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Review Microbial degradation of dyes: An overview

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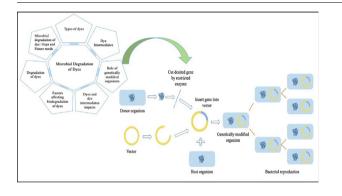
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ABSTRACT

Industrialization increases use of dyes due to its high demand in paper, cosmetic, textile, leather and food industries. This in turn would increase wastewater generation from dye industrial activities. Various dyes and its structural compounds present in dye industrial wastewater have harmful effects on plants, animals and humans. Synthetic dyes are more resistant than natural dyes to physical and chemical methods for remediation which makes them more difficult to get decolorize. Microbial degradation has been researched and reviewed largely for quicker dye degradation. Genetically engineered microorganisms (GEMs) play important role in achieving complete dye degradation of azo dye. It also compiles information about factors affecting dye(s) biode-gradation, role of genetically modified organisms (GMOs) in process of dye(s) degradation and perspectives in this field of research.

1. Introduction

Dyes are an important source in various industries such as textile, leather, paint, food, cosmetic and paper industries. There are approximately twenty-five types of dye groups available based on their chemical structure of chromophore (Sudha et al., 2014; Benkhaya et al., 2020). More than thousand dyes have been classified as textile dyes which are used to color variety of fabrics (Sponza, 2006; Abe et al., 2019). Dye intermediates are precursors of dyes. They can be obtained from raw constituents, such as naphthalene and benzene, with an aid of

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various chemical reactions (Gregory, 2000; Guo et al., 2018).

Disposal of municipal- and other industrial- effluents into water bodies cause water pollution (Kunz et al., 2002; Varjani and Upasani, 2017b). Environment is adversely affected by pollution which may cause indirect or direct health risks to all life forms on the earth (Varjani, 2017; Bencheqroun et al., 2019). Dyes can be classified on the base of their structure and application. Dyes have a great solubilizing capability in water, which makes it difficult to be removed by traditional methods (Dong et al., 2019; Lellis et al., 2019). Textile dye contains colors, which causes artistic damage as well as stops diffusion of light in the water which leads to decrease in dissolved oxygen level and affects photosynthesis rate of aquatic life (Ajaz et al., 2020).

Various methods can be used to remove dves and other pollutants from industrial effluent such as physico-chemical, biological, chemical and physical (Xu et al., 2007; Cao et al., 2019; Varjani and Upasani, 2019b; Nakkeeran et al., 2020) Biological treatment has various advantages such as, it is a simple, cheap, environmental friendly process. Also large number of microorganisms are available which are easy to maintain and also require low preparation (Crini and Lichtfouse, 2018). Apart from these dye degradation techniques periphyton biofilm or periphytic biofilm system can be also used for degradation of dyes (Li and Bishop 2004; Shabbir et al., 2017a; Shabbir et al., 2017b; Pandey and Bergey, 2018; Dias et al., 2019; Shabbir et al., 2020). Among various activities of dye industries, dye manufacturing is the main source of environmental pollution due to release of hazardous dye in water bodies. Numerous microorganisms such as algae, yeast, bacteria, and fungi possess ability to mineralize and/or decolorize various dyes (Roy et al., 2018; Tochhawng et al., 2019). Treatment of dye wastewater can be performed using pure culture or mixed microbial culture. Majorly mixed microbial culture has been reported to achieve efficient dye degradation due to synergistic metabolic actions (Kalyani et al., 2009; Mandal et al., 2010).

Genetic engineering has made a significant revolution in the field of bioremediation (Varjani et al., 2017; Kumar et al., 2020). Removal of acid red has been reported through the successful manipulation of microorganism using genetically engineering in treatment system (Jin et al., 2008). Factors like pH, temperature, structure of dye, soluble salts, heavy metals, nutrients, etc., affect the degradation of dye (Al-Amrani et al., 2014). There are various reports available which shows degradation of different dyes using microorganisms (Mane et al., 2008; Varjani and Upasani, 2016; Kiayi et al., 2019; Li et al., 2019; Pratiwi et al., 2019).

Present review intends to expand biodegradation scope of dyes. It includes types of dyes, dye intermediates and impact of dyes. It also narrates types of dye degradation techniques and through light on factors affecting biodegradation of dyes. Direct Black 38 is majorly used azo dye, hence microbial degradation pathway for Direct Black 38 has been discussed. It also provides an overview about role of genetically modified organisms (GMOs) in dye(s) biodegradation.

2. Types of dyes

There are more than three thousand azo dyes among which Sandolan Yellow, Maxilon Blue GRL and Astrazon Red GTLN are broadly applied in leather, textile, paper, food coloring and cosmetic manufacture industries (Sudha et al., 2014). From centuries fabric dyes have been used to color fabrics. More than thousand dyes are classified as textile dyes which are used to color variety of different fabrics. Nowadays most of clothes are colored with manmade or synthetic dyes. Dyes contain at least one chromophore and can absorb light in visible spectrum (400–700 nm).

Classification of dyes are carried out on the basis of their structure and application. Azo dye, nitro dye, phthalein dye, Triphenyl methane dye, indigoid dye and anthraquinone dye are classified on the basis of their structure. Whereas, acid dye, basic dye, direct dye, ingrain dye, disperse dye, moderate dye, vat dye and reactive dyes are classified on the base of their application. In this paper azo- and anthraquinone- dyes have been explained in detail.

2.1. Azo dyes

Azo dyes contain azo bond (-N=N-) and belong to class of heterocyclic and aromatic compounds, they have been reported as cacinogenic compounds (Sen et al., 2016; Yamjala et al., 2016). Maximum azo dyes are synthesized by diazotization of an aromatic primary amine and followed by coupling with one or more electron rich nucleophiles (hydroxy and amino). Several other methods are also available for synthesis of azo dyes such as oxidation of primary amines by lead tetraacetate or permanganate potassium, reduction of nitroso compounds by AlLiH₄, condonation of quinone and hydrazine, etc. (Benkhaya et al., 2020). These dyes are recorded for industrial applications and only azo dye forms 60% ratio as compared to all other types of dyes (Shah, 2014; Iark et al., 2019). Azo dyes are group of food and drug administration (FDA) certified colorants which make them safe for use in foods, cosmetics and drugs (Chung, 2016). Examples of azo dyes are Acid orange 5, Acid red 88, Methyl orange, Congo red and Direct Black 38.

2.2. Anthraquinone dyes

Second most widely used dyes after azo dyes are anthraquinone dyes, due to their good dyeing performance, easy accessibility and low price they are preferred for industrial processes. However they are highly toxic to humans and microorganisms than azo dyes. Anthraquinone dyes contain anthraquinone chromophore groups which includes benzene ring with two carbonyl group on both sides. They contain both stable as well as complex structure. Color of the dye may be influenced by different effects of substituents such as electron accepting and electron donating substituents. Common natural red colorants comprise presence of anthraquinones which are highly used in textile industries (Shahid et al., 2019). Anthraquinone dyes have been reported as the oldest dyes because they have been found thousands years back and were used in wrapping mummies. Naturally occurring anthraquinone establish the major group of natural quinoids. Several scale insects and plant roots are responsible for production of natural anthraquinones. Plants such as chai root, madder and Indian mulberry (from Rubiaceae family) and scale insects like lac, kermes and cochineal produce beautiful color palettes of red hues on different types of fibre. Color of palette is dependent on the metallic salt used for the mordant with limited color range of purple, brown and orange. Anthraquenone dyes have been divided into four categories: i) Heterocyclic Anthraquinone dyes, ii) Heterocyclic anthrone dyes, iii) Anthraquinone derivations, vi) Fused ring anthrone dyes (Li et al., 2019). Examples of Anthraquinone dyes are C.I. Reactive Blue 19, Alizarin and C.I. Acid Blue 45.

3. Intermediates of dyes

Conversion of commercial dyes with simple transformation from compounds prepared from the coal tar elements with the use of different chemical reactions are known as intermediates. Sabnis (2017), have reported dye intermediates as the raw materials used in the synthesis of organic dyes/manufacturing dye stuff. They are nearly colorless and vary in the complexity. Three types of reactions used for the production of intermediates of dyes: a) Electrophilic substitution, b) Nucleophilic substitution and c) Unit processes (Sabnis, 2017; Yu et al., 2019)

3.1. Electrophilic substitution

This reaction is used to give tetrahedral carbon atom as an intermediate, in this the initial attack of an electrophile E^+ is involved by aromatic system. However, for final product, loss of Y^+ (usually proton) from intermediate is necessary. Mono-substitution products can be achieved by attack at an unsubstituted benzene ring. In this reaction three possible sites are available for attack (Ortho, Para and Meta position), when benzene ring contained a group during electrophilic attack (Gregory, 2000).

3.2. Nucleophilic substitution

Nucleophilic reagent has an individual electron pair. They are either a neutral particle or a charged particle e.g. ammonia. This reaction includes group replacement which is activated by other substitutions within aromatic nucleus (Sabnis, 2017).

3.3. Unit processes

Unit process can be defined as production stage which requires chemical reactions. Dyes and dye intermediates are produced using a reactor followed by filtration. Then they are dried and mixed with other additives for final product manufacturing. The synthesis involves many unit processes like reduction, oxidation, nitration, sulfonation, hydroxylation, amination, alkylation, halogenation, hydrolysis, condensation, alkoxylation, esterification, carboxylation, acylation, phosgenation, diazotization and coupling. In this section we have discussed few unit processes (Gregory, 2000; Freeman and Mock, 2007; Sabnis, 2016).

3.3.1. Oxidation

Oxidation is the process which involves introduction of oxygen or removal of hydrogen from a molecule, mostly arises at an early stage of synthesis. Highly substituted particles are less responsive to oxidation (Gregory, 2000; Huang et al., 2019). Conversion of phthalic anhydride from naphthalene can be done by oxidation reaction with the use of hot V_2O_5 or KMnO₄. e.g. Hypochlorite oxidation is the production of anthranilic acid by Hofmann process (Gregory, 2000; Freeman and Mock, 2007).

3.3.2. Reduction

In reduction process conversion of compounds into an arylene diamine or arylamine from an aromatic dinitro or nitro takes place. Reduction processes such as sulphide reduction, catalytic hydrogenation and iron reduction are widely used in industrial production of dyes. eg. In preparation of indoles and pyrazolones, arylhydrazines have been used as intermediates (Gregory, 2000).

3.3.3. Nitration

Nitration is the process which introduce one or more nitro groups (serve as chromophores) into aromatic ring system and they are *meta*directing groups. Nitration reaction involves chemical agents sus as Nitric acid (HNO₃). Nitration is frequently directed by using mixed acid or nitrating mixture which is a combination of sulphuric acid (H_2SO_4) and nitric acid (HNO₃) (Freeman and Mock, 2007).

4. Impact of dyes and dye intermediates

Approximately from all color additives 50% azo dyes are extensively used as coloring substances in cosmetic, drug and food industries. This increases concern related to health and safety. Global usage of azo dye as food additive is being regulated (Jiang et al., 2020). Azo dye toxicity is based on benzidine and its counterpart like dimethoxy- and dimethylbenzidine. It may show mutagenic effect on monkeys, humans, dogs, and rodents which lead to disease like cancer (Suryavathi et al., 2005; Bencheqroun et al., 2019). Several dyes are reported to have adverse effect on ecosystem as described in table 1. Dye industrial activity negatively affects human health and environmental condition through large amount of waste discharged into open water sources (Chung, 2016; Bencheqroun et al., 2019). Use of azo dye shows undesirable effect in soil microbial populations and affects plant growth and germination (Lellis et al., 2019). De Jong et al. (2016), have used *Hydra attenuata* as a model to study ecotoxicological impact of mix pollutants in marine environment. They have reported that presence of Disperse Red 1 into fresh water affects biological functions, morphology, neurotransmitter distribution and feeding behavior of *Hydra attenuata*. *Hydra attenuata* contain antioxidant defense mechanism but at high concentration of dye morphological healthy appearance of this organism was affected, as result asexual reproduction was reduced and feeding behavior was also inhibited (De Jong et al., 2016).

5. Degradation of dyes

Complexity of dye structure (crystal ponceau 6R (502.4 g/mol molecular weight), reactive green 19 (1418.94 g/mol molecular weight), remazol red (560.5 g/mol molecular weight), Direct Blue 71 (1029.87 g/mol molecular weight)) make its degradation difficult (Ajaz et al., 2020). Removal of dye industry effluent without proper treatment is harmful for environment and human health (Oon et al., 2020). Several methods are available to treat dye effluent(s). Physical, chemical and biological treatment ((either individually or in combination) have been reported to be widely used for degradation of dyes (Lua et al., 2019; Lan et al., 2019).

5.1. Physico-chemical degradation:

Physico-Chemical degradation is a combination of chemical and physical techniques (Kumar et al., 2020). Physico-chemical treatment is the process in which physical changes are constantly present, while chemical changes in the process at different phases may or may not take place (Karimifard and Alavi Moghaddam, 2018). In this process chemicals such as Lime, Ferric chloride (FeCl₃), Ferrous sulphate (FeSO₄·7H₂O) and Alum ((Al₂SO₄)₃·18H₂O) are widely used to alter physical state of dye molecules (Ayed et al., 2020). Treatments such as flocculation, wet oxidation, membrane separations, adsorption and precipitation are examples of physico-chemical treatment (Wang et al., 2020; Kumar et al., 2020). The disadvantages of this methods are high chemical requirement, high maintenance, costly and large amount of sludge is generated which requires safe dumping (Ajaz et al., 2020).

5.2. Biological degradation

Biological degradation of pollutants is eco-friendly, shows complete mineralization of organic compounds with low sludge generation. This method has been reported as most effective method (Varjani et al., 2015; Bhatia et al., 2017; Varjani et al., 2019; Kumar et al., 2020). Biological degradation can be conducted under aerobic or anaerobic conditions (Khan et al., 2012; Bhatia et al., 2017). Various microorganisms such as bacteria, fungi, yeast and algae were used for dye degradation and decolorization (Ali, 2010; Ajaz et al., 2020). Difference in growth conditions and different metabolic mechanism of microorganisms affects degradation of dyes (Gao et al., 2018). Shabbir et al., (2017a) and Shabbir et al., (2017b), reported degradation of dyes with use of locally available biomaterial (periphyton). Reports have demonstrated importance of enzyme in degradation of dyes such as, azoreductase, laccase, peroxidase and exo-enzymes. E. gallinarum and Streptomyces S27 has been reported for degradation of azo dyes with use of azoreductase enzyme (Bafana et al., 2009; Dong et al., 2019). Laccase have great degradation potential for many aromatic compounds (Bhatia et al., 2017). Shanmugam et al. (2017), have reported maximum biodegradation of Malachite Green by Trichoderma asperellum laccase activity which converted benzaldehyde from Malachite Green via the Michler's ketone pathway. Immobilization of laccase on Glutaraldehyde-crosslinked Chitosan Beads (GA-CBs) has been reported by Nguyen et al. (2016), provided reusability and high catalytic ability which helped in degradation of sulfur dyes when concentration of

Table 1

Dyes and their impacts on environment and human health.

Dyes and	then impacts on environment an		
Sr. No.	Name of the dye	Effects	Reference
1	Disperse Red -1 and Disperse Orange -1	Increases human lymphocytes frequency of micronuclei	Ferraz et al., 2013
2	Reactive Brilliant Red	Affects activity of human proteins	Wang et al., 2008
3	Reactive Black 5	Lowers activity of urease as well as decreases rate of ammonification in earth environment	Wielewski et al., 2020
4	Direct Black 38	Causes cancer in humans such as urinary bladder.	Dewan et al., 1988
5	Direct Blue 15	Causes mutation	Zamora and Jeronimo, 2019
6	Disperse Blue 291	Casues Mutation, affects genetic structure, cellular toxins, denaturation of DNA in human cells, chromosomal instability.	Fernandes et al., 2019
7	Acid Violet 7	Causes degradation of lipid, chromosomal abnormality, breakdown of acetylcholine in mice	Mansour et al., 2010

laccase was low. Enzymatic degradation of crystal ponceau 6R (CP6R) with the help of *Brassica rapa* peroxidase has been studied which shows catalytic activity of peroxidase during dye degradation (Almaguer et al., 2018).

5.2.1. Microbial degradation

For degradation of various dyes different microbes can be used, they have different mechanisms and pathways for degradation of dyes (Cao et al., 2019; Ebrahimi et al., 2019).

Azo dyes are useful class of dyes with highest diversity of colors. Under anaerobic condition and with help of azoreductase, microorganisms degrade azo dyes and as end product they form colorless aromatic amines (Ali, 2010; Ajaz et al., 2020; Dong et al., 2019). Benzidine is generally used in construction of direct azo dyes and has been reported as potential carcinogen (Dewan et al., 1988; Ali, 2010; Sen et al., 2016). Direct dyes are inexpensive and used to dye fibers, leathers or papers without any pre-treatment. Among benzidine based azo dyes most generally used dye is Direct Black 38. Degradation of Direct Black 38 dye can be achieved using Enterococcus gallinarum (Bafana et al., 2008; Bafana et al., 2009). Direct Black 38 has three azo bonds in its structure which are the active sites for azoreductase. Direct Black 38 through metabolic reactions is converted to benzidine which upon deamination results in 4-amion phenyl. It has been reported that dyes which have benzidine as a base is highly carcinogenic as compared to the dyes without Benzidine (Yamjala et al., 2016). This is due to existence of pollutant(s) like 4-amino biphenyl and 2-4, diaminoazobenzene, which have been reported as carcinogens (Dewan et al., 1988; Ali, 2010; Bencheqroun et al., 2019).

6. Factor affecting biodegradation of dyes

Microbe based treatments for degradation of toxic environmental pollutants are economically viable, cost effective and also helps to manage environmental contaminants (Varjani and Upasani, 2017a: Rodrigues de Almeida et al., 2019; Do et al., 2020; Mishra et al., 2020). Dye industrial wastewater holds variability of azo dyes along with other dye stuff which are structurally different. It has been reported that metals, salts and other compounds make degradation of dyes more difficult and it is toxic for bacterial growth too (Ghosh et al., 2020). Factors like temperature, pH, dissolved oxygen, nutrients, dissolved organic matter, metals and organic pollutants influence water quality (Al-Amrani et al., 2014). Organic contaminants such as 2-napthole, Chloroaniline, Benzene, P-aminobenzoicacid, Ethylenedibromide, Pyrene, P-nitrophenol, etc. are commonly used in dye manufacturing and highly present in dye industry wastewater and affects growth of bacteria during wastewater treatment (Awad et al., 2019). The factors affecting dye degradation are mainly divided into two categories. i) Environmental factors, ii) Nutritional factors.

6.1. Environmental factors

6.1.1. pH

pH is important factor for growth of bacteria and also an essential characteristic for effluent treatment (Varjani and Upasani, 2017b). pH can be acidic, alkaline or neutral based on type of dyes and salts used. Rate of dye degradation in dye containing effluent may change through its pH. The problem can be solved by (a) adjusting pH of effluent to support the growth of dye degrading bacteria or (b) selecting the microbial sp. which can grow at effluent pH (Al-Amrani et al., 2014). Basutkar and Shivannavar (2019), reported maximum dye degradation at pH range of 8–10 by using *Lysinibacillus boronitolerans* CMGS-2. 98% degradation of malachite green was achieved RuO₂–TiO₂ and Pt coated Ti mesh electrodes at pH 4.5 (Singh et al., 2016).

6.1.2. Temperature

Water temperature affects activities prevailing in water such as mineralization, diffusion, chemical process which increases pH of water (Delpla et al., 2009; Varjani and Upasani, 2019b). Extreme temperatures can kill bacteria/affect the growth, if bacteria present in wastewater (Al-Amrani et al., 2014; Varjani and Upasani, 2017b). Faster rate in degradation of dye can be achieved by giving bacterial culture an optimum temperature which is generally reported as 30–40 °C for most bacteria. Das and Mishra (2017), have used bacterial consortium of *Bacillus pumilus* HKG212 and *Zobellella taiwanensis* AT 1–3 for degradation of reactive green 19 and reported highest degradation at 32.04 °C. However, few thermophilic bacteria are reported for degradation of azo dye at high temperature. Gursahani and Gupta (2011), reported 75% degradation of effluent at 60 °C by using *Anoxybacillus rupiensis*. It has been reported that decolorization rate decreases as temperature increases (Imran et al., 2015).

6.1.3. Oxygen and agitation

Environmental conditions directly affect degradation/decolorization of dye. Literature is available stating that microbial metabolism is influenced by oxygen and agitation (Varjani and Upasani, 2017a). Different microorganisms require different conditions such as aerobic condition, anaerobic and semi anaerobic. Shaking play role in aeration/ oxygen supply. Oxygenation can be improved by shaking. It is supposed that reductive enzyme activities can be increased under anaerobic condition. However, for aerobic dye degradation oxidative enzymes play important role which require presence of oxygen (Khan et al., 2012). Gonzalez-Gutierrez-de-Lara and Gonzalez-Martinez (2017), studied Direct Blue 2 dye degradation under different oxygen concentration.

6.2. Nutritional factors

6.2.1. Soluble salts

Wastewater from dye industry contains high electric conductivity due to use of high salt concentration in dying process which can be detected using conductivity meter. To increase ionic strength and

S.	Varjani,	et al.	

Degradat	Degradation of dyes using genetically modified microorganisms.	d microorganisms.					
Sr. No.	Sr. No. Genetically modified microorganism Gene Extracted from	Gene Extracted from	Gene Expressed in	Extracted Gene	Vector	Dye	References
1	Escherichia coli JM109 (pGEXAZR)	Rhodobacter sphaeroides AS1.1737	Escherichia coli JM109	Azoreductase	Vector pGEX4T-1	Acid Red GR	Jin et al., 2008
2	Escherichia coli CY1	Rhodococcus sp.	Escherichia coli DH5α	Azo-dye-decolorizing (ADD) genes	Plasmid pAZRS1	Reactive Red 22	Chang and Lin, 2001
3	Escherichia coli SS125	Bacillus latrosporus RRK1	E. coli strain DH5a	Azoreductase gene	Plasmid pAZR-SS125	Remazol Red	Sandhya et al., 2008
4	E. coli BL21 (DE3)	Halomonas elongata	E. coli DH5	Azoreductase gene	Vector pET21a	Methyl red and Remazol Black B	Eslami et al., 2016
5	Escherichia coli JM109 (pGEX-AZR)	Rhodobacter sphaeroides AS1.1737	E. coli JM109	Azoreductase gene	Vector pGEX4T-1	Direct Blue 71	Jin et al., 2009
9	Escherichia coli BL21	K. pneumoniae MGH 78,578	E. coli DH5α	AzoK gene	Vector pGEM-T	Methyl Orange	Dixit and Garg, 2019
7	E. coli BL21 (DE3)	Halomonas sp. strain GT	E. coli DH5α	AzoG gene	pET30a (+)	Azo dye wastewater	Tian et al., 2018

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Bioresource Technology 314 (2020) 123728

development of dye fixation on fabrics salts like Na_2SO_4 , NaCl and $NaNO_3$ are usually added in the dye bath. Hence, with release of dye pollutants, salts are also released in industrial wastewater. Dyes containing high salt concentration may decrease biodegradation rate by reducing biological movement (Basutkar and Shivannavar, 2019).

6.2.1.1. Carbon and nitrogen supplements. Microorganisms require nutrient supplements for quick degradation of pollutants (Varjani and Upasani, 2019a). Organic sources like peptone, yeast extract or combination of carbohydrates and complex organic sources have been reported to obtain high and quick dye degradation rate by both pure cultures and mixed cultures. Dye degradation efficiency can be increased by addition of glucose. Glucose has been reported as most effective and easily available carbon source for microbial metabolism of dyes or dye intermediates (Khan et al., 2012). Phosphorus has been reported as very important factor for growth of microorganism (Kisand et al., 2001; Varjani, and Upasani, 2019a).

6.2.1.2. Dye concentration and dye structure. Dye concentration and dye structure influence degradation/decolorization of dye. Low dye concentration may not have identified by enzymes which are secreted from dye degrading bacteria. On the other hand, high dye concentration is toxic to bacteria and also effect degradation of dye by blocking enzyme active sites. Likewise, low molecular weight and simple structure containing dyes are easy to decolorize. Whereas, high molecular weight and complex structure containing dyes have low decolorization rate (Li et al., 2019). Increased dye concentration decreases dye decolorization and/or degradation (Liu et al., 2016).

6.3. Role of genetically modified organisms

Addition of desired gene into the organism for any particular purpose (i.e. foreign gene), which is not generally part of the host system. produces genetically modified organism (GMO). Nature has selfcleaning process under environmental condition, but literature is available stating that it is insufficient and slow to remove pollutants (Peter et al., 2011; Mishra et al., 2019). Several physical, chemical and biological treatments have been reported for the degradation of hazardous pollutants such as dyes which can be used as individually or in combination (Li et al., 2019; Wang et al., 2019; Varjani et al., 2020). Nowadays, synthetic dyes are produced in such a way that they resist degradation and because of this degradation of dye by traditional techniques is becoming time and efforts consuming (Saxena et al., 2019). Each microorganism has different capability of dye degradation, detoxification and decolorization. Bacteria are most widely used for bioremediation (Kumar et al., 2020). Genetic engineering has made a significant revolution in field of bioremediation (Mishra et al., 2020). Dye degradation/decolorization can be improved using genetically modified organisms under environmental conditions. GMOs can be produced by transferring gene from one species to another species or by gene modification (Peter et al., 2011; Tahri et al., 2013; Saxena et al., 2019; Kumar et al., 2020). To design GMO, functional gene of various bacterial strains has been used such as Sphingomonas desiccabilis, Escherichia coli, Bacillus idriensis, Pseudomonas putida, Mycobacterium marinum, Ralstonia eutropha, etc. and transferred into other species (Saxena et al., 2019). Various genetic tools and techniques are available to identify expression of microbial genome such as single-stranded conformation polymorphism, randomly amplified polymorphic DNA, Polymerase chain reaction (PCR), 16S rDNA sequencing and other new sequencing technologies (Urgun-Demirtas et al., 2006; Holst-Jensen et al., 2016; Mishra et al., 2020). Sandhya (2008), produced Escherichia coli SS125 for degradation of Remazol red dye by transferring azoreductase gene form Bacillus latrosporus RRK1 to Escherichia coli DH5a and Plasmid pAZR-SS125. Jin et al. (2009), have constructed genetically modified E. coli JM109 (pGEX-AZR) in laboratory which shows decolorization of direct blue 71. It was achieved by inserting

azoreductase gene in expression vector pGEX4T-1. Vector was than expressed and transformed in *E. coli* JM109 under control of a lac operon. Ajaz et al. (2020), reported degradation of Remazol red in presence of 0.8 mg/L dissolve oxygen with help of azoreductase gene which was replicated from *Bacillus latrosporus* RRK1 and integrated in *Escherichia coli*. Degradation of various dyes using genetically modified microorganisms including details of host microorganism, donor microorganism, desired gene and vector used has been shown in Table 2

6.4. Microbial degradation of dyes: Gaps and future needs

To achieve better results in biodegradation of dyes, further research work is necessary such as (a) responsible micro-organisms, (b) limitation of experimental factors, (c) site for bioremediation and (d) degradation pathways before applying micro-organisms in the field. It would be of utmost importance to determine the nature of the degradation products and to establish their (non) toxicity to aquatic or plant life. Many microbial degradation techniques have been resisted by dyes, there is a new way to degrade dyes through genetic engineering, which opens a new arena for researchers working in this field. With the use of advanced molecular biology tools responsible genes/enzymes for dye degradation can be studied. Dye degradation may produce byproducts, nutrients and energy which can be used as resources. Complete dye degradation is a challenge for researchers. Successful application of biodegradation of dye wastewater requires a number of research studies that need to be pursued.

- Future studies on dye degradation should be aimed to reduce limitation of factors upon microbial activities.
- Re-examination of recent and early successful studies is required to improve them for enhanced efficiency.
- Effective biodegradation process should consider degradation pathways, environmental factors, degradation rate and degradation mechanisms that affect removal of pollutants. It would be highly imperative to ensure that the degraded products have no toxicity on aquatic life or plants.
- Integration of treatment technologies for dye pollutants is highly desirable for effective translation to industries.
- Study of mechanisms and theories for bacterial degradation of dye wastewater would help to explore bacterial degradation kinetics.

7. Conclusions

Disposal of wastewater generated by dye industries into environment without proper treatment impacts harmfully the soil and water environment. This demands to invent sustainable green processes to remediate the hazardous chemical compounds present in the effluent. Biological treatments offer potential benefits compared to physical and chemical treatment methods. Biological wastewater treatments have been demonstrated using microbial consortia or single microbial strain having capabilities for dye degradation. In this regard, use of genetically modified organisms could be of added advantage to enhance the process efficiency of degradation. Integration of technologies is yet another important aspect, which could bring potential benefits. Advanced technologies and materials need to be developed for effective degradation of dyes in industrial wastewater.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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S. Varjani, et al.

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