Sustainable Biodegradation of Azo Dyes: Insights into Biochar-Based Packed-Bed Bioreactors

Rakesh Kumar Gurjar[1], Kirti Mohan Sharma[2♠]
[1]Department of Environmental Science, Career Point University, Kota-324005
[2]Department of Chemistry, Career Point University, Kota-324005

Abstract—**The extensive use of azo dyes in textile and allied industries has resulted in the generation of large volumes of recalcitrant and toxic wastewater, posing serious environmental and health challenges. Conventional physicochemical treatment methods, although effective to some extent, are often associated with high operational costs, sludge generation, and incomplete mineralization of pollutants. In recent years, biological approaches have gained significant attention due to their eco-friendly, cost-effective, and sustainable nature. Among these, biochar-based packed-bed bioreactors have emerged as a promising hybrid technology integrating adsorption and biodegradation mechanisms for efficient dye removal.**

This review provides a comprehensive overview of azo dye characteristics, classification, environmental impacts, and conventional treatment technologies. It further emphasizes microbial degradation pathways under aerobic, anaerobic, and combined conditions, highlighting the role of bacteria, fungi, and microbial consortia. Special focus is given to biochar-supported packed-bed bioreactor systems, discussing their design, operational parameters, and advantages in enhancing microbial activity and dye removal efficiency. The synergistic interaction between biochar adsorption capacity and microbial degradation significantly improves decolorization and mineralization rates.

Finally, current challenges such as process optimization, scale-up limitations, and stability issues are discussed along with future perspectives. The integration of advanced materials with biotechnological approaches offers a sustainable pathway for the effective treatment of dye-contaminated wastewater.

Index Terms—**Azo dyes, Biodegradation, Biochar, Packed-bed bioreactor, Wastewater treatment**

I. INTRODUCTION

The rapid growth of industrialization, particularly in the textile, paper, leather, and dye manufacturing sectors, has led to the extensive discharge of synthetic dyes into aquatic environments. Among these, azo dyes constitute the largest and most widely used class, accounting for nearly 60–70% of total dye production worldwide. These dyes are characterized by the presence of one or more azo bonds (–N=N–) linking aromatic structures, which impart vivid colors and high chemical stability. However, the same structural stability that makes azo dyes industrially valuable also renders them resistant to natural degradation, leading to their persistence in the environment. The presence of dye pollutants in water bodies poses serious ecological and human health concerns. Even at low concentrations, dyes significantly reduce light penetration, thereby affecting photosynthetic activity in aquatic ecosystems. Furthermore, many azo dyes and their degradation intermediates, particularly aromatic amines, are known to exhibit toxic, mutagenic, and carcinogenic properties. Consequently, the treatment of dye-containing wastewater has become a critical environmental challenge.

Conventional physicochemical treatment methods such as coagulation–flocculation, adsorption, membrane filtration, and advanced oxidation processes have been widely employed for dye removal. Although these methods can achieve rapid decolorization, they are often associated with significant drawbacks including high operational costs, generation of secondary pollutants, incomplete mineralization, and complex sludge disposal issues.

♠ Corresponding author email:

These limitations have driven the search for more sustainable and eco-friendly alternatives.

Biological treatment methods have emerged as promising solutions due to their cost-effectiveness, environmental compatibility, and ability to achieve complete mineralization of dye molecules. Various microorganisms, including bacteria, fungi, algae, and microbial consortia, have demonstrated the capability to degrade azo dyes through enzymatic processes. The biodegradation mechanism typically involves reductive cleavage of azo bonds under anaerobic conditions, followed by further oxidation of aromatic amines under aerobic conditions. Despite these advantages, conventional biological systems often suffer from limitations such as low degradation rates, sensitivity to toxic shocks, and poor operational stability.

In recent years, the integration of advanced materials with biological systems has opened new avenues for enhancing dye removal efficiency. Biochar, a carbon-rich material derived from the pyrolysis of biomass, has gained considerable attention due to its high surface area, porous structure, and abundant functional groups. These properties make biochar an excellent adsorbent as well as a support material for microbial immobilization. When employed in packed-bed bioreactor systems, biochar facilitates a synergistic interaction between adsorption and biodegradation processes, significantly improving treatment performance. Biochar-based packed-bed bioreactors offer several advantages, including high biomass retention, enhanced microbial activity, reduced sludge production, and the ability to operate under continuous flow conditions. The immobilization of microorganisms on biochar surfaces not only protects them from toxic effects but also promotes efficient substrate utilization and degradation kinetics. As a result, these hybrid systems have emerged as a promising and sustainable approach for the treatment of dye-contaminated wastewater.

This review aims to provide a comprehensive overview of azo dye pollution, their classification, environmental impacts, and conventional treatment methods. It further focuses on microbial degradation mechanisms and the recent advancements in biochar-based packed-bed bioreactor technologies. Emphasis is placed on understanding the synergistic interactions between biochar and microbial communities, key operational parameters, and future prospects for large-scale applications.

1.1 Classification of Dyes

Dyes are complex organic compounds capable of imparting color to substrates by absorbing light in the visible region (400–700 nm) of the electromagnetic spectrum. This property arises due to the presence of chromophores, which are responsible for color, and auxochromes, which enhance the intensity and binding of dyes to substrates. The classification of dyes is fundamental to understanding their chemical behavior, industrial applications, and environmental impact. Dyes are broadly categorized based on their chemical structure, method of application, and origin, each classification offering critical insight into their properties and reactivity [1,2]. The energy of the absorbed radiation is given by:

$$\Delta E = E_1 - E_0 = h\nu = hc/\lambda$$

Colours contain chromophores, delocalized electron frameworks with conjugated twofold bonds, and auxochromes, electron-pulling back or electron giving substituent that cause or heighten the shade of the chromophore by adjusting the general vitality of the electron framework. Common chromophores are - C=C-, - C=N-, - C=O, - N=N-, - NO_2 and quinoid rings, regular auxochromes are - NH_3, - COOH, - SO_3H and - OH. In view of combination structure or chromophore, 20-30 unique gatherings of colors can be observed.

1.1.1 Classification Based on Chemical Structure

From a chemical perspective, dyes are primarily classified according to the nature of their chromophoric groups and molecular frameworks. Among these, azo dyes constitute the largest and most significant class, accounting for a major proportion of global dye production. Azo dyes are characterized by the presence of one or more azo linkages (–N=N–) connecting aromatic rings, which confer strong coloration and structural stability. The versatility of azo dyes allows them to produce a wide spectrum of colors, ranging from yellow and orange to deep red and blue shades. However, their stability also makes them resistant to biodegradation, and their breakdown products, particularly aromatic amines, are often toxic and carcinogenic, raising serious environmental concerns.

Another important class is anthraquinone dyes, which are based on the anthraquinone structure and are known for their excellent light fastness and chemical stability. These dyes typically produce blue, green, and violet shades and are widely used in textile applications where durability is essential. Triphenylmethane dyes represent another group, consisting of three aromatic rings attached to a central carbon atom. These dyes are known for their brilliant colors and are commonly used in paper, textile, and ink industries. However, they are generally less stable compared to azo and anthraquinone dyes. Xanthene dyes form a distinct category characterized by their fluorescent properties, making them useful in applications such as biological staining and laser technologies. Examples include fluorescein and rhodamine derivatives. Nitro and nitroso dyes, which contain nitro (–NO_2) or nitroso (–NO) groups, are less commonly used due to their relatively poor stability and higher toxicity. Phthalocyanine dyes, on the other hand, are metal-complex dyes known for their intense blue and green colors and exceptional thermal and chemical stability. These dyes are widely used in high-performance applications such as printing inks and coatings.

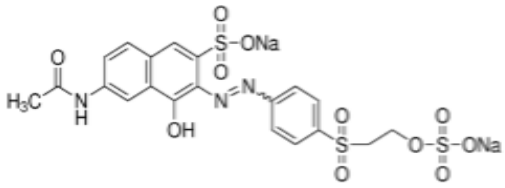
1.1.2 Classification Based on Application Method

In addition to structural classification, dyes are also categorized based on their method of application to different substrates. This classification is particularly important in industrial processes, as it determines how the dye interacts with the fiber or material being colored. Reactive dyes, for instance, form covalent bonds with functional groups present in fibers such as cellulose, wool, and silk. This strong chemical bonding results in excellent wash fastness and color durability. Direct dyes, in contrast, do not form covalent bonds but are absorbed onto the fiber surface through hydrogen bonding and van der Waals forces. These dyes are relatively easy to apply but exhibit moderate fastness properties.

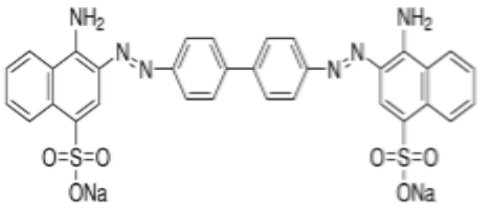
Acid dyes are typically applied in acidic conditions and are mainly used for protein fibers such as wool and silk, as well as for nylon. These dyes are anionic in nature and bind to positively charged sites on the fiber. Basic dyes, also known as cationic dyes, carry a positive charge and are primarily used for dyeing acrylic fibers and paper. Disperse dyes are non-ionic and are used for hydrophobic synthetic fibers such as polyester. These dyes are applied as fine dispersions in water and diffuse into the fiber at elevated temperatures. Vat dyes represent another important class, characterized by their insolubility in water. These dyes are chemically reduced to a soluble form during the dyeing process and subsequently oxidized back to their original insoluble form within the fiber, resulting in excellent color fastness. Sulfur dyes are widely used for cotton and are known for producing dark shades at low cost, although they may have environmental drawbacks due to sulfur-containing byproducts. Mordant dyes require the use of a metal salt, known as a mordant, to fix the dye onto the fiber, forming a coordination complex that enhances color stability.

1.1.3 Classification Based on Origin

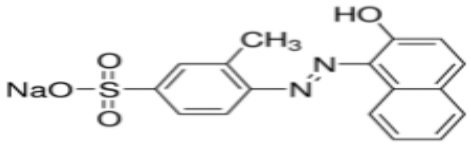
Dyes can also be classified based on their origin into natural and synthetic dyes. Natural dyes are derived from plant, animal, or mineral sources and have been used since ancient times. Examples include indigo, extracted from plant sources, and cochineal, obtained from insects. While natural dyes are generally biodegradable and environmentally friendly, they often suffer from limitations such as poor color fastness, limited shade range, and variability in quality. Synthetic dyes, which are produced through chemical synthesis, have largely replaced natural dyes in modern industry due to their superior performance characteristics. They offer a wide range of colors, high stability, and consistent quality, making them suitable for large-scale industrial applications.



Reactive Orange 16 (RO 16)



Congo Red



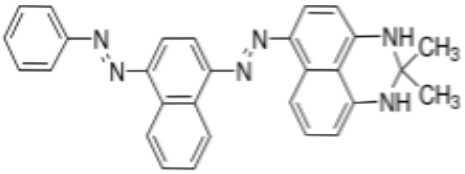
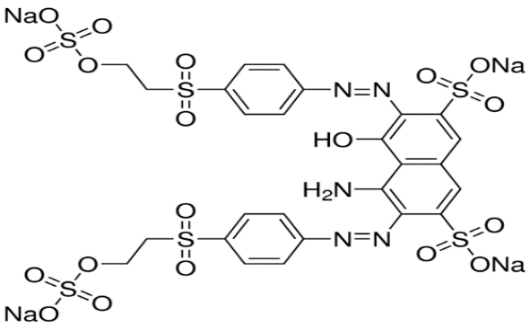
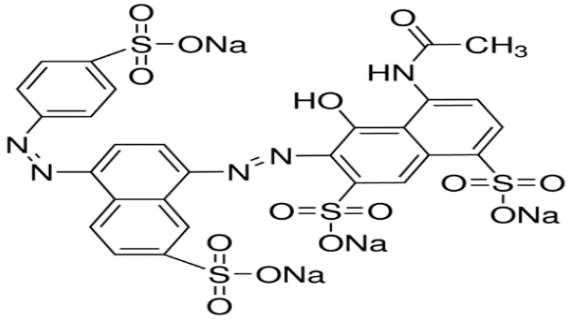


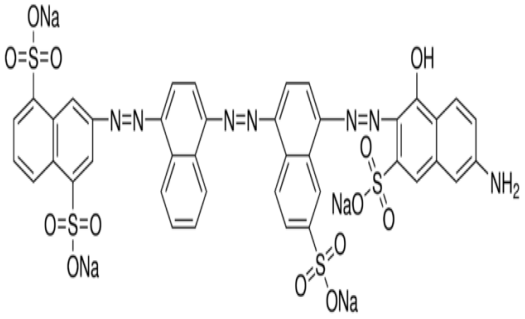
Figure 1: The chemical structure of most frequently studies azo dyes



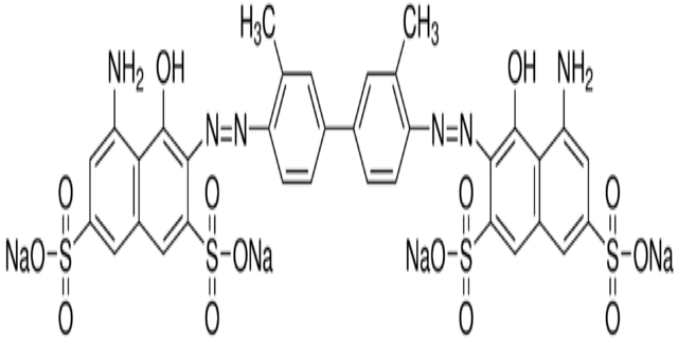
Reactive black 5



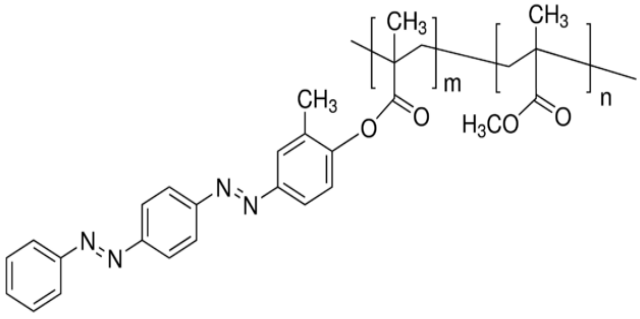
Brilliant Black BN



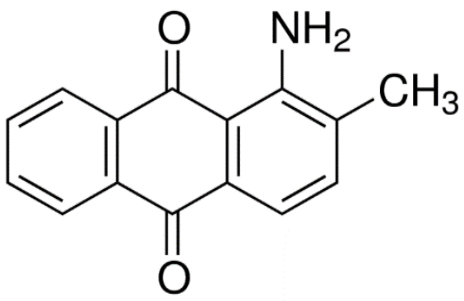
Direct Blue 71



Trypan Blue

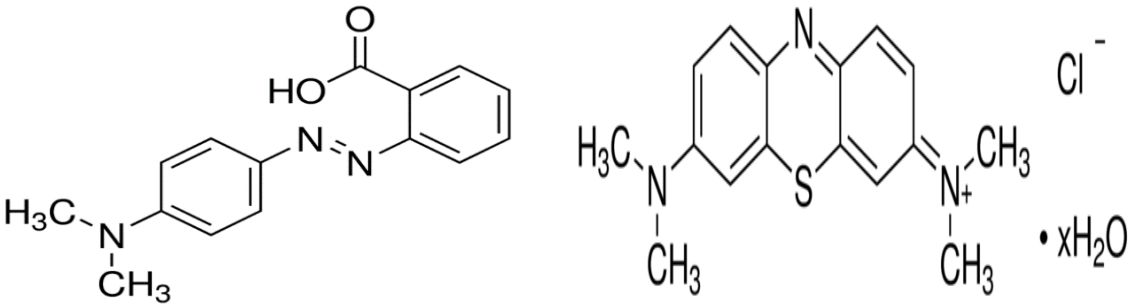


Disperse Yellow 7



Disperse Orange 11

Figure 2: The chemical structure of most frequently studies azo dyes



Methylene blue Methyl Red

Figure 3: The chemical structure of most frequently studies Synthetic dyes

Table 1: Relationship between light absorption and colour (Solomons et.al., 1996)

Colour absorbed	Colour obserbed	Absorbed radiation(nm)
Violet	Yellow-green	350-435
Blue	Yellow	435-480
Green-Blue	Orange	480-490
Blue-Green	Red	490-500
Green	Purple	500-560
Yellow-Green	Violet	560-580
Yellow	Blue	580-595
Orange	Green-Blue	595-605
Red	Blue-Green	605-750

Table 2: Effect of Azo Dyes

Name of the Dye	Effects	References
Reactive brilliant red	Inhibits function of human serum albumin	Li et al., 2010
Acid Violet 7	Induce chromosomal aberration, lipid peroxide, acetyl cholinesterase in mice	Ben Mansour Et al., 2010
Disperse Red- 1 & Disperse Orange -1	Increase the frequency of micronuclei in human lymphocytes	Chequer et al., 2009
Reactive Black 5	Decrease urease activity, arginine ammonification rate in terrestrial ecosystems	Topac et al., 2009
Disperse Blue 291	Mutagenic, Cytotoxic, genotypic effects,	Tsuboy et al., 2007
Direct Black 38	formation of micronuclei, DNA fragmentation in human hepatoma cells Urinary Bladder cancer in human	Meal et al., 1981 & Cerniglia et al., 1986
Direct Black 15	Mutagenic	Reid et al., 1984

Table 3: Applications and classes of dyes and their chemical type (after Kirk-Othmer,11 copyright# John Wiley & Sons Inc, 1993, reprinted by permission of John Wiley & Sons Inc

Class	Substrates	Method of application	Chemical type
Acid	Nylon, wool, silk, paper, inks, and leather	Usually from neutral to acidic dyebath	Az including premetallized anthraquinone, triphenylmethane, azine, xanthene, nitro, and nitro's
Azoic components & compositions	Cotton, rayon, cellulose acetate, and polyesterb	Fibre impregnated with coupling component and treated with a solution of stabilised	Azo

		diazonium salt	
Basic	Paper, polyacrylonitrile modified nylon,c polyester, and inks	Applied from acidic dyebaths	Diazacarbocyanine, cyanine, hemicyanine,diazahemicyanine, diphenylmethane,triarylmethane, azo, azine, xanthene, acridine, oxazine, and anthraquinone
Disperse	Polyester, polyamide, acetate, acrylic, and plastics	Fine aqueous dispersions often applied by high temperature pressure or lower temperature carrier methods; dye may be padded on cloth and baked on or thermofield	Azo, anthraquinone, styryl, nitro, and benzodifuranone
Direct	Cotton, rayon,a paper, leather, and nylo	Applied from neutral or slightly alkaline baths containing additional electrolyte	Azo, phthalocyanine, stilbene, and oxazine
Fluorescent frighteners	Soaps and detergents, all fibres, oils, paints, and plastics	From solution, dispersion, or suspension in a mass	Stilbene, pyrazoles, coumarin, and naphthylamides
Mordan	Wool, leather, and anodised aluminium	Applied in conjunction with chelating Cr salts	Azo and anthraquinone
Natural	Food	Applied as mordant, vat, solvent, or direct and acid dyes	Anthraquinone, avonols, avones, indigoids, chroman
Oxidation bases	Hair, fur, and cotton	Aromatic amines and phenols oxidised on the substrate	Aniline black and indeterminate structures
Pigments	Paints, inks, plastics, and textile	Printing on the fibre with resin binder or dispersion in the mass	Azo, basic, phthalocyanine, quinacridone, and indigoid
Reactive	Cotton, wool, silk, and nylon	Reactive site on dye reacts with	Azo, anthraquinone, phthalocyanine, formazan, oxazine, and basi

		functional group on fibre to bind dye covalently under influence of heat and pH (alkaline)	
Solvent	Plastics, gasoline, varnish, lacquer, stains, inks, fats, oils, and waxes	Dissolution in the substrate	Azo,triphenylmethane, anthraquinone, and phthalocyanine
Sulfur	Cotton and rayon	Aromatic substrate vatted with sodium sul®de and re-oxidised to insoluble sulfur-containing products on fibre	Indeterminate structures
Vat	Cotton, rayon,a and wool	Water-insoluble dyes solubilised by reducing with sodium hydrosulfite, then exhausted and re-oxidised	Anthraquinone (including polycyclic quinones), and indigoids

1.2 Reactive dyes

Initially showed up industrially in 1956 and were utilized to color cellulose filaments. The colors contain a receptive gathering that, when connected to a fiber in a feebly soluble color shower, frame a compound bond with the fiber. These colors are with receptive gatherings that are fit for shaping a covalent bond between carbon iotas of color atom and - OH, - NH, - SH bunches in strands. The responsive gathering is regularly a heterocyclic fragrant ring substituted with a chloride or fluoride molecule, e.g. dichlorotriazine. Another basic responsive gathering is vinyl sulfone. Receptive colors can likewise be utilized to color fleece and nylon, in the later case they are connected under feebly acidic. In the Color Index, the receptive colors frame the second biggest color class. For the most part receptive colors (80%) are azo or metal complex azo mixes yet alsoanthraquinone and phthalocyanine responsive colors are connected, particularly for green and blue shading (O'Neill et al., 1999).

The chromophore of their atoms are fundamentally the same as i.e., either azo, anthraquinone, phthalocyanine chromophore or others. The general formula for the reactive dyes structure is as follows

S------F----T----X

Where S: Solubilising group; F: Chromophore; T: Bridging group; X: Reactive system. Reactive dyes are composed of two portions: a) the chromogen and b) the reactive system (Waring and Hallas, 1990). Reactive dyes are characterized by azo bonds (N=N), and used to dye cellulose fibres. The color of the azo dyes is due to the presence of azo bond with associated chromophores (Moreira et al., 1998.). Reactive dyes are azo compounds that are linked by an azo bridge (Raymound and Dunald, 1984). Reactive dyes are typically azo based chromphores with various reactive groups, some examples for reactive dyes are Procion yellow MX- 5B, Cibacron brilliant red B, Drimaren red Z 2B, reactive black 5, Reactive yellow 2, Procion Blue MX 2G, Remazol red RB, Remazol Golden Yellow RNL, Remazol Blue B, Remazol Turquoise Blue G133, Remazol blue and Remazol orange etc., which shows different reactivity and it has different

absorbance (Table 4). Chemical structure of some dye detail in fig., 1,2,3.

Table 4. Dyes and their maximum absorbance

Dyes	Absorbance $_{max}$ (nm)
Remazol golden Yellow RNL	410
Cibacron Orange CG	489
Cibacron Red C-2G	515
Remazol Red RB	525
Remazol Blue B	590
Remazol Black B	600
Remazol Navy Blue CG	620
Remazol Turquoise Blue G133	660

1.3 Impacts of reactive dyes

Wastewater from the many industries holding various type of reactive dyes sources of serious environment pollution because, the occurrence of dyes in water is greatly observable and affects their transparency and aesthetic even if the concentration of the dyes is low (Hao et al., 2000). Reactive dyes cause respiratory and nasal symptoms (Docker et al., 1987); asthma rhinitis and dermatitis (Nilsson et al., 1993); allergic contact dermatitis (Estlander, 1988; Wilkinson and MCGeachan.,1996), mutagenicity (Mathur et al., 2005; Przybojewska et al., 1989), genotoxicity (Dogan et al., 2005; Przybojewska et al., 1989), carcinogenicity (De- Roos et al., 2005; Gonzales et al ., 1988) and teratogenicity (Birhanli and Ozmen, 2005). Adverse effects have also been detected from aquatic environment (Richardson, 1983; Michaels and Lewis, 1985). Dyes have a very low rate of removal ratio for BOD to COD (less than 0.1) (De and Radrigues, 1987). Therefore, industrial effluents containing dyes should be processed before their discharge into the environment (Wong et al., 2003).

1.4 Conventional methods

1.4.1 Importance of biological treatment relative to physicochemical methods

Dye-house effluent typically contains only 0.6–0.8 g L1 dye, but the pollution it causes is mainly due to durability of the dyes in the wastewater (Jadhav et al., 2007). Therefore it is necessary to search for and develop effective treatments and technologies for the decolorization of dyes in such effluents. Various physical/ chemical methods, such as adsorption, chemical precipitation, photolysis, chemical oxidation and reduction, electrochemical treatment, have been used for the removal of dyes from wastewater (Fig. 1). Moreover, there are many reports on the use of physicochemical methods for the color removal from dyecontaining effluents (dos Santos et al., 2007; Vandevivere et al., 1998; Wang et al., 2009a,b,c)

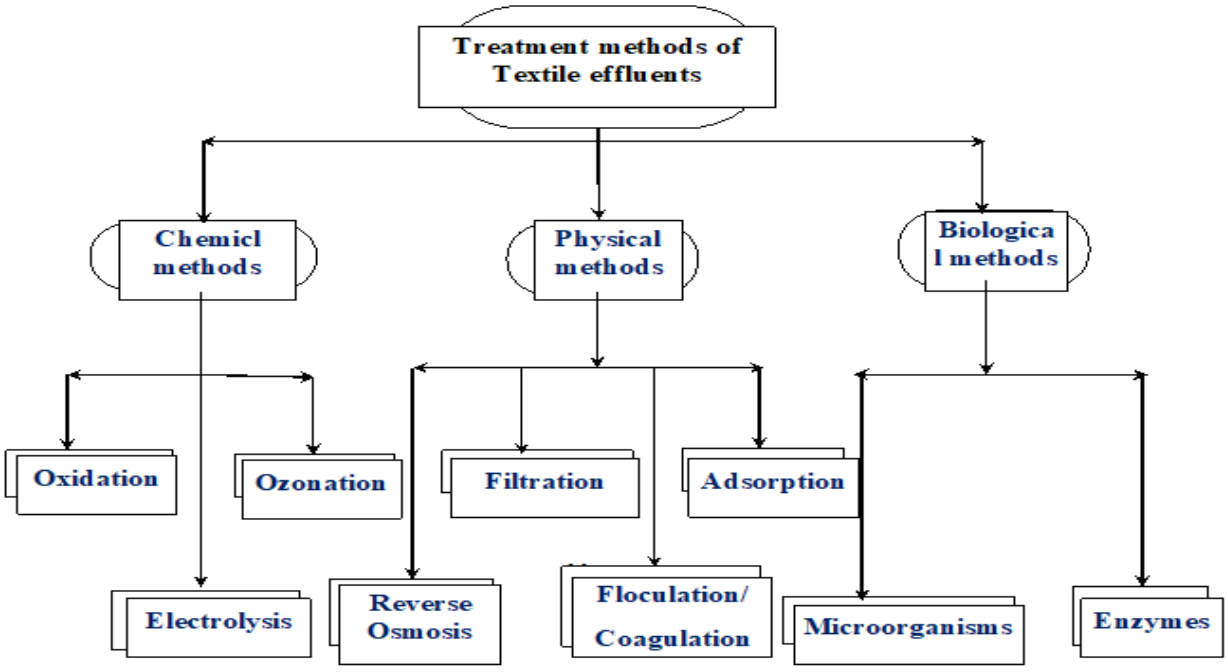


Figure 4. Treatment methods for the removal of dyes from wastewater effluent

1.4.2 Physical and chemical methods

Physical methods based on coagulation–flocculation of dyes are effective for the removal of mainly sulphur and disperse dyes, but show very low coagulation–flocculation capacity for acid, direct, reactive and vat dyes. In addition, low colour-removal efficiency and the large amount of sludge produced limit the application of these techniques (Vandevivere et al., 1998). Adsorption methods have attracted considerable interest because they efficiently remove a wide range of dyes. Adsorbent selection is based on characteristics such as high affinity, capacity for target compounds, and the possibility of adsorbent regeneration (Subramaniam et al., 2009). Although activated carbon (AC) is a very effective adsorbent for various types of dyes, it is not often used due to its high cost (Robinson et al., 2001). To make the process more economically feasible, some investigators use low cost adsorbent materials like peat, bentonite clay, fly ash, polymeric resins, ion exchangers and many biological materials such as, corn/maize cobs, maize stalks, and wheat straw for the color removal of dye wastewater (Ramakrishna and Viraraghavan, 1997).

However, the practical application of these adsorbents has been limited by problems associated with their regeneration or disposal, high sludge production, low effectiveness with regard to wide range of dyes and high cost (Anjaneyulu et al., 2005; Karcher et al., 2001). Filtration methods such as ultrafiltration, nanofiltration and reverse osmosis have been used for water reuse and chemical recovery. In the textile industry, the use of membranes provides interesting possibilities for the separation of hydrolysed dyestuffs and dyeing auxiliaries that simultaneously reduce the color, BOD and COD of wastewater, and they have also been found useful for mercerizing and bleaching it. With this approach, the selection of the type and porosity of the filter depends upon the chemical composition of the wastewater and the specific temperature required for the process (dos Santos et al., 2007).

However, membranes have some significant drawbacks, including high investment costs, potential membrane fouling and the production of secondary waste streams which need further treatment (dos Santos et al., 2007; Robinson et al., 2001). Chemical oxidation methods enable the destruction or decomposition of dye molecules, and such approaches use various oxidizing agents, such as ozone (O_3), hydrogen peroxide (H_2O_2) and permanganate (MnO_4). Modification in the chemical composition of a compound or a group of compounds takes place in the presence of these oxidizing agents, and thus the dye molecules become susceptible to degradation (Metcalf, 2003). Ozonation has been found to be effective due to its high reactivity with many azo dyes, the lack of alteration of the reaction volume due to its gaseous state, and good color removal efficiencies (Alaton et al., 2002). However its short life time, ineffectiveness towards dispersed dyes and those insoluble in water, low COD removal capacity, as well as the high cost of ozone, limits the practical application of this technique (Anjaneyulu et al., 2005). In advanced oxidation processes (AOP) (photochemical and photocatalytic), oxidizing agents such as O_3 and H_2O_2 or heterogeneous photocatalysts are used with catalysts, such as TiO_2, ZnO_2, Mn and Fe, in the presence or absence of an irradiation source which generates (OH) radicals for the destruction of hazardous dye pollutants (Anjaneyulu et al., 2005; Forgacs et al., 2004; Vandevivere et al., 1998; Wang et al., 2009a,b,c). The Fenton reaction method is relatively cheap and also has high COD removal and decolorization efficiencies for both soluble as well as insoluble dyes, but the high sludge generation associated with this approach also limits its use (Robinson et al., 2001). The H_2O_2/UV process is the most effective AOP technology, mainly because of high color removal (up to 95%), lack of sludge formation and high COD removal in a short retention time (Alaton et al., Table 5.).

Table 5: Merits and Demerits of Conventional Treatment Methods

Physical and Chemical treatment Methods	Merits	Demerits
Coagulation/ Flocculation	Simple, economic feasible	Large amount sludge production, handling and disposal problems, more amount chemicals

		required for pH adjustment
Membrane separation	All types of dyes are decolorized	High pressure, expensive, sludge generation incapable for large scale treatment
Ion exchange	Effective with no loss of redevelopment	Renewal is possible economic constraints, not effective for some dyes
Oxidation	Hasty and efficient process	High energy cost, chemical required, secondary metabolite production
Advanced oxidation process	No sludge production, little consumption of chemicals, efficiency for recalcitrant dyes	Economically impracticable
Adsorption on activated carbon	Effective	Expensive loss of absorbent
Fentons reagent	Proficient decolonization for both soluble and insoluble dyes	Sludge formation
Photochemical	No sludge production	Formation of Secondary metabolic pollutants
Irradiation	Effective oxidation at lab scale	Requires a lot of dissolved O_2

1.4.3 Biological methods

The ability of biological treatment process for decolorization of industrial effluents is ambiguous, different and divergent. Observations indicate that dyes themselves are not biologically degradable since microorganisms do not utilize the color constituents as a source of food. Furthermost presently used laboratory approaches for biodegradation contain aerobic microbes, which apply molecular oxygen as sinking comparable acceptor during the inhalation process. Yet, environmental circumstances with lack of molecular oxygen are not unusual.

Bioremediation, or the utilization of microbial methods to manage contamination, is a key research region in the ecological sciences. In such methodologies microorganisms adjust themselves to the lethal squanders and new safe strains grow actually, which then change different dangerous chemicals into less destructive structures. The instrument behind the biodegradation of obstinate mixes in the microbial framework depends on the activity of the biotransformation catalysts (Saratale et al., 2007a). A few reports exhibit the exploitation of complex natural substances which can be achieved by enzymatic instruments, for example, those related with laccase (Gopinath et al., 2001), lignin peroxidase (Duran and Esposito, 2000), NADH-DCIP reductase (Bhosale et al., 2006), tyrosinase (Zhang and Flurkey, 1997), hexane oxidase (Saratale et al., 2007b) and aminopyrine N-demethylase (Salokhe and Govindwar, 2003). Various biotechnological approaches have pulled in enthusiasm concerning handling azo color contamination in an ecoefficient way, for the most part with the utilization of microorganisms and frequently in mix with physicochemical procedures. Azo colors are xenobiotic in nature and refractory to biodegradation, and the utilization of microbial or enzymatic treatment strategy for the entire decolorization and debasement of such colors from material gushing has the accompanying favorable circumstances: (1) being earth inviting, (2) being cost-aggressive, (3) creating less emission, (4) yielding finished results that are non-dangerous or have finish mineralization; what's more, (5) requiring less water utilization contrasted with physicochemical strategies (Banat et al., 1996; Rai et.al.,2005). The adequacy of microbial decolorization relies on upon the versatility and the movement of the chose microorganisms. Thus, an extensive number of physical groups has been tried for the decolorization and mineralization of different colors as of late (Pandey et al., 2007). The separation of strong species and along these lines their corruption is one of the intriguing natural parts of profluent treatment (Chen et al., 2008). A wide assortment of microorganisms are equipped for decolorizing of an

extensive variety of colors including; microbes (Dawkar et al., 2008; Jadhav et al., 2007; Kalyani et al., 2008; Saratale et al., 2009b; Telke et al., 2008), organisms (Humnabadkar et al., 2008; Saratale et al., 2006), yeasts (Jadhav et al., 2007; Lucas et al., 2006; Saratale et al.,2009a), actinomycetes (Machado et al., 2006), green growing (Acuner and Dilek, 2004; Gupta et al., 2006; Parikh and Madamwar, 2005; Yan and Pan, 2004) and plants (phytoremediation) (Ghodake et al., 2009a; Kagalkar et al., 2009). Also, these are even able to do totally mineralizing numerous azo colors under certain ecological conditions.

1.4.4 Bacterial decolorization and degradation of azo dyes

For the most part the decolorization of azo colors happens under ordinary anaerobic, facultative anaerobic and oxygen consuming conditions by various gatherings of microscopic organisms. The instrument of microbial corruption of azo colors includes the reductive cleavage of azo bonds (R_1–N=N–R_2) with the assistance of azoreductase compounds under anaerobic conditions that brought about the arrangement of dreary arrangements containing conceivably risky fragrant amines (Chang and Kuo, 2000; Van der Zee and Villaverde, 2005). The coming about moderate metabolites (e.g., sweet-smelling amines) are additionally corrupted vigorously or anaerobically (Joshi et al., 2008). Numerous current reviews concentrate on the usage of microbial biocatalysts to expel color from the emanating (Chang et al., 2004; Hu, 2001). Broad reviews have been completed to decide the part of the differing gatherings of microbes in the decolorization of azo colors (Pandey et al., 2007). The bacterial decolorization and debasement of these colors has been of impressive enthusiasm since it can accomplish a higher level of biodegradation and mineralization, is appropriate to a wide assortment of azo colors, is reasonable and ecologically benevolent, and creates less slop (Khehra et al., 2006; Rai et al., 2005; Saratale et al., 2009c; Verma and Madamwar, 2003)

1.4.5 Using pure bacterial culture for Dye degradation

The effluents from the material industry are mind boggling, containing a wide assortment of engineered colors what's more, different items, for example, dispersants, acids, bases, salts, cleansers, humectants, oxidants, Release of these huge effluents into streams and lakes brings about a diminished disintegrated oxygen fixation, along these lines making anoxic conditions that are deadly to occupant living beings. Natural processes provide a contrasting option to existing innovations since they are more cost-effective, environmentally friendly, and don't produce huge amounts of sludge. Additionally, bacterial decolourisation is typically faster than chemical methods for decolourising and mineralising azo dyes. Mixed communities are especially valuable here, as microbial consortia can, by and large, perform biodegradation tasks that no single pure strain can carry out effectively (Nigam et al., 1996). In any case, blended societies just give a normal, naturally visible perspective of what is going on in the framework, and the results are not easily replicated, making exhaustive, powerful elucidation of the outcomes very troublesome. Endeavors to segregate immaculate bacterial societies equipped for corrupting azo colors begun in the 1970s with reports of a Bacillus subtilis, Aeromonas hydrophila and in a Bacillus cereus (Wuhrmann et al., 1980). As of late a generous measure of research regarding the matter of shading expulsion has been completed utilizing single bacterial societies like Proteus mirabilis, Pseudomonas luteola, and Pseudomonas sp., and secluded Pseudomonas sp. SUK1 has indicated exceptionally encouraging outcomes for the azo color debasement under anoxic conditions (Chang et al., 2001b; Chen et al., 1999; Kalyani et al., 2008; Yu et al., 2001). What's more, there are additionally a few reviews portraying the decolourization of responsive azo colours mediated by immaculate bacterial culture and the outcomes are summarised. Pseudomonas aeruginosa decolorized a business tannery and material color, Reactive dyes, in the imminence of glucose under vigorous conditions (Nachiyar and Rajkumar, 2005 It additionally getting to be noticeably less demanding to decide the nitty gritty systems of biodegradation utilizing the devices of organic chemistry and sub-atomic science, and this data could be helpful to direct the catalyst framework with a specific end goal to deliver changed strains with improved compound exercises. The quantitative investigation of the energy of azo color decolorization by a specific bacterial culture has been contemplated in detail (Chang and Kuo, 2000; Telke et al., 2008). In our research facility, the metabolic pathway of specific colors utilizing unadulterated culture was resolved utilizing oxidoreductive enzymatic reviews and

utilizing different explanatory procedures, for example, UV–vis spectroscopy, fourier change infrared spectroscopy (FTIR), gas chromatography mass spectrometry (GC/MS), high execution fluid chromatography (HPLC) atomic absorption spectroscopy (NMR), et cetera.

1.4.6 Using mixed bacterial cultures

Bacterial decolorization is capable, but individual bacterial strains generally can't degrade azo colors totally, and the middle of the way items are regularly cancer-causing fragrant amines, which should be additionally disintegrated (Joshi et al., 2008). In this way, treatment contexts made out of mixed microbial populaces accomplish a higher degree of biodegradation and mineralization due to the synergistic metabolic exercises of the microbial group, and have impressive points of interest over the utilization of neat societies in the exploitation of caused azo colors (Chen and Chang, 2007; Khehra et al., 2005; Saratale et al., 2010b). In a microbial consortium, the singular strains may assault the color particle at various positions or may use metabolites delivered by the existing together strains for further deterioration (Chang et al., 2004; Forgacs et al., 2004; Jadhav et al., 2008b; Saratale et al., 2009b).

Large, amid azo color exploitation starting cleavage of the azo bonds happens, which comes about into the creation of fragrant amines which are lethal in nature, however within the sight of microbial consortium, these sweet-smelling amines get debased by correlative creatures, making the procedure more powerful and productive (Moosvi et al., 2005, 2007; Tony et al., 2009a,b). Likewise, it is deadly besides, hard to separate a solitary bacterial strain from color containing wastewater tests, long drag adjustment strategies are then required for powerful decolorization and exploitation of azo colors. A few reviews concerning the biodegradation of hued wastewater utilizing co-societies and blended bacterial societies are given in Tables 6 and 7,

1.5 Mechanisms of microbial colour removal

Individually Components of microbial covering removal Azo mixes are helpless to organic degradation under both aerobic and anaerobic conditions (Khehra et al., 2005). In general microbial debasement of azo colors includes the reductive cleavage of azo bonds (R_1–N=N–R_2) with the support of an azoreductase chemical under anaerobic conditions, and this includes an exchange of four-electrons (decreasing counterparts). This then continues through two phases at the azo linkage, and in each stage two electrons are exchanged to the azo colour, which acts as the final electron acceptor, bringing about decolourization and the formation of colourless compounds (Chang et al., 2000).

The subsequent intermediate metabolites (e.g., fragrant amines) are then further degraded aerobically or anaerobically (Chang et al., 2004; Pandey et al., 2007). Decolorization of azo colors under anaerobic conditions is thought to be a moderately straightforward and non-particular process. The viability of different anaerobic treatment applications for the exploitation of a wide assortment of engineered colors has been displayed commonly (Wang et al., 2009a,b,c). Under anaerobic conditions, a low redox potential is achieved, which enables the decolourisation of azo dyes (Bromley-Challenor et al., 2004).

A few agents recommend that the nearness of oxygen more often than not restrains the azo bond diminishment movement, since oxygen consuming breath may rule usage of NADH (Nicotinamide adenine dinucleotide $C_{21}H_{27}N_7O_{14}P_2$), along these lines hindering the electron exchange from NADH (two nucleotides joined through their phosphate groups) to azo bonds (Chang et al., 2001b). It was equally reported that anaerobic azo colour decolourization is a random procedure, where azo colour may act as an electron acceptor provided by the transporters of the electron transport chain (Bromley-Challenor et al., 2004; Stolz, 2001). On the other hand, decolourization may be attributed to nonspecific extracellular responses happening between diminished mixtures created by the anaerobic biomass. (Van der Zee et al., 2001). Anaerobic decolourisation of different azo colours has been accomplished with mixed oxygen-consuming and facultative anaerobic microbial consortia (Moosvi et al., 2005; Nigam et al., 1996), and although large portions of these societies can develop vigorously, decolourisation occurs only under anaerobic conditions.

A significant part of the exploratory work including the anaerobic decolorization of colors (overwhelmingly azo colors) was led utilizing mono societies. Types of Bacillus, Pseudomonas, Aeromonas, Proteus, Micrococcus and purple non-

sulfur photosynthetic microorganisms were observed to be compelling in the anaerobic exploitation of various azo colors It was likewise revealed that in anaerobic conditions the saturation of the azo colors through natural film into the microbial cells goes about as the key rate-constraining element for the decolorization (Kodam et al., 2005). A few bacterial strains that can vigorously decolorize azo colors have been disconnected within the previous couple of years. Under oxygen consuming conditions mono-and di-oxygenase proteins catalyze the consolidation of oxygen from O_2 into the sweet-smelling ring of natural mixes before ring parting (Sarayu and Sandhya, 2010). This sort of azoreductase movement was found in Pseudomonas species strains K22 and KF46, and after cleansing also, portrayal it was watched that this compound framework was sans flavin (Zimmermann et al., 1982, 1984). These microscopic organisms can't use azo color as the development substrate, and require extra natural carbon sources (Stolz, 2001). Besides, there are few microscopic organisms that can develop on azo mixes as the sole carbon source. These microscopic organisms cut R_1–N=N– R_2bonds reductively and use amines as the wellspring of carbon and vitality for their development. Examples ofbacterial strains with this characteristic are Xenophilus azovorans KF 46 (beforehand Pseudomonas sp. KF46) and Pigmentiphaga kullae K24 (already Pseudomonas sp. K24), which can develop vigorously on carboxy orange I and carboxy orange II, individually (McMullan et al., 2001). Just couple of microscopic organisms with specific azo color diminishing compounds have been found to debase azo colors under completely high-impact conditions (Nachiyar and Rajkumar, 2003; Zimmerman et al., 1984)

1.6 Microbial degradation of reactive dyes

Effluents from the textile industries contain reactive dye in a concentration range of five 5 mg/L-1500mg/L. Processing of dye contaminated effluents is currently a primary environmental problem (Liu et al., 2009). Conventional treatment methods such as activated sludge process, chemical coagulation, carbon absorption, chemical oxidation, photo decomposition, electro- chemical treatment, reverse osmosis, hydrogen peroxide catalysis etc are difficult, ineffective (or) economic disadvantage (Gong et al., 2007; Marrakchi et al., 2008). Some of these techniques are even effective, although they have some shortcomings, they are: excess amount of chemical usage with obvious disposal problem; costly plant requirements (or) operating expenses; lack of effective color reduction; particularly for sulfonated azo dyes; sensitivity to a waste water input. Techniques by chemical oxidation using sodium hypochlorite to remove the color release a lot of aromatic amines which are carcinogenic or toxic compounds (Lorencoet al., 2000). Physical and chemical methods are effective for color removal but need of more energy and chemicals than biological processes and sometimes it causes pollution into solid or liquid side streams and it requires additional treatment or disposal. So, the treatment of dyes attention on some microbes which are to biodegrade and biosorb dye in wastewater. Number of microbes holds dye decolorizing ability like bacteria, fungi and yeast etc.

1.6.1 Azo colour decolourization under aerobic conditions

A few bacterial strains that can vigorously decolorize azo colors have been detached amid the previous couple of years. A hefty portion of these strains require natural carbon sources, as they can't use color as the development substrate (Stolz, 2001). Pseudomonas aeruginosa decolorized a business tannery and material color, Navitan Fast blue S5R, within the sight of glucose under oxygen consuming conditions. This life form was additionally ready to decolorize different other azo colors (Nachiyar et al., 2003). There are just not very many microbes that can develop on azo mixes as the sole carbon source. These microbes divide (R1 –N=N–R2) bonds reductively and use amines as the wellspring of carbon and vitality for their development. Such living beings are particular towards their substrate. Cases of bacterial strains with this quality are Xenophilus azovorans KF 46 (already Pseudomonas sp. KF46) and Pigmentiphaga kullae K24 (already Pseudomonas sp. K24), which can develop vigorously on carboxyorange I and carboxy-orange II, individually (Zimmermann et al., 1982). These living beings, in any case, couldn't develop on fundamentally practically equivalent to sulfonated colors, corrosive orange 20 (Orange I) and corrosive orange 7. Long adjustment of 4-aminobenzenesulfonate (4-ABS) corrupting Hydrogenophaga intermedia strain for development on 4-carboxy-4'- sulfoazobenzene (CSB) as the sole

natural carbon source prompted the seclusion of other strain, which lessened CSB and used the two amine metabolites (Jadav et al., 2007). Sphingomonas sp, strain 1CX, a commit aerobe, can develop on an azo color, corrosive orange 7, as sole carbon, vitality and nitrogen source (Coughlin et al. 1999). This strain corrupted just a single of the part amines (1-amino 2-naphthol) framed amid corrosive orange 7 decolorization and 4-aminobenzene sulfonate (4-ABS) debasement.

1.6.2 Azo colour decolourization under anaerobic conditions

Methanogenesis from complex natural mixes requires the planned cooperation of a wide range of trophic gatherings of microscopic organisms, including acidogenic, acetogenic and methanogenic microbes (Wuhrmann et al., 1980). Color decolorization under these conditions requires a natural carbon/vitality source. Straightforward substrates like glucose, starch, acetic acid derivation, ethanol and more intricate ones, for example, whey and custard, have been utilized for color decolorization under methanogenic conditions (Yoo et al., 2001; Isik and Sponza, 2004; Chinwetkitvanich et al., 2000; Van der Zee and Villaverde, 2005; Willetts et al., 2000; Talarposhti et al., 2001). Broad reviews have been completed to decide the part of the various gatherings of microscopic organisms in the decolorization of azo colors. (Carliell et al., 1996; Razo-Flores et al. 1997) have related the decolorization with methanogens, while contemplates by different agents demonstrated that acidogenic and additionally methanogenic microscopic organisms add to color decolorization (Chinwetkitvanich et al., 2000; Bras et al., 2001). Diminishment under anaerobic conditions gives off an impression of being nonspecific, as shifted gathering of azo mixes are decolorized, in spite of the fact that the rate of decolorization is reliant on the additional natural carbon source, and also the color structure (Bromley-Challenor et al., 2000; Stolz, 2001). Besides, there is no connection between's decolorization rate and sub-atomic weight, demonstrating that decolorization is not a particular procedure and cell penetrability is not imperative for decolorization. In this manner, anaerobic azo color decolorization is an accidental procedure, where color may go about as an acceptor of electrons provided via bearers of the electron transport chain. Then again, decolorization may be credited.

1.6.3 Combined anaerobic-aerobic treatment Degradation of dyes

The essential of (reductive) splitting of the azo linkage in azo color particles before (oxidative) assist corruption, makes a procedure in which anaerobic and oxygen consuming conditions are consolidated the most intelligent idea for the natural evacuation of azo colors (Knackmuss, 1996). The coordinated approach depends on transient detachment of the anaerobic and the high-impact stage, as in sequencing group reactors (SBR) or on the rule that dispersion of oxygen in microbial biofilms is generally restricted to 10-200 µm (Eichlerova et al., 2007) so that anaerobic and high-impact conditions coincide in a solitary domain. The evacuation of shading accomplished in the anaerobic stages was by and large high, generally higher than 70% and in a few cases right around 100%. The response time is an imperative figure the anaerobic expulsion of azo colors, diminishing the water powered maintenance time of the anaerobic stage was found to bring about lower shading evacuation efficiencies and anaerobic azo color lessening is a somewhat moderate response (Salokhe et al., 2003). The biomass focus additionally assumes a part in the anaerobic expulsion of azo colors bringing down the biomass and the strong maintenance time of a sequencing group reactor brought about a lower shading evacuation effectiveness (Lucas et al., 2006). The reviews that focused on azo color decrease prompting the arrangement of sweet-smelling amines. The recuperation rates gone between <1% to just about 100%. This wide range may mostly be clarified by the challenges experienced in investigating these regularly artificially unsteady mixes. The destiny of sweet-smelling amines in the oxygen consuming stage can't be convincingly decided. Fractional or finish evacuation of numerous fragrant amines can be associated from the decline or vanishing with the occasionally unidentified crests in HPLC chromatograms (O'Neill et al., 2000; Stolz et al., 2001; Tan et al., 2005; FitzGerald and Bishop, 1995; Kalyuzhnyi and Sklyar, 2000; Harmer and Bishop, 1992; Jiang and Bishop, 1994) and in addition from the reduction of UV absorbance (Chang et al., 2001). In addition, a vast decline of danger to oxygen consuming bacterial movement was measured

between the gushing of the anaerobic stage and the profluent of the high-impact organize (O'Neill et al., 2000; Sosath et al., 1997).

1.6.4 Review previous works on bio-degradation

Table 6: Biodegradation of dyes in coloured wastewater using pure bacterial cultures: review of studies reported in the literature

Name of the dyes and concentration	Organisms used	Cond. (pH)temp.(^{0}C), agitation)	Time (h)	Decolorization (%)	Reference
Reactive Blue 13; (200 mg/L)	Pseudomonas sp	7.0, 35, static	70	83.2	Lin et al. (2010)
Reactive Green 19 A; (50 mg/L)	Micrococcus glutamicus NCIM 2168	6.8, 37, static	42	100	Saratale et al. (2009c)
Reactive Black 5; (1 g/L	Enterobacter EC3	7.0, 37, anaerobic	36	92.56	Wang et al. (2009a,b,c)
Congo Red; (3 g/L)	Mutant Bacillus sp. ACT2	7.0, 37, static	37-48	12-30	Chen et al. (2009a
ater and oil soluble azo dyes; (6 mg/L) N	Lactobacillus acidophilus and Lactobacillus fermentum	NA, 37, anaerobic	36	86-100	Evangelista-Barreto et al. (2009
Orange II; (0.050 mM	(UCP986)Geobacillus stearothermophilus	5–6, 50, aeration (150	48	100	Hsueh et al. (2009)
		rpm)			
Reactive Red 198, Reactive Black 5, Reactive Red 141, Reactive Blue 171, Reactive Yellow 84 (300 mg/L)	Aeromonas hydrophila	30, agitation (125 rpm), 7.5	12	100	Chen et al. (2009b)
Reactive Red 141; (3.8 g/L)	Aeromonas hydrophila	7.0, 30, 200 rpm, Chemostat pulse technique	18	94	Jin et al. (2009)
Direct Blue 71; (150 mg/L)	Escherichia coli JM109(pGEX-AZR)	9.0, 30, anaerobic	36	96	Dawkar et al. (2009)
Navy Blue 2GL; (50 mg/L)	Bacillus sp. VUS	7.0, 40, static	45	91.52	Wang et al. (2009a,b,c)
Reactive Red 180; (200 mg/L)	Citrobacter sp. CK3A	7.0, 32, anaerobic	48	91.3	Ghodake et al. (2009a)
Direct Brown MR; (50 mg L1)	Acinetobacter calcoaceticus NCIM-2890	7.0, 30, static	24	88	Telke et al. (2009a)
C.I. Reactive Orange 16; (100 mg/L)	Pseudomonas sp. SUK1	7–8, 30, stati	6	96	Kalyani et al. (2008)
Reactive Red 2; (5 g/L)	Pseudomonas	6.2 –	24	94	Sarayu

	aeruginosa	7.5, 30, static			and Sandhya (2010)
Remazol Orange; (200 mg/L)	Enterococcus gallinarum	7.0, 30, static	20	100	) Bafana et al. (2009)
Direct Black 38; (100 mg/L)	Pseudomonas sp. SU-EBT	NA, NA, static	12	97	Telke et al. (2009a
Congo red; (1 mg/L)	Brevibacillus laterosporus MTCC 2298	8.0, 40, static	48	91	Gomare et al. (2009)
Golden Yellow HER; (50 mg/L)	Rhizobium radiobacter MTCC 8161	7.0, 30, static	48	90	Telke et al. (2008)
Reactive Red 141; (50 mg/L)	Comamonas sp. UVS	7.0, 30, static	13	100	Jadhav et al. (2008)
Navy Blue HE2R; (50 mg/L)	Exiguobacterium sp. RD3	6.5, 40, static	48	91	Dhanve et al. (2008)
RED RBN; (1 g/L)	Proteus mirabilis	7.0, 30, static	20	95	Chen et al. (1999)
Red RBN; (3000 mg/L)	Aeromonas hydrophila	6.5 – 7.5, 30–35, static	8	90	Chen et al. (2003)
Reactive Blue 172; (500 mg/L)	Pseudomonas aeruginosa NBAR12	5.5 – 10. 0, 20–35, NA	42	83	Bhatt et al. (2005)
Congo Red; (100–300 mg/L)	Bacillus sp.	7.0 – 8.0, 40, static	24	100	Kannappan et al. (2009
Methy1 Red; (100 mg/L)	K1ebsi1e11a pneumoniae R5-13	7.0, 37, NA	168	100	Wong and Yuen (1996)
Reactive Brilliant Red; X-3B; (50 mg/L)	Rhodopseudomonas palustris AS1.2352	6.0 – 8.0, 30, 200 rpm	24	90	Liu et al. (2006)
Azo, Triphenylmethane dyes; (5 mM)	Citrobacter sp	7–9, 35–40, static	1	100	An et al. (2002)

Table 7: Decolorization of various azo dyes by co-cultures of pure bacterial strains

Name of dye and concentration	Organisms used	Condition (pH, temp.(^{0}C), agitation)	Time (h)	Decolorization (%)	References
Green HE4BD, mixture of 6 reactive dyes; (50 mg/Leach)	(Proteus vulgaris and Micccroccus glutamicus)	8.0, 37, static	24	100	Saratale et al. (2010a)
Reactive Brillia	Penicillium sp. and	3.0, 30, static	72	87.8	Gou et al. (2009)

nt red X-3B and Acid Red B; (50 mg/L)	bacterial Sphingomonas xenophaga QYY				
Congo Red, Brodeaux, Ranocid fast Blue and Blue BCC; (10 mg/L)	(Bacillus vallismortis and B. Megaterium)	7.0, 37, agitation (130 rpm)	120	96, 89.6	Tony et al. (2009a,b)
Acid Orange 7 and many other azo dyes; (200 mg/L)	(Aeromonas caviae, Protues mirabilis and Rhodococcus globerulus	7.0, 37, microaerophilic	16	90	Joshi et al. (2008)
Scarlet R and mixture of 8 dyes; (50 mg/Leach)	(P. vulgaris and M. glutamicus	7.0, 37, static	3	100	Saratale et al. (2009b)
Direct Black 22; (100 mg L^{-1})		7.0, 45, static	12	>91	Mohana et al. (2008)
Reactive Red 22; (1 g L^{-1})	E.coli and Pseudomonas luteola	7.0, 28, 200 rpm	25	100	Chen and Chang (2007)
Acid Red 88; (100 mg L^{-1})	Four bacterial strains Bacillus cereus, Pseudomonas putida, Pseudomonas fluorescence and Stenotrophomonas acidaminiphila	7.0, using UFCR anoxic–aerobic sequential bioreacto	3 months	98	Khehra et al. (2006)
Reactive Red 195; (30 mg L1)	Enterobacter sp., Serratia sp., Yersinia sp., Erwinia sp	7.0, 37, 150 rpm	48	90	Jirasripongpun et al. (2007)
Direct Yellow 86, Basic Orange 2, Reactive Green 19, Direct Blue 54, Reactive Blue 171, Reactive Red 141, Acid Red 260 (50 mg L1 each)	Consortium of two isolated strains (BF1, BF2) and a strain of Pseudomonas putida (MTCC-1194)	9–10.5, 28, 120 rpm	168	80	Resmi et al. (2004)
Direct Fast Scarlet 4BS; (50 mg L1)	Pseudomonas 1–10) D	4–9, 20–40, NA	24	99.1	Fang et al. (2004)

Acid Red 88 Acid Red 119, Acid Red 97, Acid Blue 113, Reactive Red 120; (60 mg L1 each)	Bacillus cereus (BN-7), Pseudomonas putida (BN-4),Pseudomonas fluorescence (Stenotrophomonas acidaminiphila (BN-3)	7.0, 35, 100 rpm	24 24 24 24 24	78 99 94 99 82	Khehra et al. (2005)
Reactive Violet 5R; (100 mg L1)	Mixed bacterial consortium JW-2	6.5–8.5, 25–37, static	36	100	Moosvi et al. (2007
Ranocid Fast Blue; (100 mg L1)	Bacterial consortium SV5	7.0, 37, static	24	100	Mathew and Madamwar (2004)
Direct Red 81; (200 mg L1)	Bacterial consortium	7.0, 37, NA	35	90	Junnarkar et al. (2006)
Acid Red 97, FF Sky Blue, Amido Black 10B; (100 mg L1)	Gloeocapsa pleurocapsoides and Chroococcus minutus	NA, 27, static	26 days	78-90	Parikh and Madamwar (2005)
Reactive Black 5; (100 mg L1)	Pseudomonas aerugino sa and Bacillus circulans, and (NAD1 & NAD6	NA, 37, static, anaerobic batch	48	70-90	Dafale et al. (2008)
Direct Blue-15; (50 mg L1)	Alcaligenes faecalis, Sphingomonas sp. EBD, Bacillus subtilis, Bacillus thuringiensis and Enterobacter cancerogenus	NA, 37, static	24	92.14	Kumar et al. (2007)
Reactive Violet 5; (200 mg L1)	Bacterial consortium RVM11.1	6.5–8.5, 25–40, static	37	94	Moosvi et al. (2005)
Remazol Black B; (0.5 g L1)	Alcaligenes faecalis, Commomonas acidovorans	om temp between 12 and 20, Upflow anaerobic Filter (UAF) at batch condition 4	48	95	Oxspring et al., 1996
Direct Red 28 (10 mg L1)	(Bacillus vallismortis, B. pumilus, B. cereus, B. subtilis and B. megaterium)	.0, 37, aeration rate of 0.6 mmol/min along with a flow rate of 60	10 days	93	Tony et al. (2009a,b)

		ml/h (PBR)			

Table 8. Biodegradation of dyes in coloured wastewater using mixed bacterial cultures: review of studies reported in the literature

Name of dyes and concentration	Organisms used	Condition (pH, temp.(^{0}C), agitation)	Time (h)	Decolorization (%)	References
Reactive Black 5; (100–3000 mg/L)	Mixed culture	6.0–7.0, RT, NA	48	90	Mohanty et al. (2006
Reactive Red 2; (50–2000 mg /L)	Mixed, mesophilic methanogenicculture	7.0, 35, static	250 dsys	92-87 mg /Lday	Beydilli and Pavlostathis (2005)
Reactive Black 5 and Direct Brown 2; (0.2 and 3.2 g/L)	Granulated anaerobic mixed culture	Anaerobic process, 35, agitation	300	90	Isik and Sponza (2004)
Remazol Brilliant Orange 3R, Remazol Black B and Remazol Brilliant Violet 5R; (100 mg /L)	Mixed culture of bacteria	30, 200 rpm, combined anaerobic–aerobic system	24	78.9	Supaka (2004)
Acid Orange 7, and Direct Red 254; (60 and 300 mg /L)	Mixed anaerobic bacterial consortia	NA, 35, 100 rpm	50 dys	88	Bras et al. (2001)
Reactive Black 5; (116 mg/L)	Dried activated sludge	2.0, 20, NA	30 min	50	Gulnaz et al. (2006)
Mixture of azo- and diazo-reactive dyes	Mixed bacterial cultures	NA	96	80	Nigam et al. (1996)
Diazo-linked chromophore; (NA)	Mixed bacterial cultures	7.0, use of triple mix gas H2–10%, CO2–10% N2–80% at 308C strictly anaerobicconditions	48	85	Knapp and Newby (1995
Reactive and disperse textile dyes; (0.5 g L1)	Mixed cultures	6, 37, 120 rpm	120-240	100	Asgher et al. (2007)
Acid Orange 7; (60–300 mg /L)	Methanogenic and Mixed bacteria cultures	NA, 37, NA	140	96	Bras et al. (2001)
Methyl Red; (20–30 mg /L)	Mixed culture	6, 30, 180 rpm	24	90	Adedayo et al. (2004
Direct Black-38; (100 mg/L)	Mixed microbial culture	NA, 37, static	16	82	Kumar et al. (2007)

Reactive Red 3.1; (20–30 mg /L)	Activated sludge obtained from domestic and industrial mestic and industrial effluent treatment plants	Anaerobic packed bed reactor followed by an aerobic stirred tank reactor	240	100	Bromley-Challenor et al. (2004)
Acid Red 42, Acid Red 73, Direct Red 80, Disperse Blue 56; (200 mg L1)	Original seed sludge collected from a municipal wastewater treatment plant	NA	51	90-93	Goncalves et al. (2001)
Wastewater containing up to 15 different sulfonated azo dyes	Mixed bacterial population, with sulphate-reducing bacteria, and a methanogenic population	An anaerobic baffled reactor	80	90	Plumb et al. (2001)
Hydrolysed Reactive Orange 96	Sulphate-reducing bacteria, methane producing bacteria and fermentative bacteria in an anaerobic mixed culture	NA	NA	NA	Yoo et al. (2000)
Acid Orange 7	An uncharacterized aerobic biofilm. Strain 1CX (Sphinogomonas sp.) and Strain SAD4I (Gram-negative bacterium)	A rotating drum bioreactor containing the biofilm	40	95	Coughlin et al. (2002)
Azo dyes	Bacillus cereus, Sphaerotilus natans, Arthrobacter sp., activated sludge	NA, NA, Anoxic conditions	1	90	Wuhrmann et al. (1980)
Acid Orange 10, Acid Red 14 Acid Red 18	Anaerobic digester sludge and aeration tank mixed liquo	2-stage anaerobic-aerobic (fixed film fluidised bed/suspended growth activated sludge) reactor	NA	65-90	Fitzgerald and Bishop (1995)
Reactive dyes, diazo dyes, azo dyes, disperse dyes and phthalocyanine dyes	Alcaligenes faecalis, Commomonas acidovorans	Anaerobic condition	48	100	Nigam et al. (1996)
Various azo and diazo-	Thermophilic anaerobic	NA	48	68-84	Banat et al.

reactive dye	bacterial culture				(1996)
Simulated textile wastewater containingProcion Red HE7	Sludge from textile wastewater treatment plant (Inclined Tubular Digester) granules from paper pulp processing plant (Upflow Anaerobic Sludge Blanket)	Upflow Anaerobic Sludge Blanket (UASB)	NA	78	O'Neil et al. (1999)

Table 9. Microbial method to degrade different dyes from different Organism: review of studies reported in the literature

Name of The dye	Organism used	Conditions	Experimental conditions	Comments and References
Congo red, Methyl orange, Erich Rome black-T, Textile Effluent	Schizophyllum commune	Batch immobilization continuous	pH=4.5 150 RPM, Temp. 37°C Pump-0.7 ml/min-in continuous reactor	In 84h Dyes >96 %, textile effluent 77% removal efficiency in batch and In 120h textile effluent 56% RE in continuous phase Selvam.K et al.,(2012)
Congo red, Methyl orange, Erich Rome black-T, Textile Effluent	Lenzites eximia	Batch immobilization and continuous reactor	pH=4.5 150 RPM, Temperature 37°C Pump-0.7 ml/min-in continuous phase	In 84h Dyes >90 %, textile effluent 75% removal efficiency in batch and In 120h textile effluent 54% RE in continuous reactor Selvam.K et al.,(2012)
Reactive black 5	Laccase of Trametes versicolor in presence of syringaldehyde phenoxy	Batch immobilization	pH=7.6 150 RPM, Temperature 38°C	In 30 min RE is 98% Ismat Bibi &Haq Nawaz Bhatti., (2012)
Reactive Dyes	Pseudomonas, Bacillus sp.	Batch immobilization	pH=7 150 RPM, Temp. 40°C	In 192h 50% Removal efficiency Of dye Prasad M.P. et al.,(2013)
Textile dye & Disperse Red 54	Brevibacillus laterosporus	Batch immobilization	pH=7 150 RPM, Temperature 30°C	In 48h 100% dye Removal efficiency and 70.33% RE of Textile effluent Mayur B.Kurade et al.,2015
Textile Effluent	Pseudomonas aerugiosa, Bacillus cereus, Bacillus subtilis,	Batch immobilization	pH=7 150 RPM, Temperature 30°C	Use Individual microbes in 72h 66%>RE> 72.48% and used

	Pseudomonas fluorescens, Escherichia coli, mixed Bacteria (a+b+c+d+e)			mixed culture RE is 98.34% Of textile effluent dyes N.Sriram and D.Reetha-2015

Table 10. Continued Microbial method to degrade different dyes from different Organism: review of studies reported in the literature

Name of the dye	Organism used	Conditions	Experimental conditions	Comments and References
Reactive Orange-4, Reactive Blue, Reactive Yellow-M4G, Reactive Black 5	A)Pseudomonas fluoresces, B)Bacillus sp., C)Escherichia coli	Batch immobilization	pH=7.3 150 RPM, Temperature 30°C	In 192h A sp.RE 79%,82%, 90%, 70% B sp. RE 60%, 65%, 23%, 70% C sp. RE 75%, 60%, 15%, 25% of respectively synthetic dyes. N Sriram et al.,(2013)
Acid Red 7	Acinetobacter radioresistens	Batch immobilization	pH=7.4 150 RPM, Temperature 30°C	In 48h >70% removal efficiency Mohan
				das Ramya et. al.,(2010)
Congo Red	Proteus sp.	Batch immobilization	pH=7-9 150 RPM, Temperature 30°C	In 48h >85% removal efficiency Perumal et al.,(2012)
Ramazol Orange	Pseudomonas aeruginosa	Batch immobilization	pH=7.6 150 RPM, Temperature 30°C	In 24h > 90% removal efficiecy Sauryu and sandhya et. Al.,(2010)
Reactive Black 5 Reactive Orange 16 Direct Reactive 78 Direct Reactive 81	Pseudomonas sp.SUKI And P.rettgeri strain HSLI	Batch immobilization	pH=7 150 RPM, Temperature 30.2°C	In 30h, 12h, 18h, 24h respectively time of dye removal efficiency 98-99% Harsadlade et al.,(2015)

II. BIOCHAR-BASED PACKED-BED BIOREACTORS

Biochar-based packed-bed bioreactors represent an advanced, sustainable approach to wastewater treatment, particularly for removing recalcitrant azo dyes. Biochar, a carbon-rich material produced via pyrolysis of biomass under limited oxygen conditions, exhibits high surface area, porosity, and functional

groups, making it an excellent support matrix for microbial immobilization.

2.1 Role of Biochar in Dye Removal

Biochar contributes to dye removal through dual mechanisms: (i) adsorption of dye molecules onto its porous surface and (ii) enhancement of microbial degradation by providing a suitable habitat for biofilm formation. Oxygen-containing functional groups such as hydroxyl and carboxyl groups facilitate strong interactions with dye molecules.

2.2 Packed-Bed Bioreactor Configuration

Packed-bed bioreactors (PBRs) are widely recognised as efficient systems for treating dye-contaminated wastewater because of their simplicity, high biomass retention, and ability to operate under continuous flow conditions. In a typical configuration, the reactor consists of a cylindrical column packed with a solid support material, here, biochar, which serves both as an adsorbent and as a carrier for microbial immobilisation. Wastewater containing azo dyes is introduced either from the top (down-flow mode) or bottom (up-flow mode), allowing it to pass through the packed bed where simultaneous adsorption and biodegradation processes occur. A packed-bed bioreactor generally includes an influent distribution system, the packed column, and an effluent collection unit. Uniform wastewater distribution is critical to prevent channelling and ensure maximum contact between dye molecules and the biochar-supported microbial biofilm. The porosity and particle size of the packing material influence hydraulic characteristics such as flow rate, pressure drop, and residence time. Biochar, with its high surface area and porous structure, enhances mass transfer and provides an ideal microenvironment for microbial colonization and biofilm development.

Microorganisms immobilized on the biochar surface form a stable biofilm that plays a central role in dye degradation. This immobilization offers several advantages over suspended growth systems, including protection of microbial cells from toxic shock loads, improved resistance to environmental fluctuations, and higher cell density within the reactor. As the dye-laden wastewater flows through the system, azo dye molecules are first adsorbed onto the biochar surface and subsequently degraded enzymatically by the immobilized microorganisms. This dual mechanism significantly enhances overall treatment efficiency. Operational parameters such as hydraulic retention time (HRT), influent dye concentration, pH, temperature, and flow regime critically influence reactor performance. Optimal HRT ensures sufficient contact time for adsorption and biodegradation, while appropriate pH and temperature conditions maintain microbial activity and enzyme efficiency. Additionally, periodic backwashing or flow adjustment may be required to prevent clogging and maintain reactor performance over prolonged operation.

Packed-bed bioreactors can be operated under aerobic, anaerobic, or sequential anaerobic–aerobic conditions depending on the targeted degradation pathway. Anaerobic conditions favor reductive cleavage of azo bonds, whereas subsequent aerobic treatment facilitates mineralization of intermediate aromatic amines. This flexibility makes PBR systems particularly suitable for treating complex dye mixtures.

2.3 Mechanism of Dye Degradation

Dye degradation in biochar-based packed-bed bioreactors involves a synergistic combination of physicochemical adsorption and biological transformation processes. This integrated pathway improves overall azo dye removal efficiency by coupling rapid surface interactions with subsequent microbial degradation.

Initially, when dye-containing wastewater enters the packed-bed reactor, dye molecules adsorb onto the biochar surface. Multiple interactions govern the adsorption process, including π–π stacking between aromatic dye structures and the graphitic domains of biochar, electrostatic attraction between charged dye molecules and functional groups on the biochar surface, and hydrogen bonding. The high porosity and large surface area of biochar facilitate rapid uptake of dye molecules, effectively reducing their concentration in the aqueous phase and minimizing toxicity to microorganisms. Following adsorption, biodegradation is initiated by microorganisms immobilized on the biochar surface. Under anaerobic or oxygen-limited conditions, azo dyes undergo reductive cleavage of the azo bond (–N=N–), which is catalysed by intracellular or extracellular azoreductase enzymes. This process involves the transfer of electrons from electron donors such as NADH or NADPH to the azo bond, resulting in its breakdown

and formation of colorless but potentially toxic aromatic amines. This step is crucial as it leads to the decolorization of the dye, although it does not represent complete detoxification. Subsequently, these intermediate aromatic amines are further degraded under aerobic conditions through oxidative enzymatic reactions. Enzymes such as laccases, peroxidases, and monooxygenases play a significant role in breaking down the aromatic ring structures, leading to the formation of simpler, less toxic compounds such as organic acids, aldehydes, and ultimately carbon dioxide and water. This sequential anaerobic–aerobic degradation pathway ensures both decolourization and mineralisation of dye pollutants.

Biochar also enhances microbial activity by acting as an electron shuttle, facilitating electron transfer during redox reactions involved in dye degradation. Additionally, it provides a protective microenvironment that stabilizes microbial communities and enzymes, thereby improving degradation kinetics and system resilience. Functional groups on biochar further promote microbial attachment and biofilm formation, which are essential for sustained reactor performance. Mass transfer processes, including diffusion of dye molecules into biochar pores and transport of metabolites out of microbial cells, also play a vital role in determining the overall degradation rate. Efficient mass transfer ensures continuous interaction between pollutants and active microbial sites.

2.4 Factors Affecting Performance

The performance of biochar-based packed-bed bioreactors (PBRs) for azo dye removal depends on a combination of physicochemical and biological parameters. Optimisation of these factors is essential to achieve high decolourisation efficiency, stable operation, and effective mineralisation of dye pollutants.

One of the most critical parameters is pH, as it directly influences dye ionization, biochar surface charge, and microbial enzyme activity. Most microbial degradation processes perform optimally in a near-neutral pH range (6.5–7.5). Deviations from this range can inhibit enzymatic reactions, particularly those involving azoreductases and oxidative enzymes, thereby reducing degradation efficiency. Additionally, pH affects electrostatic interactions between dye molecules and biochar, influencing adsorption capacity. Temperature is another key factor affecting both microbial metabolism and reaction kinetics. Typically, mesophilic conditions (25–37°C) favour most dye-degrading microorganisms. Higher temperatures may increase reaction rates but can also cause enzyme denaturation and microbial inactivation, while lower temperatures slow metabolic activity and reduce overall efficiency.

The hydraulic retention time (HRT) plays a crucial role in determining the contact time between dye molecules, biochar, and microbial biofilm. Adequate HRT ensures sufficient adsorption and biodegradation; however, excessively long retention times may reduce system throughput, whereas shorter HRTs can lead to incomplete degradation. Therefore, optimizing HRT is essential for balancing efficiency and operational feasibility. Initial dye concentration significantly impacts reactor performance. At low concentrations, dyes are adsorbed and degraded efficiently; however, at higher concentrations, the system may experience inhibitory effects due to toxicity and saturation of adsorption sites. High dye loading can also hinder microbial activity and reduce enzyme efficiency, necessitating gradual acclimatization of microbial communities. Biochar properties, including surface area, pore size distribution, and functional groups, strongly influence reactor efficiency. Biochar with higher porosity and abundant surface functional groups provides more active sites for adsorption and microbial attachment. Additionally, the biomass origin and pyrolysis conditions used in biochar production determine its physicochemical characteristics and performance.

Microbial consortium composition is another determining factor. Mixed microbial cultures generally perform better than pure cultures because synergistic interactions enable complete degradation of complex dye molecules and their intermediates. The adaptability and resilience of microbial communities enhance system stability under varying operational conditions. Flow rate and hydrodynamics also affect mass transfer and contact efficiency within the reactor. Uniform flow distribution is essential to avoid channelling, which can reduce effective contact between wastewater and the biofilm. Proper reactor design ensures optimal fluid dynamics and minimizes dead zones. Lastly, dissolved oxygen levels influence the degradation pathway. Anaerobic conditions favor azo bond cleavage, while aerobic conditions are

necessary for the breakdown of aromatic amines. Therefore, maintaining appropriate redox conditions, either sequentially or simultaneously, is vital for complete mineralization.

2.5 Advantages Over Conventional Systems

Biochar-based packed-bed bioreactors (PBRs) offer several advantages over conventional wastewater treatment systems, particularly for removing recalcitrant azo dyes. One of the primary benefits is the synergistic integration of adsorption and biodegradation, where biochar rapidly adsorbs dye molecules while immobilized microorganisms subsequently degrade them. This dual mechanism enhances overall removal efficiency and reduces pollutant toxicity.

Another key advantage is high biomass retention due to microbial immobilization on the porous biochar surface. This increases microbial density, improves enzymatic activity, and enhances resistance to toxic shock loads. The biochar matrix also provides a protective microenvironment, ensuring operational stability under fluctuating conditions such as variations in pH, temperature, and dye concentration. These systems are characterized by low sludge production, which minimizes post-treatment handling and disposal issues commonly associated with physicochemical methods. Additionally, packed-bed bioreactors support continuous operation, making them suitable for large-scale and industrial applications with consistent wastewater flow.

Biochar is a low-cost, sustainable material derived from biomass waste, supporting the process's economic feasibility and environmental sustainability. Its high surface area and functional groups enhance both adsorption capacity and microbial attachment. Furthermore, PBRs require little energy and have a simple design, making them easy to operate and maintain. Their flexibility to function under aerobic, anaerobic, or combined conditions allows efficient degradation and mineralization of complex dye molecules.

Overall, biochar-based packed-bed bioreactors represent an efficient, cost-effective, and environmentally friendly technology for advanced wastewater treatment.

III. CONCLUSIONS

This review highlights the environmental challenges posed by azo dyes and evaluates various treatment strategies, with a focus on biological degradation. While physicochemical methods offer rapid decolourization, their limitations such as high cost, sludge generation, and incomplete mineralisation necessitate the development of sustainable alternatives.

Biological treatment methods, particularly microbial degradation using bacteria, fungi, and consortia, show strong potential because they are eco-friendly and can achieve complete mineralisation under optimised conditions. Integrating anaerobic and aerobic processes improves degradation efficiency by enabling both azo bond cleavage and subsequent breakdown of aromatic amines. Biochar-based packed-bed bioreactors are a promising hybrid technology that combines adsorption and biodegradation. The synergistic interaction between biochar and microbial communities significantly improves dye removal efficiency, operational stability, and cost-effectiveness.

Despite these advancements, challenges related to large-scale application, process optimization, and long-term stability remain. Addressing these issues through interdisciplinary approaches will be crucial for future implementation.

IV. FUTURE PROSPECTIVES

Future research on dye degradation should focus on developing integrated and hybrid treatment technologies that combine biological, chemical, and material-based approaches. Engineered biochar with tailored surface functionalities can further enhance adsorption capacity and microbial interaction.

Advancements in molecular biology and omics technologies can help elucidate microbial pathways and develop genetically modified strains with enhanced degradation capabilities. Additionally, the application of nanotechnology and artificial intelligence for process optimization and monitoring can significantly improve treatment efficiency. Scaling up biochar-based packed-bed bioreactors for industrial applications remains a major challenge. Additionally, pilot-scale studies and techno-economic analyses are essential to evaluate feasibility.

Sustainable biomass sources for biochar production and circular economy approaches should also be emphasized.

Therefore, integrating advanced materials, biotechnology, and process engineering holds great promise for efficient and sustainable treatment of dye-contaminated wastewater.

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