodegradation of azo dye

Because of their chemical stability and synthetic origin, reactive azo dye (AD) degrades slowly using conventional wastewater treatment technologies. They are tough to remove since anaerobic biotreatment might produce dangerous aromatic amines even though they are designed to be stable under aerobic conditions.

One of two methods by which microbes decolourize textile dyes is either dye adsorption on microbial biomass or dye biodegradation by cells or enzymes. Adsorbents include organisms such as bacteria, microalgae, and fungi, and throughout the adsorption process, the dye is not broken down into smaller pieces. In contrast to biosorption, biodegradation breaks the original dye structure and converts them into simpler and less toxic substances. Therefore, biodegradation is preferred over other available options. The dyes might become degraded by microbes and their enzymes through anaerobic and aerobic metabolism (Shah, 2014).

2.1.2. Biodegradation of dye by pure culture and mixed culture bacteria

It has been demonstrated that many bacterial species are more effective than other microbes at digesting wastewater that contains dyes (Liu et al., 2021). Azo Dyes are hazardous to the environment and public health, and a co-substrate approach is frequently a place where Azo dyes are biodegraded. Bacterial degradation of azo dyes is caused by the anaerobic splintering of azo bonds (-N=N-) by azo-reductase enzymes. This azo bond breakdown (-N=N-) creates potentially hazardous intermediates that can be addressed either anaerobically or aerobically (Palani et al., 2012). Furthermore, in terms of dye degradation, bacterial (*Pseudomonas sp.* SUK1) degradation

of reactive dye 80 is 37% higher than fungal (*Aspergillus ochraceus* NCIM-1146) degradation (Shah et al., 2012). In recent years, research on the degradation of dye-containing textile wastewater has been conducted using single bacterium cultures such as *Aeromonas hydrophila* (Lade et al., 2012) and *Enterobacter sp. EC3* (Wang et al., 2009). *Providencia rettgeri* successfully degraded dazzling crocein present in sewage sludge (Shahi et al., 2021). In the Gompertz models, using ethanol as a co-substrate could encourage the growth of *P. rettgeri* and the biodegradation of contaminants (Shi et al., 2014). Both methods have successfully degraded dyes and employed them as an energy source, despite the improvement taking considerably longer to achieve the desired level of decolorization (Srinivasan and Sadasivam, 2021). Due to their rapid growth rate and outstanding hydraulic retention time, bacteria are frequently utilized to break down dyes among microorganisms; as a result, they may be effective in the processing of highly toxic organic wastes (Ajaz et al., 2020). Temperature, pH, dye structure and concentration, and contact time are the factors that affect the bioremediation of dyes from textile effluent. Most of the studies were conducted at temperatures ranging from 30 to 40°C, pH ranging from 3 to 10, and dye concentrations varying from 20 to 5000 mg/L (Ihsanullah et al., 2020). Xie et al., 2020 investigated the effect of different physicochemical parameters on the decolorization of Reactive Black 5 (RB5) by the bacterial flora DDMY1 under microaerophilic conditions. The bacterial flora decolorizes dye (> 70%) in both acidic and alkaline effluents with pH ranging from 4.0 to 9.0. The role of several bacterial groups in decolourizing azo dyes has been the subject of extensive research.

Using a single type of bacteria culture to treat textile effluent confirms reproducibility. With molecular biology and biotechnology expertise, biodegradation pathways can be identified by a single strain, which may help develop better biochemistry knowledge and higher enzyme activities. However, individual bacterial cultures rarely degrade azo dyes completely, and the

intermediate molecules typically involve toxic aromatic chemicals that need additional degradation (Khan et al., 2014).

2.1.3. Biodegradation by fungi

A fungal (fungi) culture can adapt its metabolism to changing environmental conditions. This ability is critical to their survival. Intracellular and extracellular enzymes aid in metabolic activity. These enzymes are capable of degrading various dyes found in textile wastewater. These cultures appear appropriate for degrading the dyes in textile discharge due to the availability of enzymes. The enzymes contain lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Chandar, 2014). White-rot fungi, such *as Phanerochaete chrysosporium* are reported as color-decolourizing fungi (Dwivedi and Tomar, 2018). Moreover, the degradation of dyes in textile effluents by white-rot fungi has some serious drawbacks, such as a prolonged growth phase, the requirement for nitrogen-restricted conditions, variable enzyme production, and the need for a sizable reactor due to the lengthy holding period for a complete breakdown (Anastasi et al., 2011).

2.1.4. Biodegradation of dye by algae

Azo dye (colors can be broken down and solubilized by microalgae (El-Sheekh et al., 2009). Because of their abundance in salt and freshwater, algae are potential sustainable biosorbents (Srinivasan and Viraraghavan, 2010). Algae have gained significant interest in the current years for the degradation of dyes in textile discharge due to their abundant availability. Algae have a high capacity for biosorption and bio-coagulants due to their high surface area and generally strong binding affinities (Dönmez and Aksu, 2002; Tien, 2002). Algal species have excellent cell-wall characteristics, which play an important role in biosorption, complexation, and electrostatic attractions. Phosphate, hydroxyl, amino, and carboxylate functional groups attached

to the algal cell surface are important in decolourizing dye from textile effluent (Özer et al., 2006). According to a thorough review of the existing literature, algal degradation of dyes occurs via three distinct mechanisms (Holkar et al.,2016): (i) dye transformation, (ii) dye consumption, and (iii) chromophores adsorption on algae. Various algal strains have been utilized in textile wastewater to break down a broad spectrum of colors (El-Sheekh et al.,2009; Baldev et al., 2013; Kulkarni et al., 2018).

2.1.5. Biodegradation of dye by yeast

Yeasts have several advantages over filamentous fungi and bacteria when it comes to textile dye degradation. For example, they grow quickly and can easily withstand adverse conditions (Yu and Wen, 2005; Martorell et al., 2012). Yeasts have the potential to be utilized as an alternative for the treatment of wastewater-containing dyes because of their quick growth rates and capacity to withstand challenging environmental conditions like low pH (Khan et al., 2013; Ali et al., 2020). There has been little research on the degradation of dyes by yeasts (Saratale et al., 2009; Kuhad et al., 2004). A yeast strain can extract a high concentration of different dyes from effluent (Aksu, 2003; Tan et al., 2014; Tan et al., 2016; Martorell et al., 2017). Biosorption and reductive azo bond cleavage are the primary mechanisms for the degradation of textile colors using yeast.

2.1.6 Biodegradation of dye by enzymes

Enzymes work as bio-catalysts, typically available in the form of liquid used for the bio-degradation of dyes and organic wastes (Nguyen et al., 2016). Biocatalysts gaining popularity in various industrial applications in recent years. Textile effluent biodegradation has several benefits over conventional technologies, including substrate specificity and selectivity, green chemistry, ease of access, cheaper costs, and high efficiency by enzymatic degradation (Kushch et al., 2019; Novotny et al., 2004). The main features of enzymatic degradation processes are non-toxicity, environmental friendliness, and reusability. However, biocatalyst deactivation brought on by denaturation is one of the main obstacles to enzymatic degradation (Jun et al., 2019). Several significant operational factors, including oxygen transfer, pH, temperature, dye structure, the presence of redox mediators, and enzyme and dye concentration, have an impact on the effectiveness and degradation of enzymatic dye decolourization (Teerapatsakul et al., 2017). A biological reactor's ability to bio-remediate textile dye depends on the immobilization of enzymes. Recent research work is focused on the effectiveness of various enzyme sources for dye degradation. (Sarkar et al., 2020; Rieegas-Villalobos et al., 2020). Horseradish peroxidase (HRP) immobilization on calcium-alginate was recently tested by Bilal et al. for the performance of degrading three distinct synthetic dyes, and they found that the average removal efficiency was better than 75%. (Bilal et al., 2016).

Table 2.1: A summary of various microorganisms used in the biodegradation of azo dye

S.N	Types of azo dye	Microorganisms	Process parameter (time (h), pH, T (°C), dye concentration(mg/L)	Removal Efficiency (%)	References
1.	Acid red 18	*Pseudomonas putida*	3, 3, 35, 1000	80.5-91.2	Wang et al.,2012
2.	Remazol Blue	*Bacillus megaterium, Micrococcus luteus* and *Bacillus pumilus*	120, 7, 30 ± 1, 25	*B.megaterium*:60 .8 ±2.7 *M. luteus*: 72.9 ±3.3	Karatay et al., 2015
3.	Congo red	*Brevibacillus parabrevis*	48,7,30, 500	88.92	Abu Talha et al. (2018)
4.	Congo red	*Bacillus* sp.	-,7 -, 50	93.6	Sonwani et al. (2020ba)

5.	Congo-red	*Bacillus* sp. MH587030.1	50, -, 564, 7, 25– 100	95.7	Sonwani et al. 2020
6.	Red RBN	*Proteus mirabilis*	20, 6.5– 7.5	95	Chen et al 1999
7.	Congo-red	*Pseudomonas extremorientalis* BU118	24, 8, 30, 50	94	Kurade et al.2012
8.	Crystal Violet	*Aeromonas hydrophila*	10, 5– 10, 25– 37, 50	>90	Ren et al., 2006
9.	Orange M2R	*Lysinibacillus* sp. KMK-A	48, 7, 37, 200	98	Chaudhari et al.,2013
10.	Reactive Blue 13	*Proteus mirabilis* LAG	5, 7, 35, 100	87.6	Olukanni et al.,2010
11.	Reactive Red 159	*Rhodobacter sphaeroides, Rhodopseudomonas palustri*	8d,9,30,6500	97.68	Srisuwun et al., 2018
12.	Reactive Blue 221	*Trichosporon akiyoshidainum*	16,7,26,200	72	Nouren et al., 2017

Table 2.2: Various packing materials are used in bioreactors for the biodegradation of azo dye

S. N	Types of azo dye	Bioreactor	Packing material	Removal efficiency (%)	References
1.	Congo red	Fluidized bed bioreactor	polyurethane foam	92.0	Shalini and Setty, 2019
2.	Congo-red	Packed bed bioreactor	coconut shell bio-char	85.3	Goswami et al., 2020
3	Real textile effluents	Triple-layered fixed bed reactor	non-corrosive wire mesh (20 cm × 20 cm)	>80	Kurade et al., 2017
4,	Congo-red	Up-flow column reactor	polyurethane foam	100	Lade et al., 2015
5.	Congo-red	Moving bed biofilm reactor	low-density polyethylene–polypropylene	95	Sonwani et al., 2021
6.	Congo-red	Moving bed biofilm reactor	Polypropylene polyurethane foam	95.7	Sonwani et al. 2020

7.	Acid Orange 7	Packed-bed bioreactor	Polyurethane foam (PUF)	87.3	Swain et al. 2021
8.	Remazol Brilliant Blue R	Packed-bed bioreactor	Solid sugarcane bagasse	87.6	Torres-Farradá et al.,2018

Table 2.3: A detailed analysis of various kinetics modeling applied in azo dye biodegradation

S.N.	Types of azo dye	Types of kinetic model	Microorganisms	Kinetic parameters	References
1.	Blue dye	Monod	*Streptomyces sp. DJP15*	μ_{max} = 0. 431 per h Ks = 0.0001mg/L	Garba Uba et.al., 2021
2.	Acid orange 7.	Andrew-Haldane model	*Bacillus* sp.	μ_{max} = 0. 185 per day Ks= 49.3 mg/L Ki=133.32 mg/L	Sonwani et al.,2021
3.	Textile dye	Andrew-Haldane model	Mixed culture	μ_{max} = 0.037–0.146 per h, Ks = 651.04–1372.88 mg /L and K_i = 5681.81–18727.59 mg /L	Gnanaprag asa et al., 2010
4.	Textile dye	Monod and Haldane	Mixed culture	The values of μ, K_S, K_i, and k_d were 3.52 mg COD/mg VSS-d, 71.7 mg COD/L, 81.63 mg COD/L, and 4.9 ×	Lin and Ho,2022

				10^{-3} 1/d respectively.	
5.	Congo red	Modified Stover-Kincannon model,(MSK) and kinetic constants	*Bacillus sp.* MH587030.1	The MSK- K_B and U_{max} were 0.253 g/L·day and 0.263 g/L·day, respectively.	Sonwani et al., 2020
6.	Carmoisine	Monod	Saccharomyces cerevisiae ATCC 9763	μmax = 0.133 per h K_S = 3.7 mg/l,	Kiayi et al.,2019

2.2 Summary of the literature review and research gap

The demand for dyes is increasing continuously due to the increased demand for different types of fabrics. The increase in the demand of different types of fabrics provides thrust to the textile industries, creating more space for businesses to grow. There are several other applications of dyes in day-to-day life, and different types of dyes have been synthesized and used for different applications. There are several characteristics that influence the rate of decolorization of the azo dye.

The textile industry is the major consumer of dyes, and azo group dyes are primarily used in the textile industry. Most small textile industries can't afford the high treatment cost of dye-contaminated wastewater, so they usually discharge the effluent without proper treatment. These dyes reach the water bodies through surface run-offs and sewage lines. The average degradation rate of these dyes is very slow, and the half-life is high, so dyes may accumulate in water bodies if their source is continuous. Due to their long half-life, they may enter aquatic foods such as fish

through the water. As marine food is a significant source of protein in many societies, through aquatic foods, these dyes may enter the human body and create many illnesses. It was reported that CR is very hazardous to C. dubia because it inhibits its ability to reproduce and survive at concentrations greater than 3 mg/L, and it also had a substantial impact on the type and quantity of food consumed. According to data on toxicity, it is essential to treat wastewater produced by various dye-related industrial operations to reduce the pollution load brought on by releasing this carcinogenic pollutant. Physical, chemical, and biological approaches have been investigated and used to detoxify and decolorize CR-laden effluents to achieve the desired level of degradation.

Lot of studies are already available on the successful bioremediation of different dyes, as given in the literature review chapter of this thesis. But most of the studies are limited to batch reactors and mixed culture. Moreover, the studies on bioremediation CR dye are also limited. The available CR dye degradation research has been performed on the basis of the various microorganism, reactors, and bio carriers. Although the bioremediation process has many advantages, the area still has lot of challenges so commercial application is limited and needs a more focused approach to overcome the various challenges in this area, particularly the biodegradation of compounds having low biodegradability such as CR dye.

The following research gaps were identified based on the literature survey:

1. Limited bacterial species are reported for efficient degradation of CR dye, which provides further scope to work on isolation, characterization, and performance evaluation for more bacterial species.
2. The continuous immobilized (attached growth) system always performs better than the batch attached. There have been a few reports of bioreactors used to biodegrade wastewater discharged by the textile industry, particularly congo red dye.

3. Limited studies are available on continuous bioreactors for Congo red dye degradation.
4. The RSM technique is explored in the biodegradation process for Congo red dye.
5. The few studies are available on the growth and inhibition kinetics of the Congo red dye.

2.3 The objective of the research work

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The overall objective of the current work is to develop an efficient, cost-effective, environmentally friendly technique for the effective treatment of dye effluent from textile industries. Hence the present work objectives follow as:

1. Isolation, acclimatization, and screening of potential bacterial species having Congo red dye degradation.
2. Identification of potential bacterial species using 16S rRNA technique and its application for the biodegradation of Congo red dye.
3. Optimization of process parameters using one variable at a time (OVAT) by RSM techniques.
4. Performance evaluation of bioreactors namely PBBR and MBBR
5. Evaluation of the kinetics, growth kinetic of Congo red dye, and various modes.