CHAPTER 1

1.1 Introduction

The dye and dyestuff industry is considered one of the most polluting industries, releasing vast amounts of polluted effluents into the soil and water bodies, posing serious risks to aquatic life and the environment (Kumar Sonwani et al., 2021; Tripathi et al., 2023a). Over 10,000 different types of dyes and pigments are consumed by various industries such as textile, cosmetics, leather, paper, etc., while global dye production exceeds 7×10^5 tonnes per year (Ihsanullah et al., 2020). Water pollution caused by dye discharge from textile industries is one of the most serious threats to the ecosystem. Several major environmental agencies, including the United States Environmental Protection Agency (USEPA), have classified dyes as hazardous pollutants (Bharti et al., 2019; Premaratne et al., 2021). According to the World Bank, dyeing and finishing treatments used on fabric account for 17% to 20% of textile industry water pollution. The wastewater discharged from the textile dyeing industry contains 72 toxic chemicals, 30 of which are insoluble in waste treatment processes (Bhatia et al., 2017). The effluent from dying industries is generally characterized by high COD, high suspended solids, high salinity, alkali, acids, surfactants, saop of metals and other toxicants (Holkar et al., 2016; Premaratne et al., 2021). Due to their carcinogenic nature, the presence of dyes in water, even in low concentrations, can have adverse environmental effects. Therefore, it is of great concern to remove these toxic dyes from the environment, not only for aquatic life but for human life as well (Tripathi et al., 2023b).

1.2 Dyes: classification and environmental impact

Dyes are classified into a variety of categories based on their origin, color, chemical structure, and utilization methods as depicted in **Fig. 1.1**. On the basis of origin, dyes can be natural or synthetic, and based on chemical structure it can classify into acridine, azo, phthalocyanine, chromophoric and nitroso dyes (Vikrant et al., 2018; Yagub et al., 2014). Based on solubility dyes can be divided into two categories water-soluble (Direct, reactive, acidic, basic) and water-insoluble (disperse, vat) (Srivastava et al., 2022; Velusamy et al., 2021).

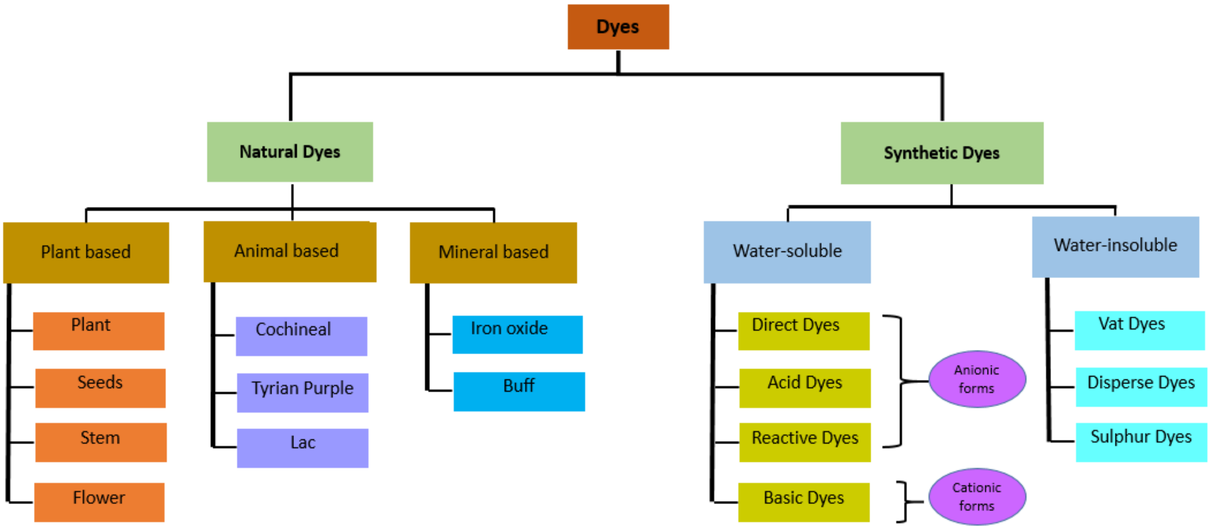

Fig. 1.1 Classification of various dyes

Azo dyes are the most extensively used synthetic dyes that contain one or more azo bonds (–N=N–) and are considered the most dominant industrial effluent product (Swain et al., 2021a). In textile industries it had been estimated that approximately 80% of total dyes are azo dyes for dyeing purposes, azo dye constitutes a very mutagenic and recalcitrant category of dye on a commercial scale (Sarkar et al., 2017). These azo dyes can be further classified into various categories (mono azo, bis azo, tris azo, poly azo) depending on the azo group present (Senthil Rathi and Senthil Kumar, 2022). Chromophore and auxochrome components play vital roles in dyes molecule (Al Prol, 2019). The color of a dye is due to the presence of the chromophores group. Some important chromophores are -*C=C*-, -*C=N*-, -*N=N*-, -NO_2, -*NO*, -*C=O*. Based on the structure of chromophores, there are 20-30 varieties of dyes. Azo, anthraquinone, phthalocyanine, and triarylmethane dyes are considered as relatively more important groups (Mohammad and Azeez, 2005; Said et al., 2020). The auxochrome is an electron-withdrawing substituent that intensifies the color of the chromophore by revamping the overall energy of the electron system. The most common auxochromes are $-NH_3$, $-COOH$, $-OH$, $-NR_2$, $-NHR$, $-SO_3H$ (Salleh et al., 2011).

The degradation of a dye is affected by its structure and properties. Furthermore, binding the dye to the fabric needs the use of numerous auxiliaries that are also related to the type of dye. A covalent bond between the dye and the fiber is created when an alkali is added, causing the dye to migrate from the outside to the inside of the fiber (Ledakowicz and Pázdzior, 2021). Therefore textile effluent results in high COD, BOD, TDS, and pH values. **Table 1.1.** Compares the characteristics of textile wastewater effluents with the standard set by World Health Organization (WHO).

Table 1.1 Characteristics of real textile wastewater and permissible limit by WHO.

S No.	Parameters	Real textile wastewater	Permissible limits as per WHO
1.	pH	5 – 12	6.5 – 8.5
2.	COD	250 – 8000 (mg/L)	100 – 300 (mg/L)
3.	BOD	100 – 5000 (mg/L)	150 – 250 (mg/L)
4.	TDS	1000 – 11000 (mg/L)	500 (mg/L)
5.	TSS	50 – 5000 (mg/L)	100 – 10 (mg/L)
6.	Chlorides	200 – 6000 (mg/L)	250 (mg/L)
7.	Sulfate	500 – 1000 (mg/L)	250(mg/L)
8.	Color	50 – 2000 Pt-Co unit	15 Pt-Co unit
9.	Temperature	30 – 45 ℃	-

Various studies have raised serious concerns about the effluent from the textile industry and its effects on the environment and human health (**Fig. 1.2**). The textile dyes significantly reduce the aesthetic appeal of water bodies, hinder photosynthesis, hinder plant growth, increase BOD and COD, and if used for irrigation purpose than it may bio accumulate in our food chain and increase toxicity, carcinogenicity, and mutagenicity which adversely affect the human health (Kishor et al., 2021b).

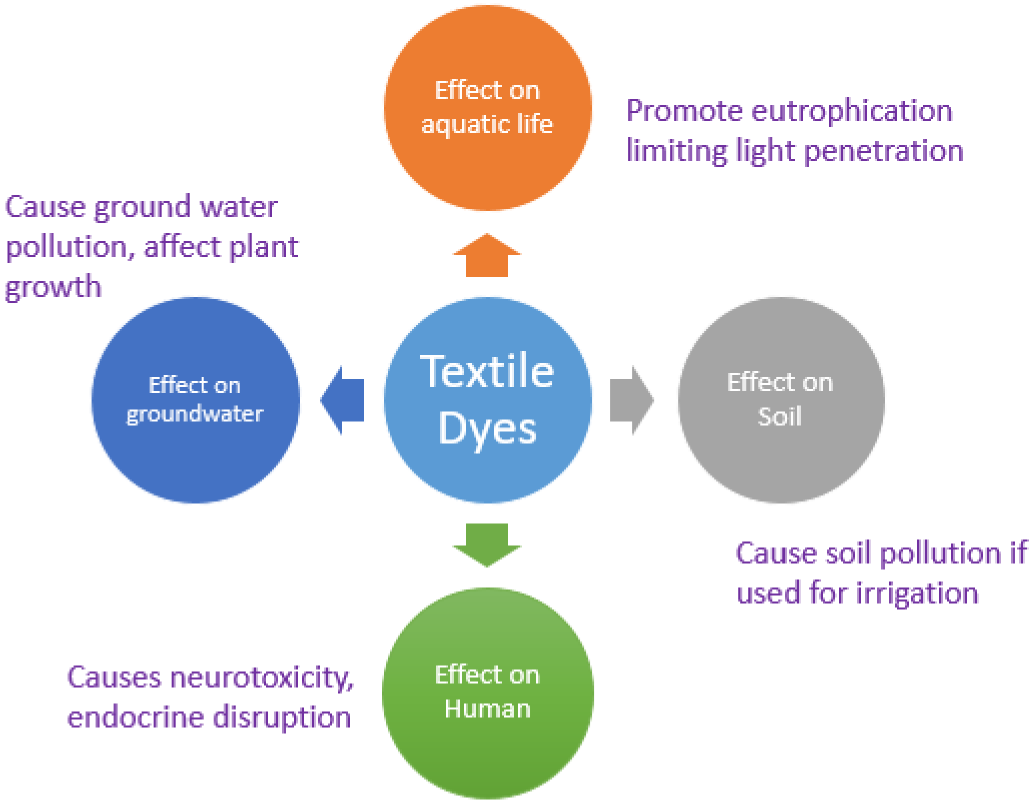

Fig. 1.2 Effect of textile wastewater effluent on the environment.

Excess dyes in water bodies can have a wide range of negative effects on the ecology and putrescence of water resources, so developing effective methods for removing them from the hydrosphere is critical.

1.3 Treatment method

The two major types of dye remediation techniques are physicochemical and biological. The physicochemical methods used for the treatment of textile effluents include adsorption, ion exchange, coagulation-flocculation, oxidation process, membrane filtration including reverse osmosis, nanofiltration, electro-dialysis, etc. The physicochemical techniques used for dye remediation have the disadvantages of being expensive, requiring a lot of energy, and producing a lot of waste. **Fig 1.3**. Summarizes all the treatment approaches for textile wastewater treatment.

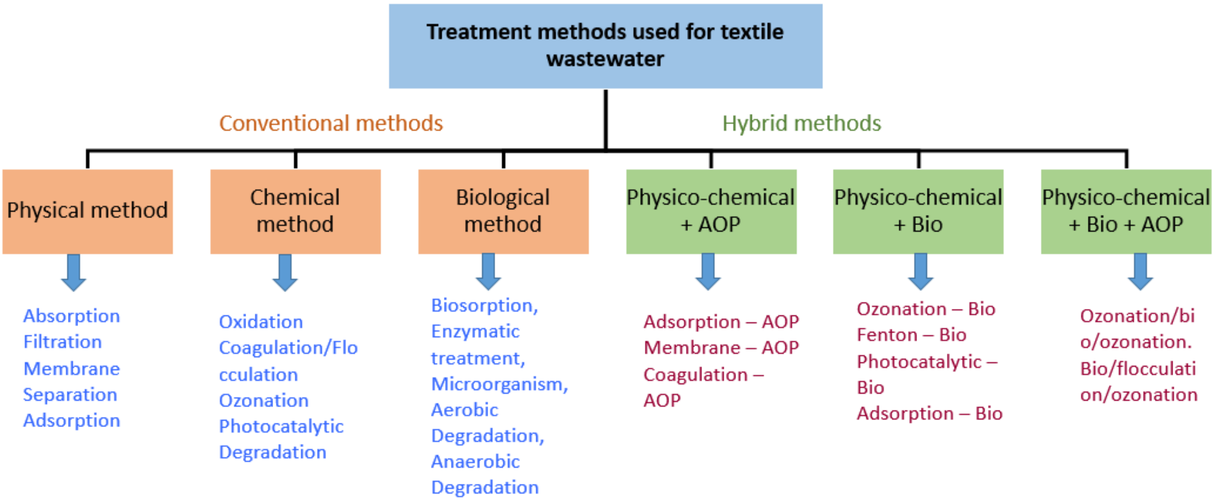

Fig. 1.3 Various treatment approaches used for textile wastewater.

1.4 Biological method of dye removal

Biological techniques for pollutant degradation are an environmentally friendly option, with complete mineralization of organic compounds and low sludge generation. This method has been reported to be very effective and environmental friendly (Corso and Maganha De Almeida, 2009; Shinkafi et al., 2016; Varjani et al., 2020a). The fundamental of biodegradation is based on microbial metabolism, which degrades the pollutants in wastewater. Overall, biodegradation is reported to be the most effective and widely used technique for decomposing textile effluents (Chaturvedi et al., 2022a). Microorganisms, particularly bacteria, play an important role in biodegradation and each microorganism has its unique degradation efficiency and operating conditions. The efficiency of biodegradation process can be enhanced significantly by optimization of the process parameters. Several process parameters such as pH, temperature, dye concentrations, and aerobic/anaerobic conditions have a remarkable impact on the biodegradation of various synthetic dyes (Srivastava et al., 2022). The conjugate double (-N=N-) bonds in azo dye are broken down by bacteria under anaerobic conditions, producing a colorless solution that contains potentially dangerous aromatic amines. These aromatic amines are then broken down aerobically (Kishor et al., 2021b). The biodegradation of dye wastewater is represented in **Fig 1.4**. Biodegradation can be classified as aerobic, anaerobic, or anoxic (both aerobic and anaerobic) depending on the nature of the microorganisms (Geed et al., 2018b). Enzymes (mainly azo reductase) primarily catalyze the dye under aerobic conditions. The azo reductase enzyme facilitates the breakdown of chromophore azo bonds, with nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH) acting as reducing agents (Jamee and Siddique, 2019). This process involves transferring four electrons (reducing equivalents) to the azo linkage in two steps, with two electrons being forwarded to the azo dye (which functions as an electron acceptor) in every step, resulting in dye decolorization and formation of a colorless liquid (Kishor et al., 2021a). It has been observed that in an anaerobic environment, a relatively lower redox potential (50 mV) forms, causing the azo dyes to decolorize effectively (Bromley-Challenor et al., 2000). For complete decolorization of an azo dye, an amalgamation of anaerobic and aerobic processes is typically preferred.

The benefits of biodegradation comprise of production of less toxic sludge, low cost, and high removal efficiency. Compared to physical and chemical processes, biological processes produce sludge that is relatively less harmful to the environment (Kumar Sonwani et al., 2021). The fact that these biological approaches can be used in situ (on-site) or ex-situ (off-site) and microorganisms can be used as a consortium or as a pure strain, and even plants demonstrate their adaptability (Ghosh et al., 2017; Vikrant et al., 2018).

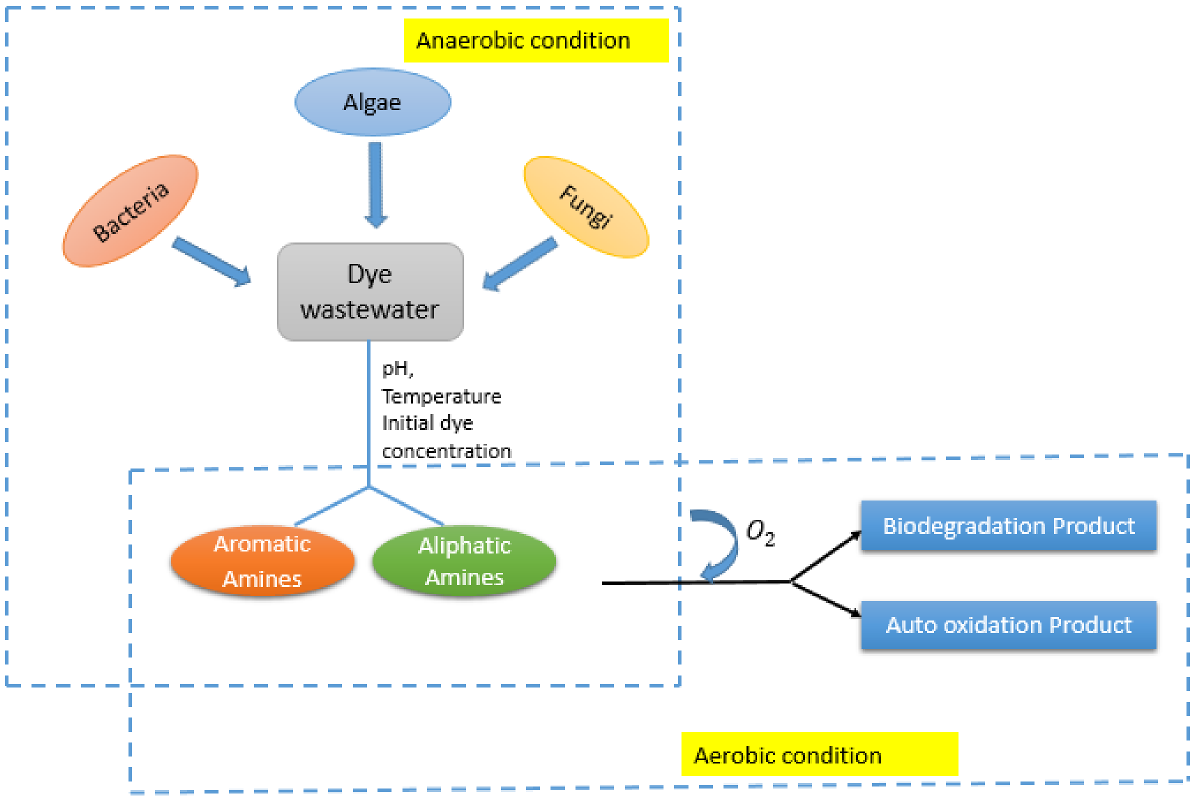

Fig. 1.4 Schematic of azo dye biodegradation under aerobic and anaerobic conditions.

1.4.1 Bacterial degradation

Various microbes can be used to degrade different dyes because they have different degrading mechanisms and pathways (Cao et al., 2019). Microbial degradation of azo dyes is an anaerobic-aerobic process that involves anaerobic decolorization via the azo reductase mechanism of the azo dyes followed by aerobic degradation of the aromatic amine intermediates, which can be more recalcitrant and mutagenic (Ajaz et al., 2020; Dong et al., 2019). The use of a single bacterium culture for textile wastewater treatment confirms reproducibility. Using knowledge of molecular biology and biotechnology, the detailed mechanisms of biodegradation caused by a single strain can be determined. However, individual bacterial cultures rarely completely degrade azo dyes, and the intermediate compounds are frequently toxic aromatic compounds that necessitate further decomposition (Holkar et al., 2016; Khan et al., 2014).

Microbial consortia are preferred over pure cultures because they can grow on a broader range of substrates. They frequently have large number of bacterial strains that can attack different locations on the molecular structure of azo dyes. Bacterial strains that can mineralize intermediates of aromatic amines are frequently found in microbial consortia (Korenak et al., 2018). More research is needed on microbes that play an important role in organic contaminant remediation and also can be used as pollution indicators for various toxicants in wastewater. The main advantage of working with bacteria is that they are easy to culture and grow faster than other microbes. Bacterial dye degradation ability can be easily improved through molecular genetic modification. Bacteria can catabolize aromatic hydrocarbon-based organic pollutants, allowing them to be breakdown by utilizing them as an energy source (carbon source) (Bhatia et al., 2017; Yang et al., 2014).

Based on the application the bacterial degradation can be further divided into two parts (i) *in-situ* (treatment of pollutants on-site) and (ii) *ex-situ* (off-site treatment). The *in-situ* degradation process generally refers to bio-stimulation, bio-attenuation, and bio-augmentation. In bio-stimulation, the nutrients, water, electron acceptor, and donors are added to enhance the biodegradation rate. During bio-attenuation, biodegradation occurs naturally with the addition of nutrients or bacteria. The addition of microorganisms with the ability to biodegrade recalcitrant molecules in polluted environments is known as bio-augmentation (Daniel et al., 2019).

Ex-Situ bioremediation is the removal of pollutants from a contaminated environment and treatment in another location. In the past few years, various organic pollutants have been degraded via *ex-situ* biodegradation or genetically engineered biodegradation (Varjani et al., 2015). Several factors such as pH, salinity, microorganism type, and bioreactor type can affect the *ex-situ* biodegradation process. In comparison to *in-situ* biodegradation, the cost of operation of *ex-situ* biodegradation is very high (Megharaj et al., 2011).

1.5 Ozonation:

Ozonation is an advanced oxidation process in which ozone is used as the oxidizing agent (O_3). Because of the numerous benefits of this process, interest in using ozone in wastewater treatment has grown significantly in recent years. Because ozone is a strong oxidant, it reacts quickly with most organic pollutants, including aromatic rings and conjugated double bonds of azo dyes (Gökçen and Özbelge, 2006; Zhang et al., 2006).

1.5.1 Ozonation mechanism

Ozone is a highly effective oxidizing agent that consists of a one-weak single bond and one strong double bond. Ozone reacts with the substances in wastewater in two ways: in an acidic environment it reacts directly as molecular ozone (O_3) and in an alkaline environment it reacts indirectly as secondary oxidants, such as hydroxyl radicals ($OH^{\bullet}$), O_2^-, $HO_2^{\cdot}$ (Arslan-Alaton and Alaton, 2007; Bilińska and Gmurek, 2021).

Hoigne, Staehelin, and Bader (HSB) described the ozone decomposition process in an aqueous solution (Staehelln and Hoigne, 1985; Venkatesh et al., 2017). The reaction sequence can be summed up as follows:

$O_3 + OH^- \rightarrow HO_2 + O_2^-$

$HO_2 \rightarrow O_2^- + H^+$ (Formation of superoxide radicals)

Now radical chain reaction takes place, during which $OH^{\cdot}$ are formed

$O_3 + O_2^- \rightarrow O_3^{\cdot -} + O_2$

$O_3^{\cdot -} + H^+ \rightarrow HO_3$

$OH^{\cdot}$ that have formed, react with additional ozone via following mechanism

$OH^{\cdot} + O_3 \rightarrow HO_4^{\cdot}$

$HO_4^{\cdot} \rightarrow O_2 + HO_2^{\cdot}$

In termination step radical scavengers which consume $OH^{\cdot}$ without generation of O_2^{-} superoxide radicals.

Thus, the remediation by ozone may be remarkably accelerated in an alkaline solution but less effective in an acidic solution. In alkaline pH, ozone can form a more powerful hydroxyl radical oxidizing agent (E^0 = 2.8 V) than its own redox potential (E^0 = 2.08 V), which can break down the complex structure of dyestuffs and result in decolorization (Gharbani et al., 2008). Some researchers prefer direct ozonation for dye degradation because molecular ozone has a relatively higher selectivity for dye when compared to the hydroxyl ($OH^{\bullet}$) radicals. Ozone has the ability to quickly break down the double bond of an azo dye, resulting in color removal in short time intervals and subsequently, it increases the biodegradability of certain dyes (Castro et al., 2017).

The ozonation process is known as one that produces relatively less sludge compared to other AOPs. The remaining ozone degrades into water and oxygen. However, ozonation is best suited for total decolorization rather than mineralization. Furthermore, ozone production is costly due to high energy consumption. That is the reason why this method must be used in conjunction with the other processes (Paździor et al., 2017; van Leeuwen et al., 2009).

1.6 Integration of Ozonation and Biodegradation

The scientific community is attempting to overcome some of the difficulties associated with biodegradation by finding and engineering effective microbial strains and integrating AOPs with biodegradation to increase overall degradation efficiency and decrease the level of toxicity of the treated dyeing wastewater (Chaturvedi et al., 2022a). AOPs are used in conjunction with biodegradation as a pre-treatment or post-treatment step. AOP may be preferred as a post-treatment step following the biological process because the aromatic amines, which are typically formed after the biological treatments of azo bonds, are resistant to biodegradation. Some AOPs, however, may produce toxic by-products that can be biodegraded if biological treatment is performed after the AOPs.

Most ozonation research is currently focused on its use as a pre-treatment in conjunction with biological processes. This is usually due to the fact that industrial effluents may contain such compounds which are harmful to the microorganisms used in biological treatment. As a result, the pre-treatment aims to improve the biodegradability of the effluent samples. Paździor et al., 2017 have found 96% toxicity removal for biodegradation followed by Ozonation in SBR and 98% toxicity removal for Horizontal Continuous Flow Bioreactor (HCFB). Dias et al., 2019 found ozonation removed color very quickly, but the mineralization achieved was quite low. Venkatesh et al. used ozonation in conjunction with anaerobic biodegradation using an up-flow anaerobic sludge blanket (UASB) and found 90% COD reduction and 94% color removal. Castro et al., 2021 used Ozonation followed by biodegradation in a moving bed bioreactor (MBBR) and found that after Ozonation, color removal was more than 97% but COD removal was only 48%. After the treatment of the ozonolysis product in MBBR the COD removal was 93%. Punzi et al., 2015 found that the integrated anaerobic-ozonation process successfully removed up to 99% of the color and COD removal was up to 85-90%, and toxicity was also reduced for 100-1000 mg/l of the Remazol Red dye.

1.7 Brief Objective

The objective of this thesis is to investigate and evaluate environmentally viable approaches to the treatment of textile dyes in wastewater. The primary focus is on using biological processes for effluent treatment. A particular emphasis was given on increasing efficiency through the integration of a hybrid process that combines ozonation and biodegradation. The study seeks to contribute to the development of practical and environmentally acceptable strategies for treating textile dye pollution in wastewater.