Isolation of Microorganisms and Its Application for Decolorization of Anthraquinone and Azo Dyes from Textile Wastewater

Ahmad Fathoni[1]*, Soo Kyoung Jeong[2], and Joong Kyun Kim[2]
[1]Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI), Indonesia
[2]Department of Biotechnology, Pukyong National University, Korea

Abstract

The treatments of textile wastewater by chemical and physical methods have some drawbacks such as its economical feasibility and its relatively high cost, its lack of effective color reduction and its formation of by-product. Conversely, biological processes have been proposed as a less expensive and less environmentally intrusive alternative. In the present study, decolorization of various dyes was studied using microorganisms isolated from textile/dye wastewater inoculated into culture medium supplemented with 0.01% of anthraquinone and azo dyes (Disperse Red 73, Lanasol Blue 3G Disperse Orange 30, Kemachrome Black T, and Mixture of dyes). The effect of medium composition was investigated by applying four different media (BSM, ME, Basal, and GYP). Out of 42 isolates, 4 isolates showed the potential for decolorizing structurally different anthraquinone and azo dyes employed in the industry. Based on the microscopic observation, the 3 isolates designated as OW1, RW8, ATSN-3 were identified as bacteria and 1 isolate designated as BSS was observed as fungus. BSS fungus isolate showed the highest ability to decolorize various reactive dyes including anthraquinone and azo dyes. BSS grew well in the ME medium, resulting in approximately 96.4% decolorization efficiency after 72 hours. The result indicates the potential for this isolate to be used in the biological treatment of textile industry.

Keywords: microorganisms, decolorization, textile wastewater, anthraquinone, azo dyes

Introduction

Wastewaters from textile industries constitute a threat to the environment in the world, as the degradation products of textile dyes are often carcinogenic. In addition, textile dyes hinder photosynthetic aquatic plants and algae to absorb the sunlight. The main important pollutants in textile effluent are recalcitrant organic compounds, color, toxicant and inhibitory compounds, surfactants and chlorinated compounds. During processing, about 5–20% of the used dyestuffs are released into the processed water (Wong & Yu, 1999; Soares *et al.*, 2001) and dye is the most difficult constituent to treat by conventional biological wastewater treatment (Chung *et al.*, 1992).

Reactive dyes, including many structurally different dyes, are widely used in the textile industries because of their wide variety of color shades, high wet fastness profiles, ease of application, brilliant colors, and minimal energy consumption. The three most common groups are azo, anthraquinone and phthalocyanine dyes (Axelsson *et al.*, 2006), which are toxic and carcinogenic (Acuner & Dilek, 2004). Disposal of these dyes into the environment causes serious damage, since it may significantly affect the photosynthetic activity of hydrophytes by reducing light penetration (Aksu *et al.*, 2007). It also may be toxic to some aquatic organisms due to their breakdown products (Hao *et al.*, 2000).

Dyes can be removed from wastewater by chemical and physical methods including absorption, coagulation-flocculation, oxidation and electrochemical methods (Lin & Peng, 1996). However, both of the physical and chemical methods have some drawbacks in application, such as high-energy costs, high-sludge production, and formation of by-products (Sarioglu *et al.*, 2007). Conversely, bio-processing can overcome these defects because it is cost saving and environmentally benign.

*Corresponding author:
Cibinong Science Center, Jln. Raya Bogor Km. 46,
Cibinong 16911, Indonesia
Phone +62-21-8754587, Fax. +62-21-8754588
E-mail: ahmad.fathoni1737@gmail.com

Fungi (Acuner & Dilek, 2004; Asgher *et al.*, 2008; Jadhav *et al.*, 2007) and algae (Daneshvar *et al.*, 2007; Mohan *et al.*, 2002) have been used in dye decolorization. Absorption rather than degradation plays a major role during the decolorization process by fungi and algae; as a result, the dyes remain in the environment. It is well-known that bacteria are able to degrade and even completely mineralize many reactive dyes under certain conditions (Asad *et al.*, 2007; Chen *et al.*, 2003; Kapdan & Erten, 2007; Moosvi *et al.*, 2005). Even better, the products of intermediate metabolism during the decolorization process, such as aromatic amines, can be degraded by the hydroxylase and oxygenase produced by bacteria (Pandey *et al.*, 2007). Some new bacterial strains capable of decolorizing a broad-spectrum of dyes have also been isolated and characterized (Deng *et al.*, 2008). Bacterial degradation of reactive dyes is often initiated under anaerobic conditions by an enzymatic biotransformation step (Carvalho *et al.*, 2008; Park *et al.*, 2006). The resulting products such as aromatic amines are further degraded by multiple-step bioconversion occurring aerobically or anaerobically (Barragan *et al.*, 2007; Xu *et al.*, 2006).

The objectives of this study were: a) to isolate and screen microorganisms from dye wastewater, and b) to test the effect of medium composition on the degrading activity of dye decolorizing isolates.

Materials and Methods

Isolation and Screening for Dye Decolorizing Microorganisms. Five litres of samples (dye wastewater) were collected from three different sites including aeration tank water 1, aeration tank water 2, and settling water of dyeing industry located in Busan-Republic of Korea, and were brought to Environmental Bioengineering Laboratory, Pukyong National University, Busan, Republic of Korea. Aliquots (1 mL) of the samples were serially diluted in sterile distilled water and inoculated into 9 mL of NaCl (0.01%) in a 15-mL tube. Samples were incubated in shaking incubator at 30°C and 150 rpm for 12 days. Samples were then streaked on agar medium supplemented with mixture of dyes (0.01%) which had purple color and were incubated at 30°C and 150 rpm until colonies were formed on the plates. The colonies were purified by repeated streaking on the same agar medium.

Those isolates were screened for their potential activity on decolorizing different dyes solution, for instance Lanasol Blue 3G, Disperse Red 73, Kemachrome Black T and Disperse Orange 30 (Figure 1–4). The screening for the dye decolorizing isolates was carried out in a 15-mL tube by inoculating 5% (v/v) of isolates into 10 mL of screening medium with the following composition (per L): 0.188 g Bacto-peptone, 0.318 g KH_2PO_4, 0.563 g sucrose, 0.344 g NH_4Cl, 0.049 g $MgSO_4$, 0.011 g $FeCl_3$, 1 g yeast extract, 1 mL minerals solution containing 3 g/L of $FeSO_4 \cdot 7H_2O$; 0.01 g/L of H_3BO_3; 0.01 g/L of $Na_2MoO_4 \cdot 2H_2O$; 0.02 g/L of $MnSO_4 \cdot H_2O$; 0.01 g/L of $CuSO_4 \cdot 5H_2O$; 0.01 g/L of $ZnSO_4$; and 0.5 g/L of ethylenediaminetetraacetic acid, and 1 mL vitamins solution containing 0.2 g/L of nicotinic acid; 0.4 g/L of thiamine-HCl; 0.2 g/L of nicotinamide; and 0.008 g/L of biotin. The culture was incubated in shaking incubator at 30°C and 150 rpm for 6 hours. After that, 3 mL of samples were centrifuged at 4,000 rpm for 10 min. Supernatant was then diluted into 10-fold dilution and filtered using 0.45 µm filters before being measured for the decolorization percentage of each dye (color).

Effect of medium composition on dye removal. In order to find out a high-performance of the isolated dye degrading microorganisms, various selective medium such as BSM medium, ME medium, Basal medium and GYP medium supplemented with 0.01% of different dyes solution were used in this experiment (Table 1). This experiment was done by applying 4 selected isolates obtained from previous experiment into the culture medium and incubating at 30°C and 150 rpm for 72 hours.

Table 1. Composition of media (per L).

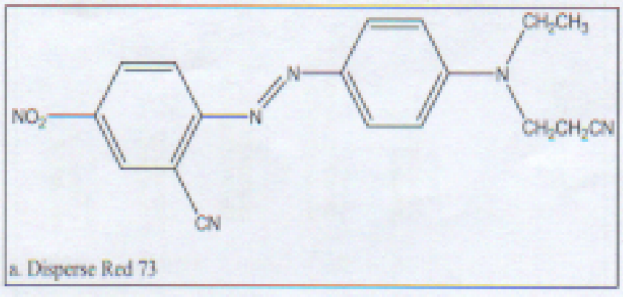
No	Media	Composition
1	BSM medium Initial pH= 6.8	0.188 g Bacto-peptone, 0.318 g KH_2PO_4; 0.563 g sucrose; 0.344 g NH_4Cl, 0.049 g $MgSO_4$, 0.011 g $FeCl_3$, 1 g yeast extract, 1 mL minerals solution, & 1 mL vitamins solution
2	ME medium, Initial pH= 5.6	20 g malt extract, 1 mL minerals solution, & 1 mL vitamins solution
3	Basal medium, Initial pH=7.0	0.28 g NH_4Cl, 0.25 g K_2HPO_4, 0.1 g $MgSO_4.7H_2O$, 0.1 g $CaCl_2.2H_2O$, & 1 ml trace element containing 0.5 g/L of $ZnSO_4.7H_2O$; 0.1 g/L of $Fe(NH_4)_2(SO_4)_2.6H_2O$; 0.25 g/L of $CuSO_4.5H_2O$; 0.005 g/L of $MnSO_4.H_2O$; 0.005 g/L of H_3BO_3 (anhydrous); and 0.005 g/L of $Na_2MoO_4.2H_2O$.
4	GYP medium, Initial pH=9.0	10 g glucose, 5 g poly-peptone, 5 g yeast extract, 1.4 g KH_2PO_4, 10.2 g Na_2CO_3, & 0.2 g $MgSO_4.7H_2O$

Decolorization Assay. Firstly, the dyes including Disperse Red 73, Lanasol Blue 3G, Kemachrome Black T, Disperse Orange 30, and Mixture of dyes (Purple) were initially scanned by an integration method previously developed by Wu *et al.*, (1998) and Wu *et al.*, (2001) to determine each of their fix wavelength. This method involved scanning the absorbance of the samples from 400 to 700 nm (Spectrophotometer Pharmacia-Biotech, Ultrospec® 3000).

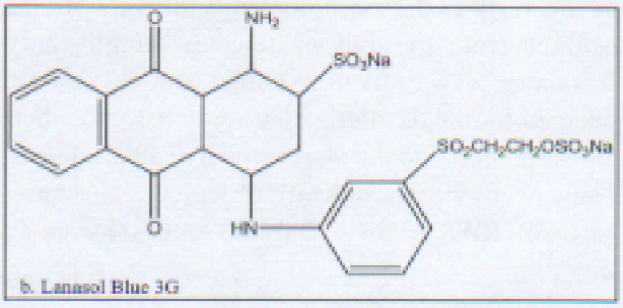
Decolorization efficiency was expressed as percentage of decolorization and was determined by measuring the absorbance of the supernatant at the fixed wavelength of each color (Red: 515 nm, Blue: 621 nm, Orange: 490 nm, Black: 535 nm, & Mixture: 591 nm) and analyzed according to Wang *et al.* (2009). Decolorization efficiency was calculated by the formula below.

$$Decolorization\ efficiency\ (\%) = \frac{OD_0 - OD_t}{OD_0} x100$$

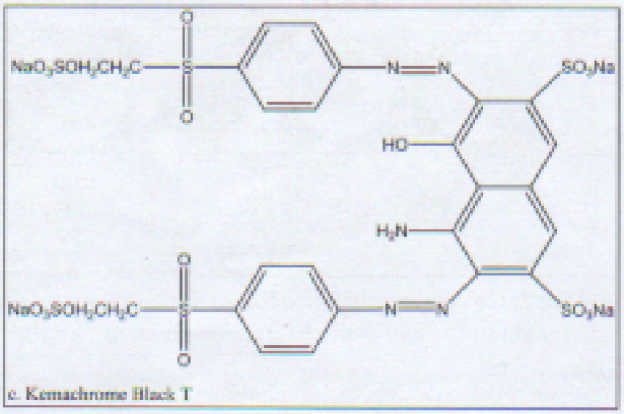
where OD_0 refers to the initial absorbance, OD_t refers to the final absorbance and t refers to the incubation time.



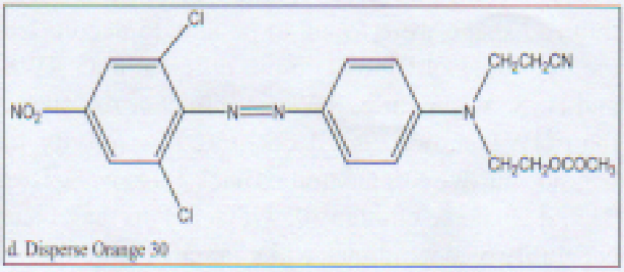
a. Disperse Red 73



b. Lanasol Blue 3G



c. Kemachrome Black T



d. Disperse Orange 30

Figure 1. Structure of dyes; a. Disperse Red 73, b. Lanasol Blue 3G, c. Kemachrome Black T, and d. Disperse Orange 30.

Results and Discussion

In the present study, a total of 42 isolates were purely isolated on agar medium supplemented with 0.01% of mixture of dyes containing Disperse Red 73, Lanasol Blue 3G, Kemachrome Black T and Disperse Orange 30, which possesses purple color. Those isolates were initially screened for dye decolorizing ability by inoculating each single isolate into the culture medium containing 0.01% of dye.

In the screening study, it was found that out of the forty-two morphologically distinct strains isolated from the dye wastewater sample, only 4 isolates (OW1, RW8, ATSN-3 and BSS) were selected for the decolorization study based on their higher potential to decolorize the dye after 6 hours. Those were microscopically observed as bacteria; i.e. OW1, RW8, ATSN-3, and BSS fungus (Figure 2).

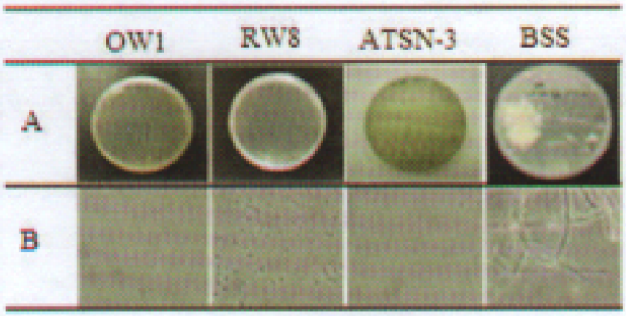


Figure 2. Colony forming of selected microorganisms on agar medium (A) and their observation under microscope (B).

The OW1 and ATSN-3 which have a small and thin rod shape were found to be able to decolorize the dye Lanasol Blue 3G more efficient than RW8 and BSS. Meanwhile, RW8 with thicker rod shape than OW1 showed good decolorizing activity to degrade the dye Kemachrome Black T, Disperse Red 73 and Disperse Orange 30, which means that it has capability on degrading a wider range of structurally different dyes. In addition, BSS fungus showed good potential as well to decolorize a wide range of the dye including Lanasol Blue 3G, Disperse Red 73, Kemachrome Black T, Disperse Orange 30, and mixture of dyes which has purple color (Figure 3).

In the similar study conducted by Arun Prasad & Bhaskara Rao (2010), out of the 30 bacterial strains isolated from the textile industry effluent, only 3 isolates were found to be potential for decolorizing the dye. The isolation of different microorganisms

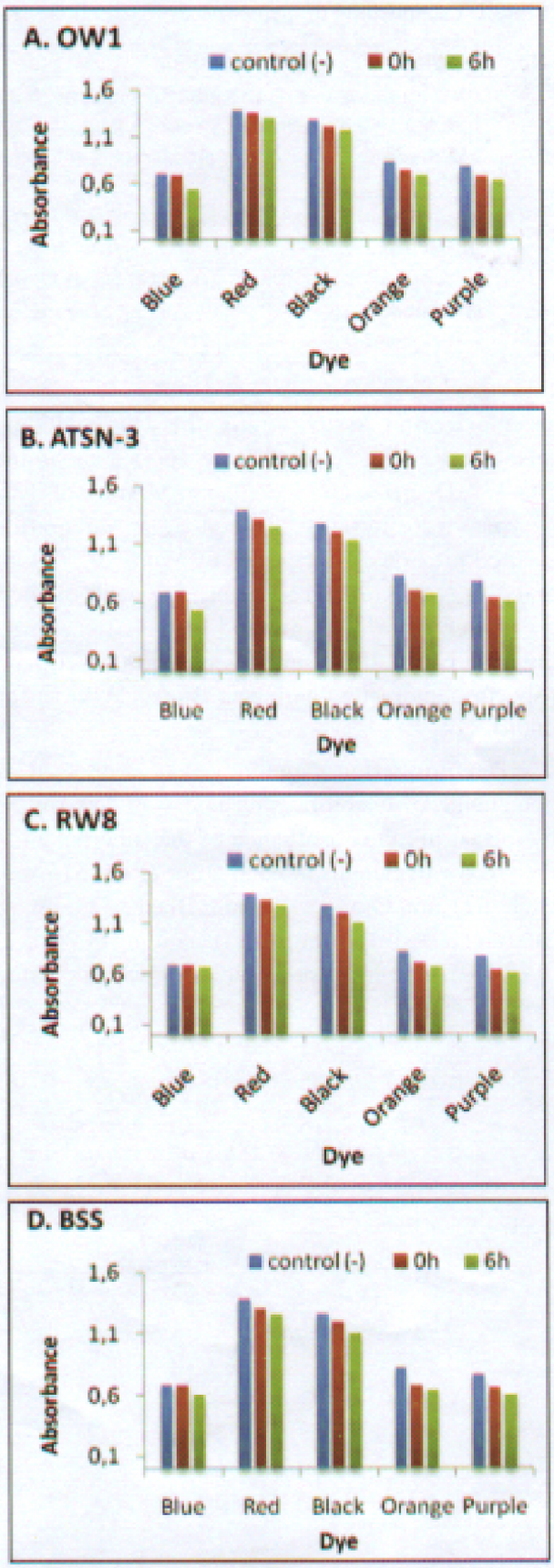


Figure 3. Decolorization of Lanasol Blue 3G, Disperse Red 73, Kemachrome Black T, Disperse Orange 30, and mixture of dyes by (A) OW1, (B) ATSN-3, (C) RW8, and (D) BSS.

from the dye wastewater sample showed the natural adaptation of each microorganism to bear in the presence of toxic compound (dye). Meanwhile, the difference in decolorization ability of the isolates is due to the dissimilarity in specificities, structure and complexity, particularly on nature and position of substituent in the aromatic and the interaction with azo bond with different azo dyes, as reported by Sani & Benerjee (1999), Radha & Raghupati (2005), Vijaykumar & Vaishampayan (2007). Sharma & Saini (2004) also reported that the difference in their rate of decolorization may due to the loss of ecological interaction, which they might be sharing with each other under natural conditions.

Afterwards, in order to maximize the decolorizing activity of the isolates, the effect of medium composition on decolorizing of the dyes was also studied. The isolates were cultivated at 30°C in 4 media, i.e. BSM medium, ME medium, Basal medium, and GYP medium. The result showed that among 4 different media composition, BSM medium and ME medium were found to be an optimum medium for the decolorization of the dyes by BSS fungus, which was shown by color change of the medium during reaction (Figure 4).

In the BSM medium, the percentage of decolorization was 88.1% after 72 hours reaction. It was lower when compared to the result obtained using ME medium (96.4%) (Figure 5).

Our results were higher than the decolorization of Reactive Red 5B exhibited by VITEF1, VITEF2, and VITEF3 (60%, 57%, 58%) (Arun Prasad and Bhaskara Rao, 2010). They were also higher than the study of *Bacillus odyssey, Morganella morganii,* and *Proteus* sp. SUK 7 by Patil *et al.* (2008) over the decolorization percentage of Reactive Blue 59, which were 89%, 90%, and 82%, respectively, that were proven lower when compared to BSS fungus activity.

This describes the adaptability of the isolates to the severe conditions of the dye wastewater and their survival in the highly contaminated water. The ability of the isolates to decolorize dye was also been attributed to their adaptability to the xenobiotic compounds, their biological activity, and chemical structure of the dyes (Zhou & Zimmerman, 1993). There are two mechanisms how the isolate or single microorganism degrades the dye molecule as reported by Coughlin *et al.* (1997). Firstly, the single microorganism may break down the molecule chain at different position, and secondly, the microorganism may use degradation product produce by another strain for further degradation. Hence, the use of isolated microorganisms in consortium to develop efficient biological process for the treatment of dye wastewater containing various dyes needs to be further studied.

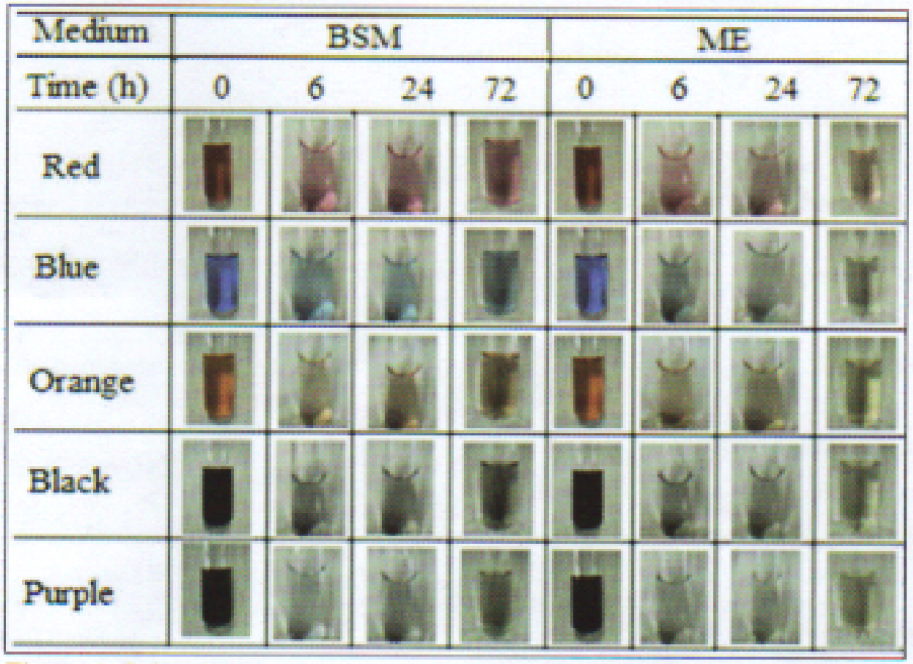
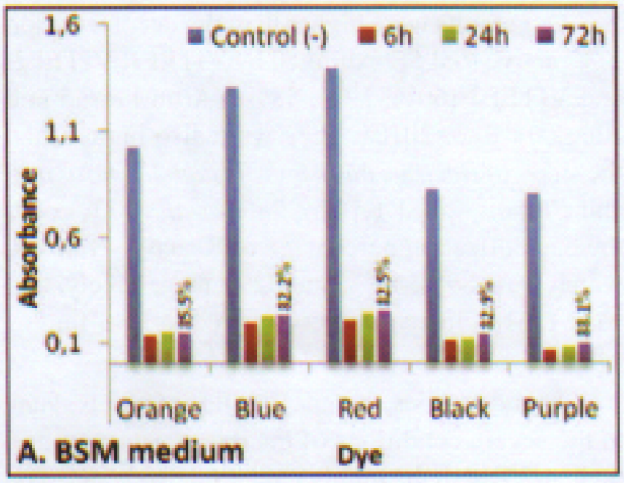


Figure 4. Color change of several dyes in the BSM and ME medium during 72 hours by BSS.



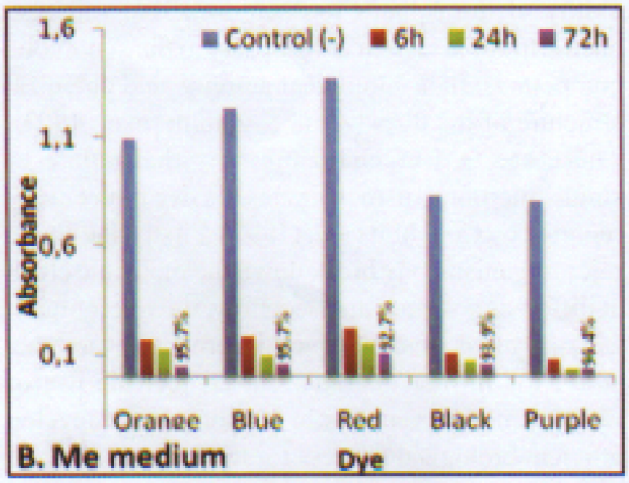


Figure 5. Decolorization of different dyes by isolated fungus in BSM (A) and ME medium (B) after 72 hours.

Conclusions

Four isolated microorganisms namely OW1, RW8, ATSN-3, and BSS from the dye wastewater in dyeing industry, Busan-Republic of Korea, were found to possess the potential to decolorize the Anthraquinone and Azo dyes. The highest dye decolorization efficiency up to 96.4% was obtained from BSS fungus using ME medium after 72 hours.

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