Performance of a newly enriched bacterial consortium for degrading and detoxifying azo dyes

Guang Guo, Fang Tian, Can Zhang, Tingfeng Liu, Feng Yang, Zhixin Hu, Chong Liu, Shiwei Wang and Keqiang Ding

ABSTRACT

To obtain a bacterial consortium that can degrade azo dyes effectively, a bacterial consortium was enriched that can degrade Metanil yellow effectively. After 6 h, 96.25% Metanil yellow was degraded under static conditions by the bacterial consortium, which was mainly composed of *Pseudomonas, Lysinibacillus, Lactococcus*, and *Dysgonomonas.* In particular, *Pseudomonas* played a main role in the decolorization process. Co-substrate increased the decolorization rate, and yeast powder, peptone, and urea demonstrated excellent effects. The optimal pH value and salinity for the decolorization of azo dyes is 4–7 and 1% salinity respectively. The bacterial consortium can directly degrade many azo dyes, such as direct fast black G and acid brilliant scarlet GR. Azo reductase activity, laccase activity, and lignin peroxidase activity were estimated as the key reductase for decolorization, and Metanil yellow can be degraded into less toxic degradation products through synergistic effects. The degradation pathway of Metanil yellow was analyzed by Fourier transform infrared spectroscopy and gas chromatography–mass spectrometry, which demonstrated that Metanil yellow was cleaved at the azo bond, producing *p*-aminodiphenylamine and diphenylamine. These findings improved our knowledge of azo-dye-decolorizing microbial resources and provided efficient candidates for the treatment of dye-polluted wastewaters.

Key words | azo dye, bacterial community, bacterial consortium, decolorization, Metanil yellow, toxic

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INTRODUCTION

Environmental pollution caused by colored textile wastewater has become a major concern in many countries. Dye wastewater causes considerable fluctuation in water quality, deep coloring, high chemical oxygen demand, and biochemical oxygen demand, which is difficult to treat. Dyes are classified into azo, anthraquinone, stilbene, indigo, triphenylmethane, and styryl dyes according to their chemical structure. Azo dye is a complex aromatic compound with multiple varieties, and constitutes 70% of the total production dye in worldwide (Tan *et al.* 2013). Approximately 10–15% of dyes are discharged as effluent in fabric production (Singh *et al.* 2015). Azo dyes are chemically, biologically and light stable, and are difficult to degrade. Aniline compound, the degradation product of azo dyes, is teratogenic, carcinogenic, and mutagenic and has been one of the most important factors threatening water's environmental safety (Wang *et al.* 2007; Kokabian *et al.* 2013). Therefore, discharging azo dye into a water body results in ecological risk in the long term. It has gained much attention for reducing environmental pollution caused by dyes and accelerating dye degradation in the environment (Khalid *et al.* 2012; Solis *et al.* 2012).

The physicochemical method is commonly used for colored textile wastewater treatment in many developed countries. Physical methods mainly include adsorption, flocculation, and filtration. Meanwhile, handling hazardous dyes absorbed in surfaces by physical methods leads to environmental problems (Naushad *et al.* 2015, 2016; Sharma *et al.* 2015; Albadarin *et al.* 2017). Chemical treatment methods mainly include ozonation, Fenton oxidation, electrochemical oxidation, sonochemical oxidation, and advanced oxidation (Kumar *et al.* 2017, 2018, 2019); the methods are applied in a limited way because of their high cost and secondary pollution (Vikrant *et al.* 2018). Compared with the physicochemical method, the biological method enables complete degradation

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of dyes, which is inexpensive and environmentally friendly (He *et al.* 2017). Bacteria, fungi, and algae can degrade azo dyes. Bacteria have the greatest application value because of their rapid reproduction, strong adaptation, and minimal pollution. Microorganisms can degrade azo dyes using numerous enzymes, such as azo reductase, laccase, peroxidases (PODs), and NADH-DCIP reductase (Bhatia *et al.* 2017). Among them, azo reductase, laccase, and PODs are the main enzymes in degrading azo dyes (Imran *et al.* 2014).

However, most degradation products of dyes, such as aniline, are difficult to degrade by pure bacterium that need to be further degraded by other microorganisms. Compared with a pure strain, bacterial consortium is more advantageous mainly because of the synergistic degradation effects among microorganisms. For instance, Tamboli *et al.* (2010) discovered that the degradation rate of a bacterial consortium is higher than that of pure bacteria. Pure bacteria cannot achieve satisfactory removal effects. The degradation rate of a bacterial consortium is usually higher than that of pure bacteria (Mishra & Maiti 2018). In actual application, the dye wastewater environment is complex. A single strain cannot meet the requirements of the degradation process, because of low substrate spectrum and adaptability, as well as low enzyme activity generally. However, synergistic effects in the bacterial consortium improved the adaptability of the microorganisms for large-scale application. Therefore, enriching a bacterial consortium to degrade azo dyes shows great research value and application prospects.

In this study, a bacterial consortium was enriched that can effectively degrade Metanil yellow, and the degradation characteristics were studied. In addition, the degradation pathways and toxicity of the degradation products were identified. The key enzyme activity in the degradation process was tested. This research provides a bacteria resource and experimental basis for intensified biological treatment of colored textile wastewater.

MATERIALS AND METHODS

Dyes and chemicals

Metanil yellow and other synthetic dyes used in the study were procured from Linyi Dyes and Chemicals Co., Ltd, Shandong, China. All other chemicals used in the study were of analytical grade.

Culture medium

The liquid basal medium (LBM) is composed of (g/L) Na_2SO_4 0.5, NH_4Cl 0.3, $CaCl_2$ 0.1, KH_2PO_4 0.2, KCl 0.5, $MgCl_2{\cdot}6H_2O$ 1.2, NaCl 2.0, and yeast powder 1.0. The pH value was adjusted to 7.0 by NaOH. LBM was sterilized for 30 min at 1×10^5 Pa, and Metanil yellow ($C_{18}H_{14}Na_3O_3S$) was added (100 mg/L).

Enrichment bacterial consortium capable of decolorizing azo dyes

10 mL of activated sludge from Jiangsu province was placed into a 90 mL LBM conical flask (the concentration of Metanil yellow is 100 mg/L) at 30 °C for static cultivation. When the decolorizing rate reached 80% and above, 10% putrid fluid (the culture medium containing the bacteria after the decolorizing rate reached 80%) was brought, and inoculated into the fresh LBM culture medium. After eight generations, the enrichment cultivation was finished.

Community composition of the bacterial consortium

Fast DNA SPIN kit was adopted for the extraction of microbial genomic DNA. The 16S rRNA gene sequence of microorganisms was obtained using the Illumina MiSeq PE250 high-throughput sequencer (Shanghai Major Biomedical Science and Technology Co., Ltd). Each 20 μL reaction system includes rTaq enzyme at 0.2 μL, 10 × PCR buffer at 2.0 μL, 20.5 mmol/L dNTPs at 2 μL, 5 μmol/L Primer-F at 0.8 μL, 5 μmol/L Primer-R at 0.8 μL, 25 mg/L template DNA at 10 ng, and aseptic ddH_2O at 20 μL. Polymerase chain reaction (PCR) conditions were 95 °C for 3 min; then 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s in 35 cycles; and 72 °C for 10 min. The primers were 515F: 5′-GTGCCAGCMGCCGCGG-3′ and 907R: 5′-CCGTCAA TTCMTTTRAGTTT-3′. Bacterial phylotypes were identified using operational taxonomic units (OTUs) at distance criterion of 0.03. Representative sequences were blasted with RDP classifier database (http://rdp.cme.msu.edu/misc/resources.jsp) and Silva database (http://www.arb-silva.de/).

Optimization of nutritional requirements and physicochemical parameters

Bacterial fluid was agitated and added into the LBM culture media with different nutrient sources, NaCl, pH values, and initial concentrations of Metanil yellow. To examine the influence of each nutrient source on the decolorization performance of the bacteria, 10 C-sources was added to the medium at 1 g/L (glucose, sucrose, maltose, lactose, soluble starch, yeast powder, urea, peptone, potassium nitrate, and beef extract). All treatments were run in triplicate.

Resuspended bacterial liquid (1 mL) was drawn and added into the LBM with different azo dyes (100 mg/L). Experiment substrates included direct fast light black G (646 nm), acid brilliant scarlet GR (510 nm), acid orange 2 (484 nm), direct blue 5B (598 nm), and acid black ATT (636 nm). The decolorization rate can be calculated using Formula (1):

$$\text{Decolorization rate: } q(\%) = (A_0 - A_t)/A_0 \times 100 \quad (1)$$

A_0 is the dye absorbance at the initial time; A_t is the dye absorbance at time t. All the experiments were repeated three times.

Enzymatic assays

Microbial bacterial consortium was collected by centrifugation and then dissolved with phosphate buffer (pH 7.2). The cell suspension was disrupted by sonication at 400 W with three strokes (5 s each with a 5 s interval for 30 min at 4 °C). The supernatant was used for the testing of enzyme activity after centrifugation of the suspension liquid (10,000 × g, 20 min at 4 °C).

Enzyme activity was tested as described by Song *et al.* (2017). Laccase activity was tested in the 100 mM acetate buffer (pH 4.0), which contained 0.5 mM of 2,2-azinobis(3-ethylbenzthiazoline-6-sulphonate) (ABTS) as the substrate and 0.6 mL of enzyme. Activity was measured by the decrease in absorbance at 420 nm over a 1 min period using a UV/VIS spectrophotometer. The reaction liquid of lignine peroxidases contained a 50 mM sodium tartrate buffer (pH 3.0) with 2 mM veratryl alcohol as substrate, 0.5 mM H_2O_2, and 0.8 mL enzyme. The azoreductase activity and protein concentrations were detected as described by Tian *et al.* (2019). All the experiments were repeated three times. Enzyme activity was calculated as the amount of substrate consumed or product generated per milligram of protein per minute.

Determination of intermediates

The degradation product was collected and extracted with 100 mL of ethyl acetate at pH of 7, 2, and 10, respectively. The extracted liquid was dried by anhydrous sodium sulfate and rotated until dry. The obtained samples were ground with KBr powder and pressed to form a uniform disk. Infrared scanning analysis was conducted by Bruker VERTEX 70 infrared spectrometer, and the scanning wave length was within 500–4,000 cm^{-1}.

Rotated degradation products were detected using Thermo Fisher ISQ LT GC-MS. The gas chromatography–mass spectrometry (GC-MS) column was a silica capillary column (30 m × 0.25 mm × 0.25 μm). The temperature rise program was 50 °C, in 5 min, and 8 °C/min until the temperature reached 280 °C and then was maintained for 10 min. The flow rate of the carrier gas (helium) was 1 mL/min, and the temperatures of the syringe and detector were both 250 °C. The mass spectra of the degradation products were scanned under 70 eV in EI mode.

Plant toxicity inspection

Phytotoxicity tests were performed to assess the toxicity of the degradation products and azo dye (Chen *et al.* 2018). The extracted degradation products and Metanil yellow were dissolved into 100 mg/L (TOC). The solution was added into quartz sand, and sterilized cucumber and *Oryza sativa* seeds were placed on the quartz sand. The glass culture dish was covered and incubated in the incubator (25 °C), in the dark. The germination rate, germ length, and root length were recorded after 7 days.

Statistical analysis

Data were analyzed by a one-way analysis of variance (ANOVA) using Tukey–Kramer multiple comparisons testing.

RESULTS AND DISCUSSION

Enrichment of bacterial consortium

After eight enrichments, a bacterial consortium that can degrade Metanil yellow was obtained, named M1. Results (Figure 1) showed that 96.25% Metanil yellow was degraded by the bacterial consortium after 6 h, in LMB culture medium. Other research obtained a bacterial consortium (Bacterial consortium AR1) that can degrade a single azo dye (Reactive Red 195) thoroughly in 14 h (Khan *et al.* 2014). The decolorization rate was remarkably lower than that achieved by M1 in the present research. In the initial decolorization stage, microorganisms can be colored. However, the coloring of microorganisms fully disappeared after degradation, indicating that the microorganisms decolorized the dye by degradation, not by adsorption. This finding is in agreement with other research (Song *et al.* 2017). The present

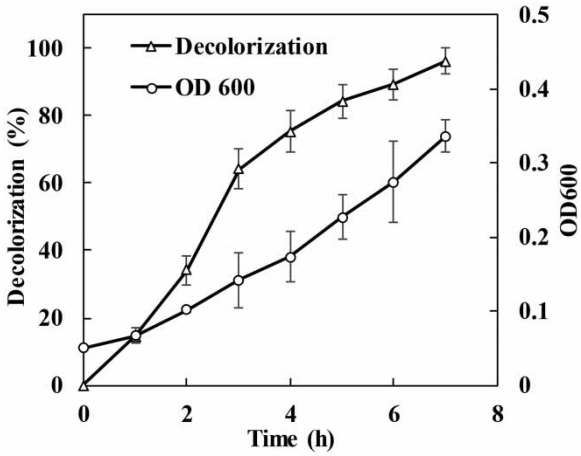


Figure 1 | Decolorization of Metanil yellow (100 mg/L) by the consortium M1 under static conditions at 30 °C, pH 7.0. Data represent averages from triplicate assays, with error bars showing the standard deviation.

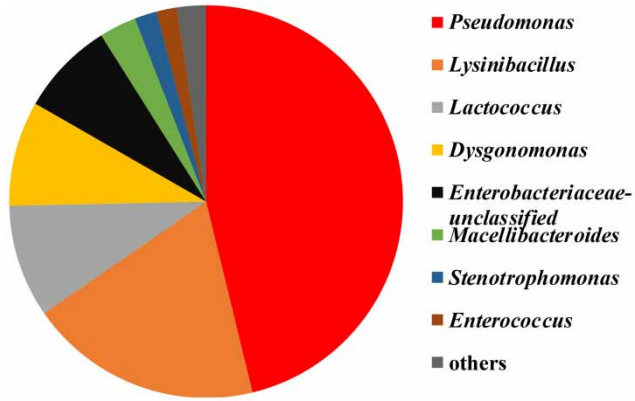


Figure 2 | Community structure of the bacterial consortium.

research also studied the influence of aerobic and anaerobic environments on the decolorization. The results showed that the decolorization rate under static conditions were more remarkable than in agitation for 6 h (results had no indications), similar to other research results (Khan *et al.* 2014). Azo dyes can be decolorized through the reduction mechanisms by microorganisms. Azo reductases are usually of high activity under anaerobic conditions because oxygen inhibits the activity of microbial azo reductase. Oxygen is a better electron acceptor than azo dyes (Singh *et al.* 2015). Microorganisms can break the azo bond and generate aromatic amines under an anaerobic situation (Rathner *et al.* 2017); therefore, all other experiments were studied in static conditions. Our results indicated that the bacterial consortium, compared with a single strain, has better adaptability and degradation rate in degrading azo dyes.

Composition analysis of bacterial consortium

High-throughput sequencing indicated that the bacterial consortium was mainly composed of *Pseudomonas*, *Lysinibacillus*, *Lactococcus*, *Dysgonomonas*, unclassified_Enterobacteriaceae, *Macellibacteroides*, *Stenotrophomonas*, and *Enterococcus*. *Pseudomonas*, *Lysinibacillus*, *Lactococcus* and *Dysgonomonas* accounted for 46.21%, 19.15%, 9.30%, and 8.66%, respectively (Figure 2). *Pseudomonas* can effectively degrade azo dyes (Mishra & Maiti 2018). For example, a number of *Pseudomonas* that can degrade Remazol black B were isolated, which could degrade azo dyes in the wastewater up to pH 9 (Mishra & Maiti 2018). In the present study, it played a main role in dye degradation. *Lysinibacillus* was a decolorization bacteria in the present study. *Lysinibacillus* sp. RGS was isolated from colored textile wastewater, which can completely degrade and detoxify 50 mg/L of dyes in 24 h (Bradford 1976). Currently, no research has been reported about the degradation of Metanil yellow by this bacterium. In the present study, *Lysinibacillus* played an important role in decolorization. *Lactococcus* is a lactic acid bacteria, which is widely used in handling petrochemical wastewater (Naushad *et al.* 2016). They are frequently found in hydrolysis acidification pools that are used for decolorizing azo dyes, such as Reactive Black 5 (Kumar *et al.* 2019). In the present research, *Lactococcus* played an important role in degrading Metanil yellow. Electricity-generating strain Dysgonomonas may be involved in the degradation of azo and anthraquinone dyes. The bacteria can also degrade Reactive black 5 and Reactive blue in the wastewater using hydrolytic acidification (Xie *et al.* 2016). *Lactococcus* and *Macellibacteroides* are both hydrolytic acidification bacteria, which can digest and decompose starch, and they are reported for decolorizing the colored textile wastewater by acidification (Kumar *et al.* 2018). *Stenotrophomonas* sp. BHUSSp X2 can degrade 97% Acid red 1 (200 mg/L) after 6 h, under static conditions (Kumari *et al.* 2016). The bacterial strain *Enterococcus* sp. L2 can decolorize the azo dye Reactive violet 5R and various types of azo dyes (Rathod *et al.* 2017). When the azoreductase gene in this bacteria is overexpressed in *Escherichia coli*, the activity of azoreductase increased remarkably (Rathod *et al.* 2017). Therefore, *Pseudomonas* was the main decolorization bacterium in the bacterial consortium.

Effect of physicochemical factors

The nitrogen and carbon sources in azo dyes are insufficient; hence, it is difficult to biodegrade azo dyes without

an external nutrient (Khan *et al.* 2013). Nutrient sources, such as yeast extract and glucose, directly influence the growth and decolorization rate. In the present study, the growth of the bacterial consortium and the decolorization rate are unsatisfactory without nutrient sources. Therefore, carbon and nitrogen sources were added to improve the nutrient environment of the bacterial consortium (1 g/L). The results are shown in Figure 3(a). Nutrient influenced the degradation rate. Yeast extract, peptone, and urea enhanced the degradation rate, which reached 96.38%, 95.62%, and 93.66% after 6 h, respectively. Meanwhile, the decolorization rate of glucose, sucrose, maltose, lactose, and soluble starch was lower than that of yeast extract. Yeast extract is the best nutrient source for bacterial growth and decolorization. During the reduction of the azo bond, the microorganism transferred the reducing equivalent from the carbon source to the reductase azo bond. Yeast extract mainly improves the activity of azo reductase and subsequently improves the decolorization rate (Imran *et al.* 2016). Nitrogen sources have more stimulative effects than carbon sources, because microorganisms prefer external carbon sources in azo dyes (Khan *et al.* 2013). Furthermore, some external carbon sources, such as peptone, beef extract, urea, and yeast extract, which can produce NADH, can also serve as electron donors for acceleration of the reduction of azo bonds by microorganisms (Khan *et al.* 2013).

Inorganic salts were added as a fixing agent to improve dyeing efficiency. Therefore, the salinity is usually high in colored textile wastewater. This could inhibit the normal metabolism of microorganisms, reducing the capability of bacteria to degrade dyes. The effect of salt concentration on the decolorization was studied, as shown in Figure 3(b). The decolorization rates reached 94.21%, 91.15%, and 75.06% after 6 h under salt concentrations of 10, 20, and 40 g/L, respectively. When the salt concentration reached 60 and 80 g/L, decolorization rates were only 52.81% and 15.68%, respectively. Salinity inhibits the decolorization capability of the bacterial consortium. Chen *et al.* found that the decolorization rate was negatively correlated with the salinity (Chen *et al.* 2018). The present research also found that the decolorization rate decreased as the salinity increased. Salty environments inhibit the growth of bacterial consortia. This phenomenon may be associated with the fact that salinity inhibits the normal metabolism of a bacterial consortium and the activity of degradation enzymes in the bacterial consortium. High salinity can increase osmotic pressure, cause plasmolysis, destroy the metabolic enzyme activity, and restrict the growth of microorganisms. When salinity increases, microorganisms consume more energy

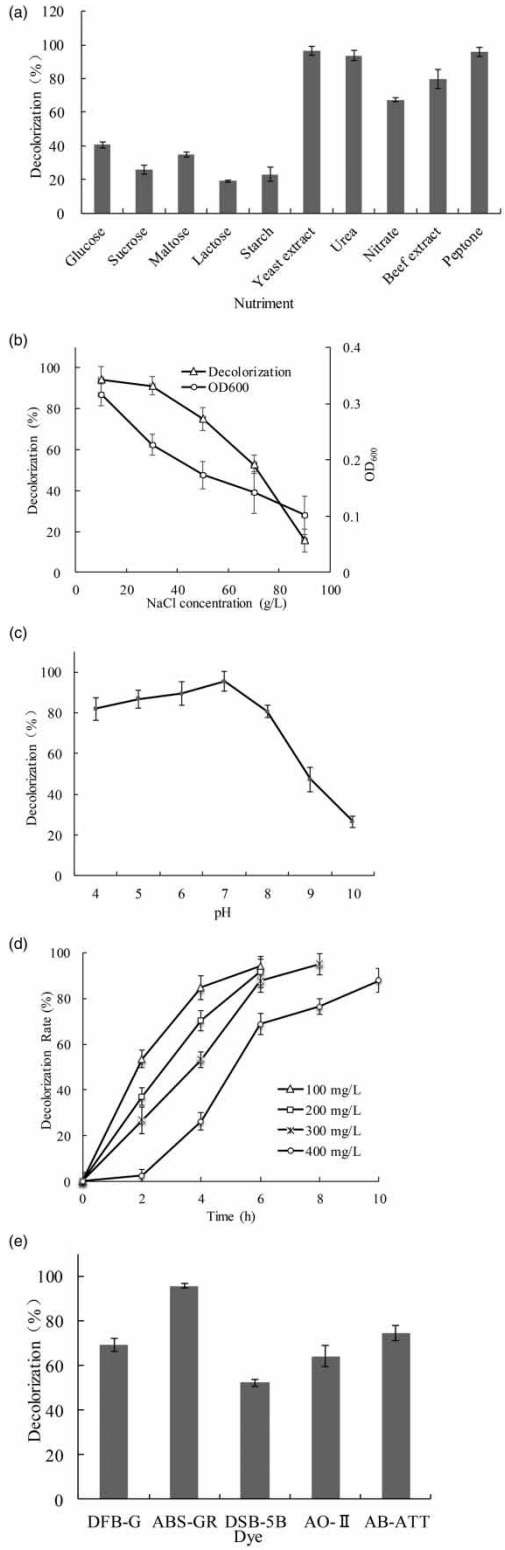


Figure 3 | Effect of co-metabolites (a), NaCl concentration (b), pH (c), initial dye concentration (d) and different structures of azo dyes (e) on dye decolorization by the bacterial consortium M1 under static conditions at 30 °C. The pH is 7.0, except parameter c. The azo dye concentration is 100 mg/L, except for parameter d.

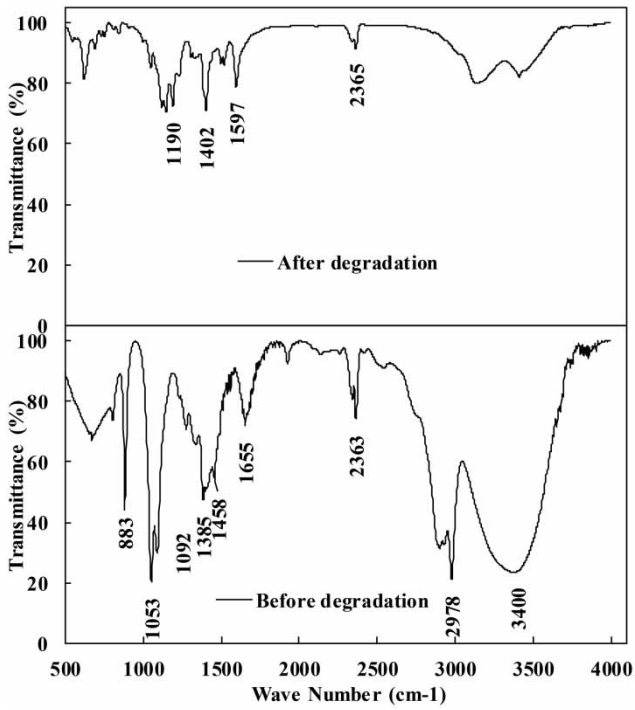


Figure 4 | Fourier transform infrared (FTIR) spectra of Metanil yellow and samples from the end of decolorization experiments conducted under static conditions at 30 °C, pH 7.0.

than that at low salinity, and the metabolic pathway involved is altered. To adapt to such an adverse environment, microorganisms have to synthesize some small osmoprotectant and secrete an extracellular polymeric substance in the body as a protectant (Tian *et al.* 2019).

pH is an important influencing factor for biodegradation. The pH value of dye wastewater is mainly determined by inorganic salts. The pH value leads to changes in the surface charge of bacterial cells, thereby affecting the penetration of dye into the cell membrane. It may also influence the structure of the enzyme, leading to denaturation and inactivation of the protein. pH can change the dissociated states of substrate molecules and influence the combination of enzyme and substrate (Chen *et al.* 2018). The present study investigated the influence of pH values on the degradation of Metanil yellow by the bacterial consortium. The results are shown in Figure 4(b). The decolorization rate at pH 7 was 95.43%, which decreased after pH 7. The decolorization rate increased with pH values of 4, 5, and 6, reaching 82.02%, 86.78%, and 89.47%, respectively. The degradation rate was remarkably reduced when the pH gradually increased to alkaline levels. When the pH reached 10, the decolorization rate was only 26.51%. High pH values remarkably inhibited the decolorization rate of the microorganisms. Generally, the pH of microbial decolorization ranges from 6 to 10. Transmembrane transportation of dyes is a limiting step in microbial decolorization, because pH affects the transmembrane transportation of dye molecules and subsequently influences the decolorization efficiency (Saratale *et al.* 2011). Each enzyme performs at the highest activity at its optimum pH value, because pH value affects the activity of the microbial enzymes and the solubility of the dyes. In the present study, the bacterial consortium is suited to an acidic environment. It can stably decolorize the dye in an acidic environment in a short time, thus having application value for acid dye wastewater treatment.

Given that azo dye is toxic, the influence of the initial dye concentration on the degradation was studied. The results are shown in Figure 3(d). The degradation rate decreased with increase in the initial concentration. The decolorization rates reached 94.11%, 91.60%, and 87.61% in the initial concentrations of 100, 200 and 300 mg/L after 6 h, respectively. Decolorization rate was only 68.82% after 6 h when the dye concentration increased to 400 mg/L, whereas the decolorization rate reached 87.89% after 10 h. Microorganisms need more time to degrade high concentrations of dyes (Vatandoostarani *et al.* 2017). The aforementioned results showed that decolorization decreased because the azo dye is toxic to microorganisms; this finding is similar to other research results (Song *et al.* 2017). In the present study, the bacterial consortium had higher resistance to high concentrations of Metanil yellow.

Given that the colored textile wastewater included variety of dyes, the bacterial consortium must degrade many types of azo dye. The substrate range of the bacterial consortium was tested with an initial concentration of 100 mg/L, such as Direct fast black G, Acid brilliant scarlet GR, Acid orange 2, Direct blue 5B, and Acid black ATT (Figure 3(e)). The bacterial consortium can degrade 95.71% Acid brilliant scarlet GR after 6 h. Moreover, decolorization rates of Direct fast black G, Acid black ATT, and Acid orange 2 reached 69.20%, 74.57%, and 64.28%, respectively. The degradation rate of Direct lake blue 5B was relatively low (52.34%) after 6 h, but its degradation efficiency can reach 85.84% after 24 h. The characteristics of the substituents and the relative substitution positions of the azo bonds influenced the decolorization rate. Hydroxy and amino contained in the aromatic ring can improve the decolorization rates, whereas methoxy, methyl, nitro, and carboxyl inhibit the dye decolorization (Imran *et al.* 2014). Among the azo dyes in this experiment, only Acid brilliant scarlet GR and Direct blue 5B have two azo bonds. Acid brilliant scarlet GR has 1 amino and 2 sulfonic groups that improved

the degradation and decolorization process. Meanwhile, direct blue 5B has 2 methoxy and 4 sulfonic groups, which may inhibit the degradation rate of the bacterial consortium to a certain degree.

Enzyme analysis

The bacterial degradation of azo dyes is usually catalyzed by the reduction of azo bonds. Azo reductase, laccase, and PODs are the main enzymes in degrading azo dyes (Singh *et al.* 2015). The enzyme activity in the microorganisms is researched as shown in Table 1. Azo reductase activity is measured under all the concentrations of salt. Highest activity occurred under 1% salinity (3.13 μM of Methyl Red reduced min^{-1} mg^{-1}protein). As salinity increased, azo reductase activity decreased, and its activity is only 1.48 μM Methyl Red reduced min^{-1} mg^{-1}protein under 5% salinity. The activities of the Lip and Lac enzymes were highest under 1% salinity but decreased at increased salinity (5%), decreasing by 58.35% and 51.90%, respectively. The result is similar to other research results (Song *et al.* 2017). These enzymes had varied contributions to the microbial degradation processes according to the type of azo dye. Azo reductase and laccase are the main enzymes, and PODs played a certain role in decolorizing azo dyes (Singh *et al.* 2015). However, only laccase and NADH–DCIP reductase activities were observed in some microorganisms, such as *Shewanella oneidensis* WL-7 (Naushad 2014). Furthermore, three enzymes showed high activity in the present research, indicating that the three enzymes played an important role in azo dye degradation.

Phytotoxicity assessment

The toxicity of Metanil yellow and its metabolites was determined in the seeds of two plants (rice and cucumber). The results are shown in Table 2. The germination rate and length of rice and cucumber after Metanil yellow treatment were lower than those in metabolites and distilled water. The germination rates of rice and cucumber in 100 mg/L Metanil yellow treatment were only 50% and 67%, respectively. Meanwhile, their root length (2.4 and 1.6 cm) and shoot length (3.4 and 2.3 cm) were both short. However, germination rates of rice and cucumber in metabolites both reached 100%, and their root length (3.6 and 1.8 cm) and shoot length (4.0 and 2.9 cm) were both relatively longer than that in Metanil yellow. The germination rates of rice and cucumber in distilled water treatment both reached 100%; their root lengths were 3.8 and 2.4 cm, respectively, and germ lengths were 4.2 and 3.6 cm, respectively. Some organisms cannot completely degrade amines, and the toxicity of azo dyes is higher than that after degradation. Conversely, some microorganisms can completely degrade and subsequently decrease the toxicity of dye (Mishra & Maiti 2018). The results showed that the bacterial consortium can decrease the toxicity of Metanil yellow. This finding is similar to other research results (Chen *et al.* 2018).

Table 1 | Intracellular enzymes activities of bacterial consortium after decolorizing 100 mg L^{-1} Metanil yellow under different salinities

Salt concentration (g/L)	Azoreductase[a]	Laccase[b]	Lignin peroxidase[b]
10	3.13 ± 0.08*	6.82 ± 1.02*	10.25 ± 1.51*
30	2.89 ± 0.05*	4.72 ± 0.86*	8.48 ± 1.23*
50	1.48 ± 0.04*	3.98 ± 0.92*	5.32 ± 1.02*

Values are mean of three experiments ± standard error of mean. Values were significantly different from control at *$P < 0.001$ by one-way ANOVA with Tukey-Ramer comparison test.
[a]μM of Methyl Red reduced min^{-1} mg^{-1} protein.
[b]U min^{-1} mg^{-1} protein.

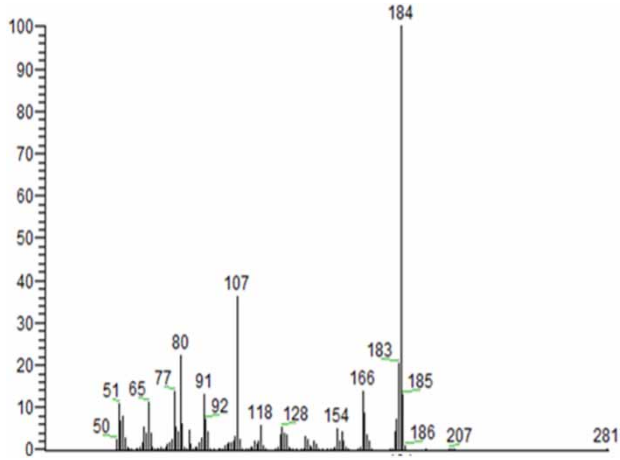
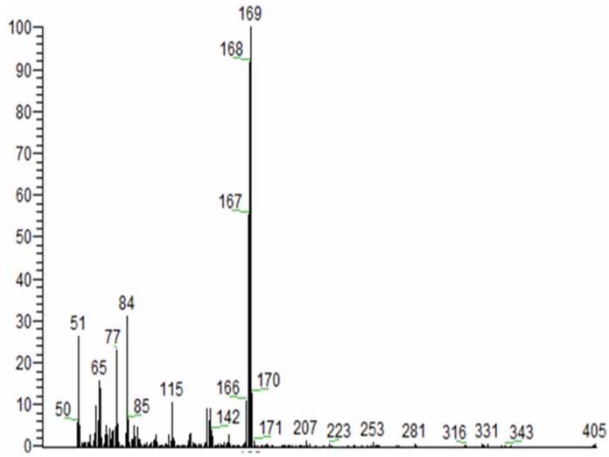
Table 2 | Phytotoxicity comparison of Metanil yellow and its extracted metabolites

Parameters studied	Distilled water	Acid Brilliant Scarlet GR	Metabolite
Cucumis sativus			
Germination (%)	100	50	100
Shoot (cm)	4.2 ± 0.8	3.4 ± 0.6	4.0 ± 0.6
Root (cm)	3.8 ± 0.6	2.4 ± 0.7	3.6 ± 0.7
Oryza sativa			
Germination (%)	100	67	100
Shoot (cm)	3.6 ± 0.6	2.3 ± 0.6	2.9 ± 0.8
Root (cm)	2.4 ± 0.5	1.6 ± 0.7	1.8 ± 0.5

Metabolite analysis by FTIR and GC-MS

The differences in the Fourier transform infrared (FTIR) spectra of Metanil yellow and its metabolites are shown in Figure 4. The peaks of the azo bond stretching –N = N– at 1,655 cm^{-1} was detected before Metanil yellow degradation, whereas the peaks disappeared after degradation, indicating that the azo bond was broken. The specific peak of N-H (3,400 cm^{-1}) decreased after degradation, suggesting that the microorganisms removed N-H. The specific peak appearing at 1,053 cm^{-1} represented -SO_3 and apparently decreased after degradation. The peak at 818 cm^{-1} for the *p*-desubstituted benzene ring vibrations also disappeared, showing that the benzene ring was fractured. Therefore, the azo bonds of Metanil yellow were degraded by the microorganisms. This finding is similar to the GC-MS research results.

Table 3 | GC–MS spectral information of metabolites obtained after decolorization of MB by bacterial consortium

Metabolite	RT (min)	Mw (kDa)	Mass spectrum (m/z)
1,4-Benzenediamine, N-phenyl-	21.11	184	
Diphenylamine	15.57	169	

The GC-MS analysis results before and after degradation (Table 3) showed that the main degradation products of Metanil yellow were *p*-aminodiphenylamine (retention time 21.11 min) and diphenylamine (retention time 17.57 min). The characteristic fragment of aminodiphenylamine was 184, and the similarity to the standard in the GC-MS NIST database was 77.13%. The result indicated that the azo bond of Metanil yellow was opened and formed primary amine, and the formed *N*-phenylphenethylamine further transformed into diphenylamine by removing the primary amine on the phenyl ring. Under anaerobic or anoxic conditions, the first step in microbial degradation of azo dyes is reduction of azo bonds by the enzymes to form colorless aniline metabolites, and the complete degradation of such products requires the participation of oxygen (Chen *et al.* 2018).

CONCLUSION

A bacterial consortium was enriched from the active sludge of colored textile wastewater, which can degrade Metanil yellow effectively. The bacterial consortium is mainly composed of *Pseudomonas*, *Lysinibacillus*, *Lactococcus*, and *Dysgonomonas*. The optimal pH value for the dye degradation induced by bacteria was 4–7, and Metanil yellow was effectively degraded mainly within 1–6% salinity. High decolorization rate was achieved at initial dye concentrations of 100–400 mg/L. The bacterial consortium directly degraded several azo dyes, such as Direct fast black G and Acid brilliant scarlet GR. High activity of azo reductase, laccase, and lignin PODs appearing in the bacterial consortium degraded the Metanil yellow into *p*-aminodiphenylamine and diphenylamine. The toxicity of the intermediates was remarkably decreased.

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