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The decolorization effect by *Aspergillus* sp. 3 on Goldfish opercular beats

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Abstract

Batik effluent had high toxicity to aquatic organisms. Dye decolorization is a process used to reduce color density. Fungi which used for decolorization was *Aspergillus* sp 3. This study aimed to investigate the ability of fungi on decolorization of 3 kinds of batik effluents (Indigosol Green dye, Indigosol Purple dye, and Naphtol Black dye) and to investigate the ability of fungi on reducing TDS level. The study was also investigated the initial toxicity of batik effluents to Goldfish (*Cyprinus carpio*). Decolorization was measured by spectrophotometry, the pH value was measured by pH meter, and the TDS value was measured by TDS meter. In addition, the decolorized batik effluent was tested for toxic effect on *C. carpio* by total opercular beats. Decolorization assay showed that *Aspergillus* sp. 3 had the ability to decolorized only 2 kinds of batik effluents. The decolorization percentage of Indigosol Purple was 60.015% and Naphtol Black was 56.679%. The pH after treatment decreased from 8.5–9 to 5.3–6. The range of pH value of Indigosol Green, Indigosol Purple, and Naphtol Black 5.3–6. Besides that, *Aspergillus* sp. 3 also had the ability to reduce the TDS level in the effluent. The TDS level on Indigosol Green, Indigosol Purple, and Naphtol Black as 4,965%, 25,307%, and 15,129%, respectively. Initial toxicity assay of effluent to *C. carpio* showed that there was a difference of total opercular beats, which exposed by decolorized and before decolorized batik effluent. The total opercular beats of *C. carpio* on decolorized batik effluents showed high value than before decolorized batik effluents. It can be concluded that *Aspergillus* sp. 3 had the ability to decolorize and decreasing the initial toxicity of Indigosol Purple batik effluents on *C. carpio*.

Keywords: *Aspergillus* sp 3. *Cyprinus carpio*, decolorization, opercular beats, TDS

Introduction

Batik effluent is one of the environmental problems caused by their toxicity content on textile effluent. According to Gola *et al*. (2015), the textile industry is also one of the most significant contributors to this toxic effluent generation, which releases high concentrations of dyes, mordants, and other auxiliaries to the environment. The examples of textile dyes that produce toxic effluent are Indigosol Green, Indigosol Purple, and Naphtol Black. The batik effluent can be decolorized by biological decolorization. According to Ali *et al*. (2009), the domain of biological remediation has been gaining a great deal of attention during the last

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couple of decades for its cost-effective and eco-friendly evolving nature. According to Karim *et al.* (2016), biological processes are advantageous as they convert organic compounds to non-toxic products. Biological methods as an eco-friendly alternative for remediation for heavy metal (Mishra & Malik 2013) and dyes pollutants (Kaushik & Malik 2009).

The example of a biological agent for decolorization is fungi. According to Kaushik & Malik (2009), fungi have been proven to be the most effective organism for textile effluent treatment and decolorization. Fungi have more advantages compared to single-cell organisms because their mycelia can dissolve insoluble substrates by producing extracellular enzymes. Fungi have greater physical and enzymatic contact with the environment because of their ratio to the cell surface. By far, the fungal decolorization degradation of dyes has been reported either through biosorption (Fu & Viraraghavan 2000) or enzymatic mineralization (lignin peroxidase, manganese peroxidase, manganese independent peroxidase, and laccases) (Ferreira *et al.* 2000; Wesenberg *et al.* 2003; Wong & Yu 1999; Zheng *et al.* 1999). Fungi offer an efficient system due to large surface area and easy solid-liquid separation (Mishra & Malik 2013). Fungi also possess multiple mechanisms for degradation of organic and inorganic contaminants (Awasthi *et al.* 2014). The example of fungi which commonly used is *Aspergillus* sp. Hefnawy *et al.* (2017) reported the fungi decolorization of dye could be achieved by treating with *Aspergillus flavus* and *Aspergillus niger*. Dewi *et al.* (2018a, 2019a) reported *Aspergillus* sp. 3 could decolorize batik effluent at 24 h, and its degradation products did not cause any toxicity. It was very effective in the removal of BOD, COD, TDS, TSS, EC, and reduce chromium, sulfide, ammonia, phenol, and fat from batik effluent (Dewi *et al.* 2018b, 2019b).

The problems of this research can be formulated how the ability of *Aspergillus* sp. 3 to decolorized the batik effluents and reducing the TDS level. The purposes of this research were consisted of investigating the ability of *Aspergillus* sp. 3 on batik effluents decolorization and reducing the TDS level.

Materials and Methods

Fungal isolate was used in this research was isolate of *Aspergillus* sp. 3, isolated from batik effluent from previous studies (Dewi *et al.* 2018b). Batik dye effluents used in this study were Indigosol Green, Indigosol Purple, and Naphtol Black. The batik effluents used in this study were diluted 10 times. The making of it was done by adding batik effluents as much as 40 mL into 360 mL of distilled water. So that the final volume of each dilution of 400 mL is obtained

Inoculant preparation

The *Aspergillus* sp. 3 was grown on Potato Dextrose Agar (PDA) medium (extract of 200 g potato, 15 g agar, 20 g dextrose in 1000 mL distilled water) in Petri dishes which were previously sterilized by autoclaving for 121 ºC and 2 atm, at room temperature for 5 days. The five culture mycelium plugs (Ø 5 mm) were inoculated into 100 mL Potato Dextrose Broth (PDB) medium in 250 mL Erlenmeyer flasks aseptically then incubated for 3×24 hours on a 75 rpm shaker.

Decolorization assay

The fungal pellets isolate of *Aspergillus* sp. 3 formed after 3×24 h incubation time, then aseptically separated from its PDB medium. The separated pellets were added with all three types of batik waste of 100 mL aseptically, then incubated using shaker by 75 rpm at room temperature for 3×24 hours. Pellet mycelium *Aspergillus* sp. 3 formed then filtered using filter paper with a pressurized filter. The supernatants were analyzed for decolorization assay, pH measurement, TDS measurement, and toxicity assays. The decolorization was

analyzed by UV-vis spectrophotometry. The percentage of decolorization was analyzed by the formula:

$$\% \text{ Decolorization} = \frac{\text{first absorbance} - \text{last absorbance}}{\text{first absorbance}} \text{x } 100\%$$

pH measurement

The pH of effluents was measured before and after decolorization using pHmeter.

TDS measurement

Batik effluents before and after decolorization were measured using a digital TDS meter. The percentage of TDS reduction is measured using the formula:

$$\% \text{ TDS level} = \frac{\text{first TDS} - \text{last TDS}}{\text{first TDS}} \text{ X } 100\%$$

Toxicity assay

The toxicity assay of effluent before and after treatment was conducted in aquarium volume 10 l. The *C. carpio* was first acclimated with water. The size of *C. carpio* was 3–4 cm. Then, the fish put into the aquarium containing the effluent before and after treatment with *Aspergillus* sp. 3 and counted the movement of the fish operculum in the first 5 minutes, second and third by repeating 3 times.

Results

Batik effluents decolorization

The decolorization was expressed by percentage decolorization (Fig. 1). The best decolorization of 3 kinds of batik effluents was evident with *Aspergillus* sp.3 on Indigosol Purple (60.015%) at 3x24 h incubation time followed by Naphtol Black (56.679%). Besides that, *Aspergillus* sp. 3 had a poor decolorization activity on Indigosol Green (-69.03%). This happens probably because Indigosol Green had a more complex structure than other dyes. Shaoxing Biying Textile Technology (2016) stated that the Molecular Formula of Indigosol Green is $C_{36}H_{20}Na_2O_{10}S_2$ and $C_{34}H_{14}Cl_2Na_2O_8S_2$ for Indigosol Purple.

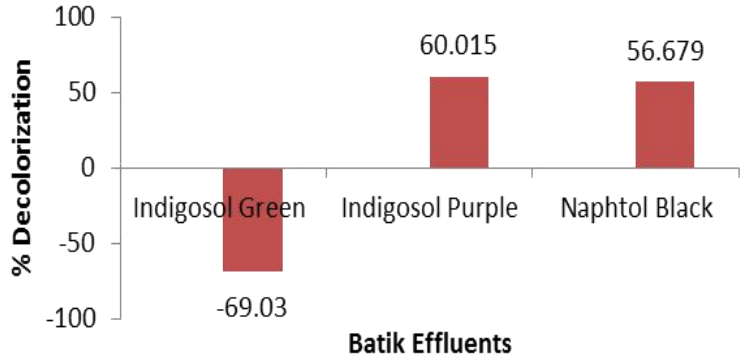

Figure 1. Histogram of decolorization of Batik effluent by *Aspergillus* sp. 3 at 3×24 hour incubation time

Besides that, the color changed of concentrated batik effluent to less dense to clear yellow were indicated that *Aspergillus* sp. 3 had the ability to decolorized batik effluents. Color changed of batik effluent from concentrated to clear yellow is suspected because the complex compounds in batik dyes have broken down into simpler compounds. The color changed of batik effluent before and after the decolorization process is presented in Fig. 2.

The result of the TDS level showed that *Aspergillus* sp. 3 had the ability to reduce the TDS of batik effluents Indigosol Green, Indigosol Purple, and Naphthol Black at 10 times dilution, but it was not optimal. This is indicated by the small average value of the percentage reduction in TDS obtained (Fig. 3). This is presumably because the batik effluent used contained a lot of organic and inorganic compounds. Therefore it was difficult to degrade.

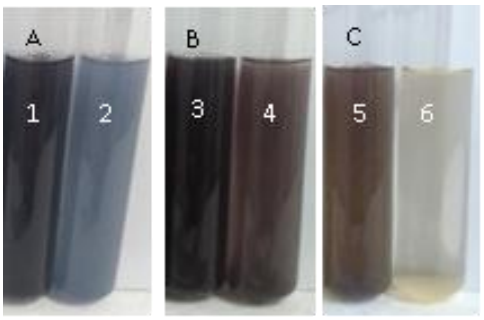

Figure 2. Decolorization of Batik effluent by *Aspergillus* sp. 3 at 3x24 hour incubation time. (A) Indigosol Green, (B) Indigosol Purple, (C) Naphtol Black.
(1) Control of Indigosol Green batik effluent by 10x dilution, (2) Indigosol Green batik effluent by 10x dilution after decolorization, (3) Control of Indigosol Purple batik effluent by 10x dilution, (4) Indigosol Purple batik effluent by 10x dilution after decolorization, (5) Control of Naphtol Black batik effluent by 10x dilution, (6) Naphtol Black batik effluent by 10x dilution after decolorization.

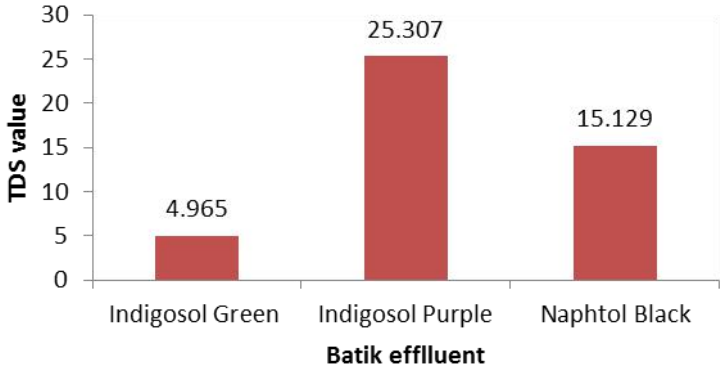

Figure 3. Histogram of decreasing of TDS value on batik effluent by *Aspergillus* sp at 3 x24 hour incubation time

The result showed that the pH of Indigosol Green, Indigosol purple, and Naphtol Black batik effluent before the decolorization process were 9, 8.5, and 8.5. The pH of batik effluent before treatment tends to be alkaline. Then, the pH value of batik effluent from Indigosol Green, Indigosol Purple, and Naphtol Black after decolorization process were 7.8–8.2; 4.2–4.3; and 5.4–5.8 at 5 times dilution. The pH value of batik effluent from Indigosol Green, Indigosol Purple, and Naphtol Black after decolorization process were 5.8–6.1; 5–5.3; and 5.5–6 at 10 times dilution. These results indicate a decrease in pH due to the decomposition of organic and inorganic compounds that produce acidic compounds (Table 1).

Table 1. pH value of batik effluents decolorization by *Aspergillus* sp. 3.

Treatments	Indigosol Green	Indigosol Purple	Naphtol Black
Before decolorization	9	8.5	8.5
After decolorization	5.8 – 6.1	5 – 5.3	5.5 – 6

Besides that, the toxicity was assayed on *C. carpio* by operculum movement observation which showed in Table 2. Based on the table showed that the movement of the *C. carpio* operculum in degraded batik effluent is higher compared to batik effluent that has not been degraded. In fact, in the second five minutes the *C. carpio* is dead whereas the *C. carpio* can survive up to the third five minutes in the degraded batik effluent.

Table 2. Opercular beats of *C. carpio* as toxicity assay

Treatment	Replication	Total of Operculum Motion		
		First 5 min	Second 5 min	Third 5 min
Water	1	700	490	762
	2	650	756	800
	3	500	930	910
Effluent	1	81	0	0
	2	38	0	0
	3	59	0	0
Neutral Effluent	1	323	42	0
	2	269	355	399
	3	170	32	0

Discussion

Application of *Aspergillus* sp. 3 had been proved as a superior on decolorization of 2 kind batik effluent. *Aspergillus* sp. 3 had the ability to decolorized Indigosol Purple and Naphtol Black. Ali *et al.* (2009) reported that *A. niger* SA1, a brown-rot fungus has similar capabilities like white-rot in the efficient and comprehensive degradation of pollutants like dyes. Asses *et al.* (2018) reported that CR was successfully decolorized and biodegraded by *A. niger.* High decolorization efficiency (97%) was obtained after six days of culture.

The decolorization process by the fungus was analyzed by UV-spectrophotometry. Decolorization of dye was followed by changes in the absorbance value. There was a decreasing in absorbance value caused by reducing the color intensity. The decolorization process by fungus causes the clear less color of batik effluents. The color of batik effluents had been changed from concentrated color into clearness color, which means that the complex compound on batik effluent had been broken into simply compound. The reducing color intensity related to decolorization percentage. Dewi & Lestari (2010) reported that the lower concentration cause, the higher of decolorization percentage. Loss of color is an obvious indicator of dye removal from solutions but the assessment of products or metabolites; their chemical nature and further evaluation of toxicity become essential (Khandare & Govindwar 2015). Dewi *et al.* (2018a) reported that *Aspergillus* sp. 3 was the best isolate for decolorizing the effluent. Degradation by *Aspergillus* sp. 3 as the most effective fungi is seen more clearly than in the other species. The mycelium, which caused degradation using the *Aspergillus* sp. 3 fungus culture, was seen to be absorbing more dye than the other cultures.

Color removal from effluent containing dyes appeared to be due to mechanisms of the fungal biomass, primarily biosorption/bioabsorption. After decolorization of dyes by the biomass mechanism and then subsequent formation, dyes were metabolically degraded by fungal strains into their derivative products (Ali *et al.* 2009). The decrease in color intensity by the activity of fungi isolates was caused by adsorption and enzymatic activity (Schiegel & Schmit, 1994). Biosorption can occur in biomass; amino, carboxyl, thiol, lipid, and phosphate groups that exist in the fungal cell walls are responsible for binding the dye molecules in the biosorption process (Kyzas *et al.* 2013). Dye decolorization caused by a ligninolytic enzyme (laccase, manganese peroxidase, and lignin peroxidase). Lac enzyme activities of *Pleurotus ostreatus* is 200.43 U/l, MnP is 9.714 U/l, and LiP is 12938.60 (Dewi *et al.* 2019c).

Textile effluent is characterized by color; varies in hydraulic flow rate; and has high pH, temperature, biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), and total suspended solids (TSS) (Banat *et al.* 1996; Ghoreishi & Haghighi 2003). *Aspergillus* sp. 3 was showed the reducing of the TDS level in the batik effluents after decolorization. This is indicated by the small average value of the percentage reduction in TDS obtained. According to Dewi *et al.* (2018b) reported *Aspergillus* sp. 3 was the best isolate for reducing TDS. Kanagaraj & Mandal (2011) reported that the reduction of TOC, TDS, and TSS of the dye had been initially treated with *A. niger*.

High TDS value reduces light penetration into the water and ultimately decreases the photosynthesis in aquatic flora. This causes a reduction in the dissolved oxygen level of water bodies, which results in extremely low purification of effluent by microorganisms (Namdhari *et al.* 2012). High TDS can disturb oxygen transfer and interfere with the biological metabolism of pollutants (Pophali *et al.* 2003).

Decolorization of batik effluent also affected by pH. The growth of fungi as a biological decolorization agent was affected by pH. Zafar *et al.* (2008) reported that solution pH is among the most significant parameters controlling the sorption. Ncibi *et al.* (2007) stated this occurs because of its impact on both the biosorbent surface binding site and the dye molecules that are ionized in the water. Aksu (2005) reported dye adsorption could change with the change of solution pH. Dye biosorption (positively charged cation) is influenced by the surface level of the adsorbent (negatively charged), which is determined by the pH of the solution. Sites with a negative charge on the adsorbent At low pH have low availability because the number of H+ ions compete with the cations of the dye for the adsorption site (Hameed *et al.* 2008) because, in the system of adsorption, there is a net positive charge due to the H_3O^+ presence. It can be interpreted that H^+ ions in such a system compete with cations of the dye, produces a protonated active site by ruling out the dye cation that binds to the surface of the adsorbent (Wang *et al.* 2008). The amount of negative charged is low at a lower pH, which is not preferably a positively charged cation of dye for adsorption due to electrostatic repulsion (Nainasivayam *et al.* 2004). Negatively charged surfaces become more available when the pH of the dye solution increased due to biosorbents that have different functional groups undergo deprotonation (Saaed *et al.* 2009).

Besides that, the toxicity assays also testes on the opercular beats of *C. carpio*. As the degree of toxicity of water increases, it produces high-stress conditions on fishes, the degree of toxicity produced by the poisonous substance is dose-dependent upon environmental conditions such as temperature, pH of water, oxygen content and presence of residue molecules (Capkin *et al.*, 2006; Singh & Mishra 2009). An increase in temperature, rise in metabolic rate of fish and hence its oxygen demand, but it decreases oxygen solubility in water. This counter tendency may result in depletion of oxygen to a lethal level (Tantarpale *et al.* 2012).

Conflict of Interest

The authors state no conflict of interest from this manuscript.

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Author Contributions

All authors have reviewed the final version of the manuscript and approved it for publication. RSD and KK designed the study, performed research, and collected the data; FAU for analysing the data and wrote the paper. RSD is the main contributor to this manuscript.

References

Aksu Z, 2005. Application of biosorption for the removal of organic pollutants: a review. Process Biochemistry 40, 997–1026.

Ali N, Hameed A, Siddiqui MF, Ghumro PB, Ahmed S. 2009. Application of *Aspergillus niger* SA1 for the enhanced bioremoval of azo dyes in Simulated Textile Effluent. African Journal of Biotechnology 8(16), 3839-3845.

Asses N, Ayed L, Hkiri N, Hamdi M. 2018. Congo Red Decolorization and Detoxification by *Aspergillus niger*: Removal Mechanisms and Dye Degradation Pathway. BioMed Research International 2018, 1-9, DOI: 10.1155/2018/3049686.

Awasthi MK, Pandey AK, Bundela PS, Wong JW, Selvam A. 2014. Evaluation of thermophilic fungal consortium for organic municipal solid waste composting. Bioresources Technology 168, 214–221.

Banat IM, Nigam P, Singh D. 1996. Microbial decolourization of textile-dye containing effluents: a review. *Bioresources Technology* 58:217–227.

Capkin E, Altmok I, Karahan S. 2006. Water quality and fish size affect toxicity of endosulfan, an organochlorine pesticide, to Rainbow trout. Chemosphere 64, 1793-1800.

Dewi RS, Lestari S. 2010. Dekolorisasi Limbah Batik Tulis Menggunakan Jamur Indigenous Hasil Isolasi Pada Konsentrasi Limbah Yang Berbeda. Molekul 5(2), 75 – 82.

Dewi RS, Kasiamdari RS, Martani E, Purwestri YA. 2018a. Decolorization and detoxification of batik dye effluent containing Indigosol Blue-04B using fungi isolated from contaminated dye effluent. *Indonesian Journal of Biotechnology* 23(2), 2018, 54-60. DOI: 10.22146/ijbiotech.32332.

Dewi RS, Kasiamdari RS, Martani E, Purwestri YA. 2018b. Bioremediation of Indigosol Blue 04B Batik Effluent by Indigenous Fungal Isolates, *Aspergillus* spp. Omni-Akuatika 14 (2): 11 – 20.

Dewi RS, Kasiamdari RS, Martani E, Purwestri YA. 2019a. Optimization of the conditions for the decolorization of batik wastewater by *Aspergillus* sp. 3. In AIP Conference Proceedings (Vol. 2094, No. 1, p. 020036). AIP Publishing.

Dewi RS, Kasiamdari RS, Martani E, Purwestri YA. 2019b. Efficiency of *Aspergillus* sp. 3 to reduce chromium, sulfide, ammonia, phenol, and fat from batik wastewater. In IOP Conference Series: Earth and Environmental Science (Vol. 308, No. 1, p. 012003). IOP Publishing.

Dewi RS, Ilyas M, Sari AA. 2019c. Ligninolitic Enzyme Immobilization from Pleurotus ostreatus for Dye and Batik Wastewater Decolorization. Jurnal Pendidikan IPA Indonesia 8(2), 220-229.

Ferreira VS, Magalhaes DB, Kling SH, Silva JG, Bon EPE. 2000. N-demethylation of methylene blue by lignin peroxidase from *Phanerochaete chrysosporium*: Stoichiometric relation for H_2O_2 consumption. Applied Biochemistry & Biotechnology 4(86): 255-265.

Fu YZ, Viraraghavan T. 2000. Removal of a dye from aqueous solution by the fungus *Aspergillus niger.* Water Quality Research Journal of Canada 35: 95- 111.

Ghoreishi SM, Haghighi R. 2003. Chemical catalytic reaction and biological oxidation for treatment of non-biodegradable textile effluent. Chemistry Engineering Journal 95(1–3):163–169

Gola D, Namburath M, Kumar R. 2015. Decolourization of the Azo dye (direct brilliant blue) by the isolated bacterial strain. Journal of Basic and Applied Research International 2, pp.1462–1465.

Hameed BH, Mahmoud DK, Ahmad AL. 2008. Sorption of basic dye from aqueous solution by pomelo (*Citrus grandis*) peel in a batch system, Colloids Surf. A: Physicochemisty Engineering Aspects 316, 78–84.

Hefnawy MA, Gharieb MM, Shaaban MT, Soliman AM. 2017. Optimization of Culture Condition for Enhanced Decolorization of Direct blue Dye by *Aspergillus flavus* and *Penicillium canescens.* Journal of Applied Pharmaceutical Science 7(2), pp. 83-92,

Kanagaraj J, Mandal AB. 2011. Combined biodegradation and ozonation for removal of tannins and dyes for the reduction of pollution loads. Environmental Science and Pollution Research 19(1), *42–52.* DOI: 10.1007/s11356-011-0534-0.

Karim E, Dhar K, Hossain T. 2016. Co-metabolic decolorization of a textile reactive dye by *Aspergillus fumigatus.* International Journal of Environmental Science and Technology. DOI: 10.1007/s13762-016-1136-7.

Kaushik P, Malik A. 2009. Fungal dye decolourization: recent advances and future potential. Environment International 35, 127–141.

Khandare RV, Govindwar SP. 2015. Phytoremediation of textile dyes and effluents: Current scenario and future prospects. Biotechnology Advances 33(8), 1697–1714. doi:10.1016/j.biotechadv.2015.09.003.

Kyzas GZ, Fu J, Matis KA. 2013. The Change From Past to Future for Adsorbent Materials in Treatment of Dyeing Wastewaters. Materials 6(11), pp. 5131–5158.

Mishra A, Malik A. 2013. Recent advances in microbial metal bioaccumulation. Critical Reviews in Enviromental Science and Technology 43, 1162–1222.

Nainasivayam C, Kadirvelu K. 1994. Coir pith, an agricultural waste by-product for the treatment of dying wastewater. Bioresources Technology 48, 79–81.

Namdhari BS, Rohilla SK, Salar RK, Gahlawat SK, Bansal P, Saran AK. 2012. Decolorization of Reactive Blue MR, using *Aspergillus species* Isolated from Textile Waste Water. ISCA Journal of Biological Science 1(1), 24-29.

Ncibi MC, Mahjoub B, Seffen M. 2007. Kinetic and equilibrium studies of methylene blue biosorption by *Posidonia oceanica* (L) fibers. Journal of Hazardous Material 280–285.

Pophali G, Kaula S, Mathur S. Influence of hydraulic shock loads and TDS on the performance of large-scale CETPs treating textile effluents in India. Water Research 37, 353–61.

Saeeda A, Iqbal M, Zafar SI. 2009. Immobilization of *Trichoderma viride* for enhanced methylene blue biosorption: Batch and column studies. Journal of Hazardous Materials 168, 406–415.

Shaoxing Biying Textile Tecnology, 2016. http://www.worlddyevariety.com/vat-dyes/solubilised-vat-green-1.html

Schiegel HG, Schmit K. 1994. *Mikrobiologi Umum.* Diterjemahkan oleh Baskoro, R. M dan Watemena, J. R., Yogyakarta: Gadjah Mada University Press.

Singh S, Mishra RN. 2009. Occurrence of organochlorine pesticides residues in Kuano river of eastern Uttar Pradesh. Journal of Environmental Biology 30:467-468.

Tantarpale VT, Rathod SH, Kapil S. 2012. Temperature Stress on Opercular Beats and Respiratory Rate of Freshwater Fish *Channa Punctatus*. International Journal of Scientific and Research Publications 2(12), 1-5.

Wang XS, Zhou Y, Jiang Y, Sun C. 2008. The removal of basic dyes from aqueous solutions using agricultural by-products. Journal of Hazardous Material 157, 374–385.

Wesenberg D, Kyriakides I, Agathos SN. 2003. White-rot fungi and their enzymes for the treatment of industrial dye effluents. *Biotechnology Advances* 22: 161-187

Wong YX, Yu J. 1999. Laccase catalyzed decolorization of synthetic dyes. Water Research 33: 3512-3520.

Zheng Z, Levin RE, Pinkham JL, Shetty K. 1999. Decolorization of polymeric dyes by a novel *Penicillium* isolate. Process Biochemistry 34: 31- 37.