



Review:

Bacterial degradation of anthraquinone dyes*

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Abstract: Anthraquinone dyes, which contain anthraquinone chromophore groups, are the second largest class of dyes after azo dyes and are used extensively in textile industries. The majority of these dyes are resistant to degradation because of their complex and stable structures; consequently, a large number of anthraquinone dyes find their way into the environment causing serious pollution. At present, the microbiological approach to treating printing and dyeing wastewater is considered to be an economical and feasible method, and reports regarding the bacterial degradation of anthraquinone dyes are increasing. This paper reviews the classification and structures of anthraquinone dyes, summarizes the types of degradative bacteria, and explores the possible mechanisms and influencing factors of bacterial anthraquinone dye degradation. Present research progress and existing problems are further discussed. Finally, future research directions and key points are presented.

Key words: Anthraquinone dyes; Bacterial degradation; Degradation mechanism; Influencing factor
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1 Introduction

Synthetic dyes are used extensively in textile, paper, leather, plastic, pharmaceutical, cosmetic, food, and other fields (Yagub et al., 2014; Wang et al., 2018). With over 100000 commercially available dyes, more than 7×10^5 metric tons of dyes are produced annually worldwide, of which the textile industry accounts for two-thirds (Das and Mishra, 2017). Because of low utilization efficiency, approximately 2%–50% of these dyes remain unused and are present in processed effluents (Cui MH et al., 2016). These synthetic dyes, which are either discharged directly or

processed incompletely and then discharged into the environment, are highly visible and affect the aesthetic merit, water transparency, and gas solubility of the water body (Banat et al., 1996). Moreover, some toxic substances in the dyes have threatened human health by causing bleeding, skin ulceration, nausea, and dermatitis (Lee et al., 2006).

The structural diversity of synthetic dyes is due to different chromophore groups such as azo, anthraquinone, and triphenylmethane (Mishra and Maiti, 2018). Anthraquinone dyes are the second most used dyes after azo dyes because of their low price, easy accessibility, and good dyeing performance. Anthraquinone dyes have a complex and stable structure and are more toxic to microorganisms and human cells than azo dyes (Novotný et al., 2006). There are various treatment methods for anthraquinone dye degradation which include Fenton oxidation (Khataee et al., 2016), photocatalytic oxidation (Zhang and Lu, 2018), ozone oxidation (Lovato et al., 2017), ultrasonic catalytic oxidation (Samanta et al., 2018) and

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microwave catalysis (Park et al., 2018). However, compared to these chemical methods, biological methods are inexpensive and do not cause secondary pollution, although biodegradation can be unstable and time-consuming (Solís et al., 2012; Rybczyńska-Tkaczyk et al., 2018). Microorganisms have been used to degrade anthraquinone dye, including algae (Otto and Schlosser, 2014), fungi (Zhang et al., 2016; Šlosarčíková et al., 2017), actinomycetes (Linde et al., 2014), and bacteria (Parmar and Shukla, 2018). In comparison with filamentous fungi and algae, some bacteria grow more rapidly at extreme pH and temperature, with low nutrient concentrations and high pollutant concentrations (Chen et al., 2007). Therefore, bacteria may be more useful than fungi and algae for anthraquinone dye degradation.

Several articles have reviewed the microbial decolorization and degradation of synthetic dyes. For example, Crini (2006) has reviewed the decolorization and/or bioadsorption of various dyes in wastewater by (dead or living) biomass. Ali (2010) described that fungi, bacteria, yeasts, and algae could efficiently degrade dyes. Recently, Mishra and Maiti (2018) summarized three major classes of reactive dyes (azo, anthraquinone, and triphenylmethane dyes) that could be degraded by various bacteria. In spite of these available review articles, a comprehensive study on bacterial anthraquinone dye degradation is not available. Bacterial anthraquinone dye degradation is a complicated process that involves adsorption, degradation, and enzyme catalysis. However, previous reviews on anthraquinone dye degradation focused either on a summarized variety of biological species or on very specific pathway. More importantly, many other researchers have focused on the isolation of highly-efficiency bacteria for anthraquinone dye degradation and have achieved good decolorization effects. However, the mechanisms and optimal conditions of anthraquinone dye degradation remain unknown. This restricts industrial application. Therefore, a more integrated review containing degrading bacteria and their universal mechanisms of bacterial anthraquinone dye removal is needed. In this review, anthraquinone dye degradation by pure or mixed bacteria is debated. Degradation mechanisms and their main influencing factors are also discussed. The review should provide useful information for anthraquinone dye wastewater treatment.

2 Structure and classification of anthraquinone dye

Anthraquinone dyes contain anthraquinone chromophore groups. The anthraquinone chromophore comprises two carbonyl groups on both sides of a benzene ring (Table 1, a). Diverse anthraquinone dyes are formed by changing the substituents (amino, hydroxyl, halogen, and sulfonic acid substituents) that are present. In addition, the properties and locations of the substituents affect the anthraquinone dye color. Electron-donating and electron-accepting substituents have different effects, with the introduction of the latter having little influence on color. In contrast, electron-donating substituents (i.e. amino or substituted amino groups) significantly impact dyes with color changing from yellow to red, purple, or blue (Table 1, a–d) (Duval et al., 2016). Anthraquinone dyes have been divided into four categories: (1) anthraquinone derivatives, such as Disperse Red 3B (Table 1, e) and Acid Green 25; (2) heterocyclic anthraquinone dyes, such as Vat Yellow 28 (Table 1, f); (3) fused ring anthrone dyes, such as Vat Green 1 (Table 1, g); and (4) heterocyclic anthrone dyes, such as Pigment Yellow 24 (Table 1, h). Anthraquinone dye color varies greatly because of its substituents, and this is also the main reason why the same strains have different degradation efficiencies for various dyes.

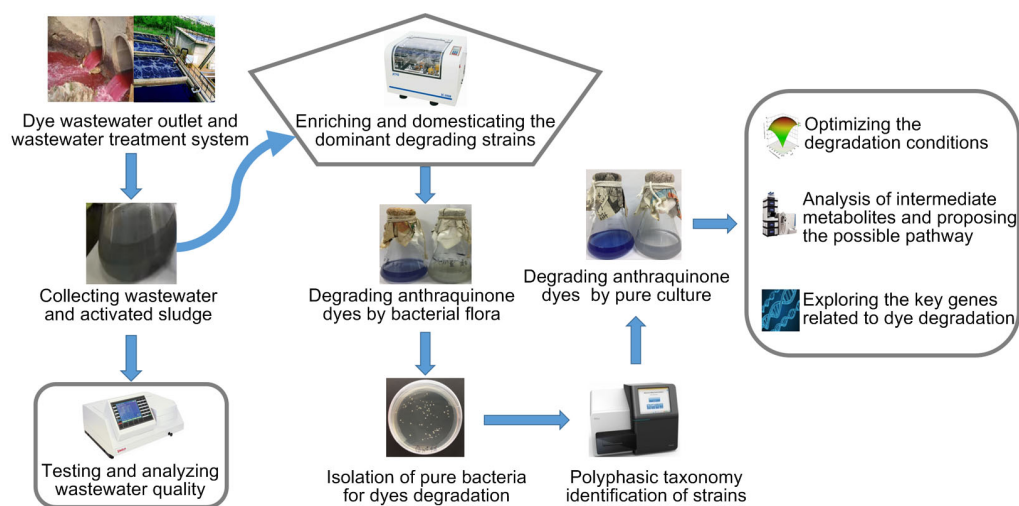
3 Bacterial degradation of anthraquinone dyes

In previous studies, researchers have typically isolated anthraquinone dye-degrading strains from activated sludge and dyeing wastewater collected near dyeing wastewater treatment systems or outlets. An increased number of domesticated degradation strains can accumulate in the sites contaminated with dyes. The universal stepwise procedure for isolating dye-degrading strains under laboratory conditions is shown in Fig. 1.

A range of indigenous bacteria, including *Bacillus* sp. (Wang et al., 2009), *Pseudomonas* sp. (Forss et al., 2017), *Shewanella* sp. (Wang et al., 2015), *Aeromonas* sp. (Ren et al., 2006), *Rhodococcus* sp. (Roberts et al., 2011), and *Klebsiella* sp. (Xie et al., 2016), have been isolated to degrade anthraquinone

Table 1 Basic structures of several anthraquinone dyes

| No. | Chemical structure | Dye | CAS |
|-----|--------------------|-------------------------------------------------------------------|------------|
| (a) | | Anthraquinone (Yellow) | 84-65-1 |
| (b) | | 1-(Methylamino) anthraquinone (Red) | 82-38-2 |
| (c) | | 1-[(2-Hydroxyethyl) amino]-4-(methylamino) anthraquinone (Purple) | 86722-66-9 |
| (d) | | 1-Amino-4-(methylamino) anthracene-9,10-dione (Blue) | 1220-94-6 |
| (e) | | Disperse Red 3B | 84-65-1 |
| (f) | | Vat Yellow 28 | 4229-15-6 |
| (g) | | Vat Green 1 | 128-58-5 |
| (h) | | Pigment Yellow 24 | 475-71-8 |

**Fig. 1 Stepwise procedure for isolating dye-degrading strains under laboratory conditions**

dyes. Some of these pure or mixed bacteria and their degradation conditions are summarized in Table 2. The main types of dyes that have been studied include anthraquinone derivatives and heterocyclic anthraquinones, such as Remazol Brilliant Blue R and Reactive Blue 19; as the bacterial degradation of fused ring and heterocyclic anthrone dyes is yet to be reported, their biodegradation may be difficult. The reported optimum conditions for the bacterial degradation of anthraquinone dyes have been in accordance with their growth conditions. Most strains require additional carbon and nitrogen sources to co-metabolize

dyes, and degradation efficiency varies among different strains. Velayutham et al. (2018) isolated a strain identified as *Staphylococcus* sp. K2204 that could efficiently and completely degrade 100 mg/L of Remazol Brilliant Blue R within 12 h. Tian et al. (2016) isolated five strains from soil and sludge, including *Serratia* sp. JHT01, *Serratia liquefaciens* PT01, *Pseudomonas chlororaphis* PT02, *Stenotrophomonas* sp. PT03, and *Mesorhizobium* sp. PT04, and *S. liquefaciens* PT01 was the best strain as it could decolorize 50%–60% of Remazol Brilliant Blue R within 336 h in Luria-Bertani (LB) medium.

Table 2 Anthraquinone dye-degrading bacteria strains

| Bacterial name and reference | Dyes (concentration (mg/L)) | Conditions (pH, temperature, shaker speed) | Time (h) | Degradation rate (%) [*] | Culture medium | |
|------------------------------------------------------------------------------------------------------------------|-----------------------------------|--------------------------------------------|----------|-----------------------------------|--------------------------------------------------------------------------------|------|
| <i>Pseudomonas</i> strain GM3 (Yu et al., 2001) | Reactive Blue 2 (100) | pH=7, 35 °C, 150 r/min | 48 | 14–24 | 5 g/L peptone, 2.5 g/L yeast extract, 2.5 g/L NaCl and mineral solution | |
| | Acid Green 27 (100) | | | 60–84 | | |
| <i>Aeromonas hydrophila</i> DN322 (Ren et al., 2006) | Reactive Brilliant Blue K-GR (50) | pH=7.5, 30 °C, 150 r/min (microaerophilic) | 36 | 85±7 | M9 synthetic medium | |
| | Acid Blue 25 (50) | | | 60±5 | | |
| | Acid Blue 56 (50) | | | 21±6 | | |
| <i>Shewanella decolorationis</i> S12 (Xu et al., 2006) | Reactive Brilliant Blue K-GR (50) | pH=8, 30 °C, 150 r/min | 11 | 93 | Lactate medium | |
| <i>Bacillus cereus</i> strain DC11 (Deng et al., 2008) | Acid Blue 25 (100) | pH=7, 37 °C, 150 r/min | 6 | 96±4 | M9 synthetic medium | |
| | Disperse Red 11 (50) | | | 24 | | 90±8 |
| | Reactive Brilliant Blue K-GR (50) | | | 24 | | 85±5 |
| | Acid Blue 56 (50) | | | 48 | | 20±4 |
| <i>Bacillus subtilis</i> (Olagnathan and Patterson, 2009) | Vat Blue 4 (300) | pH=10, 37 °C, 150 r/min | 12 | 100 | Nutrient broth | |
| | Vat Blue 4 (500) | | | 24 | | 100 |
| | Vat Blue 4 (700) | | | 36 | | 100 |
| <i>Enterobacter</i> sp.F NCIM 5545 (Holkar et al., 2014) | Reactive Blue 19 (50) | pH=7, 30 °C, 150 r/min | 108 | 25 | Nutrient broth | |
| | Reactive Blue 19 (50) | | | 90 | | |
| <i>Sphingomonas xenophaga</i> QYY (Lu et al., 2015) | Bromaminic Acid (100) | pH=7, 30 °C, 150 r/min | 10 | >96 | ABAS-LB medium | |
| <i>Escherichia coli</i> DH5α (Cerboneschi et al., 2015) | Acid Blue 277 (300) | pH=7, 37 °C, 150 r/min | 16 | >60 | LB medium | |
| | Acid Blue 260 (300) | | | >80 | | |
| | Acid Blue 40 (300) | | | >60 | | |
| | Acid Blue 25 (300) | | | >50 | | |
| | Acid Blue 43 (300) | | | >20 | | |
| | Acid Blue 324 (300) | | | >60 | | |
| <i>Serratia liquefaciens</i> PT01 (Tian et al., 2016) | Remazol Brilliant Blue R (125) | pH=7, 30 °C, 150 r/min | 336 | 50–60 | LB medium | |
| <i>Escherichia coli</i> CD-2, <i>Klebsiella pneumoniae</i> , <i>Bacillus subtilis</i> WD23 (Cui DZ et al., 2016) | Reactive Blue 19 (100) | pH=7, 37 °C | 12 | <40 | Basal medium: 5 g/L glucose, 2 g/L NH ₄ Cl and many inorganic salts | |
| <i>Staphylococcus</i> sp. K2204 (Velayutham et al., 2018) | Remazol Brilliant Blue R (100) | pH=7, 37 °C (static) | 12 | 100 | Nutrient broth | |
| <i>Staphylococcus hominis</i> subsp. <i>hominis</i> DSM 20328 (Parmar and Shukla, 2018) | Reactive Blue 4 (50) | pH=7, 37 °C, 60 r/min | 24 | 97 | Nutrient broth | |

^{*} Data are expressed as range, mean, or mean±standard deviation (SD)

Generally, the degradation efficiency of mixed bacteria is better than that of a pure bacterium under the same conditions, and this is likely because of collaboration among the bacteria. Yang et al. (2017) used a bacterial community (in which the dominant bacteria were Proteobacteria and Firmicutes) to decolorize Active Blue 19 in nutrient broth. The decolorization and mineralization rates of 150 mg/L dye within 48 h were 89.2% and 13.0%, respectively, indicating that the bacterial flora could not completely degrade the dye, but instead degraded the macromolecular dye into intermediate small metabolites. Chaudhari et al. (2017) found that aerobic bacterial granules (ABGs), containing predominantly Proteobacteria and Firmicutes, could degrade and tolerate high concentrations of Reactive Blue 4 of up to 1000 mg/L with a maximum decolorization rate (V_{\max}) of (6.16 ± 0.82) mg/(L·h). As shown in Table 2, most of the isolated degradative bacteria can grow and degrade dyes under aerobic conditions and produce decolorization enzymes under anaerobic conditions. Firmicutes have been reported to be the dominant bacteria under anaerobic or microaerobic conditions (Balapure et al., 2014), and they could promote anthraquinone dye degradation in the hydrolysis stage (Wang et al., 2011). In addition, almost all functional bacteria decolorize and degrade dyes only in a nutrient medium, such as LB and nutrient broth medium. A few strains can utilize dyes as sole carbon and nitrogen sources, and their decolorization rate is low.

4 Mechanisms of bacterial anthraquinone dye removal

The decolorization of dyes by bacteria may result from adsorption or biodegradation. In the process of adsorption, cell mats become deeply colored, whereas those that retain their original color undergo biodegradation (Ren et al., 2006). Many studies have reported degradation pathways for azo dyes, but there is little research focused on the bacterial degradation mechanisms of anthraquinone dyes. Therefore, most proposed pathways and intermediates of bacterial anthraquinone dye degradation have not yet been clearly elucidated.

4.1 Bacterial anthraquinone dye adsorption mechanisms

The bacterial adsorption of dyes occurs when dyes adhere to bacterial surfaces through covalent, electrostatic, or molecular forces. In the process of bacterial anthraquinone dye removal, the dyes are usually adsorbed through the bacterial surface before further degradation occurs; therefore, adsorption is one of the crucial steps in dye degradation. Peptidoglycan has been reported to be an important factor that affects the bacterial adsorption of dyes. The mass fraction of peptidoglycan in the cell wall of Gram-positive bacteria ranges from 40% to 90%, while that of Gram-negative bacteria is only approximately 10%; consequently, the adsorption capacity of the former is 5–10 times greater than that of the latter (Cai et al., 2008). In addition, bacterial adsorption does not involve chemical reactions in which the functional groups on the surface of the cell wall play a major role in the adsorption process. Du et al. (2012) tested the functional groups of live and heat-treated *Pseudomonas* sp. DY1 using potentiometric titration and Fourier transform infrared spectroscopy (FTIR) and revealed the main functional groups as CH_2OH , C-O , P-O_2 , CONR_2 , CO-O-C , NH_2 , C-N , COO^- , and PO_2^- , while NH_2 played a prominent role in dye biosorption.

Current research on the adsorption properties of dyes only compares living and dead cells. To investigate the dye adsorption of inactivated bacteria, the decolorization properties of Acid Blue 277 by *Bacillus gordonae*, *Bacillus benzeovorans*, and *Pseudomonas putida* (autoclaved at 110 °C for 15 min) were studied, giving biosorption rates of 13%, 19%, and 18%, respectively (Walker and Weatherley, 2000). Autoclaving increased the cell membrane permeability and enhanced the adsorption capacity (Ogugbue et al., 2012); however, the decolorization rate was still mostly due to living cells rather than dead cells. Few scholars have studied adsorptive decolorization due to the production of large amounts of sediment, which not only need to be treated further but also cause secondary pollution. In future studies, reducing the pollution caused by adsorption and using molecular biology techniques to explore the mechanism of bacterial dye adsorption would be necessary.

4.2 Bacterial anthraquinone dye degradation mechanisms

Biodegradation of dyeing wastewater is more significant than adsorption for environmental protection. The bacterial degradation of anthraquinone dyes is due to a reduction reaction in which the catalytic cracking of the conjugated dye bonds is performed by a reductase. After the loss of the chromophores, the remaining complex polycyclic aromatic hydrocarbons are decomposed into single rings or simple polycyclic aromatic hydrocarbons (naphthalene, anthracene, etc.) and are then finally completely decomposed into carbon dioxide and water under aerobic conditions.

The first step of bacterial anthraquinone dye degradation involves the dissociation of the small molecular groups around the anthraquinone rings from the parent compound under aerobic conditions (Andleeb et al., 2012). For example, the main metabolites of Reactive Blue 4 degradation by ABGs were 4-amino-9,10-dihydro-9,10-dioxoanthracene-2-sulfonic acid and 2-(4,6-dichloro-1,3,5-triazin-2-ylamino)-4-aminophenol, as detected by high performance liquid chromatography-high resolution mass spectrometry (HPLC-HRMS). The proposed pathway is displayed in Fig. 2 (Chaudhari et al., 2017). The anthraquinone ring is gradually broken, forming much smaller molecular compounds through oxidation and hydrolysis. *Sphingomonas herbicidovorans* FL can degrade 1-amino-4-bromoanthraquinone-2-sulfonic acid into phthalic acid, 2-amino-3-hydroxy-5-bromobenzenesulfonic acid or 2-amino-4-hydroxy-5-bromobenzenesulfonic acid; a probable pathway was proposed and is illustrated in Fig. 3 (Fan et al., 2008). Andleeb et al. (2012) analyzed the complete degradation pathway of Brilliant Blue K2RL using liquid chromatography-mass spectrometry (LC-MS) and found that the parent compound was initially broken at the attachment site of the reactive group, releasing the difluorochlorotriazine ring and anthraquinone moiety. Further cleavage of the anthraquinone metabolites led to the formation of small molecules such as benzoic acid. The above studies indicated that the differences in anthraquinone decolorization by bacteria were mainly due to the different functional groups of anthraquinone. However, the bacteria exhibited similar metabolic pathways, including demethylation, hydrogenation reduction, dehydroxylation, and other biochemical processes.

The anthraquinone ring was then broken into single benzene rings, and the benzene rings were ultimately cleaved to form linear small molecules.

However, several problems in the research of dye degradation pathways still exist. First, different kinds of anthraquinone dye degradation pathways have not yet been elucidated, such as those of fused ring and heterocyclic anthrone dyes, which may be because of the complex structures and toxicities of these dyes. Secondly, possible anthraquinone dye degradation pathways have been suggested in the literature, but many of these pathways are incomplete. The dye degradation process may occur quickly under eutrophic conditions, resulting in many intermediate products that are difficult to detect; thus, these proposed pathways are based on speculated intermediates. In addition, the detection of countless chemical structures derived from anthraquinone dyes is difficult, making the capture of intermediate metabolites challenging. Further studies should be conducted to explore the degradation mechanisms of other new anthraquinone dyes instead of the limited number of simple anthraquinone dyes, such as Remazol Brilliant Blue R, Reactive Blue 19, and Acid Blue.

4.3 Enzymatic anthraquinone dye degradation mechanisms

Anthraquinone dyes (such as Reactive Brilliant Blue 19 and Reactive Brilliant Blue 114) contain an antioxidant dianthrone group, which is difficult to degrade catalytically. Most anthraquinone dye degradative enzymes have been isolated and purified from fungi, but a few have been obtained from bacteria. Here, we discuss several enzymes that have been found to decolorize anthraquinone dyes efficiently. For example, the intracellular enzymes of *Pseudomonas* GM3 mainly contribute to dye reduction (Yu et al., 2001). A purified *Anabaena* peroxidase was found to efficiently decolorize anthraquinone dyes such as Reactive Blue 5, Reactive Blue 4, Reactive Blue 114, Reactive Blue 119, and Acid Blue 45 with decolorization rates of 262, 167, 491, 401, and 256 $\mu\text{mol}/(\text{L}\cdot\text{min})$, respectively (Ogola et al., 2009). Uchida et al. (2015) also identified a dye-decolorizing peroxidase protein from *Vibrio cholerae* (VcDyP), and its activity was assayed by monitoring the degradation of Reactive Blue 19. To identify the radical site of VcDyP, each of nine tyrosine or two

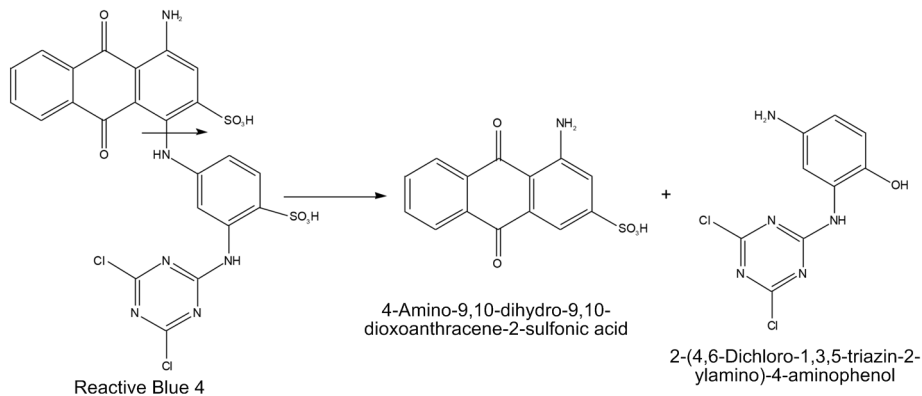


Fig. 2 Proposed pathway for the biotransformation of Reactive Blue 4 by aerobic bacterial granules (ABGs)
This figure is modified from Chaudhari et al. (2017)

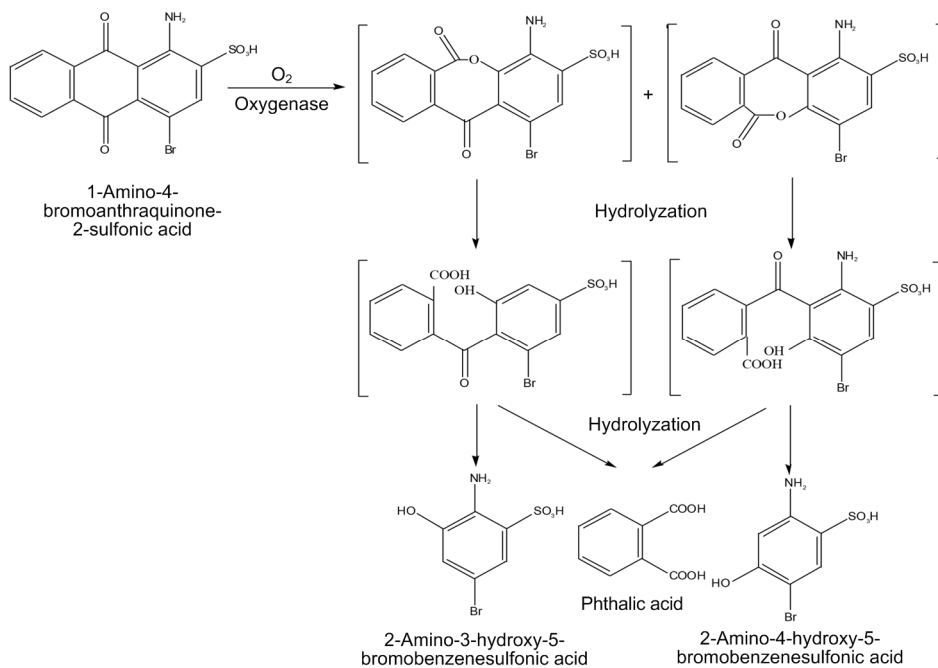


Fig. 3 Proposed pathway of 1-amino-4-bromoanthraquinone-2-sulfonic acid degradation by *Spingomonas herbidovorans* FL

This figure is modified from Fan et al. (2008)

tryptophan residues was substituted, and the results indicated that two distal residues, aspartic acid (Asp) 144 and arginine (Arg) 230, were essential for the DyP reaction. These radicals moved to Tyr129 and Tyr235 during the degradation of dyes at pH 3–5 and to Tyr133 at pH 6–10 (Fig. 4). Liu et al. (2017) found a new bacterial laccase from *Klebsiella pneumoniae* that could efficiently decolorize anthraquinone dyes. More enzymes with potential value for the biocatalysis of anthraquinone dyes are needed, because of their high efficiency. Future research should focus on exploring

the mechanism of anthraquinone dye degradation by enzymes rather than testing only enzyme activity.

5 Factors that affect bacterial anthraquinone dye degradation

Anthraquinone dye degradation is significantly influenced by a variety of operational parameters including intrinsic factors, such as bacterial strain characteristics, and extrinsic factors, such as pH,

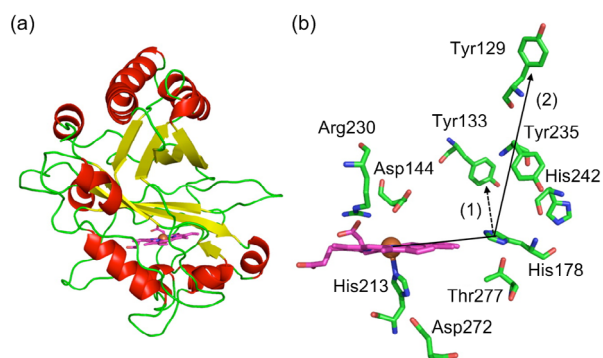


Fig. 4 Crystal structures of VcDyP

(a) α -Helices and β -sheets of a VcDyP subunit are colored red and yellow, respectively. The heme is colored pink. (b) Close-up of the heme-binding site and putative pH-dependent radical transfer pathways at a lower pH (1) and higher pH (2). Reprinted with permission from Uchida et al. (2015), copyright 2015, American Chemical Society

temperature, carbon and nitrogen sources, availability of oxygen, dye concentration and structure, metal ions, ventilatory capacity, reducing power, and electron donors (Krishnan et al., 2017). In general, researchers have investigated the impacts of different factors to improve the efficiency of anthraquinone dye degradation.

5.1 Effect of pH on bacterial anthraquinone dye degradation

pH has a major effect on the decolorization efficiency of dyes, and the optimal pH for color removal is often between 6.0 and 8.0. Otherwise, the decolorization rate would rapidly decrease under strong acidic or alkaline conditions (Kobayashi et al., 2017). For example, the VcDyP expressed in *Escherichia coli* could degrade Reactive Blue 19 at a lower pH (under which conditions Tyr129 and Tyr235 are in the active site of the dye degradation reaction); however, it lost its activity under neutral or alkaline conditions because of a change in the radical transfer pathway (Uchida et al., 2015). The pH affects the decolorization and degradation rates in two main ways: (1) pH changes the state of dye molecules in aqueous solution, which further affects dye absorption (He, 2009), and (2) pH might also affect the cell membrane permeation efficiency, which has been found to be the limiting step for bacterial dye degradation (Kodam et al., 2005). A buffer is usually added to adjust the pH value during biological processes in wastewater treatment plants, and this could enhance bacterial

activity and improve the decolorization rate. However, this method has greatly increased the cost of bacteria degradation. Improving the pH resistance of bacteria is necessary to enable their activity under variable pH conditions.

5.2 Effect of temperature on bacterial anthraquinone dye degradation

During the process of dyeing wastewater treatment, the growth, reproduction rate, and biodegradation activities of microorganisms, and also the solubility of pollutants are affected by temperature (Pearce et al., 2003). In general, the optimum temperature range for bacterial decolorization of dyes is in a narrow range and is consistent with bacterial growth: temperature ranges between 25 and 37 °C (Cui DZ et al., 2016; Velayutham et al., 2018). The decolorization rate of Reactive Blue 4 by *Staphylococcus hominis* subsp. *hominis* DSM 20328 increased with rising temperature from 25 to 37 °C (the maximum rate was observed at 37 °C) and then decreased with raised temperature to 55 °C, which may have resulted in the loss of cell viability or the deactivation of enzymes responsible for degradation (Parmar and Shukla, 2018). At present, the biological treatment systems of printing and dyeing wastewater treatment plants usually stop working in the cold season because the low temperature reduces bacterial activity and influences the degradation efficiency. In future research, isolating strains that can tolerate extreme temperatures will be essential.

5.3 Effect of external carbon and nitrogen sources on bacterial anthraquinone dye degradation

The traditional activated sludge system used in treating dyeing wastewater has a few dye-degrading strains. The strains that are available have low activity and require the addition of some nutrients, such as carbon and nitrogen sources, to promote bacterial growth and maintain the diversity of the microbial community. Carbon and nitrogen sources typically used in the laboratory include L-rhamnose, creatinine, beef extract, glucose, sucrose, D-maltose, KNO₃, (NH₄)₂SO₄, tryptone and yeast extract (Kurade et al., 2016). However, more economical nutrients, such as sodium acetate, industrial flour, urea, animal waste, and ammonium chloride, are usually chosen for dyeing wastewater treatment systems. This is one of

the reason why the strains work efficiently in the laboratory but not in actual treatment systems. In future, laboratory studies identifying inexpensive and readily available carbon/nitrogen sources for degrading anthraquinone dyes will be important.

In contrast, excess carbon and nitrogen sources can also inhibit dye biodegradation efficiency, perhaps through carbon catabolite repression (Crabtree effect), wherein higher external glucose concentrations limit the microbial tricarboxylic acid cycle (Sadykov et al., 2013). Holkar et al. (2014) researched the effects of different concentrations of glucose (2–12 g/L) on the degradation of Reactive Blue 19 by *Enterobacter* sp.F NCIM 5545, and they found that the decolorization rate was low when the glucose concentration was high, such as at 8 and 12 g/L. The same results were obtained in other research (Wang et al., 2009). In the process of anthraquinone dye degradation, various strains have different carbon and nitrogen sources; thus, balancing the exogenous carbon and nitrogen sources to enhance the dye decolorization rate and to reduce the inhibition of carbon metabolism is necessary.

5.4 Effect of oxygen on bacterial anthraquinone dye degradation

Oxygen is essential for the growth of aerobic dye-degrading bacteria. While oxygen is converted into water as an electron acceptor to generate energy, it has a serious inhibitory effect on anaerobic bacteria and even results in the loss of their physiological functions. In the laboratory, the effects of oxygen content on strain growth and dye degradation rates have been generally studied using different fluid volumes, shaker speeds, or anaerobic incubators. There has been a lot of work on anthraquinone dye degradation by aerobes, but little on anaerobes, as shown in Table 2. In fact, there are some strains that can decolorize anthraquinone dyes efficiently under anaerobic conditions. For example, the decolorization rate of Acid Blue 25 (100 $\mu\text{mol/L}$) by *Bacillus cereus* DC11 is more than 55% under anaerobic conditions and below 5% under aerobic conditions, indicating that the oxygen of the carbonyl group in the anthraquinone dye is a preferable terminal electron acceptor under anaerobic conditions (Deng et al., 2008). In actual printing and dyeing wastewater treatment systems, aerobic and anaerobic intermittence technolo-

gies (such as anaerobic-oxic (A/O), anaerobic-anoxic-oxic (A²/O), and moving-bed biofilm reactor) are often used to improve biodegradation efficiency. Anaerobic processes are also important for printing and dyeing wastewater treatment; therefore, further understanding of anaerobic dye degradation mechanisms will allow future low energy consumption wastewater treatment systems and improved biodegradation properties.

5.5 Effect of dye concentration and structure on bacterial anthraquinone dye degradation

Dye concentration has a significant effect on the dye degradation rate. If the dye concentration is too low, the enzymes secreted from the degradative bacteria may not effectively identify the dyes; conversely, if the concentration is too high, the dyes may be toxic to the bacteria or block the enzyme active sites, reducing dye degradation efficiency (Jadhav et al., 2008). In general, the dye decolorization rate decreases with increasing dye concentration. Liu et al. (2017) reported that the decolorization rate of Remazol Brilliant Blue R reached more than 70% at low concentrations (10–30 mg/L) and then declined sharply, ultimately reaching only 15% at 200 mg/L. Printing and dyeing wastewater can have variable water quality, and the chromaticity difference can reach more than 4000 times, which requires researchers to cultivate and domesticate degradative bacteria that can adapt to a wide range of dye concentrations.

Dye structure also significantly influences the decolorization rate. In general, dyes with simple structures and low molecular weights are easily decolorized, while the decolorization rate is relatively low for dyes with high molecular weights or complex molecular structure groups. In addition, the nature and number of the anthraquinone dye substituents as well as their positions play a major role in the degradation rate. Hitz et al. (1978) suggested that the influence of single substituents on dye decolorization is: $-\text{SO}_3 > -\text{NO}_2 > -\text{Br} > -\text{Cl} > -\text{H} > -\text{NH}_2 > -\text{OCH}_3 > -\text{CH}_3 > -\text{COOH} > -\text{OH}$. Itoh et al. (1993) found that the pigment Violet 12, which has two hydroxyl groups, was more readily decolorized than Disperse Violet 1, which has two amino groups. New anthraquinone dyes are researched and developed every year, but laboratory studies are often limited to classic dyes;

thus, little research has been conducted on the relationship between anthraquinone dye structure and degradation efficiency. In the future, the biodegradation of new dyes in large quantities should be considered.

6 Conclusions and outlook

The properties of dyeing wastewater include complex composition, large chromaticity, strong acidity/basicity, and high chemical oxygen demand. It is therefore difficult to biodegrade when it contains a matrix of dyes. Among the economical technologies of anthraquinone dye removal, microbial systems were the most practical in running expenses and manpower requirements. In this regard, research has mainly focused on the isolation of highly efficient degradative bacteria, the purification of enzymes, and the study of their characteristics. However, there is quite a bit of research on degradation mechanisms and this is crucial for the practical application of efficient strains. Some studies have used molecular biology methods to express a variety of specific functional gene fragments in strains to generate bacteria with stronger flocculation abilities and higher dye tolerances, but the environmental hazards of these bacteria are unpredictable. In addition, nutrient broths, such as LB broth, are often used in the laboratory to isolate and study these highly degradative bacteria. When these bacteria are used for actual dyeing wastewater treatment, these strains demonstrated high specificity for specific dyes, easily assimilated by indigenous communities, and required constant addition of specific nutrients, increasing the cost of treatment.

Based on the importance of bacterial metabolism and the main limitation of recent research, future studies should focus on isolating strains that can utilize anthraquinone dyes as the sole carbon and nitrogen source and also tolerate extreme conditions. In addition, there should be more focus on new anthraquinone dyes which are being developed and applied in dyeing plants every year. Finally, laboratory results at pilot and large-scale studies to decolorize dyeing wastewater should be implemented.

Contributors

Hai-hong LI and Yang-tao WANG co-discussed and wrote the manuscript. Yang WANG, Hai-xia WANG, and

Kai-kai SUN were responsible for searching the literatures. Zhen-mei LU contributed to the study design, writing and editing of the manuscript.

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Compliance with ethics guidelines

Hai-hong LI, Yang-tao WANG, Yang WANG, Hai-xia WANG, Kai-kai SUN, and Zhen-mei LU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题目: 细菌降解蒽醌染料研究进展

概要: 本文综述了近年来细菌降解蒽醌染料的研究进展及机理, 以期为蒽醌染料废水的实际处理提供理论依据。目前主要利用物理、化学及生物法处理工业印染废水中的各种染料。与前两者相比, 生物法具有经济且环保的特点。本文以蒽醌染料的分类及结构为基础, 总结近年来已报道的蒽醌染料高效降解细菌的多样性; 初步探讨细菌吸附、降解蒽醌染料的机理与主要影响因素; 根据目前的研究进展及存在问题, 提出细菌降解蒽醌染料的研究方向。

关键词: 蒽醌染料; 细菌降解; 降解机理; 影响因素