Microbial Degradation of Dyes: An overview

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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. This paper provides scientific and technical information about dyes & dye intermediates and biodegradation of azo dye. It also compiles information about factors affecting dye(s) biodegradation, role of genetically modified organisms (GMOs) in process of dye(s) degradation and perspectives in this field of research.

Keywords: Biodegradation; Decolorization; Genetically modified organism; Dye intermediates

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 various chemical reactions (Gregory, 2009; 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 dyes 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). Biological treatment has various advantages such as, it is a simple, cheap, environmental friendly process. Also large number of

67 microorganisms are available which are easy to maintain and also require low preparation
68 (Crini et al., 2019). Apart from these dye degradation techniques periphyton biofilm or
69 periphytic biofilm system can be also used for degradation of dyes (Li and Bishop 2004;
70 Shabbir et al., 2017a; Shabbir et al., 2017b; Pandey and Bergey, 2018; Dias et al., 2019;
71 Shabbir et al., 2020). Among various activities of dye industries, dye manufacturing is the
72 main source of environmental pollution due to release of hazardous dye in water bodies.
73 Numerous microorganisms such as algae, yeast, bacteria, and fungi possess ability to
74 mineralize and/or decolorize various dyes (Roy et al., 2018; Tochhawng et al., 2019).
75 Treatment of dye wastewater can be performed using pure culture or mixed microbial
76 culture. Majorly mixed microbial culture has been reported to achieve efficient dye
77 degradation due to synergistic metabolic actions (Kalyani et al., 2009; Mandal et al., 2010).
78
79 Genetic engineering has made a significant revolution in the field of bioremediation
80 (Varjani et al., 2017; Kumar et al., 2020). Removal of acid red has been reported through
81 the successful manipulation of microorganism using genetically engineering in treatment
82 system (Jin et al., 2008). Factors like pH, temperature, structure of dye, soluble salts, heavy
83 metals, nutrients, etc., affect the degradation of dye (Al-Amrani et al., 2014). There are
84 various reports available which shows degradation of different dyes using microorganisms
85 (Mane et al., 2008; Varjani and Upasani, 2016; Kiayi et al., 2019; Li et al., 2019; Pratiwi et
86 al., 2019).
87
88 Present review intends to expand biodegradation scope of dyes. It includes types of
89 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 contains 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 also contain carcinogenic properties (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 tetra-acetate or permanganate potassium, reduction of nitroso compounds by $AlLiH_4$, condonation of quinone and hydrazine, etc. (Benkhaya et al., 2020). These dyes are recorded for industrial applications and only azo dye contains 60% ratio as compared to all other types of dyes (Shah, 2014; Iark et al., 2019). Azo dyes make up 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 but it is 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 dye has been reported as the oldest dyes because they have been found thousands years back and were used in wrapping mummies (Gurses et al., 2016). 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, 2009).

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, 2009; Freeman et al., 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, 2009; 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 $KMnO_4$. e.g. Hypochlorite oxidation is the production of anthranilic acid by Hofmann process (Gregory, 2009; Freeman et al., 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, 2009).

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 (HNO_3). Nitration is frequently directed by using mixed acid or nitrating mixture which is a combination of sulphuric acid (H_2SO_4) and nitric acid (HNO_3) (Freeman et al., 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 dimethyl- benzidine. 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 ($FeCl_3$), Ferrous sulphate ($FeSO_4.7H_2O$) and Alum ($(Al_2SO_4)_3.18H_2O$) 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 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).

271 *5.2.1. Microbial degradation* - For degradation of various dyes different microbes can be
272 used, they have different mechanisms and pathways for degradation of dyes (Cao et al.,
273 2019; Ebrahimi et al., 2019).

274 Azo dyes are useful class of dyes with highest diversity of colors. Under anaerobic
275 condition and with help of azoreductase, microorganisms degrade azo dyes and as end
276 product they form colorless aromatic amines (Ali, 2010; Ajaz et al., 2020; Dong et al.,
277 2019). Benzidine is generally used in construction of direct azo dyes and has been reported
278 as potential carcinogen (Dewan et al., 1988; Ali, 2010; Sen et al., 2016). Direct dyes are
279 inexpensive and used to dye fibers, leathers or papers without any pre-treatment. Among
280 benzidine based azo dyes most generally used dye is Direct Black 38. Degradation of
281 Direct Black 38 dye can be achieved using *Enterococcus gallinarum* (Bafana et al., 2008;
282 Bafana et al., 2009). Direct Black 38 has three azo bonds in its structure which are the
283 active sites for azoreductase. Direct Black 38 through metabolic reactions is converted to
284 benzidine which upon deamination results in 4-amion phenyl. It has been reported that dyes
285 which have benzidine as a base is highly carcinogenic as compared to the dyes without
286 Benzidine (Yamjala et al., 2016). This is due to existence of pollutant(s) like 4-amino
287 biphenyl and 2-4, diaminoazo-benzene, which have been reported as carcinogens (Dewan
288 et al., 1988; Ali, 2010; Bencheqroun et al., 2019).

289

290 **6. Factor affecting biodegradation of dyes**

291

292 Microbe based treatments for degradation of toxic environmental pollutants are
293 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*

316 *boronitolerans* CMGS-2. 98% degradation of malachite green was achieved RuO_2–TiO_2
317 and Pt coated Ti mesh electrodes at pH 4.5 (Singh et al., 2016).
318
319 6.1.2. *Temperature* - Water temperature affects activities prevailing in water such as
320 mineralization, diffusion, chemical process which increases pH of water (Delpla et al.,
321 2009; Varjani and Upasani, 2019b). Extreme temperatures can kill bacteria/affect the
322 growth, if bacteria present in wastewater (Al-Amrani et al., 2014; Varjani and Upasani,
323 2017b). Faster rate in degradation of dye can be achieved by giving bacterial culture an
324 optimum temperature which is generally reported as 30-40^{o}C for most bacteria. Das and
325 Mishra (2017), have used bacterial consortium of *Bacillus pumilus* HKG212 and *Zobellella*
326 *taiwanensis* AT 1-3 for degradation of reactive green 19 and reported highest degradation
327 at 32.04^{o}C. However, few thermophilic bacteria are reported for degradation of azo dye at
328 high temperature. Gursahani and Gupta (2011), reported 75% degradation of effluent at
329 60^{o}C by using *Anoxybacillus rupiensis*. It has been reported that decolorization rate
330 decreases as temperature increases (Imran et al., 2015).
331
332 *6.1.3. Oxygen and agitation* - Environmental conditions directly affect
333 degradation/decolorization of dye. Literature is available stating that microbial metabolism
334 is influenced by oxygen and agitation (Varjani and Upasani, 2017a). Different
335 microorganisms require different conditions such as aerobic condition, anaerobic and semi
336 anaerobic. Shaking play role in aeration/oxygen supply. Oxygenation can be improved by
337 shaking. It is supposed that reductive enzyme activities can be increased under anaerobic
338 condition. However, for aerobic dye degradation oxidative enzymes play important role

339 which require presence of oxygen (Khan et al., 2012). Gonzalez-Gutierrez-de-Lara and
340 Gonzalez-Martinez (2017), studied Direct Blue 2 dye degradation under different oxygen
341 concentration.
342
343 *6.2. Nutritional factors*
344
345 *6.2.1. Soluble salts* - Wastewater from dye industry contains high electric conductivity due
346 to use of high salt concentration in dying process which can be detected using conductivity
347 meter. To increase ionic strength and development of dye fixation on fabrics salts like
348 Na_2SO_4, NaCl and $NaNO_3$ are usually added in the dye bath. Hence, with release of dye
349 pollutants, salts are also released in industrial wastewater. Dyes containing high salt
350 concentration may decrease biodegradation rate by reducing biological movement
351 (Basutkar and Shivannavar, 2019).
352
353 6.2.2. *Carbon and nitrogen supplements* - Microorganisms require nutrient supplements
354 for quick degradation of pollutants (Varjani and Upasani, 2019a). Organic sources like
355 peptone, yeast extract or combination of carbohydrates and complex organic sources have
356 been reported to obtain high and quick dye degradation rate by both pure cultures and
357 mixed cultures. Dye degradation efficiency can be increased by addition of glucose.
358 Glucose has been reported as most effective and easily available carbon source for
359 microbial metabolism of dyes or dye intermediates (Khan et al., 2012). Phosphorus has
360 been reported as very important factor for growth of microorganism (Kisand et al., 2001;
361 Varjani, and Upasani, 2019a).

362 *6.2.3. Dye concentration and dye structure* - Dye concentration and dye structure
363 influence degradation/decolorization of dye. Low dye concentration may not have
364 identified by enzymes which are secreted from dye degrading bacteria. On the other hand,
365 high dye concentration is toxic to bacteria and also effect degradation of dye by blocking
366 enzyme active sites. Likewise, low molecular weight and simple structure containing dyes
367 are easy to decolorize. Whereas, high molecular weight and complex structure containing
368 dyes have low decolorization rate (Li et al., 2019). Increased dye concentration decreases
369 dye decolorization and/or degradation (Liu et al., 2017).
370
371 **7. Role of genetically modified organisms**
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373 Addition of desired gene into the organism for any particular purpose (i.e. foreign
374 gene), which is not generally part of the host system, produces genetically modified
375 organism (GMO). Nature has self-cleaning process under environmental condition, but
376 literature is available stating that it is insufficient and slow to remove pollutants (Peter et
377 al., 2011; Mishra et al., 2019). Several physical, chemical and biological treatments have
378 been reported for the degradation of hazardous pollutants such as dyes which can be used
379 as individually or in combination (Li et al., 2019; Wang et al., 2019; Varjani et al., 2020).
380 Nowadays, synthetic dyes are produced in such a way that they resist degradation and
381 because of this degradation of dye by traditional techniques is becoming time and efforts
382 consuming (Saxena et al., 2019). Each microorganism has different capability of dye
383 degradation, detoxification and decolorization. Bacteria are most widely used for
384 bioremediation (Kumar et al., 2020). Genetic engineering has made a significant revolution

385 in field of bioremediation (Mishra et al., 2020). Dye degradation/decolorization can be
386 improved using genetically modified organisms under environmental conditions. GMOs
387 can be produced by transferring gene from one species to another species or by gene
388 modification (Peter et al., 2011; Tahri et al., 2013; Saxena et al., 2019; Kumar et al., 2020).
389 To design GMO, functional gene of various bacterial strains has been used such as
390 *Sphingomonas desiccabilis, Escherichia coli, Bacillus idriensis, Pseudomonas putida,*
391 *Mycobacterium marinum, Ralstonia eutropha,* etc. and transferred into other species
392 (Saxena et al., 2019). Various genetic tools and techniques are available to identify
393 expression of microbial genome such as single-stranded conformation polymorphism,
394 randomly amplified polymorphic DNA, Polymerase chain reaction (PCR), 16S rDNA
395 sequencing and other new sequencing technologies (Urgun-Demirtas et al., 2006; Holst-
396 Jensen et al., 2016; Mishra et al., 2020). Sandhya (2008), produced *Escherichia coli* SS125
397 for degradation of Remazol red dye by transferring azoreductase gene form *Bacillus*
398 *latrosporus* RRK1 to *Escherichia coli* DH5a and Plasmid pAZR-SS125. Jin et al. (2009),
399 have constructed genetically modified *E. coli* JM109 (pGEX-AZR) in laboratory which
400 shows decolorization of direct blue 71. It was achieved by inserting azoreductase gene in
401 expression vector pGEX4T-1. Vector was than expressed and transformed in *E. coli* JM109
402 under control of a lac operon. Ajaz et al. (2020), reported degradation of Remazol red in
403 presence of 0.8 mg/L dissolve oxygen with help of azoreductase gene which was replicated
404 from *Bacillus latrosporus* RRK1 and integrated in *Escherichia coli*. Degradation of various
405 dyes using genetically modified microorganisms including details of host microorganism,
406 donor microorganism, desired gene and vector used has been shown in Table 2
407

8. 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 by-products, 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.

4. 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.

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Table Legends

Table 1 Dyes and their impacts on environment and human health

Table 2 Degradation of dyes using genetically modified microorganisms

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Table 1: Dyes and their impacts on environment and human health

Sr. No.	Name of the dye	Effects	Reference
1	Disperse Red -1 and Disperse Orange -1	Increases human lymphocytes frequency of micronuclei	Ferraz, 2013
2	Reactive Brilliant Red	Affects activity of human proteins	Wang, 2008
3	Reactive Black 5	Lowers activity of urease as well as decreases rate of ammonification in earth environment	Wielewski, 2020
4	Direct Black 38	Causes cancer in humans such as urinary bladder.	Dewan et al., 1988
5	Direct Blue 15	Causes mutation	Zamora et al., 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

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Table 2: Degradation of dyes using genetically modified microorganisms

Sr. No.	Genetically modified microorganism	**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
6	*Escherichia coli* BL21	*K. pneumoniae* MGH 78578	*E. coli* DH5α	*Azo*K 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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