



Review article

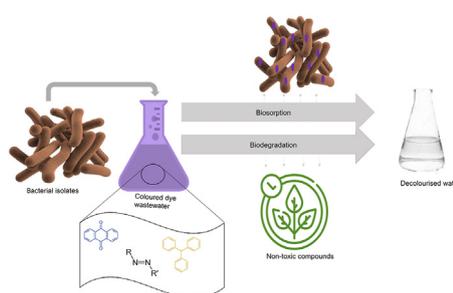
Use of bacterial isolates in the treatment of textile dye wastewater: A review

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HIGHLIGHTS

- Bacterial isolates offer environmentally friendly solution to dye degradation.
- Pure and mixed bacterial cultures can remove textile dyes in optimised conditions.
- Dyes are removed through biosorption or biodegradation mechanisms.
- Latest technologies provide more effective dye removal options.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

Bacterial isolates
Textile wastewater
Dye degradation
Dye decolourization

ABSTRACT

The textile industry uses large amounts of dyes like reactive, azo, anthraquinone, and triphenylmethane to colour textiles. Dyes that are not used up during the colouration process usually end up in water bodies as waste leading to the pollution of the water bodies. This makes the industry to be one of the major contributors to water pollution in the world. Bacterial agents isolated from various sources like dye contaminated soil and textile wastewater have shown to have the ability to effectively decolourise and degrade these dye pollutants leading to improved water quality. This review discusses bacterial isolates that have been used successfully to degrade and decolourise textile dyes, their mode of dye removal as well as the factors that affect their dye degradation ability. It further looks at the latest wastewater treatment technologies that incorporate bacterial microorganisms to treat dye wastewater.

1. Introduction

The textile industry is one of the oldest and important manufacturing industries in the world that has contributed immensely to the development of many economies, employing approximately 35 million people around the world [1, 2]. Despite its undeniable importance the textile industry is unfortunately one of the highly polluting industries. The wet processing sector of the textile industry is responsible for preparatory processes like desizing, scouring, bleaching, dyeing as well as finishing of

textiles. These processes consume large amounts of chemicals in the form of alkalis, salts, surfactants, dyes, pigments, and water [2]. Water is an important yet scarce resource needed for human survival and it is estimated that globally one in three people lack access to clean water, and one of the main contributing factors to the lack of clean water is partly due to industrial pollution [3, 4]. An average textile mill producing about 8 000 kg of fabric per day consumes about 1.6 million litres of water with dyeing and printing sections consuming 24% of this total [3]. The textile and dyeing industry is estimated to consume about 700 000 tonnes of

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Received 24 August 2021; Received in revised form 25 October 2021; Accepted 26 May 2022

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dyes per year and of all the dyes used, between 10-15% remain unfixed, ultimately finding their way into water bodies [5]. Textile dyes when discarded into water bodies not only affect the appearance of the water but also the stability of aquatic environments as the dyes prevent light from penetrating through the water. This leads to reduced oxygen levels in the aquatic environment which negatively affects aquatic animals inhabiting that environment [6]. The disposal of untreated textile dye wastewater has therefore become a major concern in many countries due to the dangers that these dye pollutants pose both to human and aquatic life.

A number of physicochemical, electrochemical and biological treatment methods like adsorption, coagulation-flocculation, membrane technologies, fenton-oxidation, ozonation, electrolysis have been used to remove dyes in textile effluents [7, 8]. These methods have their own advantages and disadvantages in relation to efficiency, cost-effectiveness and byproduct waste production as summarized in Table 1, which might explain why a combination of different methods is normally used in the treatment of textile dye wastewater to achieve maximum efficiency. In an attempt to come up with more environmentally friendly and economic treatment methods, there has over the past few years been a major interest in the use of microbial agents to treat dye effluents [9, 10]. This has largely been due to the advantages that biological wastewater treatment methods have over traditional wastewater treatment methods as shown in Table 1.

A quick search (use of bacterial agents to degrade dyes) in the Science Direct platform shows that over the past five years there has been over 9 000 research papers published on the subject as shown in Figure 1, highlighting the interest that the subject has generated amongst researchers.

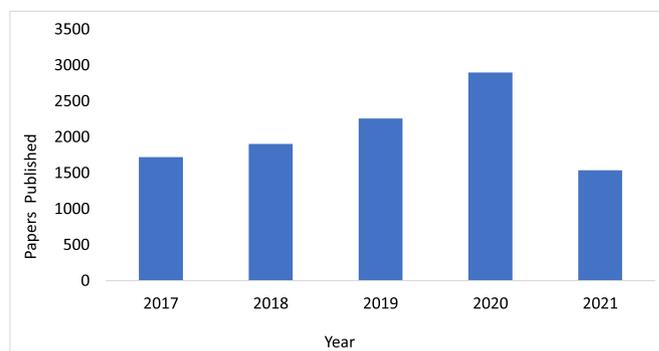


Figure 1. Distribution of research papers on bacterial degradation of dyes published between 2017-2021 on the Science Direct platform.

There are various microbial organisms that have been used to treat dye wastewater like algae, yeast, fungi, and bacteria. Algae, yeast, and fungi have traditionally been the preferred microbial agents for dye wastewater remediation. However, due to their longer growth cycles and low efficiency in decolourising dyes, bacterial organisms have since gained more attention [21, 22]. Bacterial organisms have the advantage of shorter growth duration as well as the ability to degrade and mineralize dyes. Several studies have been reported on the optimization of bacterial organisms for wastewater treatment as shown in Table 2. Some of the studies have focused on identifying bacterial strains that can be used to degrade and decolourise dye wastewater as well as try to understand their mechanism of action. Therefore, the main objectives of this review are to 1) Explore the use of bacteria in pure or mixed cultures

Table 1. Advantages and disadvantages of different dye wastewater treatment methods.

Method	Rationale of method	Advantages	Disadvantages	Reference
Biological Methods	Uses different microbes like bacteria fungi, algae, yeast and enzymes to degrade and decolourise dyes	<ul style="list-style-type: none"> - Simple, economically attractive and environmentally friendly process - Large number of species can be used in consortiums or pure cultures e.g. bacteria, fungi - Good dye removal efficiency - High removal of biochemical oxygen demand and suspended solids (BAS) - Anaerobic bacteria are suitable for large scale application 	<ul style="list-style-type: none"> - Requires optimally favourable environment - Requires management and maintenance of the microorganisms and/or physicochemical pre-treatment - Slow process - Generation of biological sludge and uncontrolled degradation products 	[10, 11, 12]
Adsorption	Uses different types of adsorbents like plant biomass and activated carbon to remove pollutants through the process of adsorption	<ul style="list-style-type: none"> - Good dye removal efficiency - Short reaction time 	<ul style="list-style-type: none"> - Challenge in regeneration of adsorbents, - Generates toxic by-products: no mineralisation of dyes - Not applicable to all dyes - Requires precise control of process conditions e.g pH 	[11, 13, 14]
Coagulation	Uses coagulants such as alum to form flocs that settle colloidal particles together with dye molecules	<ul style="list-style-type: none"> - Technologically simple process - Highly effective in eliminating metals - Good dye removal efficiency 	<ul style="list-style-type: none"> - Large chemical consumption - Produces sludge - Requires precise control of process conditions e.g. pH 	[11, 15, 16]
Advanced oxidation	Uses free radicals mainly hydroxyl radicals generated from ozone, UV radiation and hydrogen peroxide to degrade dye molecules	<ul style="list-style-type: none"> - High dye removal efficiency - possibility of dye mineralisation - Short reaction time - Low chemical consumption - No production of sludge - Dye mineralisation 	<ul style="list-style-type: none"> - Cost intensive - Produces undesirable by-products - Requires precise control of pH 	[11, 17]
Membrane filtration	Uses pressure to remove pollutants by passing them through a membrane with a defined pore size either through macrofiltration, microfiltration, nanofiltration or reverse osmosis	<ul style="list-style-type: none"> - Simple process - Highly efficient - Environmentally friendly: no chemicals are used - Easily applicable in actual industrial applications 	<ul style="list-style-type: none"> - Membrane fouling - High energy and maintenance costs - High initial setup costs - Poor dye mineralisation 	[11, 18]
Electrochemical treatment	Uses oxidants generated in-situ via redox reactions on the surface electrodes to remove pollutants	<ul style="list-style-type: none"> - Highly effective in removing organic pollutants - Economic process - Easy to control process pH - Process does not use chemicals 	<ul style="list-style-type: none"> - Cost intensive: High energy consumption - Generates secondary products 	[11, 19, 20]

Table 2. List of bacterial strains that have been used to successfully decolourise and degrade textile dyes.

Bacterial Strain	Target Dye(s)	Treatment Conditions (Temp, pH, Initial conc, Time)	Efficiency	Reference
Consortium: <i>Neisseria</i> sp., <i>Vibrio</i> sp., <i>Bacillus</i> sp., <i>Bacillus</i> sp. and <i>Aeromonas</i> sp	Reactive dyes: Novacron Orange FN-R, Novacron Brilliant Blue FN-R, Novacron Super Black G, Bezema Yellow S8-G and Bezema Red S2-B	37 °C, pH 7, Static conditions	Monoculture: 0–90% Consortium 65%–90%	[46]
<i>Alcaligenes faecalis</i> AZ26, <i>Bacillus cereus</i> AZ27 and <i>Bacillus</i> sp	Novacron Super Black G (NSB-G),	37 °C, pH 8.0, 200 mg/L 96 h, static conditions	90%	[47]
<i>Pseudomonas aeruginosa</i> (RS1) and <i>Thiosphaera pantotropha</i> ATCC 35512	Reactive Yellow 145 (RY145)		96 and 72 h	[48]
<i>Staphylococcus</i> sp. K2204	Remazol Brilliant Blue R (RBBR),	100 mg/L 37 °C, 12 h, static condition	100%	[38]
<i>Bacillus megaterium</i> KY848339.1	Acid Red (AR337)	pH 7, 30 °C, 24 h, 500 mg/L	91%	[49]
<i>M.yunnaensis</i>	Methyl Orange	pH 7, 30 °C, 100 mg/L	98%	[50]
<i>Acinetobacter baumannii</i> JC359	Reactive Black 5 (B-GDN), Reactive Red 120 (RP) and Reactive Blue 19 (RNB)	PH7,7, 9 (RP, B-GDN and RNB) 37 °C, 48 h, 500 mg/L	98.8% for B-GDN, 96 % for RP and 96.2% for RNB	[51]
<i>Acinetobacter baumannii</i>	Reactive Red 198	pH 7, 37 °C, 72 h, 200 mg/L	96.20%	[52]
<i>Bacillus stratosphericus</i> SCA1007	Methyl Orange	pH 7, 35 °C, 12 h, 150 mg/L static conditions	100%	[53]
<i>Streptomyces</i> DJP15	Reactive Blue 222	pH 7, 35 °C, 48 h, 50 mg/L, static conditions		[54]
<i>Bacillus pseudomycolides</i>	Acid Black 24	pH 7, 37 °C, 24 h, 40 mg/L	96%	[55]
<i>Escherichia coli</i> NG188	Remazol Brilliant Blue	pH 8, 40 °C, 72 h, 50 mg/L, static conditions	72%	[56]

in the biodegradation of the three mainly used dye types in the textile and dyeing industry i.e., azo, anthraquinone, and triphenylmethane dyes. 2) Identify bacterial strains that have been successfully isolated and used to degrade these types of dyes and their mechanism of dye removal. 3) Discuss the latest trends in bacterial dye degradation. 4) Discuss the factors that affect bacterial biodegradation efficiency. This article has reviewed relevant literature sourced from scholarly scientific databases in relevant fields. Several articles were accessed and screened resulting in a total of 156 articles that were deemed relevant, with distribution by discipline of 7.7% Textile Science and Engineering, 43.3% Environmental Science and Management, and 43.3% Biological Sciences, and 5.8% Chemical Engineering and Technology.

2. Types of dyes used in the textile industry

There are different types of dyes used in the textile industry. These are most commonly classified according to their: source, chemical structure, and industrial application [23]. The two sources of dyes are: natural and synthetic dyes. Natural textile dyes are obtained from plants, insects and minerals, while synthetic dyes are derived from organic and inorganic compounds, generally prepared from petroleum by-products or earth mineral types of synthetic resources [24]. Synthetic dyes are the most utilised in the textile industry compared to natural textile dyes. Their popularity emanates from their solubility in water, ease of absorbance and very fast colouration compared to natural textile dyes [21]. Natural textile dyes also have numerous other challenges like a low degree of fixation, poor colour fastness, and narrow shade range [25]. However, natural textile dyes may still be utilised for their beneficial functional properties to textiles such as: anti-microbial, anti-bacterial, anti-inflammatory, anti-insect, UV protection and deodorising characteristics [26].

Classification of dyes by chemical structure focuses on the nature of the chromophore grouping; these classes include: triphenylmethane, phthalein, triphenyl methyl, nitrated, anthraquinone, azo, indigo, azine, xathene, nitro, oxazine, phthalocyanine and triarylenethane, azo, anthraquinone, indigo, xanthene, phthalocyanine, nitrated and nitrosated, diphenylmethane, triphenylmethane, azine, xathene, nitro, oxazine,

diarylmethane and polymethic dyes [24]. Figure 2 shows dye structures of some commonly used dyes in the textile dyeing industry. Amongst these dye classes azo, anthraquinone and triphenylmethane dyes are the most widely used. The main challenge with the structure of these dyes is their stability due to their aromatic nature, which makes them difficult to degrade.

2.1. Textile dyes and the environment

The textile industry is one of the global economic drivers; however, it is also one of the largest consumers of high amounts of chemicals, energy and water [27]. The textile industry uses over 8 000 chemicals, most which are hazardous to the environment and human health. The negative environmental impact of the textile industry is mostly as a result of untreated effluent discarded into the water bodies which accounts for 80% of the total industry emission [28]. What further complicates the situation is the presence of non-biodegradable organic compounds from textile dyes in the effluent. According to Yaseen and Scholz [29] textile wastewater consist of dyes, suspended solids, raw materials, inorganic salts and other chemicals. Without proper and effective wastewater treatment processes, the dyes and the other constituents of textile wastewater escape into the environment [23]. The colouring in wastewater is due to incomplete exhaustion of dyes during the dyeing process leading to excess dyestuff being discharged into the environment [30]. In addition to dyes, wastewater also contains other toxic substances such as sulphur, naphthol, nitrates, acetic acid, soaps, chromium compounds, and heavy metals like copper, nickel, arsenic, lead, cadmium, mercury and cobalt, formaldehyde based fixing agents and hydro-carbon based softeners. These pollutants, when washed-off into water bodies, are detrimental to the environment, cause deterioration of the ecological balance and are also hazardous to humans [31].

Textile effluent affects the environment in a number of ways: the dyes and pigments in the textile effluent change the water colour, and pH, cause high levels of biological and chemical oxygen demand (BOD, COD) as well as an increase in total organic carbon and suspended solids. All these effects interrupt and inhibit the ecological processes of aquatic life [31], affect the aesthetic quality and transparency of water bodies, which

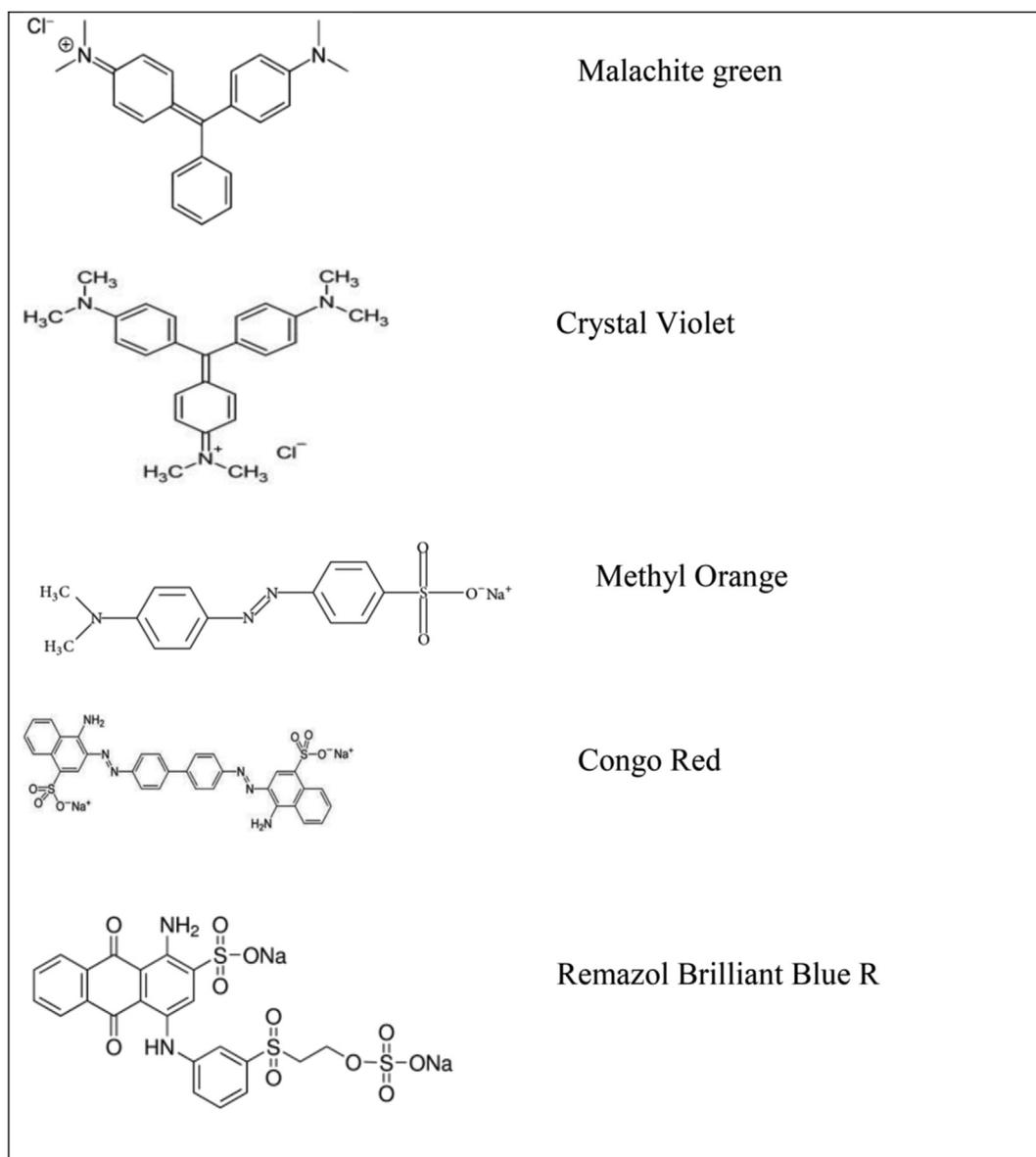


Figure 2. Some common textile dye structures.

obstructs light penetration into deeper layers of water bodies reducing photosynthesis [23]. Consequently, the above effects cause oxygen depletion and under oxygenation of stagnant aquatic environment, deterioration of water quality, lower gas solubility which may be toxic to the aquatic flora and fauna [23]. Other negative impacts include bacterial proliferation, and pestilential odours. The contamination of the environment with dyes may accumulate biologically throughout the food chain in aquatic organisms posing, a danger to species at the high end of the food chain such as humans [32]. These toxic substances from dyeing effluent in wastewater persist in the water bodies due to their non-responsiveness to biodegradation, light, temperature changes, detergents, chemicals, soaps, and bleaches. The toxicity of dyes in the wastewater is also capable of altering the soil by destroying the micro-organisms which influences agricultural productivity. Irrigating with the liquid effluent of the textile industry may clog soil pores and harden the texture, thus preventing root penetration [33].

Textile dyes and textile industry pollutants are also highly toxic, carcinogenic and mutagenic posing high risk to human health [27]. Exposure to these dyes and pigments in water leads to respiratory sharpening which in extreme cases may also affect a person's immune

system. Symptoms of the respiratory problems include itchy and watery eyes, sneezing and general symptoms associated with asthma such as coughing and wheezing. These together with skin irritation, may also be caused by other chemicals contained in dyes [33]. Some of the synthetic substances used in the textile industry like some optical whiteners, soda ash, caustic soda and bleach as well as formaldehyde-based resins, ammonia, acetic acid, and some shrink-resistant chemicals are other chemicals with health related side effects. When absorbed through the skin they may result in allergic reactions such as irritated and blocked noses, sniffing, and sore eyes [31]. Furthermore dye effluents contain trace metals which can also cause severe skin irritation, dermatitis, ulceration of skin and nausea on exposure.

Globally there are wastewater regulatory organs that seek to control the number of hazardous substances discharged into the environment. The standards and industry guidelines vary in terms of the methods used to measure wastewater constituents. Effluent discharge parameters are also influenced by the source, characteristics, and intended use of the treated wastewater. The wastewater quality parameters are: pH between 5.5-9, Carbon Oxygen Demand of 20 mg/l, Biological Oxygen Demand of less than 60 mg/l, oil and grease of 10 mg/l, and total suspended

substances not exceeding 100 mg/l. The aim of treating wastewater is to minimise the number of hazardous substances such as Nonylphenol Ethoxylates (NPEOs & NPEs), azo dyes, brominate and chlorinated flame retardants, chlorobenzenes, chlorophenols, chlorinated solvents, heavy metals, organotin compounds, perfluorinated chemicals, phthalates, and short-chained paraffins to allowable levels [34]. The documented textile industry's undesirable effects on the environment and mankind indicate a need to adopt sustainable dyeing practices. These may include optimising the dyeing process, improving the wash fastness, identifying and utilising innovative, effective and economical methods for treating textile wastewater prior to discarding it into the environment. Reducing the process temperature may also economise energy.

3. Bacterial strains used in degradation and decolourisation of textile dyes

Bacterial strains have been used successfully to degrade and decolourise dye containing textile effluents with focus being targeted at azo dyes [35, 36, 37], anthraquinone [38, 39, 40] and triphenylmethane based dyes [41, 42], largely because of their hazardous nature and their large consumption rates within the industry. Most of the bacterial species that have been used to degrade and decolourise dyes have been isolated from soil collected from areas surrounding the textile manufacturing plants [43], textile effluent sludge waste [44], and dye contaminated textile wastewater [45]. Bacteria from such locations, due to its exposure to the extreme conditions created by the textile effluent waste become acclimatised to these conditions rendering them potential dye degrading agents. A variety of bacterial strains have been identified as potential candidates for the degradation and decolourisation of different dyes. Table 2, shows some of the strains that have been successfully used to degrade different types of dyes. Most of the identified strains have been shown to achieve effective dye degradation efficiencies at optimum conditions. Since dye decolourisation and degradation are dependent on various environmental and nutritional factors, the optimization of these factors plays an important role in the effective degradation of dyes. It is also important to note that optimized simulated conditions might not be applicable to real industrial textile wastewater which is more complex as it contains various chemicals that affect the interaction between the bacteria and the dye compounds.

It is important to note that the bacteria chosen to degrade a particular dye should be able to decolourise and degrade the dye to its simplest compounds, in order to render it non-toxic to both animal and plant life. The biodegradation of dyes by microorganisms is made possible by enzymes found in the cell structures of the organisms. Different types of enzymes have been identified in the biodegradation of textile dyes, with laccases, lignin peroxidase and azoreductase enzymes having been shown to have the greatest potential in degrading different types of synthetic dyes [57]. Azo reductase enzymes are responsible for the degradation of azo dyes through the reduction of the $-N=N-$ azo bond under anaerobic conditions into colorless toxic aromatic amines. The amine metabolites can be further reduced aerobically or anaerobically into corresponding simpler environmentally friendly metabolic compounds [58]. Ewida et al. [49], through Liquid Chromatography-Mass Spectrum analysis, showed that *Bacillus megaterium* KY848339.1, a bacterial strain isolated from textile wastewater, was able to completely degrade Acid red 337 azo dye into small aliphatic compounds and CO_2 . The bacterium was able to remove 91% of the dye in 24 h in an initial dye concentration of 500 mg/L. This is an ideal result because azo dyes are toxic and the ability of bacteria to degrade the dyes into non-toxic metabolites makes them even more attractive as dye biodegradation agents. Similarly, Sivasubramani et al. [59], discovered that an azo dye Reactive blue 194 could be degraded with an efficiency of 92.3% by a strain from the same family, *Bacillus megaterium* PNS 15 using azoreductase and laccase enzymes. As already pointed out, these enzymes have been identified as the ones responsible for the degradation of azo dyes, further, Solis et al. [37] indicated that

any microbial species capable of secreting oxidase and reductase enzymes are able to degrade azo dyes.

In another study, a reactive dye, Reactive blue 160 (RB160) was decolourised using a bacterial strain identified through gene sequencing as *Bacillus firmus*. Toxicology studies performed on the degraded products of the RB160 revealed that the dye was broken down into non-toxic compounds shown by low toxic levels of RB160 on root cells of *Allium cepa* and human skin cell line (CRL 1474) compared to high toxicity levels of the undegraded dye on the *Allium cepa* and CRL 1474. The study also showed that the degraded dye compounds showed a significant development in zebra fish embryos as compared to the undegraded dye. It was therefore concluded that the bacterial species were able to successfully decolourise and degrade RB160 into nontoxic compounds that were more environmentally friendly compared to the undegraded dye [60].

Anthraquinone dyes on the other hand have previously been known to be resistant to biodegradation by bacteria due to their stable aromatic structure [61]. However, work that has been done recently has identified possible bacterial agents that can degrade anthraquinone dyes. Laccases and peroxidases excreted by these bacterial isolates have shown to effectively degrade anthraquinone dyes [62, 63]. Farraj et al. [64] isolated *Hortaea* sp from petroleum contaminated soil and studied its ability to degrade solvent green (SG). The study found that the fastest degradation time (24 h) of the dye by the bacteria was achieved at the lowest initial concentration of 10 mg/L. It was also reported that 1,2-dioxygenase and laccase enzymes were responsible for the biodegradation of the dye to its simpler non-toxic compounds. The addition of copper in the reaction medium is believed to have enhanced the laccase enzyme activity. Laccases have in previous studies been shown to have the capability to degrade anthraquinone dyes into non-toxic metabolites by oxidizing the aromatic rings of phenolic compounds.

In the case of triphenylmethane (TPM) dyes, bacteria also play an important role in their removal from textile wastewater. Bacterial isolates through their enzymes systems are able to remove these dyes through either degradation or biosorption. Adenan et al. [65] showed that various actinobacteria isolated from soil were capable of degrading four commonly used TPM dyes, namely Malachite green (MG), Crystal violet (CV), Methyl violet (MV) and Cotton blue (CB) with good degradation efficiencies. Of the isolated bacterial strains *N. alba* showed the highest degradation efficiency of 97.0%, 95.1%, 95.8% and 83.8% for MG, MV, CV and CB respectively in a period of 14 days. The extent of degradation was noted to be higher in live cells compared to dead cells for all the studied dyes. The researchers did not conduct enzyme analysis therefore no conclusions were made in terms of the enzymes that were responsible for the degradation of the TPM dyes. It was however suggested that the differences in the degradation efficiencies between live and dead cells were an indication that live cells were able to secrete enzymes capable of degrading the dyes. Previous studies have shown that TPM dyes are susceptible to degradation by triphenylmethane reductase, laccase and peroxidase enzymes [66, 67]. In another study Cao et al. [68] used *C. davisae* bacterial strain to degrade Crystal violet dye. The bacterial strain was able to degrade the dye with an efficiency of 97%. The study of the reaction kinetics revealed that dye degradation followed first-order kinetics with maximum degradation taking place between 25-35 °C. The pH of the culture medium also influenced the rate of degradation, with an optimum pH of 7 showing the highest degradation efficiency.

To improve the efficiency of bacteria in degrading dyes, researchers have shifted focus from using individual bacterial isolates to using bacterial consortia. It has been shown that using a consortium of bacteria instead of using single bacterial cultures produces better dye degradation efficiency [69]. This could be a result of the synergistic metabolic networks created in consortia as compared to pure single cultures. Another advantage of using a consortium is that it allows for a one-pot treatment system. Most dyes can only be completely degraded in a two-stage process: anaerobic followed by either aerobic or anaerobic

reduction. The use of mixed cultures allows two or more bacteria to coexist within the same culture medium increasing the possibilities of dyes being degraded due to the synergistic effect of the bacteria in the consortium. As a result, most research is now focused on using consortiums instead of pure single cultures in the treatment of textile dye wastewater. Dissanayake et al. [70] used a microaerophilic mixed bacterial consortium to degrade Methyl orange and Congo red in a single-step process into simple non-toxic compounds. The biodegradation studies showed that the consortium was able to degrade and decolourise the dyes with an efficiency of over 95% within 50 h. The success of the biodegradation of the dyes was attributed to the synergistic influence of the co-metabolic activities of the bacteria within the consortium. Similarly [71], found that a bacterium consortium that consisted of *Zobellella taiwanensis* AT 1–3 and *Bacillus pumilus* HKG212 was able to effectively decolourise Reactive green 19 with a degradation efficiency above 97% at optimum conditions compared to 86.80% and 89.50% degradation efficiency recorded for the pure cultures of *Zobellella taiwanensis* AT 1–3 and *Bacillus pumilus* HKG212 respectively under similar conditions. The use of mixed cultures is useful when degrading azoic dyes as it can eliminate the need for a two stage anaerobic-aerobic sequence that is usually required to degrade azoic dyes. Mohanty et al. [72] also found that a bacterial consortium-BP of *Bacillus fexus* TS8 (BF), *Proteus mirabilis* PMS (PM), and *Pseudomonas aeruginosa* NCH (PA) was able to decolourise an anthraquinone dye Indanthrene Blue RS with a decolourisation efficiency of 100% within 9 h compared to the pure cultures of the bacterial strains which completely degraded the dyes within an average of 19 h. The researchers attributed the fast degradation of the dye by the bacteria in the consortium to the combined effect of the oxidoreductive enzymes in the reaction medium.

In a continued effort to improve the efficiency of bacteria dye wastewater remediation, algal-bacterial consortiums have recently emerged as an economic wastewater treatment method capable of removing nutrients, heavy metals as well as mineralising and decolourising dyes from wastewater [73, 74]. Algae has been previously used to treat wastewater [75, 76], therefore their use in combination with bacteria harnesses the advantages provided by both microbes to provide better systems for dye wastewater treatment. Three mechanisms are responsible for dye decolourisation in algal bioremediation: assimilative utilisation of chromophores for the production of algal biomass, CO₂ and H₂O transformation of coloured molecules to non coloured compounds and adsorption of chromophores on the algal biomass [22]. There are studies that have also shown that the use of algal-bacterial consortiums provides an environment capable of withstanding extremes in temperature, pH and concentration of dissolved inorganic compounds [77, 78] all of which can be limiting factors in bacterial biodegradation of dye wastewater. Ayed et al. employed a consortium containing algal-bacterial probiotic strains (*Pseudomonas putida*, *Chlorella* and *Lactobacillus plantarum*) to treat real textile wastewater containing CI Reactive blue 40 (CI RB 40). The study found that the consortium managed to remove COD and colour from the wastewater with efficiency of 89 and 99% respectively. Phototoxicity studies confirmed the ability of the consortium to degrade the dye into nontoxic compounds [79].

4. Latest advances in bacterial dye degradation

4.1. Use of immobilized bacteria in dye wastewater treatment

The challenge with the use of pure and bacterial consortiums in bioremediation applications is that the bacteria can be easily affected by environmental factors like pH, temperature, and initial dye concentration among others which often leads to reduced dye decolourisation. To overcome these challenges and improve the bioremediation process, immobilization techniques have gained momentum within the field of wastewater treatment. The use of immobilization techniques provides various advantages compared to using free bacterial cells like improved bacterial stability, survivability as well as reusability of the bacteria

without loss of enzymatic activity [80], leading to improved dye decolourisation and degradation. During immobilization, bacterial organisms are cultured and immobilized using one of the different methods like microencapsulation, matrix entrapment, covalent binding, and adsorption onto a suitable support system before being exposed to the dye contaminated wastewater. To be able to achieve maximum biodegradation efficiency the support material chosen for the immobilization of the bacteria must be able to protect the bacterial organisms from toxic environmental conditions, provide operational stability, be lightweight, cost-effective, mechanically, and chemically stable, inert, and have good diffusivity. Natural and synthetic polymers like alginate, chitosan, cellulose, polyurethane, polyvinyl alcohol, polyacrylamide, polyethylene, and polyvinyl chloride among others have been used as support materials for bacterial immobilization [81].

In an effort to understand the differences in the degradation ability of immobilized bacteria and free cells, [82] isolated *Pseudomonas guariconensis* from paddy rhizosphere to evaluate its ability to degrade reactive dye Reactive red 190. The study compared the ability of free *pseudomonas guariconensis* bacterial cells and calcium alginate immobilized bacterial cells. The results of the study showed that the immobilized cells were able to degrade the dye with an efficiency of 91% compared to 86% with the free cells. Immobilisation of bacterial cells has been shown to improve the enzyme activity and stability of bacterial cells, which could explain the differences in the efficiency of the dye degradation by the bacterial strain. Similarly, Cai et al. [83] immobilized *Bacillus* sp. JF4 into polyvinyl alcohol–calcium alginate–activated carbon beads for the decolourisation of Reactive blue 19 (RB 19). The immobilization of the bacterial strain proved effective as it achieved a degradation efficiency of 100% compared to 92.1% achieved by free bacteria. The immobilized bacteria also showed good tolerance to high RB 19 dye concentrations. Increased dye concentration is one of the limiting factors in bioremediation, as an increase in dye concentration tends to inhibit bacterial enzyme activity due to dye toxicity to the bacteria.

Immobilisation has also been shown to occur naturally in bacterial organisms that can form biofilms. Bacterial biofilms are surface-associated cells immobilized in self-produced extracellular polymeric substances [84], and depending on the type of bacteria, some biofilms can degrade various types of dyes. The attractiveness of biofilms is rooted in their low cost, reusability, and their ability to adapt to toxic environments, compared to pure and mixed bacterial cultures [85]. The use of biofilms as a bioremediation alternative to the traditional wastewater treatment techniques has recently gained interest as researchers continue to search for more environmentally friendly wastewater treatment systems. According to different studies, biofilm-forming bacteria have been successfully used to treat textile wastewater [86, 87]. Tahir et al. [88] utilized biofilm-forming *Staphylococcus* and *Bacillus* sp. to decolorize and transform Mordant black 11 dye. They found that both bacterial strains were able to degrade the dye with efficiencies greater than 50%, with *Bacillus* sp showing a decolourisation efficiency of 75. 2% in a glucose supplemented medium. However, the study found that the bacterial strains were affected by high Mordant black 11 concentrations, noting a decrease in dye degradation with an increase in dye concentration from 50mg/l-150 mg/l, with maximum degradation observed at 50 mg/l. Similar studies [89, 90] have shown that increased dye concentration in the culture medium creates a toxic environment that leads to enzyme inhibition, consequently decreasing the dye degradation efficiency. Other studies have also been reported [91, 92, 93] showing that biofilms perform better in dye degradation when compared their free forms under similar conditions.

4.2. Microbial fuel cells

Microbial fuel cells (MFC) represent a promising bio-electrochemical process that is capable of degrading both organic and inorganic pollutants whilst simultaneously generating electric energy. MFC's make use of microbes that act as catalysts to oxidise organic compounds. The

oxidation of the organic compounds produces electrons and protons at the anode that through an external circuit travel to the cathode completing the electrochemical reaction leading to the production of electric energy [94]. Electron transfer plays a crucial role in the decolourisation of the dyes, as part of the generated electrons are donated to the dye for the reductive decolourisation of the dye and the other part to the anode for the generation of electricity [95]. MFC's have been shown to have great potential in degrading azo dyes [96, 97].

Recently, Reyes et al. employed an indigenous bacterial consortium to degrade four anthraquinone dyes, Acid blue 62, Acid blue 25, Acid blue 40 and Reactive blue 19 at 50 mg L⁻¹ initial dye concentration using a microbial fuel cell. The study showed that the consortium was able to degrade the dyes with dye removal efficiencies of 48%, 17%, 9% and 5% respectively. The results showed a decrease in dye removal efficiency as the complexity of the dye structures increased. Reactive blue 19 had the lowest dye removal efficiency at 5% due to the presence of sodium 2-((3 aminophenyl)sulfonyl)ethyl sulfate substituent which created a more efficient charge transfer throughout the dye chemical structure, increasing its conjugation length and consequently its stability. This shows that Reactive blue 19 was the most recalcitrant amongst the studied dyes and the bacterial consortium failed to effectively degrade the dye [98]. Similarly, in another study, Saba et al. investigated the decolourisation of an azo dye Reactive black 5 (RB 5) and an anthraquinone dye Reactive blue 4 (RB4) under an open circuit microbial fuel cell with a microbial consortium extracted from bovine rumen fluid. The results of the study revealed that RB5 was decolourised with efficiency greater than 90% in 120, 165, and 225 min at 50, 100, and 200 mg L⁻¹ concentrations, respectively. RB4 dye at 50 and 100 mg L⁻¹ took 225 and 300 min to decolorize, while 200 mg L⁻¹ dye was not decolorized at all. The differences in the decolourisation efficiency of the dyes by the bacterial consortium indicate that the degradation of the dyes was more rapid in RB5 than in RB4. This could have been due to the difference in the structures of the dyes. Reactive Blue 4 contains dichlorotriazine group which together with the C=O chromophore group makes the dye more stable rendering it difficult to degrade. The study also found that under closed-circuit conditions dye decolourisation increased with a decrease in external load [99]. Anthraquinone dyes contain anthraquinone chromophore groups which when compared to other chromophore types e.g. azo, have been shown to be more stable due to their complex structures rendering their biodegradation difficult [100].

4.3. Membrane bioreactors

Membrane bioreactors (MBR) combine the technology of biodegradation with membrane technology. This has a number of advantages compared to the traditional wastewater treatment systems like production of less sludge, high effluent quality, low maintenance and high biocatalyst retention [101, 102, 103]. However, the main challenge with membrane bioreactors is the fouling of the membrane caused by the deposition of microorganisms, colloids, and solutes on the membrane surface which affects the trans-membrane pressure and the membrane permeate flux [104], necessitating the need for continuous membrane cleaning which leads to reduced membrane lifespan [105]. Recently research on membrane bioreactors has shifted to focus on other membrane bioreactor derivatives such as anaerobic membrane bioreactors (AnMBR), biofilm membrane bioreactors (BFMBR) and membrane aerated biofilm reactors (MABR). These derivatives when compared to the traditional aerobic membrane bioreactors have shown to reduce 1) environmental impact due to energy related emissions, 2) eutrophication of fresh water resources 3) eutrophication of marine environments [106]. Liu et al. treated Methyl orange in an anaerobic baffled membrane bioreactor which combined an anaerobic baffled reactor and a membrane bioreactor. The result was a baffled membrane bioreactor that was capable of dye degradation with minimal membrane fouling. The study found that the Methyl orange was discoloured completely after passing 4 cells of anaerobic treatment before being fed into the membrane

bioreactor. Analysis of the seven different degradation intermediates from both the anaerobic and aerobic treatment showed that the dye degradation followed two different pathways which included demethylation and desulfonation. The system was operational for a total of 110 days, and during that time the membrane accumulated 0.19–0.32 gMLSS/m² of filter cake which could have been due to the pretreatment in the anaerobic baffled reactor which degraded most of the possible membrane foulants [107].

In another study, Berkessa et al. employed an anaerobic dynamic membrane bioreactor to treat anthraquinone Remazol brilliant blue R (RBRR) dye wastewater. The results of the study showed that the reactor was able to effectively remove more than 97% of both colour and COD. The membrane also showed good rejection of compounds with a high molecular weight, with a total suspended solid rejection >98.8%. It is worth noting that prior to the treatment of the wastewater in the bioreactor the dye wastewater was pretreated using the ozonation oxidation process. This facilitated the degradation of the dye molecules especially the aromatic compounds in the dye which are difficult to biodegrade under anaerobic conditions [108]. It has been shown that some dye molecules are hazardous and resistant to biodegradation [109], therefore, pretreatment of dye wastewater plays a huge role in ensuring that high-quality effluent is achieved after the final biological post-treatment process. The pretreatment process therefore could explain the reason for the high dye removal efficiency that was achieved by the researchers.

In order to improve membrane performance, researchers have come up with a system that combines MBR's and electrochemical systems termed electrically enhanced membrane bioreactors (eMBR). These systems have been shown to reduce membrane fouling in MBR's [110, 111], improve phosphorus and micro pollutant removal [106]. Electrophoresis, electroosmosis and electrocoagulation have been used in MBR's to reduce membrane fouling as well as improve pollutant removal [112, 113]. Hawari et al. demonstrated that the dielectrophoretic (DEP) motion of particles in an inhomogeneous electrical field could suppress membrane fouling in submerged MBR's. The study found that fouling-suppression performance-related closely to the intensity and frequency of the electrical field. A stronger electrical field was found to better recover the filtrate flux due to the stronger DEP force acting on the biomass particles closer to the membrane's surface. Above an intensity and frequency value of 130 V and 1 kHz, respectively the permeate flux was reduced due to an electrothermal effect [114]. Belli et al. reported on the performance and membrane fouling of a laboratory-scale eMBR for the treatment of wastewater collected from the textile industry. The researchers found that the electrocoagulation process significantly improved the colour removal efficiency of the reactor with efficiencies of 50 and 70 % when using an electrical current density of 10 A m⁻² and 15 A m⁻² respectively. In addition, the study found that the bioreactor was able to remove NH₄⁺-N with efficiency above 90%. COD removal was constant throughout the experiment ranging between 70-75%. The application of the electric current was found to create better-mixed filterability conditions which resulted in a lower membrane fouling rate and demand for membrane chemical cleaning [115].

4.4. Bio-advanced oxidation processes

Advanced oxidation processes (AOP's) utilize in-situ generated hydroxyl or sulfate radicals to degrade both organic and inorganic pollutants [116]. Fenton oxidation and ozonation are the AOP's that have been used in combination with biological treatment to degrade dyes [117]. Each of these advanced oxidation processes has previously been shown to have good dye removal capabilities [118, 119].

It has however been noted that the use of AOP's can be costly, difficult, and in some cases ineffective [120]. Biodegradation also has some limitations especially at a large scale because; 1) the process is time-consuming due to its dependence on microbial metabolic processes 2) not all microorganisms can degrade all types of dyes due to the specificity of the enzymes and 3) biodegradation is possible only on

pollutants that are biodegradable [121]. Therefore, to overcome the challenges of both AOP'S and biodegradation researchers have come up with wastewater treatment processes that make use of both AOP's and biodegradation in a combined system. Each of these systems can be used as either a pretreatment or post-treatment step, depending on the ease of degradability of the dye or the by-products from each of the treatment processes, for example, if a dye is not biodegradable but its by-products are, then biodegradation might be used as a post-treatment step, and one of the AOP's capable of degrading the dye used in the pretreatment step and vice versa.

In a study by Castro et al. an aqueous solution containing Reactive orange 16 (RO16) was subjected to a sequential treatment of ozonation as a pretreatment followed by a biological treatment in a moving-bed biofilm reactor (MBBR). The study found that 97% of the dye was removed after 5 min of ozone exposure but the process showed poor COD removal, with only 48% COD removal, an indication of incomplete dye mineralization. Further treatment in the MBBR achieved (COD) and ammonium removal of 93 ± 1 and $97 \pm 2\%$, respectively [122]. Goswami et al. combined a packed bed bioreactor (PBBR) containing *Arjuna* (*Terminalia Arjuna*) seeds biochar immobilized with *Providencia stuartii* with ozonation for the decolorisation of Congo red (CR) dye. The biological treatment was used as a pre-treatment step and it achieved 92% dye removal efficiency. The follow-up ozonation of the biologically treated sample was able to completely remove the residual dye molecules in 30 min [123]. In a study by Thanavel et al. which targeted the removal of Remazol yellow RR dye from textile wastewater using a bacterial strain *Aeromonas hydrophila* SK16 (*A. hydrophila*) and AOP's-H₂O₂, the researchers found that when used independently *A. hydrophila* had a 90% dye removal efficiency while AOP-H₂O₂ had a 63.07% under ambient conditions within 9 h at a pH of 6. In comparison, the researchers reported that the bio-AOP combined process, where the biologically treated dye was further subjected to AOP with 4% H₂O₂ showed maximum dye removal within 3 h, and it was also able to remove 84.88% and 82.76% of BOD and COD respectively [124].

In another bio-AOP combined process, Shanmugam et al. studied the degradation of Acid blue 113 (AC113) using Fenton oxidation combined with biodegradation using a bacterial consortium. The study was conducted on simulated and actual dye baths. Pre-treatment with Fenton oxidation in the simulated dye bath achieved maximum dye degradation efficiency of 85%, analysis of the treated sample revealed the presence of lower molecular weight aromatic amine compounds. This was an indication that the oxidation was able to break the dye into smaller metabolites, however, the identified compounds: benzene acetic acid, diethyl phthalate, and n-hexadecanoic acid are not considered environmentally friendly and therefore required further treatment to break them down into smaller nontoxic intermediates. This was done by employing biological degradation as the post-treatment step. The biological degradation process was able to further degrade these compounds into more environmentally friendly metabolites namely benzoic acid, 4-ethoxy-, ethyl ester, pyrrolo [1, 2-a] pyrazine1,4-dione, pyrazine1,4-dione, hexahydro-3 (2methylpropyl), and hexadecanoic acid. The Fenton oxidation of the actual dye bath achieved a lower dye removal efficiency (89.5%) and the analysis of the oxidised sample revealed the presence of naphthalene phthalic anhydride, phenol, 3, 5-bis (1, 1-dimethyl ethyl), phthalic acid, butyl hept-4-yl ester and phenol, 4, 4'-methylenebis. After the final biological treatment the sample showed the presence of biodegradable alkenes and hydrocarbons. The combined process was, therefore, able to convert AC113 into smaller easily biodegradable compounds [125].

5. Dye removal mechanism

Textile dyes are decolourised by bacteria through biosorption, biodegradation, or a combination of both mechanisms. During biosorption, the dye is adsorbed onto the cells of the bacteria impacting the colour of the dye onto the bacterial cells. This is characterized by the

reduction in the absorption spectrum of the dye in proportion to the adsorbed dye by the bacterial cells. Alternatively, when biodegradation has occurred, the dye structure is broken down into its simplest compounds characterized by the disappearance of the dye absorption peaks from the dye spectrum or the formation of new peaks if new metabolites are formed during the biodegradation process [126]. Other studies have also associated the disappearance of absorption peaks from the spectrum of treated dyes to the dye degradation mechanism [127, 128]. Degradation is therefore a more favourable mechanism as it produces metabolites that are less hazardous. Adenan et al. [129] investigated the effect of both dead and live cells from *Streptomyces bacillaris* bacterial species on the degradation of triphenylmethane dyes (Malachite green (MG), Methyl violet (MV), Crystal violet (CV), and Cotton blue (CB) and concluded that both mechanisms were responsible for the removal of the dyes. The intensity of the adsorption peaks of the triphenylmethane dyes treated with live cells was significantly reduced (MG, MV, and CV) or removed completely (CB), which is associated with the dye degradation mechanism. In contrast, the study found that triphenylmethane dyes treated with dead cells were removed through adsorption as all dye absorption peaks of the treated dye remained visible due to the dye structure remaining unchanged during the dye removal process and the bacterial cells changed into the colour of the target dye. Similarly [130], in their study on the decolourisation of an anthraquinone-based dye reactive blue 19 by the strain *B. cohnii* LAP217 concluded that the dye removal mechanism was due to biosorption of the dye on the surface of the bacterial cells.

6. Factors influencing the degradation of dyes by bacteria

The efficiency of the biodegradation of textile dyes by bacteria is influenced by environmental factors such as pH, temperature, oxygen and agitation, initial dye concentration as well as nutrient composition.

6.1. Effect of pH and temperature

The nature of textile effluents is very complex due to the variety of chemicals that are used in textile processes. The effluent is largely characterized by high pH, dye molecules and additives like salts and detergents. Biodegradation of dyes has been shown to be affected by effluent pH, with most dyes degrading effectively under neutral to slightly alkaline pH [9, 131], Table 2, reveals the range of pH required for maximum dye removal by different bacterial strains. This shows that pH is an important element for the growth of bacteria; if the effluent pH does not support the growth of the bacteria then dye degradation will not be possible as a wrong pH will hinder the bacteria inactive. To ensure that dye degradation takes place, the effluent pH must be adjusted to suit the bacteria being used or suitable bacteria that can withstand the effluent pH must be chosen for the degradation process. In their study Ewida et al. [49] found that the bacterial strain *Bacillus megaterium* KY848339.1 was able to decolourise the azo dye Acid red 337 at an optimum pH of 7 with an efficiency of 91% at an initial concentration of 500 mg/l within 24 h. Similarly [131], studied the decolourisation of azo dyes Methyl red and Navy blue by *Bacillus* sp. under different pH conditions (5–8). It was found that the maximum dye removal efficiency for both dyes was achieved at an optimum pH of 7. In another study to determine the biodegradation efficiency of *Enterobacter* sp. CV-S1 bacterial strain on a triphenylmethane based dye Crystal violet, Roy et al. [132] found that the bacterial strain was able to completely decolourise crystal violet at a pH of 6.5.

Temperature is another parameter that plays an important role in the decolourisation and degradation of dyes. Bacterial organisms need optimum temperature to support their growth, survival and metabolic activities. Low temperatures or temperatures above optimum levels might lead to cell deactivation or inactivation in the bacteria which will affect their biodegradation capabilities [133]. Studies have shown that the optimum temperature for dye degradation by bacteria in both pure

cultures and consortia lies in the range of 30–40 °C [134]. Parma and Shukla [76], tested the decolourisation of an anthraquinone based dye C.I Reactive blue 4 by *Staphylococcus hominis* Subsp. *hominis* DSM 20328 bacterial strain and found that the maximum decolourisation efficiency was achieved at a temperature of 37 °C. They discovered that an increase in temperature above 37 °C led to a decrease in the decolourisation efficiency which they attributed to the loss of cell viability in the bacteria due to the high temperatures. Naseer et al. showed that a bacterial consortium used to degrade and detoxify Navy blue CBF dye was temperature dependent, with the decolourisation efficiency increasing with an increase in temperature up to an optimum of 35 °C above which the decolourisation efficiency was seen to decrease greatly [135]. Similarly [136], studied the decolourisation of Crystal violet, a triphenylmethane dye by a bacterial strain *A. hydrophila* and found that the maximum decolourisation efficiency of the bacteria was 35 °C and it decreased with an increase in temperature above 35 °C.

6.2. Initial dye concentration

The concentration of dyes in the reaction medium during the degradation or decolourisation of dyes affects the efficiency of the degradation or decolorization process. The concentration of dyes in textile wastewater has been reported in a wide range of values, with some studies reporting concentrations of 10–50 mg/L [137] and other studies reporting values between 10–250 mg/L [138]. It is therefore important that any potential organisms should be able to effectively degrade or decolourise the target dyes without any inhibition at these dye concentrations for the bacteria to be effective in industrial applications. Various studies have shown that an increase in dye concentration leads to a reduction in the degradation efficiency of the bacteria, due to the toxicity of the dyes at high concentrations which inhibits the bacteria's metabolic activities [139, 140]. The other reasons for the reduced degradation efficiency at high dye concentration have been reported to be inadequate biomass to dye ratio as well as the blockage of enzyme active sites by the excess dye molecules [141]. Parma and Shukla [76], studied the effect of different dye concentrations on the degradation of an anthraquinone based dye C.I Reactive blue 4 by *Staphylococcus hominis* subsp. *hominis* DSM 20328 and found that an increase in dye concentration led to a subsequent decrease in the dye degradation efficiency. The findings showed that the maximum degradation efficiency of 97% was achieved at a concentration of 50 mg/L and the lowest at a concentration of 1 000 mg/L. Similarly [142], reported a maximum decolourisation efficiency of 96% in their study to determine the effect of bacteria isolated from a textile waste dump on the degradation of an azo based dye Procion red H-3B when an initial dye concentration of 50 mg/L was used. The degradation efficiency decreased sharply to 30% when the dye concentration was increased above 600 mg/L. However, there have been other strains that have been shown to degrade dyes at high concentrations. Masarbo et al. [143], studied the effect of methyl orange concentration on the decolourisation potential of three bacterial strains *Bacillus* spp. AK1, *Lysinibacillus* spp AK2 and *Kerstesia* spp. VKY1. The results showed that the three strains were able to decolourise the dye at different concentrations ranging from 600–800 mg/L, beyond which the decolourisation efficiency decreased.

6.3. Carbon and nitrogen sources

Studies have shown that the addition of carbon and nitrogen sources to growth cultures improves the degradation efficiency of most bacteria as most dyes do not have enough carbon to provide sufficient energy for bacterial growth [144, 145, 146]. The commonly used carbon and nitrogen sources include glucose, sucrose, starch, yeast extract, beef extract, peptone, tyrosine, ammonium chloride and ammonium sulphate [147]. In the degradation of azo-based dyes, the addition of carbon in the culture media not only acts as an energy source but also as an electron donor which assists in the reduction of the azo bonds. Similarly, the

addition of nitrogen sources helps to regenerate the reducing agent nicotinamide adenine dinucleotide (NADH), which acts as an electron donor in the reduction of the azo bonds [148].

Different bacterial strains respond differently to various carbon and nitrogen sources, with some strains assimilating the added carbon or nitrogen sources instead of using them as electron donors [149]. This negatively affects the dye degradation efficiency as the addition of such supplementary carbon and nitrogen sources does not bring about significant changes in the reduction of the dyes and consequently, poor degradation efficiencies are obtained. It must also be noted that excess carbon or nitrogen sources negatively impact the degradation efficiency of the bacteria, as high concentration of these supplements can lead to the inhibition of the bacterial cells. It is therefore important that an optimum amount of carbon and nitrogen be found to enhance the dye degradation capability of the bacteria.

Guadie et al. found that *Bacillus* sp. strain CH12 was able to degrade reactive dyes effectively when supplemented with different carbon sources, showing the highest degradation efficiency of 95–100% compared to the carbon-free culture which showed an efficiency of 27–51%. In the same study, it was found that the addition of organic nitrogen sources, yeast, and peptone resulted in the highest degradation efficiency (>90%) compared to the inorganic sources, where maximum degradation efficiency achieved was <30%, the lowest being NaNO₃, with maximum efficiency of 8% [150]. The low efficiency exhibited by NaNO₃ is consistent with studies that have shown that inorganic nitrogen sources have an insignificant effect on dye decolourisation or degradation efficiency [44]. Future research must focus on the use of more sustainable carbon and nitrogen supplements to move dye degradation systems towards being greener.

While most bacteria require the addition of supplementary carbon and nitrogen sources to the nutrient broth, some studies have shown that there are bacteria that are capable of degrading dyes without any additional carbon or energy sources. Roy et al. extracted two potential bacterial strains *Enterobacter* spp. CV-S1 and *Enterobacter* spp. CM-S1 from textile effluent which they used to decolourise malachite green (MG). The results showed that the two strains could completely decolourise 15 mg/L of the dye in 78 and 144 h respectively at optimized pH of 6.5 and temperature of 35 °C without the need of additional carbon and protein sources [151]. This means that these species can use the dye as their carbon and energy source for their growth.

6.4. Shaking and static conditions

Shaking or static conditions also affect the biodegradation efficiency of bacteria [140]. Under shaking conditions, there is an increase in the amount of dissolved oxygen which supports microbial enzyme activity [152]. This can lead to improved bacteria dye degradation capabilities due to the high metabolic activity of the bacterial enzymes. Even though shaking conditions lead to increased degradation efficiency, it is important to note that the presence of excess oxygen in the reaction medium can inhibit the activity of reductive enzymes like azoreductase due to a more positive redox potential created by excess oxygen decreasing degradation efficiency of the bacterial organisms due to the reduction of the redox mediator instead of the dye [147]. This means that shaking conditions can only be favoured by aerobic oxidative enzymes, while static conditions by anaerobic reductive enzymes. Kabeer et al. [153] analysed the ability of *B. vietnamensis* sp. MSB 17 to decolourise Malachite green, a triphenylmethane dye under both static and shaking conditions. The study found that the highest decolourisation efficiency was obtained under static conditions where an efficiency >99% was achieved compared to 80% under shaking conditions. Azo dyes have also been shown to degrade effectively under static conditions [154, 155]. Maniyam et al. in their study of the decolourisation and biodegradation of Methyl red by two *Rhodococcus* strains UCC 0016 and UCC 0008 found that the decolourisation efficiency of the dye was higher under static conditions compared to shaking conditions for both bacterial strains

[126]. On the other hand, [156], compared the degradation efficiency of three reactive dyes, Reactive orange -16, Reactive black -B, and Reactive yellow- MR under shaking and static conditions using a consortium of bacteria and discovered that the maximum decolourisation of all the dyes was under shaking conditions. This shows that different bacteria respond differently to similar culture conditions.

7. Conclusions and perspectives

The disposal of dyes in water sources has hazardous effects on both human and animal life and there is a need to find environmentally friendly and cheaper methods to remove dye pollutants from water sources. There are different physical, chemical, and biological methods that can be used to remove dye pollutants from water, with each method having its own advantages and disadvantages. The shift in research towards finding more environmentally friendly processes has made biological water treatment methods the focus in dye wastewater treatment as biological methods have been deemed less harsh to the environment, economic and do not produce waste products like sludge. In this regard, bacterial organisms have been identified as one of the potential agents in dye removal studies. This review, therefore, focused on the use of bacteria in removing dye pollutants from textile wastewater. It was shown that at laboratory scale bacteria in both pure and mixed cultures can effectively degrade different types of dye molecules from water sources with meaningful removal efficiencies into less toxic compounds. Recent advances in research have seen new technologies in the form of bacterial immobilization, microbial fuel cells, membrane bioreactors and bio-advanced oxidation processes being employed to treat dye wastewater. The removal of dyes from wastewater is dependent on several factors like dye concentration, pH, temperature, presence of oxygen, and the nutrients present in the culture medium. Therefore, for effective dye degradation, it is important that these factors are optimized. However, there is still a lot of work that needs to be done in the area; therefore going forward the following is being recommended:

- More studies must be conducted to identify bacteria that can degrade and mineralize a combination of dyes as actual textile dye wastewater consists of different dyes. It was noted during literature review that most studies focused on the treatment of model wastewater containing a single dye type e.g. azo dyes.
- The focus should shift towards using real textile Industry effluent as opposed to model effluents as model effluents used in laboratories do not give a true reflection of how the bacterial isolates will perform under actual industrial applications. Therefore model effluents might be misleading as the bacteria might not be effective when used in industrial effluents which are complex in nature. By scaling up laboratory studies to actual industrial systems a full picture of the possibility of these bacterial isolates as dye degradation agents can be obtained.
- To be able to fully understand the effectiveness of biodegradation processes in protecting the environment and be able to develop effective dye degradation systems it is relevant that degradation pathways undertaken by the identified bacteria be understood. It was noted that literature provides very few such studies. Therefore going forward focus has to be on understanding the degradation pathways of dye degrading bacteria.
- For bacteria to be successfully integrated into actual industrial treatment systems research has to focus on combined and hybrid treatment technologies where bacteria can be used in conjunction with other technologies. This will enhance the effectiveness of bacteria in degrading dyes. It is important that focus is spread across the different dye types and not limited to azo dyes as has been the trend over the years.
- The literature review did not reveal any studies focusing on disposal or recovery of the bacterial isolates after the degradation studies as the bacterial isolates can become potential pollutants themselves. It is

therefore important that in future, studies are done to determine the possibility of recovery and or disposal methods of these bacterial isolates after dye degradation has been done.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

No data was used for the research described in the article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors would like to thank Professor M.T. Masarirambi and Professor L. Wangatia for helping with the editing of the document.

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