Chapter 1

1.1. Introduction

1.1.1. Sources and pollution

The term "natural dyes" refers to any dye produced from a natural source, such as plants, animals, or minerals. Natural dyes are frequently insubstantial and must be applied to fabrics using mordants, which are typically metallic salts that have an affinity for the coloring agent and the fiber (Samanta and Agarwal, 2009). Synthetic dyes are widely employed in various industries, with textile processing industries being the largest users (Keharia and Madamwar, 2003). Perkin was the first to produce mauve, the man-made organic dye, in 1856. Woulfe produced picric acid by processing the natural dye indigo with nitric acid in 1871, making it the first synthetic organic dye. Since then, numerous new chemical dyes have been introduced to the ever-expanding list of dyes (Mathur et al., 2006).

Around 100,000 commercially accessible dyes are available, with over $7x10^7$ tonnes of dyestuff manufactured annually worldwide. The textile sector is the largest consumer of dyestuffs, accounting for over 70% of global usage. These dyes are used by a wide range of industries, with textiles using the most dyes. Other users are food, cosmetics, and paper printing-related industries (Silveira et al.,2009). The textile industry uses synthetic organic dyes like direct dyes, processing dyes, reactive dyes, and other types of dyes. Dyes are highly complex organic compounds and difficult to degrade naturally. Environmental issues associated with the manufacture and use of dyestuffs have grown significantly over the past ten years, and this is the key factor influencing the growth and cost textile dye industry in the modern day (Mathur et al., 2006). According to Chang et al., 2001 azo dyes are synthetic dyes widely utilized in the textile, food, paper, printing, leather, and cosmetic sectors, making up about 50% of all dyes produced. Azo dyes contain different types of structures, but the existence of an azo linkage, i.e., N=N, is the essential structural property. This linkage may be repetitive; mono-azo colors have one azo linkage, whereas diazo and triazo dyes have two and three, respectively. The textile industry generates 14% of India's industrial production (Ekambaram et al., 2016). Several dyes used in the textile industries can be divided into different categories based on chemical composition. Metal complex dyes, anthraquinone base, basic, and other natural products dyes that are cationic, di-azo, and azo. The azo, xanthenes (Artificial dyes), and anthraquinone dyes are highly toxic to living things (Daneshvar et al., 2007)

1.1.2 Environmental issues related with textile dye

Textile dyes, like many other industrial pollutants, are extremely hazardous and highly toxic (Sharma et al., 2018), so they are associated with the degradation of the ecosystem and a variety of diseases in humans and animals (Khan and Malik, 2018). Lellis et al., 2019 observed that textile dyes degrade water quality, limit photosynthesis, increase BOD and COD levels, inhibit plant growth, recalcitrance and bio accumulative, may affect the food chain, and toxicity, carcinogenicity, and mutagenicity may increase.

1.2. Classification of dyes

Dye is the most prevalent coloring substance that absorbs electromagnetic energy in the visible range (350-700 nm). Auxochromes and chromophores are present in dye molecules. –C=C–, –C=N, –C=O, –N=O, –N=N, $–NO_2$, quinoid rings, etc., are examples of chromophores, while $–NH_2$, –COOH, $–SO_3H$, –OH, etc., are examples of auxochromes, which change the total energy of the electron available in the molecular structure to increase the color of the chromophore (Zollinger

1987). The classification of dyes based on chemical nature and application method is shown in Table 1. Monoazo, diazo, triazo, poly azo, and azoic, the number of azo bonds present in a single dye molecule determines how azo dyes are categorized. The classifies dyes according to the chromophore, such as nitro, Nitroso, azo, anthraquinones, xanthenes, indigoids, etc., which corresponds to their chemical structure shown in Table 2 (Sudha et al.,2014; Barragan et al., 2007). Azole dyes are assigned numbers ranging from 11,000 to 39,999 in the Colour Index (CI) system (Table 3). For dye classification, the Association of Dyers and Colorists' (ADCI) color index number is used (Sandhya 2010).

Table 1.1: Classification of dye

Basis of the chemical nature and application method			
Type of dye	**Example**	**Method of Application**	**Substrate**
Direct dye	Phthalocyanine, azo, oxazine, and stilbene	Provided from baths that are neutral or slightly alkaline and contain extra electrolytes.	Leather, nylon, rayon, silk, cotton, and paper

Basic	Hemicyanine, azo, cyanine, diazahemicyanine, azine diphenylmethane, xanthene, triarylmethane, acridine, anthraquinone, and oxazine are some examples of chemicals.	Acidic dye baths	Inks, medicine, modified nylon, paper polyester
Acid	Anthraquinone, azine, azo (including premetallized), triphenylmethane, xanthene, nitro, and Nitroso.	When using neutral to acidic dye baths	Cosmetics, food, leather, nylon, modified acrylic, paper printing, ink, silk, and wool.
Reactive	Formazan, azo, basic triphenylmethane, oxazine, anthraquinone, and phthalocyanine	Interaction between a functional fiber group and a dye's reactive spot. Under the effect of heat and an alkaline pH, covalent bonding occurs.	Cellulosic, cotton, nylon, silk, and wool.
Disperse	Azo, anthraquinone, nitro, styryl, and benzodifuranone	Fine aqueous dispersions are frequently applied using high-pressure or low-pressure carrier techniques;	Acetate, nylon, polyamide, polyester, polyester-cotton, acrylic fibers, cellulose, cellulose acetate, and plastic

		dye can be padded on fabric and thermofixed.	
Sulfur	Indeterminate structures Leuco Sulphur Blue 11	The aromatic substrate was evaluated with sulfidulphide and then reoxidized to produce products on the fiber that included insoluble sulfur.	Cotton, leather, paper, polyamide fibres, rayon, Silk and wood
Solvent	Solvent Red 146	Substrate dissolution	lubricants, oils, plastics, stains, varnishes, inks, lacquers, gasoline, and waxes
Vat	Indigoids and anthraquinone	Water-insoluble dyes solubilized by reducing with sodium hydro sulfite then exhausted on fiber and reoxidized	The dye can be made soluble in water, dropping in sodium hydrogen sulfite, and applied fiber

Table 1.2: According to the chemical structure

According to the chemical structure	
Type	**Structure**
Azo dyes	-N=N-
Nitro dyes	O -N=O
Nitroso dyes	-N=O

Table 1.3: Azo dye in Colour Index (Gurses et al.,2016)

S.N.	Types of the azo group	Color index no.
1.	Monoazo	11000-19999
2.	Disazo	20000-29999
3.	Trisazo	30000-34999
4.	Polyazo	35000-36999
5.	Azoic	37000-39999

1.3. The Current treatment methods for dye degradation

Physical, chemical, and biological treatment techniques are used to treat water contaminated with dye or any ccoloringagent. Traditional physical and chemical methods for treating textile effluents include bleaching, membrane filtration, ion exchange, flocculation, coagulation, precipitation, irradiation/ozonation, and adsorption. The physical and chemical dye treatment methods have some drawbacks, such as less efficiency, high cost, increased demand for energy, and generation of secondary waste. Aside from these traditional procedures, bioremediation has lately gained popularity as it is a comparatively inexpensive and effective treatment alternative for textile waste. The biological approaches for azo dye cleanup are briefly summarized in Figure 1.1.

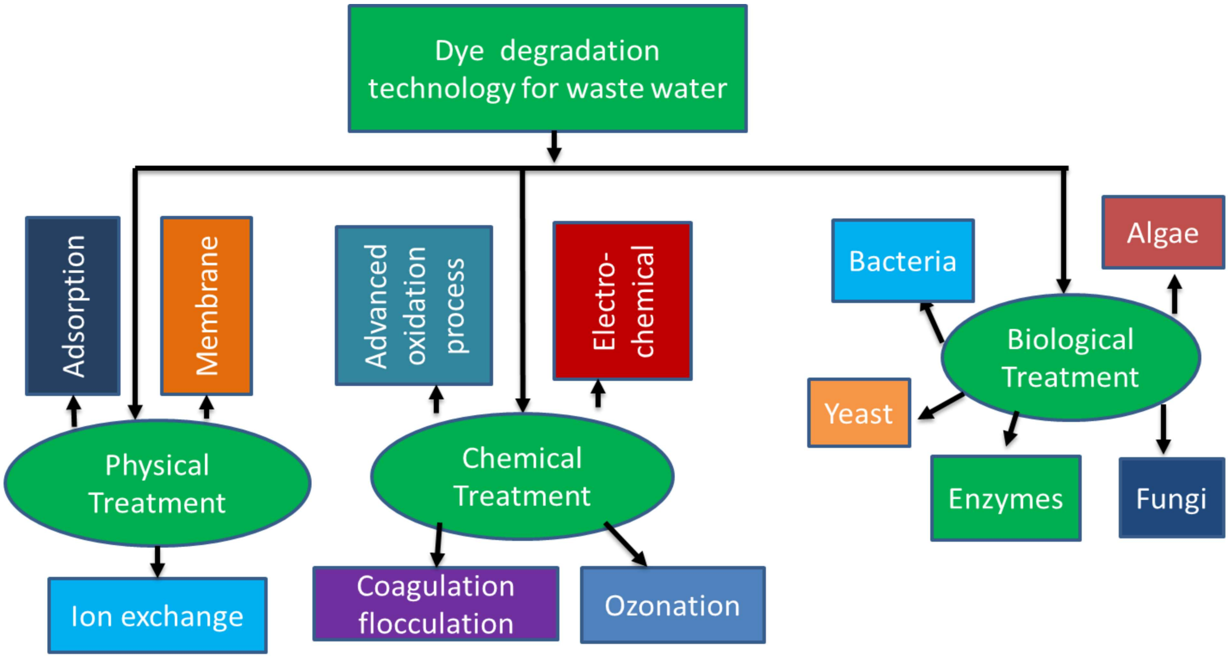

Figure. 1.1: The Current technology used for dye degradation.

1.4. Biological methods for dye removal in wastewater

Biological treatment is a low-cost, environmentally favorable method of reducing sludge production. It uses fewer chemical reagents, saves energy, is cost-effective in underdeveloped nations, and produces higher yields of mineralization of the dyes in their entirety (Pandey et al., 2007). Only the dissolved components are removed from textile wastewater using the biological method. The removal effectiveness is influenced by the ratio of organic load to dye, microbe load, system temperature, and oxygen concentration. The effectiveness of the biological process for degradation is determined by the capacity of the selected bacteria to adapt. As a result, many microbes and enzymes were isolated and tested for the removal of different colors "The isolation of potent bacteria and their use of them for degradation is an essential biological aspect of textile effluent. Several Microbes, including bacteria, fungi, and algae, have been identified that can break down a wide spectrum of color found in textile wastewater" (Holkar et al., 2016).

1.5. Biodegradation of azo dyes

The decoloration of azo dyes can be accomplished utilizing a variety of bacteria, with the reductive cleavage of the –N=N– bond being the first step in the process. The subsequent section describes the various biodegradation processes of azo dyes performed under different experimental conditions. Several bacterial types, including Clostridium, Eubacterium, yeasts, and fungi, can produce non-specific nicotinamide adenine dinucleotide hydrogen (NADPH/NADH). Azo benzene reductases are enzymes that can break down azo linkages (Pandey et al., 2007).

1.6. Biological processes of dye degradation

1.6.1. Anaerobic condition of azo dye degradation

The anaerobic process occurs in the absence of free or mixed oxygen. On the other hand, anaerobic treatment processes are carried out by microorganisms, which do not require air to decompose organic contaminants. The byproducts of anaerobic treatment for organic digestion include biomass, methane, and carbon dioxide gas (Arun., 2011).

Anaerobic dye degradation in wastewater is a potential method since it is a comparatively simple and nonspecific azo-dye reduction process. Non-specific azo-reductase bacteria directly catalyzed azo-dye reduction. However, the existence of this enzyme has yet to be formally proven, and the reduction mechanism, which relies on cofactors and redox intermediates created during the dye degradation process, must also be established (Selvaraj et.,2021). The outcome mechanism could depend on the redox intermediates generated during the anaerobic breakdown of azo dyes. The azo-bonds in the dyes are cleaved throughout the process, resulting in aromatic amines (azo-bond reduction), which remove the color of the wastewater (Chen et al.,1999).

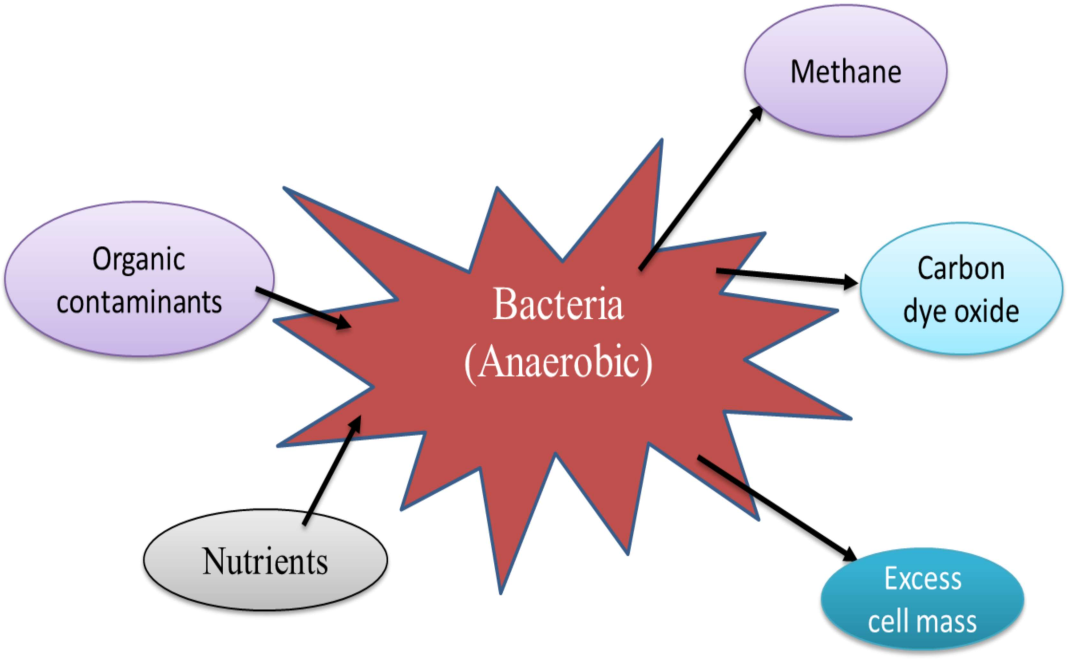

Figure. 1.2: Anaerobic process dye degradation in wastewater

1.6.2. Aerobic condition of azo dye degradation

The word "aerobic" refers to being in the presence of air (oxygen). Aerobic degradation occurs in the presence of air and employing microorganisms that assimilate organic pollutants by using molecular/free oxygen to transform them into carbon dioxide, water, and biomass (Arun, 2011). Aerobic bacteria modify themselves to play a role in the degradation process. This adaptation requires longer aerobic development in continuous culture, specifically for azo molecules. The bacteria produce an azo reductase specific for this molecule, which, under controlled situations, can reductively break the azo group in the presence of oxygen. (Stolz, 2001).

Under aerobic conditions, the enzyme azo-reductase is primarily responsible for dye degradation. According to reports, several aerobic microorganisms release azo reductase enzyme to increase the dye breakdown. Several factors, including dye content, enzyme concentration, temperature, and the rate of intermediate complex formation, affect the reduction of azo-dyes (Li et al., 2018). The rapid degradation of textile effluent by aerobic conditions in the reactor is ineffective in degrading xenobiotic compounds such as dyes (Panswad et al.,2001).

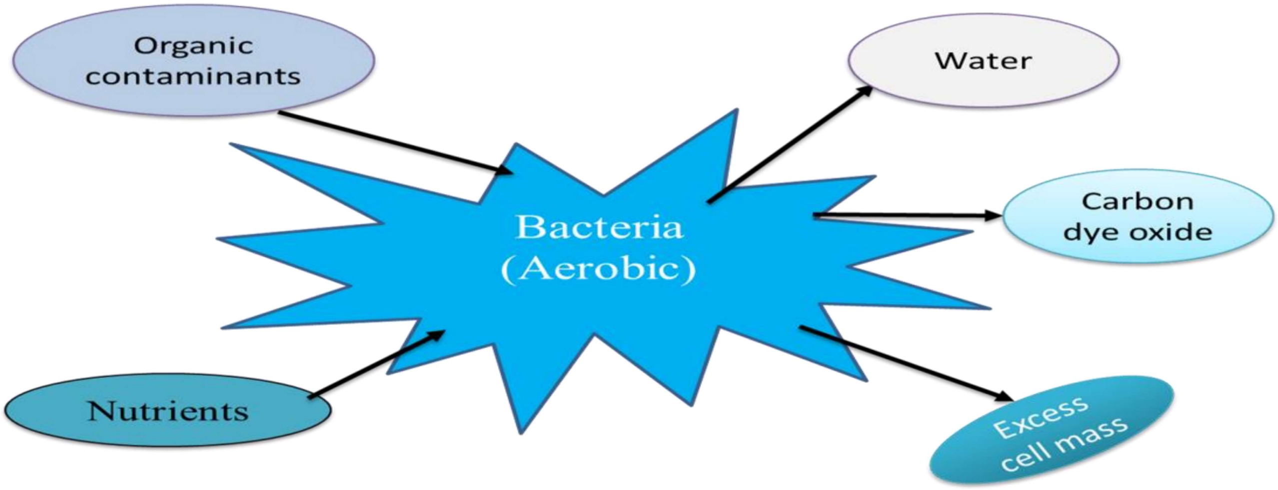

Figure.1.3: Aerobic bacteria process

1.6.3. Suspended growth bioreactors

The most popular method is to degrade effluent in suspended growth bioreactors. This technology uses free cell culture suspended in liquid media for pollutant biodegradation. There are several advantages of using growth bioreactors due to pollutants' direct contact with microbial cells. They are affordable, simple to construct, and have low mass transfer resistance. Despite these advantages, microbial activity, viability, and dispersal in suspended growth bioreactors are not certain, especially in severe conditions (Partovinia and Rasekh, 2018). Due to substrate inhibition, the performance of bacterial tends to be decreased at high substrate loading rates, resulting in reduced substrate biodegradation (Geed et al., 2018). Suspended growth bioreactors have been utilized for wastewater biodegradation, including the continuous stirred tank bioreactor (CSTR), MBR, and airlift bioreactor (Mudliar et al., 2010). This kind of bioreactor is available in numerous designs, each with unique advantages and disadvantages when it comes to the biodegradation of particular pollutants.

1.6.4. Attached growth bio-reactors

Attached growth bioreactors have emerged as a promising and successful approach for biodegradation of wastewater effluent in recent years. Attached growth is the word used to describe how bacteria or enzymes bind to carriers' surfaces and pores (Shen et al., 2015). In the attached growth bioreactors, the microbial cells form a thin biofilm (10 m to 1 mm) through which nutrients and substrate are diffused (Mudliar et al., 2010). When the biofilm ruptures or breaks, the metabolic byproducts are released, returning to the liquid phase. Due to its better surface area, good mechanical and chemical stability, low HRT, and high tolerance to high pollutant concentrations, an attached-growth bioreactor usually show better removal efficiency for AHCs (Sonwani et al., 2021; Shen et al., 2015). PUF, PVA, PVC (polyvinyl chloride), luffa, charcoal, and various agro-waste materials have been successfully employed as carriers for bacterial growth. Starch, collagen, agar, and alginate can also be used as a support matrix (Sneha et al., 2019; Lin et al., 2014). The attached growth bioreactor has been used in different reactors such as PBBR, MBBR, FBBR, and trickling filters.

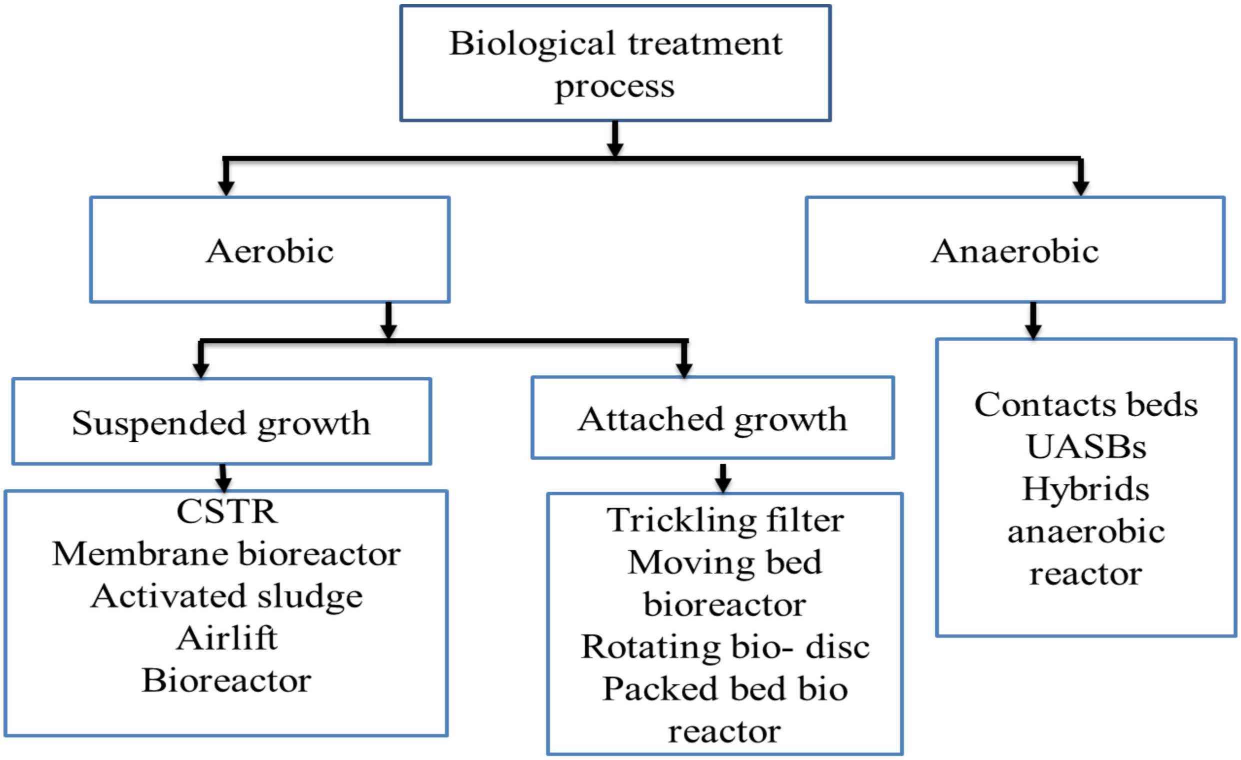

Figure.1.4: Biological processes for dye degradation

1.7. Main factors that influence the biological process of bacterial degradation

The Physical and chemical operational parameters that directly control bacterial breakdown include oxygen, temperature, pH, dye concentration, dye structure, the concentration of carbon and nitrogen sources, amount of electron donor, and a redox mediator. As a result, to improve the efficiency and speed of microbial degradation, it is required to assess the impact of each parameter on bio-degradation.

Table 1.4: Below outlines the various operational parameters that can be used to improve biodegradation.

S.N	**Parameters**	**Effect of parameter on microbial degradation**
1.	Oxygen	• Higher reductive enzyme activity results in better degradation under strictly anaerobic conditions (Cervantes and Dos Santos, 2011). • The oxidative enzymes responsible for decolourizing azo dyes require a small amount of oxygen. • Oxygen is necessary for the breakdown of the simple aromatic compounds formed during the reduction reaction of azo dyes (Parshetti et al., 2010; Jain et al., 2012) • After such an aerobic treatment, use an anaerobic treatment (Saratale et al., 2009; Olukanni et al., 2010).
2.	Carbon and nitrogen source	• Bacterial degradation of dyes observed after addition of extra carbon or nitrogen sources. • Azo dye degradation usually requires different carbon sources with concentrations of 1 g/L and organic nitrogen sources (0.5 g/L) (Ponraj et al., 2011; Garg et al., 2012; Shah et al., 2013) • Starch, fructose, and glucose are the best co-substrates. • Efficient degradation is observed for yeast extract.
3.	pH	• The pH range between 3.0 and 10.0 is optimal for degradation (Ayed et al., 2011).

		• The degradation rate was better at a pH of 7 (Anjaneya et al., 2011). • Under strongly acidic or strongly alkaline pH, the degradation rate declines quickly (Ayed et al., 2011).
4.	Temperature	• Up to the optimum temperature of 37° C, azo dye degradation increases by 50–70%. • At temperatures above or equal to 42°C, there is a slight loss of cell activity in a degradation-related enzyme, resulting in a drop in degradation activity between 80 and 90%. • For some complete microbial cell preparations (Dermacoccus abyssi MT1.1^{T} strain), the azo reductase enzyme can remain active for an hour at temperatures as high as 60 °C (Lang et al., 2014).
5.	Dye concentration	• Bacterial cultures show sound dye degradation for concentrations between 50 and 400 ppm. • Availability to the toxicity of specific dyes on bacteria or an insufficient ratio of biomass to dye concentration, higher dye concentration slowly reduces the efficiency of degradation (Holkar et al., 2014; Phugare et al., 2011). • The high dye concentrations, reactive azo dyes with sulfonic acid (SO_3H) groups prevent the growth of microorganisms (Kalyani et al., 2009)

6.	Redox mediator	• The reduced pace of lowering equivalents transfer (anaerobic reduction process) between an initial electron donor and a final electron receiver. • The reducing equivalents are transported to the terminal electron acceptor and are enhanced by redox mediators at concentrations between 0.005-0.02 mM (Sun et al., 2013).
7.	Dye structure	• The degradation rate is higher for simpler dyes like ethyl violet, crystal violet, and malachite green, which have < 500 g/mol molecular weights. • There is a lower degradation rate for dyes with electron-extracting groups in the para position of the aromatic, compared to the azo bond[for dyes with a molecular more than 500 g/mol. (Lade et al., 2012; Holkar et al., 2014). • Diazo and triazo dyes degrade more quickly than monoazo dyes do. (Garcia-Segura et al., 2011)