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Biodecolorization of Azo Dyes by Microorganisms Isolated From Serdang and Merambong Soils

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Abstract

Several local microorganisms were isolated and screened for their capabilities to decolorize selected azo dyes. Two isolates, NHG and NH2 have shown their capabilities to decolorize azo dyes i.e. Reactive Black 5 (RB5, diazo) and Metanil Yellow (monoazo), respectively, under agitated condition at room temperature. Isolate NHG was capable to decolorize RB5 99.2% under shaking condition and only 43.88% under static condition in 24 hours. The use of glucose and yeast extract as carbon and nitrogen sources, respectively, has provided optimal decolorization of RB5 by isolate NHG. Isolate NH2, which was isolated from Merambong shoal soil sample, can decolorize 93.3% of Metanil Yellow in 24 hours. Crocein Orange G and Orange II, both have exactly the same molecular weights, were 25.9% and 47.8% decolorized, respectively by the same isolate under agitated conditions. No correlation between degradation rate and molecular weight, number of azo bonds or presence of aromatic molecules has been observed.

INTRODUCTION

Azo dye compounds are synthetic colorants that contain azo group (N=N) and are commonly used in textile industries, food industries, cosmetic, leather, printing and pharmaceutical products. The usage of azo dye by industry is reported around 60 – 70% from 10000 pigments [1] and it is estimated approximately up to 15 % of dyes are released into environment during the dying process [2]. The continuous discharge of industrial dye wastewater can change the water quality by reducing sunlight penetration into the river and at the same time may cause toxicity to the aquatic organisms due to breakdown by-products of dyes [3]. Low penetration of sunlight will disturb the photosynthesis process, thus resulting low oxygen level in the water and a high level of BOD. The dye colors may also produce unsightly appearances and foul smell in river.

A number of physical and chemical methods such as activated carbon, bagasse, peat, wood chip, ion exchange, coagulation, ozonation, precipitation, and photocatalysis has been utilized to remove dye from wastewater [4], but these methods have some limitations. Physical and chemical methods consume high energy cost, high operational cost and produce large amount of sludge [5]. Biological method is generally considered as an effective pollution control strategies because they are cheap, and

environmentally friendly. Azo dye bond can be broken down by enzymes such as hydrolase and oxygenase that are produced by bacteria [6].

In this study, microorganisms that have capabilities to decolorize azo dye from various soil samples were isolated. The bacterial strains were characterized and the requirement for carbon and nitrogen sources was optimized for optimal decolorization of selected azo dye.

MATERIALS AND METHODOLOGY

Microorganism and culture conditions

Soil samples that are collected around Universiti Putra Malaysia, Serdang and Merambong shoal (01°19.979'N, 103°35.965'E) were cultured in nutrient broth for 24 hours at room temperature. The screening was carried out in universal bottles by mixing soil sample, dye and mineral salt media (MSM), which consisted of the following (g/L): K₂HPO₄ (0.4), KH₂PO₄ (0.2), (NH₄)₂SO₄ (0.4), NaCl (0.1), yeast extract (1.0), MgSO₄ (0.1), MnSO₄·H₂O (0.01), Na₂MoO₄·2H₂O (0.01) and Fe₂(SO₄)₃·H₂O (0.01). The pH of the media was adjusted to 7.0 [7]. Prior to decolorization tests,

the MSM was supplemented with 10 g/L glucose. For soil samples from Merambong shoal, the concentration of NaCl was adjusted up to 1 g/L.

The bacterial cultures in universal bottle were incubated under two conditions; on orbital shaker with 150 rpm agitation and in static condition. Both cultures were incubated at room temperature. After 48 hours of incubation, a loop-full of medium was streaked onto MSM agar with 50 ppm of dye and incubated at room temperature for 48 hour. The colonies that show ability to decolorize dyes were separated and re-streak on agar medium to be purified. The pure cultures of selected individual bacterial strains were maintain by streak on agar slant and stored.

Chemical

Azo dyes that are commonly used in industry were tested in this study and are presented in Table 1.

Table 1. Azo dyes, their percentages of purity, molecular weights, number of azo bonds, maximum wavelength used in this study.

Azo dye	Purity (%)	Molecular Weight	Azo bond	$\lambda_{\max}(\text{nm})$
Reactive Black 5	55	991.82	2	597
Direct Blue 71	50	965.94	2	594
Amaranth	85-95	604.47	1	523
Tartrazine	>85	534.36	1	427
Metanil Yellow	70	375.38	1	414
Crocein Orange G	90	350.32	1	482
Orange II sodium salt	85	350.32	1	483
Reactive Red 120	60	1469.98	2	517

Screening of azo dye with selected individual bacterial strain

The bacterial strains that have been isolated were screened with four types of azo dye; Reactive Black 5 (RB5), Direct Blue 71, Amaranth and Tartrazine. A bacterial strain isolated from soil collected from Merambong shoal was tested with Metanil Yellow. From all sample tested, three bacterial strains were isolated and further screened for azo dyes degradation and only one isolate was selected due its fast decolorization rate of RB5. The isolate was named isolate NHG. The bacterial strain isolated from Merambong shoal was named isolate NH2.

The bacterial strains were grown in MSM supplemented with glucose. After 24 hour, the 10% bacterial strain was added into 100mL of MSM with dye (50 ppm) and incubated at room temperature for 24 hour. The percentage of decolorization for every dye was determined by using UV-visible spectrophotometer [8]. The bacterial strain that shows best decolorization rate was selected for further studies and the percentages of decolorization extent were calculated using the following equation:

$$\text{Decolorization Extent (\%)} = \frac{OD_i - OD_f}{OD_i} \times 100$$

where OD_i and OD_f are the initial and final absorbance readings, respectively.

Identification of selected bacterial strain

Isolate NHG was Gram-stained using standard procedure. The catalase test was also conducted. A small amount of growth bacteria strain from 24-hour culture was placed on the glass slide labeled 'test' by sterile loop. A few drops of hydrogen peroxide were carefully added onto the test and control slides. Then the fluid over the smears was observed for the appearance of gas bubbles. Positive result will shows a rapid evolution of oxygen gas by bubbling. While negative result will shows no bubbles or only a few scattered bubbles.

Optimizing carbon and nitrogen sources for RB5 decolorization

For optimizing the carbon source for isolate NHG in decolorizing of RB5, MSM with 50 ppm RB5 was used with 10 g/L of carbon source. The carbon sources tested are arabinose, fructose, galactose, glucose, glycerol, maltose, raffinose, starch and sucrose. The carbon sources and MSM were sterilized separately and was mixed after autoclaved. The cultures were incubated on orbital shaker at room temperature for 24 hour. The percentage of decolorization for every carbon source was then determined. A similar medium was used for optimization of nitrogen source included the 10 g/L of carbon source and the nitrogen source (1g/L) of either yeast extract, ammonium chloride, glycine, L-alanine, L-glutamic, tryptone, and urea. The bacterial culture was inoculated and incubated under same conditions previously described. The percentage of decolorization was taken after 24 hour.

RESULTS AND DISCUSSION

Isolation and identification of dye degrading bacteria

Isolate NHG colony was observed to be flat, circular, smooth and yellow white. It yields both positive result on Gram staining and catalase test (Figure 1). This shows that isolate NHG contains teichoic acids and lipoids. The lipoteichoic acids in the bacteria cell wall act as chelating agents and also for certain types of adherence [9]. The positive catalase test shows isolate NHG possesses catalase enzyme which is important in protecting the cell from oxidative damage by reactive oxygen species [10].

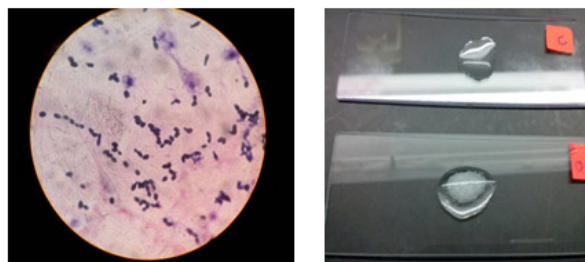


Figure 1. Identification of isolate NHG by using (a) Gram-staining and (b) catalase test. Isolate NHG was determined to be Gram positive and yield positive catalase test.

Decolorization of various azo dyes by isolate NHG

Decolorization of four types of azo dye using isolate NHG is shown in Figure 2. Among the four types of azo dye, isolate NHG was able to decolorize RB5 up to 99.2% under shaking condition and only 43.88% under static condition. This result suggests that isolate NHG requires oxygen to decolorize dye. Under aerobic condition, isolate NHG yield low decolorization rate for Tartrazine and Amaranth, even though both of these dyes have only single bond of azo. Elisangela et al. (2009) reported that the number of azo bonds may affect the decolorization rates and normally, longer times were required for decolorize the compounds with high amount of azo bonds [11]. Nonetheless, isolate NHG is capable to decolorize only 61.4% and 11.14% of Amaranth (monoazo) and Tartrazine (monoazo) respectively, while can decolorize up to 99.2% of RB5 which is a diazo. Figure 3 shows the decolorization of RB5 by isolate NHG under both static and shaking conditions.

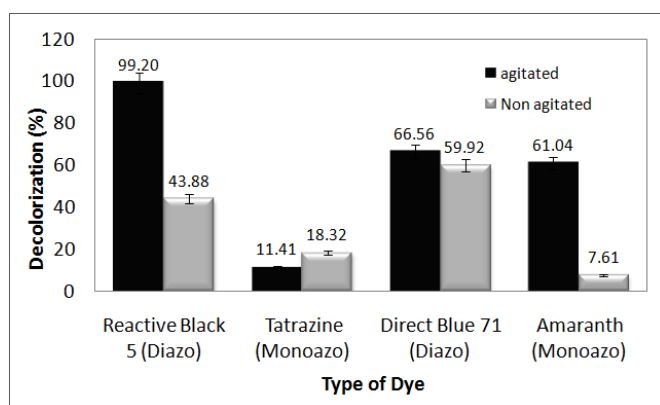


Figure 2. Decolorization of four types of azo dyes (50 mg/L) by isolate NHG in 24 hours

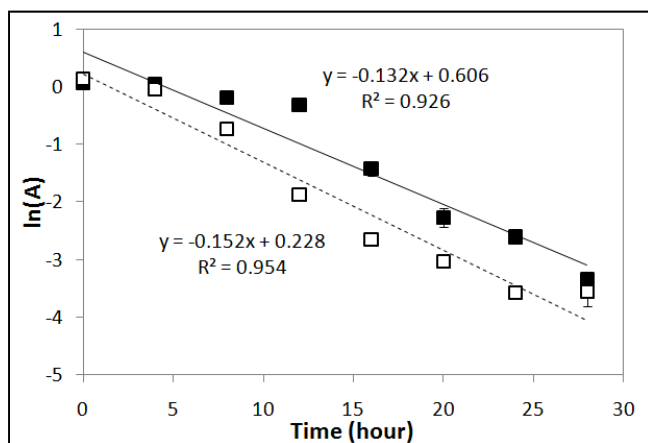


Figure 3. Decolorization of RB5 by isolate NHG under static (■) and shaking (□) conditions. The absorbance (A) readings were taken at 597 nm. Study was conducted in triplicates and bars indicated standard error. Initial concentration = 50 mg/L. Decoloration rates were 0.1324 hr⁻¹ and 0.1529 hr⁻¹ under static and shaking conditions, respectively.

Table 2 shows the decolorization of various dyes by isolate NH2. Decolorization rates of dyes by bacteria may be affected by the differences in molecular structure and weight or presence of conjugated group, such as sulfonic group ($-\text{SO}_3^-$) [12]. Isolate

NH2 has been observed to decolorize 25.9% of Crocein Orange G and only 47.8% of Orange II when tested with both dyes which have exactly equal molecular weight. Similar observations were recorded in another study with different isolates [13, 14].

Table 2. Decolorization of various azo dyes (50 mg/L) by isolate NH2 in 24 hours.

Azo dye	Purity (%)	Molecular Weight	Azo bond	λ_{max} (nm)	% decolorization
Direct Blue 71	50	965.94	2	594	27.12
Amaranth	85-95	604.47	1	523	31.55
Tartrazine	>85	534.36	1	427	12.19
Metanil Yellow	70	375.38	1	414	93.26
Crocein Orange G	90	350.32	1	482	25.89
Orange II sodium salt	85	350.32	1	483	47.75
Reactive Red 120	60	1469.98	2	517	4.52

Optimizing carbon and nitrogen sources for RB5 decolorization

Figure 4 shows the effect of nine carbon sources on RB5 dye decolorization by isolate NHG. According to Nigam et al., the degradation of azo dyes depends on the presence of co-substrate, which acts as an electron donor [15]. RB5 was decolorized up to 94.44% with present of glucose, while only 12.72% was decolorized with present of starch. Decolorization percentages for other carbon source are below 90%. In this study, glucose was observed to be the most suitable carbon source to achieve optimal decolorization of RB5.

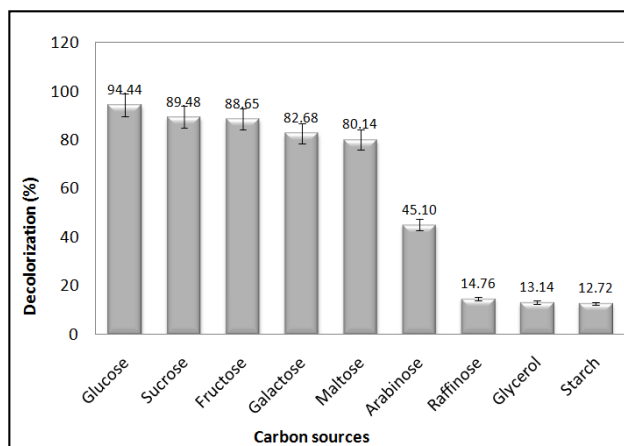


Figure 4. Effect of different carbon sources on the decolorization of RB5 by isolate NHG.

Figure 5 shows the effect of various nitrogen sources on RB5 decolorization. From seven nitrogen sources, yeast extract was observed to be the best for RB5 decolorization. Tryptone and ammonium chloride showed low percentage for RB5 dye decolorization. Yeast extract was also reported to be the most effective nitrogen source for decolorization of True Blue dye by *Aspergillus flavus* [16]. Another study indicated that ammonium sulfate is the best nitrogen source for *Pleurotus ostreatus* to achieve maximum decolorization of Solar Golden Yellow R dye [17]. The presence of various carbon and nitrogen sources in the

medium might assist or inhibit the induction of enzyme system which involved in dye decolorization [18]. Consumption of nitrogen source depends on the bacteria itself to obtain optimum dye decolorization. In this study, decolorization of RB5 by isolate NHG was highest when utilizing yeast extract as nitrogen source.

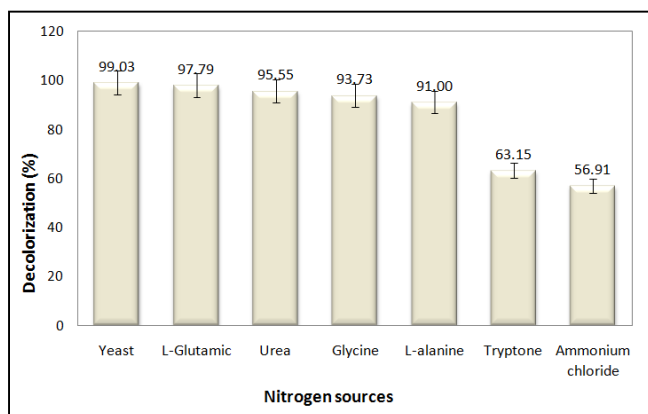


Figure 5. Effect of different Nitrogen sources on the decolorization of RB5 by isolate NHG.

Biodecolorization and/or biodegradation of azo dyes by microorganisms can be an efficient option for textile wastewater treatment. The anaerobic decolorization of RB5 by *Rhodopseudomonas palustris* strain showed that the production of various daughter products can be further degraded and detoxified [19]. The cleavage of azo bond of Metanil Yellow, for example, produces metanilic acid and *p*-aminodiphenylamine, which also can be further biodegraded [20].

CONCLUSION

Two local isolates were capable of decolorizing structurally different azo dyes under agitated and static conditions. Molecular weights, number of azo bonds, complexity of the structures and the presence of various conjugated groups do not affect the decolorization rate. Toxicity analysis of the byproducts produced by these local isolates may govern the biodecolorization or biodegradation rates of azo dyes

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