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Bioremediation of Indigosol Blue 04B Batik Effluent by Indigenous Fungal Isolates, *Aspergillus* spp.

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ABSTRACT

Effluent from the local batik home industry is a serious problem, because the effluent discharge generated is spread in different places. Untreated effluent can cause environmental pollution, such as in groundwater reservoirs,because most is discharged into rivers. The aim of this research was to evaluate the bioremediation potential of indigenous fungi in liquid culture media with Indigosol Blue 04B (IB) batik effluent. The fungi isolates tested were *Aspergillus* sp. 1, *Aspergillus* sp. 2 and *Aspergillus* sp. 3, isolated from dye effluent soil and batik effluent, and compared to white rot fungi (*Phanerochaete chrysosporium*) as a positive control. The physiochemical properties of IB batik effluent before and after fungal treatment were investigated. All of these parameters before the fungal treatment were above the recommended standard values based on the Governor regulation of Yogyakarta *Special* Region No. 7/2010. The level of biochemical oxygen demand *(*BOD*),* chemical oxygen demand *(*COD*)*, total dissolved solids (TDS), total suspended solids (TSS), and electrical conductance (EC) was reduce by *Aspergillus* spp. The highest percentage reduction was achieved by *Aspergillus* sp. 3, namely 88.34% BOD, 89.11% COD, 75.77% TSS, 85.85% TDS and 71.21% EC, after 3 days of incubation. These results show that the positive control isolate had the lowest value. The study confirms the ability of indigenous fungi isolates in the remediation of IB batik effluent and their potential for future analysis in the treatment of all types of batik effluent.

***Keywords** : Bioremediation, Indigosol, Batik Effluent, Aspergillus* spp.*, physiochemical.*

1. Introduction

One source of local revenue in Banyumas Regency comes from the batik industry. Banyumas batik consists of several home industries incorporating groups of batik craftsmen. Besides having positive impacts on the region, the industry also has negative ones. One problem is centered on the the absence of effluent treatment installations to treat the waste. Therefore, the batik craftsmen dispose ofthe effluent directly into rivers near the production site. This discharged waste contains pollutants with very high levels of organic materials, causing color change in the river water.

The environmental capability of rivers to accommodate the pollutants is limited and eventually damage can be caused to the environment it self. The various chemical agents used in the dyeing process can be harmful to aquatic life,notably when the effluent is released without any prior treatment (Kusumastuti and Syamwil, 2014). The presence of effluent in rivers threatens fishlife and other aquatic biota, and causes environmental pollution (Arimoro et al., 2007 and Velz, 1985). Environmental pollution caused by the level of effluent discharged into water bodies does not meet the maximum permissible quality standard limits for batik industry activity set by the Governor regulation of Yogyakarta Special Region No. 7/2010. Nindita et al. (2012) found that production of 40 pieces of batik cloth produces about 202.4 l of dye effluent. Only 0.6% of this is used during the dyeing, while the remainder is directly discharged without any prior treatment (Fajri, 2013).

The batik industry produces effluent from the process of dyeing or immersion. It should be noted that not all types of dye can be used in

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batik techniques, as batik dyeing is done without heating, as it uses wax, which is not heat-resistant. The use of wax is to allow the batik pattern to form perfectly. Batik wax will be melted at high temperatures. Synthetic dyes that can be used in batik processing include vat dyes (Sunarto, 2008; Kusumastuti & Syamwil, 2014). Indigosol colours are solubilized vat dyes (cKinetics, 2018), with Indigosol belonging to the type ofvat dye which is derived from the indigo complex (Jagson Colorchem Ltd, 2011). Indigosol batik waste comprises Indigosol dye powder, sodium nitrite and hydrochloric acid (Budiyonoet al., 2008). About 5% of the 60, 000 tons/year world consumption ofdyes is Indigo dye (Paradise, 1999; Spadaro et al., 1994), with annual production of this dye estimated at 22,000 tons (Schrott, 2001). The very high level of use use is responsible for wastewater. According to Balan and Monteiro (2001), Indigo dyes are considered as recalcitrant substances that cause environmental damage. Therefore, it is necessary to conduct a degradation process before discharging them into the environment.

Effluent from dye processing causes serious environmental problems. The use of these chemicals can increase BOD and COD in water resources (Rashidi et al., 2012). Laboratory test results show that the quality of river water contaminated by batik effluent does not meet the quality standards for batik effluent set by government regulations, especially the textile effluent quality standards TDS, TSS, EC NH , sulfide, chrome total (Cr), phenols, oils and fats. The BOD value was 869 mg/l and the COD value2200 mg/l (Aryani et al., 2004).

Efforts made to overcome the negative impact of pollution of from batik industry effluents include treatment before their discharge into rivers. Fungal bioremediation is one of the most effective ofthe various water treatment technologies, being an easy to implement and economical effluent treatment alternative. However, the biomass produced during the effluent treatment by fungi is of a much higher value than that fromthe bacterial process (Sankaran et al., 2010). Bioremediation by fungi compared with bacteria has been shown to be significantly higher for the degradation of synthetic dyes (Shahid et al., 2013). Fungi have been proven to be the most effective organism for textile waste treatment and decolorization. Fungi have more advantages compared 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 (Kaushik & Malik, 2009).

Based on previous research, white-rot fungus *Phanerochaete chrysosporium* is an ideal model for bioremediation by fungi, since it is more efficient than other fungi or microorganisms in degrading toxic or insoluble materials (Rhodes, 2014). *P. chrysosporium* URM 6181 has proven to be effective in the treatment of textile effluent. Effluent treated by *P. chrysosporium* URM 6181 accumulated a mutagenic metabolite derived from indigo dye (Rita de Cássia et al., 2013).

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In recent years, various studies have been conducted on fungi which are able to biosorp and biodegrade dyes in effluent (Ambrosio and Campos-Takaki, 2004; Eichlerova´ et al., 2006). Most bioremediation research using fungi has focused on *P. chrysosporium*, which is known for its ability to degrade a variety of recalcitrant compounds and xenobiotics (Cameron et al., 2000). Other fungi,such as various *Aspergillus* spp., have been shown to decolorize several dyes. The *Aspergillus sojae* B10 isolate has shown to decolorize the azo dyes Congo red, amaranth and Sudan III after 3–5 days of incubation in nitrogen-poor media (Ryu and Weon, 1992). *Aspergillus oryzae* and *Aspergillus fumigates* G-2–6,capable of decolorizing various structurally different dyes, were isolated and found to be more effective than *P. chrysosporium* (Knapp et al., 1995). The aim of this research is to evaluate the bioremediation potential of indigenous fungi (*Aspergillus* spp.) in Indigosol Blue 04B (IB) batik effluent.

2. Materials and Methods

Samples and Chemicals

The samples of Indigosol Blue 04B (IB) batik effluent used for the bioremediation in this investigation were collected from several domestic batik effluent discharge drains and kept in a refrigerator at 4°C. The indigenous fungal isolates used were *Aspergillus* sp. 1, *Aspergillus* sp. 2 and *Aspergillus* sp. 3, isolated from dye effluent soil and batik effluent. One culture of white rot fungus (*P. chrysosporium*) InaCC F206 from Indonesian Culture Collection (InaCC), Research Center for Biology, Indonesian Institute of Sciences (LIPI) was also used in the study. Media components used were Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB).

Preparation of Culture Conditions

The isolates were maintained in a PDA medium at room temperature until utilization, and the characteristics of the microscopic fungi were carried out by the slide culture technique. In this method, *P. chrysosporium* was employed as a positive control and the IB batik effluent was employed as a negative control. A comparison was made between the isolated fungi.

Physiochemical Analysis

In this work, we only study the reduction in BOD, COD, TDS, TSS, EC, temperature and pH. Wastewater treatment objective were based on the reduction of BOD, TSS, and other parameter (Metcalf & Eddy, 1991).The temperature and pH of the effluent samples were measured at the collection site. The BOD, COD, TDS, TSS and EC were analyzed in the laboratory according to the methods prescribed by Indonesian National Standard (abbreviated to SNI), which are the only standards that apply nationally in Indonesia (Salar et al.2012).

IB Batik Effluent Bioremediation.

One hundred milliliters of PDB medium were transferred to 250 mL Erlenmeyer flasks.The flasks were inoculated with 2% (v/v) fungal spore suspension containing 10^6 CFU and incubated by shaking at temperature room for 3 days. The medium amended with each of the tested IB batik effluents. Control experiments were performed inthe same conditions as described above, but without the fungi.The percentage of physiochemical reduction was calculated by the following equation:

Basic Equation: (treated/untreated Effluent) *100.

3. Results and Discussion

IB batik effluent was polluted with organic loads in the form of BOD and COD, and also with dissolved and suspended solids in the formof TSS and TDS (Table 1). The concentrations of BOD, COD, TDS and TSS untreated fungally were very high compared to the maximum permissible effluent limits for the for batik industry activity by the Governor regulation of Yogyakarta Special Region No. 7/2010 regarding effluent level standards for industrial health, health services and tourism services. Theseresults agree with the findings of Rochma and Titah (2017), who obtained values of BOD, COD and TSS duringthe dyeing process of 1777.5, 16654.8 and 208 mg L-1 respectively. The effluent was treated in amycological operation with three species of fungi. BOD, COD,TDS and TSS concentrations were measured before and after the fungal treatment.

Table 1. Physicochemical analysis of IB batik effluentand permissible limits

Parameters	Maximum permissible limits of Quality Standard	Values of effluent
Biochemical oxygen demand (mg L-1)	85	1470.68
Chemical oxygen demand (mg L-1)	250	15800
Total dissolved solids (mg L-1)	2000	16546.67
Total suspended solids (mg L-1)	60	374.6
Electrical conductance (mS cm^{-1})	1.56	17.44
Temperature (°C)	± 3 °C from air temperature	27
pH	6-9	5

Biological Oxygen Demand (BOD)

BOD concentration decreased in the IB batik effluent treated with *Aspergillus* spp. compared to the positive and negative controls (Figure 1). The results show that IB batik effluent treated with indigenous fungal isolates displayed lower BOD value than those observed in the positive control but otherwise on negative control. The results indicate that indigenous fungal isolates were effective in the reduction of BOD from the effluent, even when compared to the positive control that is known to have such capabilities. The reduction of BOD by *Aspergillus* sp. 1,2,3 ranged from 1470.68 mg L^{-1} (*negative controls*) to 495.48 mg L^{-1}, 1145.48 and 171.48 (fungally treated),respectively. Furthermore, *Aspergillus* sp. 3 displayed superiorability over the other isolates inreducing BOD.

Chemical Oxygen Demand (COD)

The results of the average values of COD in IB batik effluent after treatment with

Aspergillus spp. are presented in Figure 2. COD concentrations after treatment with*Aspergillus* sp. 1, 2, 3 were 6280 mg L-1, 1720 mg L-1, and 6240mg / L, respectively. These values show that COD has decreased from the initial characteristics of IB batik effluent (1470.68 mg/l). Figure 2 also shows that *Aspergillus* sp. 3 has superior qualities in reducing COD concentration.

According to the quality standards of the Governor regulation, the maximum permissible limit of BOD is 85 and that of COD 250. Although its are still above the limit,there is the possibility of reducing the COD value further.

BOD as a measure of the amount of oxygen used by the microbial population contained in water in response to the influx of organic matter that can be decomposed (Mays, 1996). Boyd (1990) stated that the BOD is interpreted as an overview of the amount of biodegradable organic material in water. Organic material in BOD is readily decomposable organic matter. COD (chemical oxygen demand) is the amount of oxygen required to decompose all the organic matter contained in water. COD describes the total amount of organic matter present in water, which is organic material that is bothdifficult and easy to decompose.

The overall BOD rates shown in Figure 1 decreased after treatment using *Aspergillus* spp. This means that the amount of organic matter easily decomposed in the untreated effluent (negative control) has decreased. Based on Figure 2, COD levels after treatment using *Aspergillus* spp decreased. This data shows a decrease in the total amount of organic matter present in untreated wastewater effluent (negative control), both organic materials that are difficult to digest and easy to decompose.

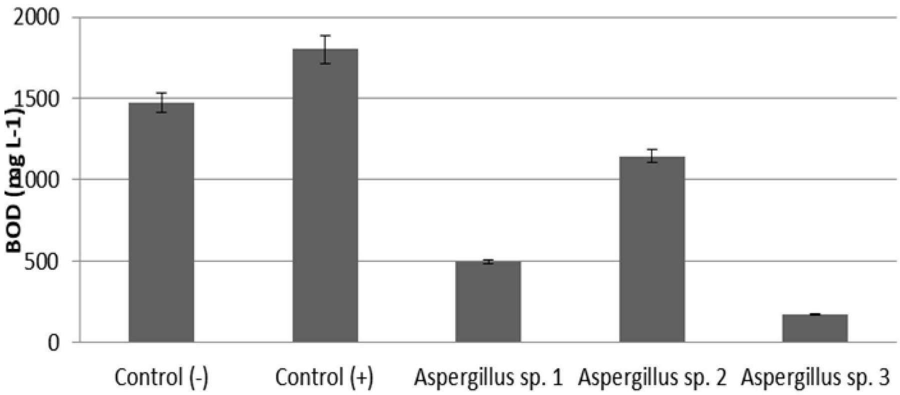

Figure 1. Effect on BOD of different fungal isolates

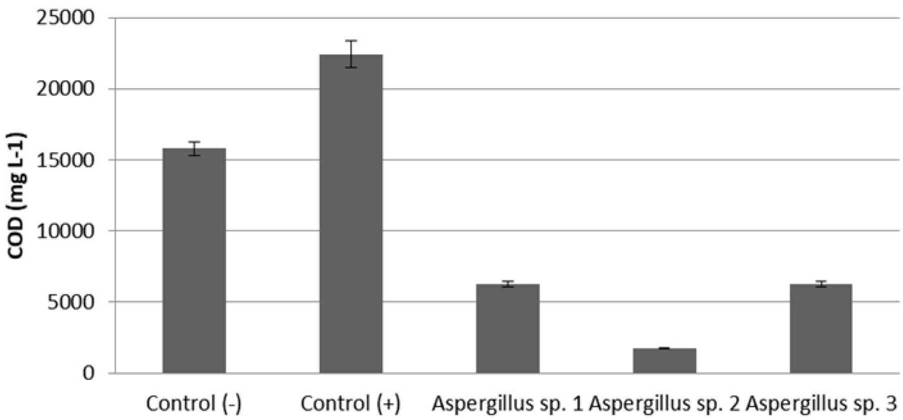

Figure 2. Effect on COD of different fungal isolates

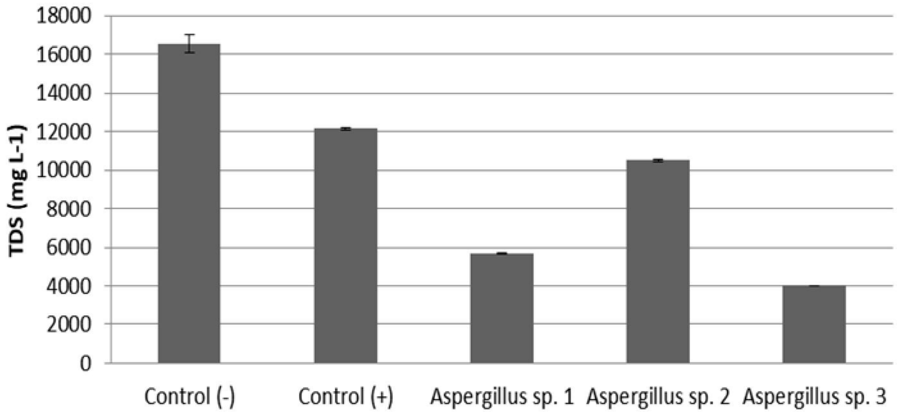

Figure 3. Effect on TDS of different fungal isolates.

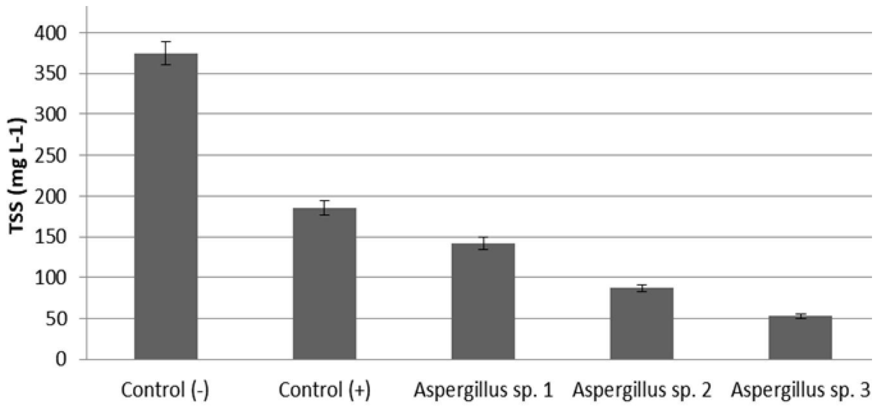

Figure 4. Effect on TSS of different fungal isolates.

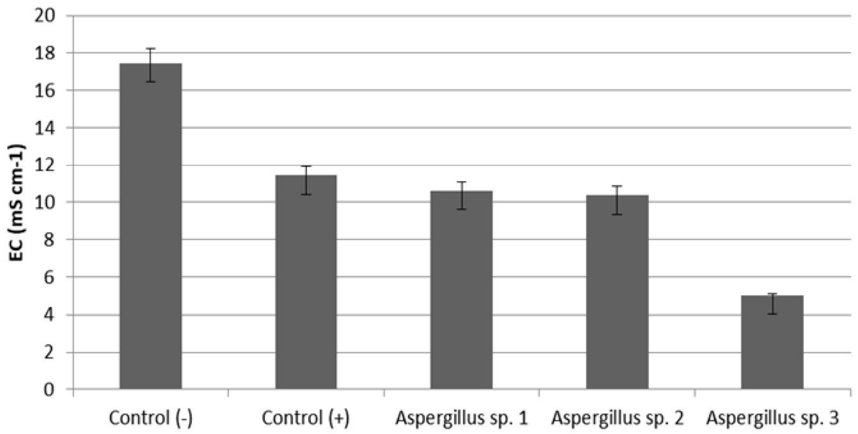

Figure 5. Effect on EC of different fungal isolates.

Total Dissolved Solids (TDS)

The TDS values from the research are shown in Figure 3. Values of 5673.33 mg L-1, 10493.33 mg L-1, and 4010 mg L-1, for the determination of TDS were obtained after the application of *Aspergillus* sp. 1, *Aspergillus* sp.2, and *Aspergillus* sp. 3 respectively. The TDS values of *P.chrysosporium* showed lower concentrations compared to the other isolates. Figure 4 also shows that*Aspergillus* sp. 3 had the highest TDS value. This indicates that *Aspergillus* sp. 3 was the best isolate for reducing TDS.

Total Suspended Solids (TSS)

Figure 4 shows good values of 141.8, 87.2 and 53mg $L^{-1}$for TSS after *Aspergillus* sp. 1, *Aspergillus* sp.2, and *Aspergillus* sp. 3 treatment, respectively. Figure 4 also shows that the TSS values of theindigenous fungal isolates are greater than the TSS values of *P.chrysosporium* treatment. This indicates that *Aspergillus* sp. 3 is superior to the other fungi.

Electrical Conductance

The value of EC of the negative control in the untreated IB batik effluent was 17.44 mS $cm^{-1,}$ while the recommended value according to government regulations is 1.56 mS cm^{-1}. This indicates that the effluent contains heavy metals at a high enough level to conduct an electrical current.

The level of EC clearly decreased in the IB batik effluent after *Aspergillus* sp. 1, *Aspergillus* sp.2 and *Aspergillus* sp. 3 treatments processes (10.61, 10.38 and 5.02mS cm^{-1}) compared to the *P.chrysosporium* treatment (11.44mS cm^{-1}) (Figure 5). It can be said that *P.chrysosporium* treatment contains heavy metals at a relatively higher level than when using *Aspergillus* spp. The percentage of EC decrease is shown in Figure 7. The decrease in the level of EC could be due to the decomposition of some heavy metal compounds in the effluent following the fungal treatment.

Temperature Measurements

The growth of fungi is affected by temperature, with an optimum temperature of 25-30^{o}C. The temperature of the IB batik effluent was 27.1^{o}C ± 0.1^{o}C (Table 2). According to the quality standards of the Governor regulation, the maximum permissible temperature is air temperature± 3 °C. This indicates that the temperature of the fungal isolate treatment was within the standard range.

pH Measurements

Initial pH in the negative control (7.5± 0.1.) decreased after treatment by *Aspergillus* sp. 1, *Aspergillus* sp.2, and *Aspergillus* sp. 3, 6.2 ± 0.2, 6 ± 0.02 and 6 ± 0.1, respectively, while the treatment with *P. chrysosporium* also showed a decrease in initial pH, 6.9± 0.1 (Table 3).

Table 2. Results of temperature measurements on IB batik effluent [Control (-)],*P.chrysosporium* [Control (+)] and fungal isolate treatment.

Replication	Control (-)	Control (+)	*Aspergillus* sp. 1	*Aspergillus* sp. 2	*Aspergillus* sp. 3
1	27.1	25.7	25.9	25.6	26
2	27.2	25.6	26	25.7	26
3	27.3	25.5	26.1	25.6	26.1

Table 3. Results of pH measurements on IB batik effluent [Control (-)],[Control (+)] and fungal isolate treatment.

Replication	Control (-)	Control (+)	*Aspergillus* sp. 1	*Aspergillus* sp. 2	*Aspergillus* sp. 3
1	7.5	6.9	6.2	6	6.1
2	7.4	6.8	5.9	5.9	6.1
3	7.3	6.7	5.8	6.1	6.15

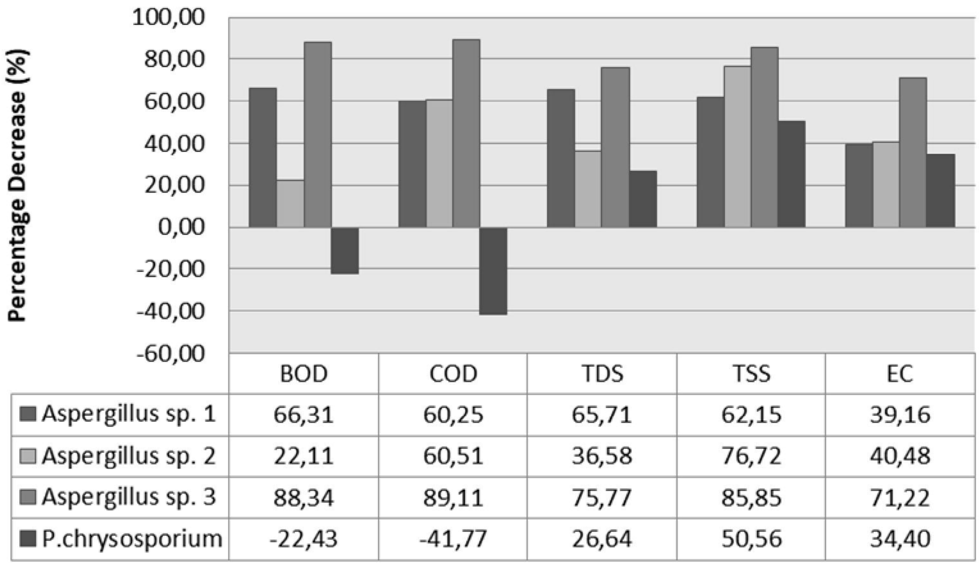

Figure 6. Percentage reduction of physiochemical properties by fungal isolates (*percentage reduction in physiochemical properties)*

The percentage BOD, COD, TDS, TSS and EC reduction by *Aspergillus* sp. 1, *Aspergillus* sp. 2, *Aspergillus* sp. 3 and *P.chrysosporium* is shown in Fig. 6.

In this study, *Aspergillus* sp. 3 also showed excellent pollutant reduction capabilities, and also showed the best resultsin thereduction of physiochemical properties.The effect of treatment on BOD, COD, TDS, TSS and EC reduction was studied as a function of treatment by *Aspergillus* sp. 3,as shown in Figure 6. All the parameters decreased in the treated, as compared to the untreated, IB batik effluent. The results also show that *Aspergillus* sp. 3 was very effective inthe removal of BOD, COD, TDS, TSS and EC from the IB batik effluent. It can be seen in figure 6 that *Aspergillus* sp. 3 is appropriate for reducing the physiochemical properties in IB batik effluent, presenting superior BOD, COD, TSS, TDS and EC percentage reduction (88.34%, 89.11%, 75.77%, 85.85% and 71.22 %, respectively), when compared to *P.chrysosporium* isolate as the positive control (corresponding figures of -22.43%, -41.77%, 26.64%, 50.56% and 34.4%) and the other types of *Apergillus* sp.

The reduction in BOD by *Aspergillus* sp. 3was 1470.68 mg L^{-1} (untreated effluent) to 171.48 mg L^{-1} (treated effluent) (Figure 1), with a mean removal efficiency of 88.34 % (Figure 6). Similar results were reported by Kshirsagar (2013), who assessed the reduction in BOD and COD of domestic wastewater samples from the sewage wastewater treatment plant Bopodi in Pune city, India using the fungal isolates *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus* *nigricans, Rhizopus nigricans* and *Cunninghamella*, which were isolated from the Mula river in Pune andefficiently utilized for remediation. *Aspergillus terreus* and *Aspergillus niger* showed excellent pollutant removal capabilities.

BOD and COD removal efficiency was a similar manner as dye removal efficiency. Reduction in COD and BOD is indicated by a degradation of the dye in the simulated effluent as a result ofthe activity of the fungal system, making these fungal strains a promising starter for the in situ bioremediation process of textile effluent treatment (Kshirsagar, 2013). The color removal of IB batik effluent is related to the decrease in the parameter value of the quality standard. This occurs because of biosorption and biodegradation mechanisms. Knapp and Newby (1995) state that biosorption is the primary dye removal mechanism. This research is line with the previous study of Aksu and Tezer (2000), who reported that the biosorption mechanism using free cell fungal was linked to the electrostatic pull between the positively charged cell wall components and the negatively charged dyes. Another description of the biosorption mechanism for binding dyes is given by Kurniasih (2017),who states that theactivity mechanism occurs from the interaction between a cationic chain material and negativity of macromolecules on the surface of the fungal cell. In this case, macromolecules on the surface of the fungal cell caused interaction between the dye cationic chain and negativity capacity on the fungal cell surface. Gnanadoss et al. (2013) are ofthe same opinion, stating thatthe cell wall plays a vital role in dye adsorption. Increased cell biosorption can be a result ofthe higher surface area available for the dye to bind.

The subsequent mechanism after biosorption is biodegradation. These observations are in line with the findings of Balan and Monteiro (2001), who reported on the removal of indigo dye by absorption and extracellular degradation of fungal. Reductions in BOD and COD levels, along with color removal, prove that the mechanisms of biodecolorization and biodegradation occurred simultaneously (Andleeb et al., 2012). Fujii et al. (1988) reported on the role of enzymes from *A. terreus*in catalyzing the vat dye ring cleavage reaction.

The percentage reduction in the case of *Aspergillus* spp. indigenous fungus was up to 22%,compared to -22.425 % and -41.7722 % by *P.chrysosporium.* It has been proven that the indigenous fungi were better than the positive control,with a great ability to degrade amounts of the xenobiotic compound.

On the other hand, it has been observed that *P.chrysosporium* achieved the lowest percentage reduction inBOD and COD. This may mean that it secretes certain organic materials in its metabolism during the incubation time, which increases the amount of organic material and thus increases the BOD and COD values.

The TDS, TSS concentration and EC value of the *P.chrysosporium* were lower than the negative control, but the values of BOD and COD were higher. The increased BOD and COD valueswere due to the performance of the *P.chrysosporium*, which was not optimum to produce enzyme. This indicates that the isolate can only partially break down bonds of organic compounds in IB batik effluent. Otherwise, it has been shown that the fungal isolates are more effective than *P.chrysosporium.*

4. Conclusion

It is concluded that all the fungal isolates from the dye effluent soil and batik effluent show promise for effluent treatment. Using such indigenous fungal isolates, water quality parameters can be effectively reduced from the IB batik effluent. The values of BOD after treatment using *Aspergillus* sp. 1, *Aspergillus* sp. 2 and *Aspergillus* sp. 3 were 22.1, 66.31and 88.34 respectively. Other parameters, such as COD, TSS, TDS and EC, showed high levels of reduction, which decreased when using indigenous fungal isolates. *Aspergillus* sp. 3 is the best isolate, showing the highest percentage reduction inBOD, COD, TSS, TDS, and EC. The comparison isolate, *P. chrysosporium*, had the lowest value for all the parameters. The indigenous fungal isolate *Apergillus* spp. was very effective in the efficient treatment of the IB batik effluent and has potential for future analysis in the treatment of all types of batik effluent.

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