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Review

Sustainable Solutions: Reviewing the Future of Textile Dye Contaminant Removal with Emerging Biological Treatments

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Abstract: Synthetic dyes, exceeding 100,000 types on the market and produced at a global scale of over 700,000 tons annually, are extensively used in the textile industry. This industry, a leading contributor to water contamination, relies on dyes like reactive, azo, anthraquinone, and triphenylmethane, resulting in substantial water usage and significant effluent generation. A significant modern challenge is the pollution caused by dye-mixed wastewater, releasing hazardous chemicals into water bodies and posing threats to ecosystems, plants, and human health. Traditionally, physicochemical techniques have addressed textile dye-containing wastewater, but their drawbacks, including cost, inefficiency, and potential secondary pollution, have steered attention towards biological alternatives. Utilizing microorganisms and enzymes, these biological methods, such as microbial cell enzyme immobilization, the biofilm technique, bioreactors, biofuel/bioelectricity production, and genetic engineering, have emerged as promising, cost-effective, and environmentally friendly solutions for efficient dye removal from wastewater. This review paper specifically highlights advanced biological techniques and emphasizes their efficacy in addressing the challenges posed by synthetic textile dyes. Through a systematic review of recent research papers, published results, and observations, this review paper provides insights into emerging biological treatment strategies for effectively removing synthetic textile dyes and contaminants from wastewater.

Keywords: synthetic dyes; textile industry; wastewater treatment; biological methods; environmental impact

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1. Introduction

The surge in technology, industry, and urbanization has notably impacted the purity of water bodies worldwide [1]. Water pollution is a pressing global issue, with industries contributing significantly, accounting for 17–20% of the problem [2]. Although dyes play a crucial role in enhancing the aesthetics of various products, their discharge during the dyeing process, where fixing efficiency ranges from 60% to 90%, emerges as a substantial environmental hazard [3]. The discharge of wastewater from the textile industry, housing toxic dyes originating from both the textile and dyestuff sectors, constitutes a major environmental challenge. Annually, approximately 280,000 tonnes of dyes are released into the environment, contributing to water pollution on a global scale [4]. The evolution of synthetic dyes since the discovery of the first one in 1856, coupled with the rapid industrialization of textile production, has led to the release of lethal textile dye effluents, impacting aquatic ecosystems adversely [5].

Azo dyes, representing over 70% of synthetic dyes globally, pose a substantial environmental risk, particularly in textile wastewater [6]. Approximately 10–15% of these dyes

are lost or spilt during processes, exacerbating the challenge of pollution [6]. Azo dyes, characterized by aromatic structures and one or more –N=N– bonds, are the most common synthetic dyes and pose risks to both human health and aquatic life [7]. The colouration and intensity of synthetic dyes result from the interaction between chromophore and auxochrome groups. These groups include essential chromophores such as N=N, CO, NO_2, quinoids, NH_3, OH, SO_3H, and CO_2H [3].

The environmental impact of the textile industry has prompted a closer examination of mechanisms for dye removal. Biological procedures, in particular, have gained attention as an alternative to chemical and physical treatments due to their environmental advantages. However, current biological treatment methods face challenges, including low efficiency, large volumes of living sludge, high energy consumption, and associated costs [8]. As a result, ongoing research in dye biodegradation emphasizes improving degradation rates by selecting more efficient microbial species, optimizing process parameters, and choosing effective reactor systems [9]. This holistic approach aims to address the environmental consequences of synthetic dye usage and wastewater discharge in the textile industry.

Environmental and Health Implications of Azo Dyes in Textile Industries

Azo dyes, extensively used across various industries, especially in textiles, have evolved into a significant environmental concern [8]. Textile manufacturing contributes to environmental degradation by discharging untreated dyestuffs and wastewater, posing severe threats to humans, plants, and animals. Textile wastewater contains a myriad of substances, including dyes, disinfectants, halogen carriers, solvents, hazardous heavy metals, carcinogenic amines, chlorine bleaching agents, biocides, pentachlorophenol, salts, softeners, surfactants, solvents, and free formaldehyde [10]. The discharge of these substances, particularly high concentrations of colours, into rivers and lakes from industrial effluents results in water pollution characterized by low biodegradability and high toxicity [7]. The intricate structure of synthetic dyes renders them highly resistant to degradation, inhibiting light penetration through water and disrupting the aquatic ecosystem food chain [4,11]. Synthetic dyes alter the chemical composition of water bodies, disrupt the ecological balance, and elevate chemical oxygen demand (COD) and biological oxygen demand (BOD) due to pH changes [12,13]. Direct irrigation with untreated dyeing effluents has significant environmental repercussions, including decreased soil fertility, seed germination, chlorophyll content, and plant protein content.

Azo dyes pose serious health risks to both humans and aquatic life. Exposure can lead to skin irritation, chemosis, contact dermatitis, exophthalmos, lacrimation, rhabdomyolysis, permanent blindness, vomiting, gastritis, acute tubular necrosis, hypertension, vertigo, and edema of the neck, face, tongue, pharynx, and larynx in humans (Figure 1) [3]. These dyes have harmful effects ranging from diminished growth, bladder cancer, and neurosensory impairment in humans to fish mortality [14]. Additionally, they exhibit mutagenic, carcinogenic, genotoxic, and teratogenic properties, causing severe health issues in humans, including renal dysfunction, digestive tract malfunction, and damage to the cerebral, hepatic, cutaneous, and central nervous systems [15,16].

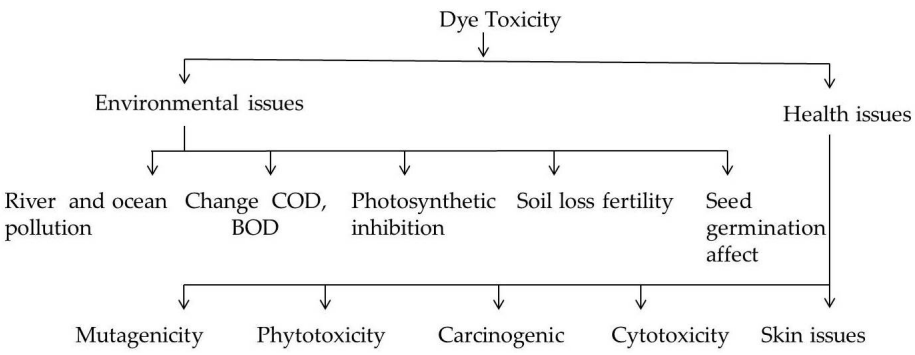

Figure 1. Common toxicity of synthetic dyes.

2. Dye Degradation Strategies

The textile industry generates hazardous pollutants, such as hydroxylated derivatives, naphthoquinone, phenolic compounds, organic acids, and aromatic amines, posing ongoing environmental challenges [17]. Dye wastewater treatment typically employs physical, chemical, and biological methods. Physical methods utilize agents with physical properties, chemical methods involve chemical agents, and biological dye degradation relies on biological agents [18].

Various physicochemical techniques for azo dye decolourization have been applied, encompassing adsorption, chemical treatment, and ion pair extractions. However, these processes are expensive and result in significant sludge volumes post-treatment. In response to these limitations, advanced oxidation processes (AOPs) and biological techniques are emerging as viable solutions for treating dye-containing wastewater. Effective removal of COD has been achieved through Fenton or photo-Fenton oxidation coupled with microbiological systems [19].

Biotransformation enzymes play a crucial role in the microbial system's biodegradation of problematic substances. Dye decolourization can occur through either adsorption on microbial biomass or the biodegradation of dyes by cells. These innovative approaches address the challenges posed by traditional physicochemical methods, offering more cost-effective and environmentally friendly alternatives for the treatment of dye-containing wastewater.

2.1. Challenges and Opportunities in Azo Dye Wastewater Treatment

Various physicochemical methods, including sonication, electrochemical oxidation [16], ozonation, photocatalytic degradation, chemical degradation, coagulation, neutralization, lime softening, precipitation, membrane filtration, ion exchange, adsorption, and oxidation, have been employed for the removal of Azo dyes from wastewater (Table 1). However, these methods present limitations due to their high reagent or energy requirements, the need for expensive equipment and monitoring systems, partial metal removal, hazardous sludge formation, and the use of toxic chemicals requiring proper disposal methods [20,21].

Table 1. Various physiochemical methods of dye degradation.

Physicochemical Methods	Advantages	Limitations
Flocculation/coagulation	Simple operation process	Sludge production
Adsorption	Excellent removal of various dyes	Transfer contaminants
Electrochemical oxidation	No consumption of chemical	Cast of electricity
Ozonation	Fast and effective dye removal method	Short half-life
Ion exchange	High cation exchange capacity	Not suitable for all dyes
Fenton oxidation	Suitable for soluble and insoluble dyes	Sludge production
Photochemical	No sludge production	Formations of byproducts
Membrane filtration	Low cast	Sludge disposal

Coagulation and flocculation, while effective, are costly physicochemical procedures that generate significant amounts of sludge, necessitating safe disposal after treatment [22]. Physical techniques based on dye coagulation–flocculation demonstrate efficiency in removing sulphur and dispersion dyes, but their capability is deficient for acid, direct, reactive, and vat dyes. Filtering technologies such as ultrafiltration, nanofiltration, and reverse osmosis have been successfully utilized for water reuse and chemical recovery [23]. The choice of method depends on the specific dye type and the environmental and economic considerations of each treatment approach.

2.2. Biosustainable Solutions for Synthetic Dye Degradation

The biodegradation of synthetic dyes involves breaking down dye molecules into smaller intermediates and subsequent decolourization [24]. Biological procedures, including bacterial and fungal decolourization, phytoremediation, and enzymatic methods, contribute to this process [25]. Actinomycetes, fungi, algae, yeast, aerobic, and anaerobic bacteria [26], as well as plants and their enzymes, have all successfully decomposed a wide range of dyes [27]. Many microorganisms are easy to maintain and require minimal preparation [28]. This analysis considers the impact of operational factors on decolourization, addresses toxicity at various phases of biological treatment, and illustrates the biochemical pathways involved in anaerobic–aerobic sequential biodegradation of azo dye, as shown in Figure 2 [27]. In the methanogenic system, the azo dye can penetrate cells, tightly attaching to them, blocking electron transfer channels and impeding the release of metabolic products and substrate intake, influencing azo dye decolourization [29].

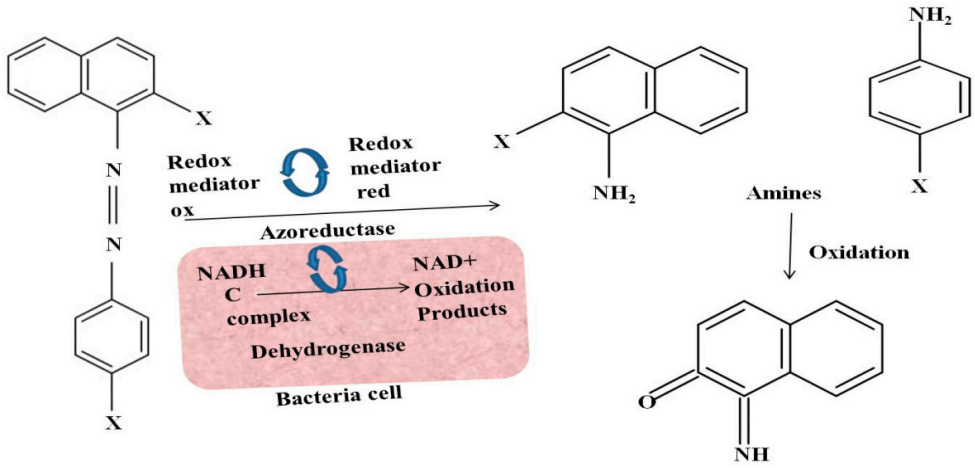

Figure 2. Biodegradation of azo dye by bacteria.

Enzymatic dye degradation represents a complete mineralization process [30]. Transitional metabolites, such as aromatic amines, undergo anaerobic or aerobic metabolism in these processes [7]. A minimal redox potential facilitates significant decolourization of azo dyes in anaerobic circumstances. However, in aerobic degradation, respiration may surpass NADH consumption, impeding electron transfer from NADH to azo bonds [31]. On the other hand, the biological degradation of synthetic colours requires maintaining proper process parameters, such as pH, temperature, nutrient concentration, and moisture content, for optimal microbial growth and activity.

Recent studies on synthetic dye bioremediation have focused on using effective microbial species and developing suitable bioreactors. Biological dye degradation offers advantages over physicochemical techniques, including cost-effectiveness, environmental tolerance, sludge-free characteristics, and the generation of fully mineralized or non-toxic end products. This approach could help minimize extensive water usage [16,22,23,28]. Regarding enzymatic degradation, azo-reductases, peroxidases, and phenol oxidases can all contribute to dye degradation [32]. Therefore, for the most efficient effluent treatment, an anaerobic approach followed by aerobic treatment can be employed to decolourize dye-containing wastewater and enhance its biodegradability [33]. The mechanisms of azo dye reduction include direct enzymatic azo dye reduction, mediated biological azo dye reduction, and azo dye decolourization by biogenic inorganic compounds [34].

3. Advanced Biological Approaches

Environmental pollution has increased due to the recent rapid advancement of industrialization. Because industrial pollutants affect surface- and groundwater as well as human health, they constitute one of the largest environmental issues. Wastewater from the dye industry has been reported to be treated using a variety of technologies. The latest technical

and scientific achievements of wastewater treatment for the dye industry are presented and discussed in this study [35]. All of these methods of dye treatment of wastewater and water are discussed in the present review paper. The primary objective of this study is to present an in-depth analysis of the most recent research on the various techniques used to remove different colours from industrial effluents.

3.1. Phytoremediation

Biological techniques such as phytoremediation can be applied on-site to clean up contaminated environments. Phytoremediation can be used for a variety of reasons, including the treatment of wastewater, the purification of surface- and groundwater, the elimination of excess nutrients from water reservoirs, and the reclamation of soil that has been contaminated by natural catastrophes [36]. Plant bioactive molecules may include hydroxylic groups, aldehydic groups, carboxylic groups, amino groups, etc. The bioactive compounds could include amino groups, carboxylic groups, aldehydic groups, hydroxylic groups, etc. These organic constituents can come from a variety of sources, including sugars, polymers, vitamins, proteins, amino acids, and liquid plant extracts [37,38].

Rich in polyphenols, plant extracts promote the degradation of the organic chemicals they include. The ability of plants to reduce metal ions in various organs and tissues, both distance from the ion penetration site, as well as on their surface, has led to the use of these plants for the extraction of metallic nanoparticles (NPs) [39]. It can be difficult to find clean, safe drinking water, particularly in underdeveloped nations, and the methods used to purify it are extremely chemical-intensive and bad for the environment. For the treatment and purification of water, absorbents and catalysts based on nanotechnology (NT) offer an environmentally acceptable substitute for these toxic dyes [39]. Hydrothermal and solvothermal procedures are two examples of physical and chemical processes that can be used to create nanoparticles, but they have some disadvantages as well, like the need for hazardous chemicals and solvents and their high costs. The need for eco-friendly, affordable, and non-toxic synthesis methods to meet the growing demand for nanoparticles has led to the employment of microbes and plants in biological or green synthesis. Numerous preparative methods for the production of diverse metallic and non-metallic nanoparticles using microbes and plants have been previously reported [40]. Because biological procedures do not result in secondary pollution, using plants for environmental cleanup may be more effective than conventional methods based on the chemical extraction of xenobiotics [37].

3.2. Biofilm Reactors

Biofilms, intricate communities of microorganisms adhering to solid surfaces, consist of cells, extracellular polymers, and both organic and inorganic components. The emerging biological treatments of effluent dyes are summarized in Figure 3. These colonies form on various surfaces and are characterized by a complex process of detachment influenced by factors such as aqueous medium hydrodynamics, flow velocity, biofilm shape, and support properties [41]. Passive immobilization occurs through spontaneous adsorption and multilayer development of cells around or inside solid support materials, creating a "biofilm". Key advantages of biofilm reactors include highly active attached biomass and enhanced resistance to contamination [42].

The production of extracellular polymeric substances (EPSs) is crucial for biofilm formation, with gene products playing a significant role. EPSs form the structural basis of the biofilm, creating water-filled channels for nutrient movement within the biofilm. Detachment of newly produced cells and dispersion of biofilm aggregates occur due to flowing effects or quorum sensing (Figure 3) [43]. Biofilm techniques, such as Moving Bed Biofilm Reactor (MBBRs), Aerobic Fluidized Bed Bioreactors (AFlBRs), and Sequence Biofilm Bioreactors (SBBRs), offer advantages like reduced head loss, no sludge recycling, operational stability, improved mass transfer, and efficient use of tank capacity compared to traditional methods like activated sludge or fixed bed [44].

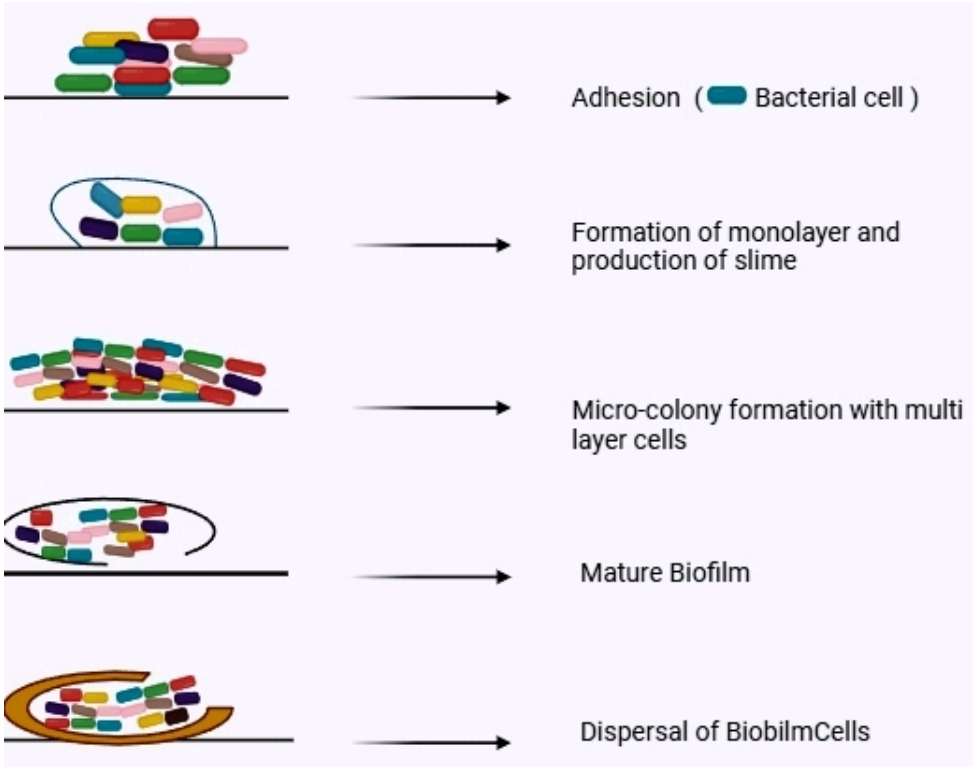

Figure 3. Biofilm formation by bacterial cells.

In the treatment of textile dye wastewater, biofilm techniques have been successful, employing dye-degrading bacterial strains such as *Lysinibacillus fusiformis* strain ZB2, *Brevibacillus panacihumi* strain ZB1, *Bacillus cereus* strain ZK2, and *Bacillus pumilus* strain ZK1. These biofilms demonstrated significant COD and dye removal efficiencies throughout the day [45]. Additionally, studies in 2021 highlighted biofilm consortia's effectiveness, with four distinct consortia showing remarkable decolourization rates, ranging from 96.9% to 99.5%, under static conditions after 72 h of incubation at 28 °C [46]. Notably, a biofilm containing *Dysgonomonas* displayed the potential to degrade the Reactive Black 5 dye [47].

3.3. Microbial Fuel Cells (MFCs)

Microbial fuel cells (MFCs) stand as innovative devices harnessing energy through the oxidation of organic and inorganic compounds in biomass via microorganisms acting as catalysts [48]. Representing an appealing method for generating bioelectricity from diverse wastewaters, MFCs operate with an anode chamber (anaerobic/anoxic) and a cathode chamber (aerobic/oxic), separated by a proton exchange membrane or an appropriate distance, employing oxidation and reduction processes [49]. MFCs exhibit two major structures, namely biocontrol and bioprocess, with the potential to increase power output by stacking similar-shaped MFC units. In contrast to traditional methods that produce significant sludge and necessitate additional equipment, MFCs directly convert waste into bioelectricity with minimal sludge production [50]. Research in this realm has paved the way for affordable microbial fuel cell devices, demonstrating efficacy in treating dye industrial effluent while concurrently generating electricity. Operating with a granular activated carbon (GAC) bioanode and biocathode, this MFC processed genuine dye wastewater without additional alterations, utilizing bacteria specific to the dye wastewater industry [48].

Bioelectrochemical systems (BESs) function by accelerating organic/pollutant decomposition at the anode while facilitating electron flow through a cascade of redox potentials (Figure 4). However, anaerobic conditions can hinder microbial metabolism when electron acceptors are lacking, slowing the rate of waste degradation. The production of H_2, instead

of H_2O, at the cathode, functioning as an electron donor for pollutant reduction, demands electricity. The limited effective electron transfer to the solid electron acceptor is attributed to the enzymes responsible for this transfer being enclosed in an insulating protein matrix. Electroactive bacteria (EAB) play a crucial role in oxidizing contaminants at the anode, releasing electrons under redox-favourable conditions [49].

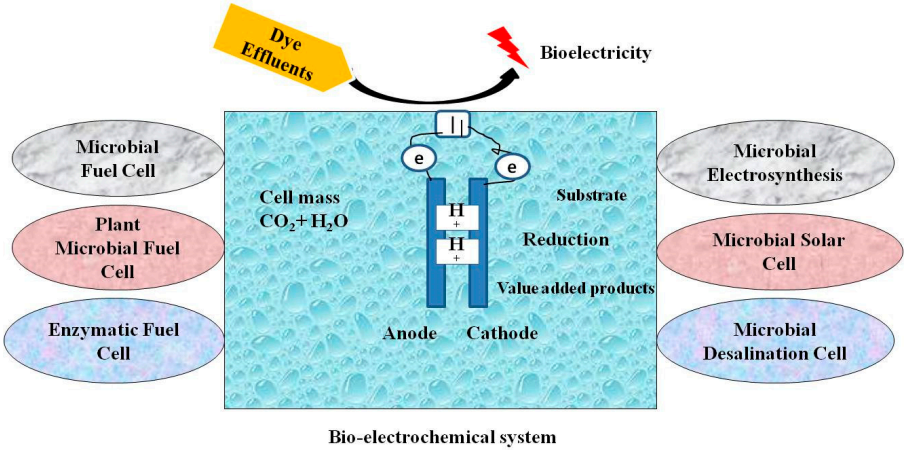

Figure 4. Microbial fuel cell system units.

MFCs, consisting of an anode, cathode, separator, and external circuit, integrate microbial and electrochemical processes, converting chemical energy into electricity through the oxidation of organic matter. While microbial activity near the cathode allows for the physiological conversion of nitrate to nitrite, denitrifying and phosphorus-accumulating bacteria thrive near the cathode, and ammonia-oxidizing, nitrite-oxidizing, and anammox bacteria predominantly concentrate at the anode [51].

In an MFC system, electrochemically active microbes in the anode compartment oxidize various organic components of coloured effluents, producing protons and electrons. These are then transported to the cathode compartment, utilized to reduce O_2 to H_2O. While the technology holds immense potential, its current limitation lies in the need for widespread implementation due to lower electricity output and higher material costs, particularly in the bioremediation of colours from textile and dye manufacturing sector effluents. Typically, double-chambered MFCs separate anaerobic and aerobic cathode chambers with a proton exchange membrane, maintaining a medium pH of 7. A combination system involving adsorption, chemical oxidation, and biodegradation is proposed for azo dye remediation from textile wastewater [52]. The process uses bacteria to generate electrical power, offering a sustainable future energy source [53].

In the realm of MFC applications, a study explored bioenergy generation and dye decolourization from dye wastewater, showcasing a power density of 940.61 ± 5 mW/m^2, a voltage output of 790 ± 5 mV, and 83% decolourization. MFCs provide multiple benefits, including waste management, electricity generation, and reduced sludge production [54]. Combining biological catalytic activity with conventional electrochemical reactions, MFCs have proven efficient in decolourizing azo dyes and producing bioelectricity. Despite the ability of air-cathode MFCs to reduce azo dyes, anaerobic conditions still promote the degradation of hazardous dye intermediates. As a solution, an MFC and aerobic sequential reactor coupled system has been suggested for complete azo dye degradation and removal of aromatic amines under aerobic conditions. The production of bioelectricity involves transferring electrons from microbial cells to the anode through processes like direct electron transfer, mediator electron transfer, and nanowires. Redox mediators enhance dye clearance and bioelectricity production in MFC systems [55]. Exoelectrogenic bacteria in anaerobic environments facilitate electron transfer from organic matter oxidation to the electrode, enhancing microbial development and metabolism. This process produces electron transfer intermediates during azo dye breakdown, amplifying the recovered energy [47].

Investigations by Oon et al., 2021 highlight the efficient decolourization of azo dyes in MFCs, reaching 90% efficiency under different operating conditions. Different MFC designs exhibit varying effectiveness in dye decolourization, with a two-chamber biocathode MFC system demonstrating superior efficiency compared to a single-chamber air cathode MFC system [55]. Microbial fuel cells continue to emerge as a promising technology for the degradation and decolourization of contaminants in textile wastewater, showcasing their potential in wastewater treatment and sustainable energy generation [56].

Utilizing microbial fuel cells for the treatment of dye wastewater demonstrates a multifaceted approach, showcasing the simultaneous generation of bioenergy and decolourization. These findings open avenues for sustainable technologies in wastewater treatment, emphasizing the potential of MFCs in providing a dual solution for environmental and energy challenges.

3.4. Microalgae through Dye Treatment and Biodiesel Production

Textile wastewater contains dyes (carbon source), nitrates (nitrogen source), phosphates (phosphate supply), and metals (micronutrients), all of which are important for microalgae development. Proteins and carbohydrates in microalgae cell walls offer functional groups for bonding with cations like metals and basic dyes. As a result, future research and development efforts should be refocused on microalgae biodiesel production. *Oscillatoria* sp. [57], *Chlorella pyrenoidosa* [58], *Desmodesmus* sp. [59], *Chlorella vulagris*, *Spirulina* sp., and *Oscillatoria tenuisin* decolourize dye effluent by degrading azo dyes into simple aromatic amines. The method of degradation of textile dye using various microalgae is shown in Table 2. Microalgae have more potential than other microorganisms because they can remediate textile effluent by absorbing nutrients and dyes and depositing lipids that can be converted to biodiesel. Biodiesel is a long-term, environmentally beneficial byproduct of the microscopic algae bioremediation. Microalgae harvesting is an essential stage in the biodiesel manufacturing process. The critical processes in harvesting include separating algal biomass from the suspension and thickening it [60].

Table 2. Textile dye degradation using different microalgae.

Microalgae Species	Dyes	Decolonization %	References
CKW1 (*Spirogyra* sp.) and PKS33 (*Cladophora* sp.)	Textile effluent dyes	90%	[61]
Chlorella vulgaris	Methylene Blue	99.7%	[60]
Chroococcus minutes	Amido black 10B	55%	[62]
Chlorella pyrenoidosa	Textile wastewater	80%	[58]
Chlorella sp. Wu-G23 (G23)	Textile wastewater	77.9%	[63]
Spirogyra sp.	Cr(IV)	30%	[64]
Oscillatoria sp.	Textile wastewater	76%	[57]
Desmodesmus sp.	Methylene Blue,	98%	[59]
	Malachite Green,		
	Reactive Red-120	99%	

3.5. Bioreactors

Eyvaz et al., 2016 investigated aerobic and anaerobic bioreactors in evaluating the treatment efficiency of textile effluents containing azo dyes [65]. Membrane bioreactors contain greater startup and ongoing expenses than traditional biological mechanisms. However, they produce extremely high-quality discharge water and have a high possibility of reusing valuable components found in textile effluent [66]. Designing and developing a suitable bioreactor for a specific treatment process generally requires a thorough examination of the biosystem, including cell growth, genetic manipulation, and metabolism, as well as the establishment of suitable experimental parameters such as operational stability, O_2 transfer, scale-up, startup cost, and soon. Membrane-based reactors, air-pulsed bioreactors, aerobic anaerobic sequential reactors, MFCs, hybrid bioreactors, and semicontinuous biore-

actors are only a few examples of these types of biological reactors which are depicted in Table 3 [67]. MBR stands for membrane bioreactor, which combines a membrane process such as ultrafiltration or microfiltration with a sustained-growth bioreactor. In MBRs, the removal of nitrogen and phosphorus is also more efficient. To exceed the discharge restrictions or remove salt from the MBR system's discharge, two phases of membrane configuration, nanofiltration or reverse osmosis, can be utilized [66].

The researchers investigated the adsorption of five reactive dyes occurring in a synthetic textile dye effluent on barley husk particles in process systems designed as a static batch-mode reactor and a packed-bed reactor (CFPBR) able to operate with a continuous flow of effluent, with the goal of treating huge amounts of water. The efficacy of these biofilm-containing support systems in decolourizing a continuous-flow packed-bed column bioreactor (CFPBR) system was investigated. As a result, compared to a batch treatment process, a continuous effluent treatment process might run longer and treat more wastewater [68]. Microbial remediation has been described in various bioreactors, including suspended carriers, slurry and fixed beds, and membrane and fluidized bed reactors. Many of these elements must be regulated and managed for optimum reactor performance while designing and operating bioreactors in cleanup. Environmental parameters such as temperature, pH, moisture, pollutant mix, pollutant concentration, and macronutrient are all elements that impact microbial growth and activity in bioreactors. Size, configuration, and mode of operation are all factors of reactor design. Any bioreactor type designed for a specific purpose and application should be simple to run and maintain. For the treatment of gaseous or volatile air pollutants, airlift bioreactors may be an appealing treatment option. MBRs benefit from using fewer tanks and ensuring that particles are removed from treated effluent due to the filtering function of the membrane. Furthermore, membranes are usually costly, making the procedure overpriced. Membrane bioreactors (MBRs) (Figure 5) combine a filtering mechanism with a biological process. The treated effluent from the membrane bioreactor is of higher quality than that obtained through other methods, allowing the secondary treatment system to perform at its best [69]. Congo red dye undergoes a 95% colour loss under anaerobic conditions in a UASB reactor. The high dye concentration in the colour waste may be successfully degraded by the isolated bacteria *Brevibacillus parabrevis*, according to Talha et al., 2017. The dye degradation experiments were conducted in a cylindrical borosilicate-packed bed bioreactor. Up to a concentration of 500 ppm, significant dye sample degradation was seen, reaching 77.82% in a free cell and 88.92% in an immobilized cell [9]. This MBR is now commonly applied for textile wastewater treatment, with a COD removal efficiency of >90%. For anaerobic MBRs (AnMBRs), based on wastewater properties, such as COD content, biogas generation can be produced. The mineralization of organic substances in an anaerobic MBR system normally consumes little energy. Eyvaz et al., 2016 evaluated the use of MBRs (anaerobic and aerobic) for the remediation of synthetic textile effluents [70]. Membrane bioreactors are an attractive prospect for the treatment of wastewater [71]. The samples treated by nanofiltration comprised parts of the biological and MBR treatment effluent [72]. MBR stands for membrane bioreactor, which combines a membrane process with a suspended growth bioreactor. Membrane bioreactors have greater initial and ongoing expenses than traditional biochemical processes. However, they produce extremely high-quality discharge water and allow for the reuse of valuable elements found in textile waste [66].

The treatment of synthetic wastewater was established using a new anaerobic–aerobic algal–bacterial photobioreactor. Overall, 99.1% of dispersed orange, 3%, and 96.3% of dispersed blue 1 could be decoloured using this approach. The symbiotic relationship in a photobioreactor is based on the reciprocal exchange of CO_2 and O_2 between bacteria and microalgae [56]. Malachite green degradation in a membrane bioreactor [MBR] was bio-augmented by *Aeromonas hydrophila* LZ MG14 to be more effective [36]. The textile effluent was sequentially treated anaerobically and aerobically in an An-SBR, then aerobically in an MBBR. With 100 mg/L of mixed azo dyes, the dye degradation percentage of the first cycle's anaerobic phase was 97%; it then decreased to 96% in the aerobic phase

before increasing again to 98%. The bioreactors were cycled for two cycles, totalling 96 h each. Reactive Orange 16 was removed using an MBBR in an aerobic environment and 97% decolourization was reported by [73]. In a study by Hameed and Ismail published in 2020, the use of immobilized mixed cells for the sequential anaerobic–aerobic decolourization, biodegradation, and detoxification of reactive yellow dye (RY15) in textile effluent was studied [74]. The capacity of immobilized *Proteus vulgaris* for C.I. Reactive Blue 172 decolourization and degradation at an initial concentration of 50 mg/L in 8 h with a reduction in COD of nearly 80%. *Enterobacter* sp. SXCR, a recently discovered bacterium isolated from soil samples contaminated with petroleum, was studied by Prasad and Aikat in 2014 for its potential to decolourize textile dyes, particularly the azo dye Congo red [74]. Degradation of azo dye a batch bioreactor was used to study the ability of *Enterobacter aerogenes* ES014 to degrade azo dye after it was isolated from wastewater. A 100 ppm dye concentration, pH 7.5, 1.5% glucose, and 0.8% beef extract were used, and the decolourizing percentage ranged from 82.3 to 78.2 and 81.5, respectively. The batch reactor was used to extract acid orange from the wastewater. The colours utilized were acid orange, methyl orange, and Congo red for *E. aerogenes* ES014. At a pH of 7.7 0.4, the medium contained 87.5 1.3 mg/L of acid orange [75]. Polyacrylamide and sodium alginate beads were used for immobilization under static and shaking circumstances [74]. The residual COD and colour in the anaerobic effluent were reportedly effectively degraded using an aerobic MBR connected to an anaerobic SBR. Because of this, membrane bioreactors are an appropriate form of bioreactor to use for the aerobic step of the combined anaerobic–aerobic process for treating dyeing wastewater. A continuous-flow stirred packed-bed reactor with an aerobic MBR was utilized to decolourize acid orange 7 with sodium acetate as the carbon supply. In an anaerobic process, the average total COD elimination was around 77–7.9% [76]. The phyla *Euryarchaeota*, *Caldiserica*, and *Proteobacteria* dominated the microbial population in the AnMBR, whereas Proteobacteria predominated in the downflow hanging sponge (DHS) reactor. According to reports, several genera discovered in the DHS and AnMBR can degrade RB5 dye and decrease azo dyes, respectively (sulfonated aromatic amines). BOD3, COD, and colour removal values from the AnMBR-DHS process were 97.3 1.8%, 94.4 4.8%, and 95.0 1.6%, respectively [77].

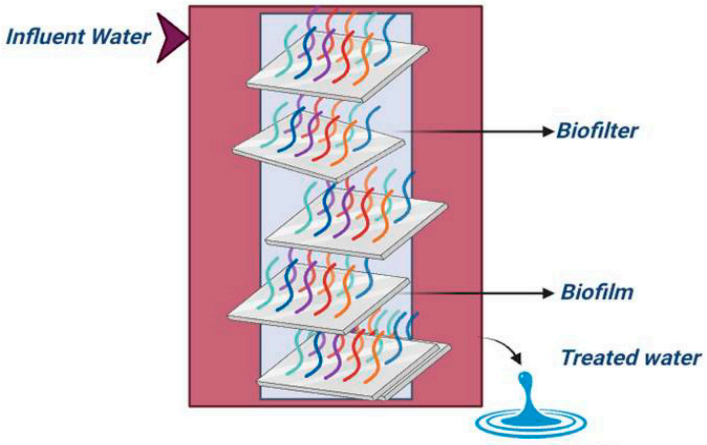

Figure 5. Wastewater treatment using biofilm bioreactors.

Table 3. Degradation of dyes using different bioreactors.

Type of Bioreactor	Microbial Strains	Dye	Efficiency, pH	Time/Temp	References
Anaerobic sequential batch reactor (An-SBR) and MBBR		Reactive Red, Reactive Black, and Reactive Brown 100 mg/L	88%	96 h	[73]
MBSBBR and SBR		Black B 32.3 mg·L^{-1}, Black WNN 64.5 mg·L^{-1} and Red 3BS 96.8 mg·L^{-1}	79.9 ± 1.5%,	15 and 25 days 30 °C	[76]
Batch bioreactor	*Enterobacter aerogenes* ES014	Acid orange, methyl orange and Congo red 100 mg/L	(82.3 ± 3.6%) (78.2 ± 3.3%) (81.5 ± 3.2%) pH 7.5	35 °C, 24 h	[75]
Anaerobic Membrane Bioreactor and downflow hanging sponge reactor	*Euryarchaeota*, *Caldiserica*, and *Proteobacteria*	Reactive Black 5	95.0 ± 1.6%	12–24 h, pH 12, 30 °C	[77]
Immobilized batch and continuous packed-bed bioreactor	*Brevibacillus parabrevis*	Congo red dye 150 ppm	95.71%	30 °C, 6 days	[9]
Upflow fixed-film microaerophilic–aerobic bioreactor	*Bacteroides*, *Sulfurospirillum*, *Pseudomonas*, *Macellibacteroides*	Raw textile effluents	73.08%	25–30 °C	[78]
Airlift bioreactor	*Ganoderma* sp. KU-Alk4	Indigo Carmine	100%	2 h	[79]
Packed-bed bioreactor	bacterial species	Acid orange 7 dye 300 mg/L	7.5, 87.31%	21.0 h	[80]
Pilot-scale aerobic reactor	*Bacillus* sp.	Acid orange 7 125 mg/L	98.7%, (25.0–45.0 °C) pH (5.0–9.0)	22 days	[81]
Moving-bed biofilm reactor		Congo red 25 to 300 mg/L	99.2% 37 °C	20 days	[81]
Packed-bed bioreactor	*Providencia stuartii*	Congo red 100 mg/L	85.3%		[81]
Air-lift bioreactor	*Bjerkandera adusta* OBR105	Red 120, blue 4, orange 16, and black 5) and acid dyes (red 114, blue 62, orange 7, and black 172) −200 mg L^{-1}	91–99%, 28 °C, pH 5	10–15 h	[82]
Anaerobic–aerobic biological reactor system		Reactive Red, Reactive Black, and Reactive Brown 100 mg/L	88%	96 h	[73]
Bench-scale bioreactors and lab-scale bioreactors	*Bacillus*, *Pseudomonas*, and *E. coli*	Reactive yellow 10 mg/L	100%	30 h	[73]
MBSBBR and SBR		Black B Black WNN Red 3BS 80 mg·L^{-1}	85.7% 94.2% 91.4%	60 days	[76]
Fixed-film bioreactor	*Alcaligenes* sp. BAB3053, *Bacillus* sp. BAB2731 (BDN2, KF500594), *Escherichia* sp. BAB2734 (BDN3, KF500595), *Pseudomonas* sp. BAB3054 (BDN4, KF500596), *Providencia* sp. BAB2749 (BDN5, KF500597), *Acinetobacter* sp. BAB2750 (BDN6, KF500598), *Bacillus* sp. BAB2751 (BDN7, KF500599), *Bacillus* sp. BAB3055 (BDN8, KF500600)	Reactive Red 2, Reactive Red 198, Reactive Red 120, Reactive Blue 160, Reactive Blue 13 Reactive Blue 172, 300 mg/L	99.5%, pH 7.0, 37 °C	24 h	[83]

Table 3. *Cont.*

Type of Bioreactor	Microbial Strains	Dye	Efficiency, pH	Time/Temp	References
Packed-bed reactor	*Bacillus cohnii* RAPT1	Reactive Red 120 200 ppm	pH 8.0, 35 °C, 100%	36 h	[84]
Batch and column reactor	*Bacillus subtilis*	Methylene Blue 0.25 g	30 °C, 96.17–90.11%	3 h	[85]
Continuous upflow packed-bed bioreactor	*Bacillus fexus TS8*, *Proteus mirabilis PMS*, and *Pseudomonas aeruginosa* NCH	Indanthrene Blue RS dye 100 and 300 mg L^{-1}	90%, pH-10.0, 30 °C	20 days	[86]
Packed-bed reactor	*Bacillus cohnii* RAPT1	Reactive Red 120	100% pH 8.0 and 35 °C	4 h	[84]

3.6. Enzyme Immobilization for Efficient Dye Decolorization

Immobilization is a well-known technology that aims to make it easier to separate and reuse enzymes while preserving the most active conformation. The use of immobilization enzymes, microbial strains, matrices, techniques, percentage of dye removal, and time in dye removal are given in Table 4. The enzyme is "securely attached" to the molecular rigidity-providing supports, typically solid. The capacity to reuse enzymes, streamline downstream processing, improve enzyme operational stability, and enable cascade reactions are just a few of the major benefits of immobilization, which ultimately provide cost-effective solutions. Strong biocatalysts suitable for industrial use and the environment can be made with the aid of immobilization [87]. Recently, synthetic dyes have been biodegraded using immobilized microbial cells (Figure 6). There are two types of cell immobilization: entrapment and attachment [88]. Azolereductase is typically responsible for decolourizing an azo dye, and oxygen inhibits this enzyme's activity. According to these findings, oxygen's negative effects could be reduced in the immobilized cell system, indicating that cell entrapment might provide an environment with low oxygen levels conducive to azo dye decolourization. In addition to immobilized cells, dye adsorption to the matrix material may also be a significant factor in removing dye content, affecting the effectiveness of decolourization. The extracellular environment affects how well a particular bacterial strain can remove dyes [18]. According to a previous report, a nicotinamide adenine dinucleotide (NADH)-dependent azoreductase is located in the outer membrane under anaerobic conditions. In most situations, it can operate as an electron shuttle between azo dyes and this enzyme. The reduced redox mediator will be preferentially oxidized by O_2 rather than by azo dye in an aerobic extracellular environment, which will cause O_2 to impede this reduction pathway. Immobilized cell systems could be used to solve this issue. Bacterial decolourization of azo dyes is excellent for cell immobilization by entrapment within natural or synthetic matrices because it produces a local anaerobic environment favourable to oxygen-sensitive decolourization. The immobilized cell system has several benefits over suspension culture, including the ability to be reused, a high cell concentration, the elimination of cell washout issues at high dilution rates, the provision of favourable micro-environmental conditions, an improvement in genetic stability, mechanical strength, a decrease in operational costs, and continuous removal of toxic metabolites [18,89]. Alginate, carrageen, and synthetic gels such as polyvinyl alcohol and polyacrylamide have all been utilized as cell immobilization matrices for decolourization areas. In addition to immobilized cells, dye adsorption to the matrix material may also be a significant factor in removing dye content, affecting the decolourization effectiveness [42]. The potential for anthraquinone dyes, or effluents in general, might be increased industrially by first treating them with immobilized oxidoreductases and then proceeding with advanced oxidation processes (AOPs) to reduce the energy cost of AOPs and avoid overusing biocatalysts. Researchers used immobilized laccase in epoxy-activated Sepabeads to test the decolourization of two structurally similar antraquinone dyes, Reactive Blue 19 and Acid Blue 25, and the results were remarkably different, almost 0% for Reactive Blue 19 and about 40%

for Acid Blue 25 [87]. Removing refractory contaminants from textile wastewater becomes possible by immobilizing desired particular bacteria. The effective diffusion coefficient of a substrate and the intrinsic kinetic characteristics of immobilized cells are necessary for the intelligent design of a biochemical reactor that may optimize the benefit of immobilized cells [90]. The azo dye Orange II is decolourized by unidentified white-rot fungus cells that are free and immobilized in alginate. They discovered that the immobilized fungus outperformed the free one and could be repeatedly used for over two months with a high efficiency of 97% in 24 h [88]. Methylene blue and malachite green dyes can be effectively decoloured using the *Desmodesmus* sp. (Green Algae) in free and immobilized conditions [91].The ability of immobilized cells to decolorize the reactive dyes RR195, R.O. 72, R.Y. 17, and R.B. 36 were evaluated. The white-rot fungus *Funalia trogii* (*F. trogii*), which is immobilized in alginate beads, was used to decolorize the dye Acid Black 52. Immobilized cultures were shown to be more efficient than free ones, with efficiencies of 98.8% and 88%, respectively [92]. When immobilized on chitosan beads, laccase enzyme from *Pleurotus ostreatus* IBL-02 was found to degrade Sandalfx Golden Yellow CRL dyes very effectively. The absorption of malachite green dye by *Cerrena* sp. laccase immobilized on various substrates was successful. It was discovered that immobilizing laccases on carbon supports made from lignocellulosic waste effectively degrades acid orange dye. In less than 24 h, laccase immobilized on silica with amino functions was able to remove more than 96% of reactive violet 1 dye. Numerous synthetic dyes have been cleaned up largely due to the immobilization of laccases from various sources. Immobilized laccase plays a significant role in dye discolouration, and this was demonstrated by treating the synthetic dye indigo carmine (50 g/mL) with the immobilized laccase enzyme. It was discovered that the dye was completely decoloured within 8 h, as opposed to the free laccase, which, under similar conditions, only decoloured the dye to 56%. It was shown that *Brevibacterium halotolerans* laccase immobilization in the alginate–gelatin gel had a beneficial effect on the enzyme's thermal and pH stability. It was seen that the immobilized enzymes removed Congo red dye very effectively. Additionally, it was found that after seven continuous cycles, these immobilized enzymes could still maintain up to 65% of their initial activity [93]. *F. trogii* cultures immobilized on *Luffa cylindrica* sponge could successfully decolourize Reactive Black 5 dye. Maximum uptakes of 257.3 and 180.7 mg/g dry beads were achieved by free and immobilized decarboxylated *C. glutamicum*, respectively, at pH 4 for reactive black five biosorption. The continuous biosorption of reactive black five by immobilized beads was demonstrated in column studies, with the decarboxylated biomass measured at 78.6 mg/g dry beads. The polysulfone immobilized decarboxylated biomass maintained strong reactive black five absorption values of >74.1 mg/g dry beads across all three cycles, despite earlier breakthrough and later exhaustion periods being seen with increasing cycles. The polysulfone-immobilized esterified *Corynebacterium glutamicum* performed well in the biosorption of reactive orange 16, exhibiting a higher experimental uptake of 248.1 mg/g compared to Reactive Black 5′s 174.1 mg/g. While esterified *Corynebacterium glutamicum* immobilized in polysulfone maintained comparable Reactive Black 5 uptakes in both single- and dual-dye immobilized systems, the uptake of Reactive Orange 16 was about 2.5 times more inhibited in the presence of Reactive Black 5, in contrast [94]. Using immobilized bacterial cells as the biocatalyst, the results of dye decolourization by immobilized *E. cloacae* strain SXCR cells will be helpful for further developing an efficient bioprocess for decolorizing Congo red dye [18]. Advantages and disadvantages of the immobilized system are shown in Table 4.

Table 4. Immobilization enzymes in dye removal.

Enzyme	Microbial Strains	Immobilization Matrices	Immobilization Technique	Dye	Decolorization %	Time	Reference
Horseradish peroxidase	*C. vulgaris* and *D. magna*	Cryogels		Direct Blue-6	99.6 to 7.3% 4.0–8.0	60 min	[95]
Laccase	*Trametes versicolor*	Chitosan beads		Sulphur blue 15 200 mg/L	81.6, 30 °C; 6.5;	12 h	[96]
Manganese peroxidase;	*Ganoderma lucidum* IBL-05	Calcium alginate beads		Sandal-fx black CKF 100 mg/L	60 °C; 4; 95.7	12 h	[97]
Laccase	*Alternia tenuissima*	Calcium alginate beads with chitosan beads		Dye	58%		[98]
Laccase	*Trametes pubescens*	Chitosan beads	Cross-linker glutaraldehyde	Acid Black 172	60%.	4 h	[99]
Laccase	*Trametes versicolor* IBL-04	Calcium alginate beads	Cross-linking	Reactive T Blue dye	92%	72 h	[100]
Laccase	*Trametes pubescens*	Genipin	Cross-linker	Synthetic dye	>55%	14 h	[101]
Laccase	*Ganoderma cupreum*	Amino functionalized nanosilica	Cross-linking with nanosilica	Reactive violet dye	96.76%	12 h	[102]
Laccase		Magnetic polynano-composite		Reactive blue 19	80%	1 h	[103]
Horseradish peroxidase		Activated kaolin	Adsorption	Acid red 109/40 mg/L	87%	40 min	[104]
Horseradish peroxidase		Cross-linked enzyme aggregates	Cross-link	Acid violet 109/30	70–90%	40 min	[104]
Horseradish peroxidase		Chitosan and APTS	Covalent adsorption	Alizarin red 200 mg/L	50%	4 h	[105]
Horseradish peroxidase		Adsorption	Hitosan	Reactive blue 19/100 mg/L	70%	4 h	[106]

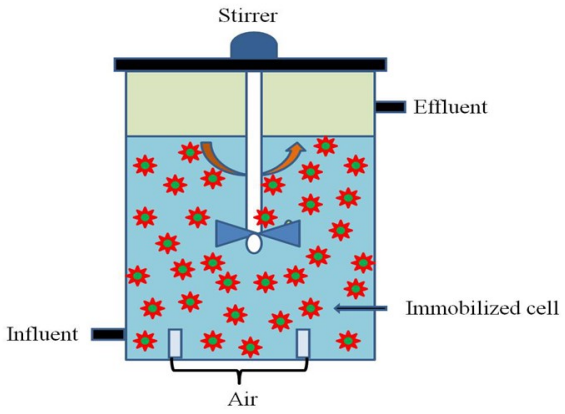

Figure 6. Immobilized cell bioreactor for dye removal.

3.7. Genetically Modified Microorganisms and Their Products

The use of genetically modified bacteria or bacteria with recombinant and overexpressing genes has gained popularity because of its ability to effectively degrade and remove environmental pollutants derived from textile dyes (Table 5) [107]. This process converts harmful substances into non-harmful forms and occasionally new products. An almost complete breakdown of synthetic dyes, including reactive black and carmine, was shown by an alkaline laccase made from a recombinant strain of *Bacillus licheniformis* in less than an hour. It was shown that the recombinant laccase decolorized more than 93% of the tested dyes in 4 h at pH 9.0 [108]. The true advantage of GMOs is their excellent capacity to hasten the process of colouration. At the same time, their drawbacks include undetermined long-term health impacts, decreased biodiversity, cross-pollination, and environmental damage brought on by horizontal gene transfer. Using genetically modified organisms (GMOs) to treat TIWWPs is a successful therapeutic strategy. Compared to other treatment options, this approach has a stronger ability to decolourize and detoxify a variety of textile dyes and wastewater. However, it negatively impacts the ecosystem because of horizontal gene transfer [56]. A strain of genetically modified *E. coli* has more active azo reductase. By employing genetically modified organisms in an environmental setting, dye degradation/decolourization can be improved. Genes can be transferred from one species to another or modified through gene editing to create GMOs. *Sphingomonas desiccabilis*, *Escherichia coli*, *Bacillus idriensis*, *Pseudomonas putida*, *Mycobacterium marinum*, *Ralstonia eutropha*, etc., are

just a few examples of the bacteria whose functional genes have been exploited to create genetically modified organisms (GMOs). Single-stranded conformation polymorphism, randomly amplified polymorphic DNA, PCR, 16S rDNA sequencing, and other modern sequencing technologies are only a few of the genetic tools and methods that can be used to determine the expression of microbial genomes [107].

There is a novel way to degrade dyes by genetic engineering, which provides a new sector for researchers working on this subject. Advanced molecular biology technologies can be used to investigate the genes and enzymes that cause dye degradation [28]. A laccase gene (Lac4) from the endophytic bacterium *Pantoea ananatis* Sd-1 was also cloned by Shi et al., 2015 and produced in *E. coli*, which has a high potential for bioremediation of the azo dyes Remazol Brilliant Blue R (35%) and Congo red (89%). By heterologously overexpressing the genes azo (azoreductase) from *Enterococcus* sp. L2 and fdh (formate dehydrogenase) from *Mycobacterium vaccae* in several bacterial cultures, Rathod et al., 2021 aimed to increase the efficacy of azo dye degradation. Additionally, Liang et al., 2018 created two *Synechococcus elongatus* PCC7942 recombinant bacteria by heterologously expressing the CotA laccase gene from *Bacillus subtilis* to degrade various textile azo dyes [108]. Since it is widely known that the enzyme azoreductase, which is encoded by the gene azoA, plays a vital role in the degradation of azo dyes, ref. [109] developed the bacterial strain *Escherichia coli* JM109 (pGEX-AZR), which demonstrated improved azoreductase activity in the degradation of C.I. Direct Blue 71 dye.

To decolourize sulfonated azo dyes, ref. [110] combined two photosynthetic bacterial strains—*Rhodobacter sphaeroides* AS1.1737 and *Rhodopseudomonas palustris* AS1.2352—with a gene-engineered *E. coli* Y.B. strain expressing an azoreductase gene with ten times better azoreductase activity [107]. Azoreductase gene from *Bacillus latrosporus* RRK1 was transferred to *Escherichia coli* DH5a and Plasmid pAZR-SS125 by [111] to develop *Escherichia coli* SS125 for the breakdown of Remazol red dye. Ref. [7] described how the azoreductase gene, which was copied from *Bacillus latrosporus* RRK1 and inserted into *Escherichia coli*, helped to degrade Remazol red in the presence of 0.8 mg/L to dissolve oxygen. *Anoxybacillus rupiensis* used at 60 °C resulted in 75% wastewater degradation. According to reports, the rate of decolourization declines as temperature increases [28]. Consequently, an efficient treatment approach involving genetically modified organisms (GMOs) may provide a potential treatment. Many scientists have created many GMOs (transgenic strains) that are utilized to treat TIWW. For example, the LacTT gene from *Thermus thermophilus* SG0.5JP17-16 was introduced into *Pichia pastoris*, increasing its ability to remove reactive black, Congo red, reactive black WNN, and Remazol brilliant blue R. Bromophenol blue, mordant black 9, cotton blue, azo phloxine, reactive brilliant blue K-GR, Congo red, reactive brilliant blue KN-R, malachite green, and reactive dark blue M-2GE from TIWW were all rapidly degraded using a thermo-alkali-stable laccase gene that was purified from *Klebsiella pneumoniae* [56]. *Escherichia coli* JM109 (pGEX-AZR), introduced gene(s), azo dyes, and C.I. Direct Blue 71 are examples of genetically modified bacteria, according to [109]. To decolourize wastewater from the textile printing industry, recombinant CueO from *E. coli* K12 was expressed in *Pichia pastoris*. Recombinant *E. coli* CotA laccase can lighten simulated textile effluents (STEs). In pure and unpurified forms, seven structurally different dyes were successfully decoloured by recombinant CotA laccase. Pure and crude CotA laccase decolourization rates were greater when STE was buffered at neutral pH [112]. Cloning, heterologous expression, random mutagenesis, site-directed mutagenesis, gene recombination methods, directed evolution, rational design, and metagenomics are examples of molecular biology tools that can speed up evolution processes and improve bioremediation. When compared to other laccases, the Lac gene from the white-rot fungus *Trametes* sp. was cloned in *Pichia pastoris*, and the purified recombinant Lac had a greater ability for decolorizing diverse dyes, such as Methyl Orange and Bromophenol Blue [68]. Moreover, genetic engineering or recombinant DNA technology can be employed to clone genes of previously identified derivative enzymes into a single microbe to increase the decolourization efficiency [113].

Although it is widely accepted that an azoreductase enzyme produced in azo-dye-degrading bacteria stimulates decolourization, the genes encoding for azoreductase and other potential decolourization-related proteins, such as transport proteins, have yet to be found or sequenced, e.g., selection, cloning, and expression for *E. coli* azoreductase.

Establishing a bank of genetic modules expressing wide-specificity enzymes or pathway segments, which may be joined to build new or enhanced degradation pathways, has facilitated the development of GEMs with numerous degradation pathways in *E. coli*. Mobilizing certain genes that encode nonspecific multifunctional degradative sequences might significantly boost the natural synchronic community's degradative ability against manufactured dyes. The mineralization of dyes by a single organism can be accomplished by constructing hybrid degradative pathways via natural or artificial gene transfer [114]. Genetic engineering aimed to create a host system with changed enzyme activity to allow for resistance and on-site breakdown of the desired contaminant. To develop GEMs to degrade environmental pollutants, bioreporter sensors have been proposed [14].

The following significant aspects influence how GEMs are used in the environment:

- Natural microbial community acquisition of foreign genes;
- Survival efficiency and stability in natural ecosystems.

Four primary methods are relied on: (1) changes in enzyme nature; (2) management of metabolic processes that cause degradation; (3) creation, evaluation, and regulation of a biological pathway; and (4) affinity development [14].

Genetic engineering is a valuable approach for improving the capacity of microorganisms to produce bioremediate wastes in the environment [115]. Microorganisms and enzymes have been genetically modified to improve biogas generation. For enhanced decolourization and detoxification of azo dyes, several bacterial strains should be researched to create the transformation of fungal laccases or oxidase genes into the bacterial genome. *Comamonas testosteroni* VP44 has been found to reduce harmful environmental pollutants by genetically modified bacteria employing diverse recombinant DNA technologies, pathway change, and substrate specificity conversion. By putting known genes of helpful enzymes into a fast-growing bacterial strain, genetic engineering techniques can be utilized to improve microorganisms' biodegradation capabilities [110]. As a result, genetic profiling/engineering may be used to create well-directed optimal hybrid strains that can degrade or change a particular type of dye [116].

Table 5. Decolouration of azo dyes using genetically modified microorganisms.

Genetically Modified Microorganism	Dye	Gene Expressed from	Extracted Gene	Vector	Gene Extracted in	References
E. coli BL21 (DE3)	Azo dye wastewater	*Halomonas* sp. *strain* GT	AzoG gene	pET30a (+)	*E. coli* DH5α	[6]
Escherichia coli BL21	Methyl Orange	*K. pneumoniae* MGH 78,578	AzoK gene	Vector pGEM-T	*E. coli* DH5α	[117]
E. coli BL21 (DE3)	Methyl red and Remazol Black B	*Halomonas elongata*	Azoreductase gene	Vector pET21a	*E. coli* DH5	[22]
Pichia pastoris	Amido Black 10B (62%), Remazol Brilliant Yellow (58%), Rhodamine 6 G (43%), crystal violet	*M. mycetomatis*	M. mycetomatis laccase-coding genes	pPICZαA and pPICZA	*P. pastoris* X33	[12,118]
E. coli DH5	Synthetic dyes	*E. coli* DH5	Laccase CotA	pMD18-T	*B. subtilis*	
E. coli K12	Malachite green	*P. pastoris* GS115	Laccase CueO	pHBM905BDM	*E. coli* K12	
Escherichia coli CY1	Reactive red 22	*Rhodococcus* sp.	Azo-dye-decolorizing genes	Plasmid pAZRS1	*Escherichia coli* DH5*a*	[119]
Escherichia coli SS125	Remazol red	*Basillus latrasporus* RRK1	Azoreductase gene	Plasmid pAZR-SS125	*E. coli strain* DH5*a*	[111]
Escherichia coli JM109 (pGEX-AZR)	Direct blue 71	*Rhodobacter sphaeoides* AS1.1737	Azoreductase gene	pGEX4T-1	*E. coli* JM*109*	[109]

Synthetic dyes, chemicals, acids, salts, and the solutions associated with them have a profound impact on the environment. Increasingly, future research will delve into the realms of environmental pollution, degradation, and the formulation of innovative strategies to address these challenges. The development of high-potential advanced technologies will play a pivotal role in conducting industrial-level biological treatment of waste, providing a more accessible means to combat environmental pollution.

Future endeavours must focus on identifying novel, highly efficient microbial species capable of controlling dye waste pollution. Additionally, efforts should be directed towards expediting the biodegradation process, enhancing research output in this field. While past research laid foundations and recent studies aimed at boosting the efficiency of dye degradation, there is a critical need for more innovative and comprehensive reviews. A compelling imperative lies in pioneering novel strategies for dye degradation at an industrial scale, building upon laboratory-level waste treatment practices from the past. Future work should explore methods to preserve and reuse water quality adversely affected by dye waste. The examination of alternative biological species, reactive enzymes, metabolites, influencing factors, and the release of toxic or non-toxic substances into the environment demands thorough investigation in the dye waste treatment domain.

Advancements in genetic engineering, biochemistry, molecular biology, and genomics will spearhead the future of dye degradation research. Recent studies have unveiled the dual functionality of microalgae, showcasing its potential to remediate industrial wastewater, such as textile wastewater (TWW), while concurrently producing biodiesel. Although many published articles remain at the laboratory- or pilot-scale level, indicating the need for further exploration, these findings open promising avenues for future research. CO_2 balance in the environment: Algae biodiesel has the potential to be a carbon-neutral fuel source as it consumes carbon dioxide from the surrounding air while growing. Renewable energy and electricity production: Algae are abundant providers of renewable energy that might decrease our dependence on non-renewable fossil fuels. Effective use of land: By allowing algae to grow where traditional crops cannot, biofuel cultivation can make use of land that would otherwise go unused. Biofuel could become a more affordable fuel alternative. Waste use and production: Waste materials from other industries, such as wastewater and carbon dioxide, can be used to grow algae. Consequently, the development of algal biomass production in wastewater and the application of biomass for the creation of commercially important products determined the efficacy of the use of microalgae.

4. Comprehensive Study of Advanced Biological Techniques

Comparative studies, scientific challenges, and possibilities related to wastewater treatment using microorganisms are listed in Table 6.

Table 6. Comprehensive study of advanced biological methods.

Methods	Advantages	Limitations	References
Phytoremediation	No requirement for maintaining isolation and preparing culture media, cost-effective, stable, safe, non-toxic, ecologically friendly, laboratory setup is not necessary.	The short root structure of aquatic plants, which limits the depth of soil treatment by the rhizosphere, is a major drawback of phytoremediation. Time-consuming in the slow progress of the environmental remediation procedure.	[35]

Table 6. *Cont.*

Methods	Advantages	Limitations	References
Biofilm Reactors	Fast and cheap. The moving-bed biofilm reactor uses less electrical energy than the activated sludge process. Reducing the biological reactor's volume or handling a higher organic load in the same reactor volume is one of the key advantages of the MBBR system.	The drawbacks of bacteria living in biofilms are as follows: (a) In comparison to free-living planktonic bacteria or actively moving bacteria, bacteria that are fixed in biofilms appear to be more vulnerable to grazing. (b) The reduction in reactor volume is limited because the biomass concentration in practice has a limit because of the complex structure and dimensions of biofilms, which lead to different types of gradients. (c) Needs a specific quantity of coagulants to be added.	[120,121]
Microbial Fuel Cells (MFCs)	High output. Produces valuable products, including biofuels. Applies to wastewater treatment.	Heterogeneous catalysts always require long reaction times because the presence of three immiscible phases at the beginning of the reaction significantly increases the mass transfer limitation within the system.	[122]
Microalgae through Dye Treatment and Biodiesel Production	Our dependence on non-renewable resources can be decreased and the fight against climate change increased by the use of this renewable energy source. Capable of growth in any kind of situation. Put in place a productive cycle for cycling nutrients. Reduce back on greenhouse gas emissions.	Open-pond farming activities are severely impacted by high temperatures. In order for algae biodiesel to be produced, transported, and used effectively, large financial investments must first be made. Challenges of stability and contamination. Requirements for using water and land. With the amount of cash and time it takes to cultivate, its manufacturing might become costly.	[123,124]
Bioreactors	The advantages of handling MBR include controlling the biofouling effect, reducing energy consumption during operation, and reducing water costs by reusing the treated water for other processes. It also helps to establish the ideal conditions for operating MBR for high-strength industrial wastewater and shock-loading rate.	Contamination chance. Concerning limitations are those related to pH, temperature, pressure, and some caustic chemicals. Excessive membrane cost, which raises the expense of operations and maintenance. The cost of the membrane includes cleaning procedures and replacement of severely fouled or corrupted membranes during maintenance.	[125]
Immobilization System	Simple retention, simple enzyme removal from the product. The enzyme can be reused. Prolonged durability. Enhanced tolerance to external stressors. Enhanced functionality efficiency. Enhanced degree of continuous operation. improved process control enhance the catalytic mechanism.	These limitations, which can be summed up as mass transfer effects, result in less effectiveness. High cost-to-income ratio. Limited application. Limits connected to carriers. System inactivation brought on by heat generation.	[126]
Genetically modified microorganisms or enzymes	The excellent capacity of GMOs to speeds up the colouring process is the real advantage.	Their disadvantages include decreased biodiversity, cross-pollination, unknown long-term health effects, and environmental harm from horizontal gene transfer.	[56]

Challenges of Azo Dye Wastewater Treatment

The primary challenges for the foreseeable future will be to strike a balance between the demands of algal development, rising production costs, and higher-value uses of the algal biomass generated. Large-scale algal systems can have several issues, such as excessively high productivity, pollution, evaporative water loss, and lack of control. Therefore, research should concentrate on reducing these disadvantages. Running a PBR at a reasonable cost is another difficulty. We can clear the path for more commercially successful and environmentally friendly production methods by tackling the issues raised, such as high production costs, restricted scalability, and energy-intensive harvesting. To improve the total efficiency of biodiesel production, for example, the development of cost-effective harvesting systems and optimized use of algae culture techniques would be

needed. Overall, the results highlight the difficulties and possibilities involved in producing algae biodiesel. The optimization of cultivation and harvesting processes is essential for it to be financially feasible. However, the best technique would be chosen based on several factors, including effluent conditions, dye type, operating situations, required treatment quality, prices, flexibility, and environmental impact. The biological treatment method is an unpredictable and time-consuming one. Overall, the practical integration of algae biodiesel into our energy environment is highly promising when the study's conclusions are applied. We may promote a greener, more sustainable energy future that depends on renewable resources and decreases our reliance on non-renewable fossil fuels by facing the challenges and recognizing the opportunities that lie ahead. During immobilization, a biofilm will develop to serve as a barrier and limit the mobility of the microalgae cells. Encapsulated biomass, on the other hand, exhibits reduced cell leakage but has diffusional constraints. Encapsulated biomass, on the other hand, exhibits reduced cell leakage but has diffusional limits. One major drawback of using aquatic plants for phytoremediation is that their short root systems mean that the rhizosphere can only treat soil to a limited depth. A further drawback is the lengthy, possibly over ten-year process of environmental cleaning, which proceeds slowly.

5. Conclusions

The textile industry predominantly employs synthetic and azo dyes, contributing significantly to environmental threats amidst global industrialization. Over the last two decades, the surge in textile production has intensified concerns about environmental issues. Discharging untreated dye waste directly into the environment poses a substantial risk to soil and water quality. Degradation, involving the breakdown of the structure and composition of dyes and their chemical derivatives in wastewater, becomes imperative for environmental preservation. Synthetic dye and its waste not only pollute water sources but also exert detrimental effects on aquatic ecosystems, flora, and human health. To address this critical issue, primarily physical, chemical, and biological methods are employed. While chemical and physical treatments exhibit high efficacy, they are often environmentally inappropriate, costly, and generate highly toxic dye secondary metabolites through complete mineralization. To overcome these drawbacks, there is a demanding need for environmentally friendly, cost-effective, highly efficient degradation strategies, and green technologies.

The biological method emerges as the optimal alternative for dye waste treatment. Enzymatic dye degradation through complete mineralization stands out as it avoids the production of secondary polluted intermediates. Bacteria, fungi, algae, plants, and yeasts play pivotal roles in dye degradation through biodegradation. Microbial degradation in both aerobic and anaerobic conditions yields favourable results. Biodegradation strategies, including enzymatic catalysis, biosorption, and bioaccumulation, contribute to the biological reduction of dyes. Immobilizing microbial cells in calcium–alginate beads is a technique employed to shield them from the adverse effects of dyes or changes in environmental conditions. Enzyme-based nanotechnology is evolving, evident in its continued application across various domains using diverse combinations of enzymes, support materials, and immobilization techniques. Enzyme immobilization is a beneficial technique for achieving both financial and scientific goals. Enzyme reuse made possible by immobilization results in significant savings in costs.

Accumulation of dyestuff and dye effluent not only pollutes the environment but also poses health and cosmetic concerns. Future investigations should focus on developing bioremediation methods based on pure and mixed cultures, where microbial strains are genetically engineered to express desired traits, utilizing tools and techniques from genetic engineering, proteomics, and metabolic studies. This approach holds promise for advancing sustainable and effective solutions to tackle dye-related environmental challenges. One of the most significant biological techniques for removing the colourful dyes found in textile effluent is phytoremediation. One of the main producers in aquatic environments like

rivers, lakes, and oceans is algae. Algae absorb nutrients from water and solar energy for photosynthesis, which turns carbon dioxide into organic matter. Thus, there has been an increasing interest in the use of microalgae and other emerging biological techniques for wastewater treatment.

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