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Corrections

Ratna Stia Dewi <ratna.dewi0509@unsoed.ac.id> Sat, Jan 23, 2021 at 11:08 AM
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Mechanisms of Indigosol Blue O4B batik dye wastewater degradation by *Aspergillus* sp. 3 and its product analysis

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Abstract
The role of fungi in the treatment of dye wastewater has been widely investigated, but not many have reported in full that about the mechanisms and degradation products. The purpose of this study was to determine the mechanism that occurs when microscopic isolate of Aspergillus sp. 3 was isolated from the disposal of batik industrial waste in degrading the Indigosol Blue O4B (IB) batik dye wastewater and deciding the degradation products that were formed. The ligninolytic test using tanic acid was carried out for qualitative tests. To prove the mechanism of degradation of IB batik wastewater, the biosorption mechanism assay was carried out (with changes in the color of mycelium formed and measurement of adsorption %) and the biodegradation mechanism assays of both crude *and* purified enzymes *(Lakase, MnP, LiP enzyme).*

The products of degradation were identified using UV-Vis spectrophotometer and FTIR absorption measurements. The isolate produced clear zones on agar media with addition of tannic acid. The isolate mechanism was superior in degrading batik IB wastewater by biosorption (adsorption percentage of 33.39%; 50.49%; 68.55% at 24, 48, 72 hours) and enzymatic degradation (laccase enzyme specific activity, MnP, LiP in the amount of 75.087 U/mg; 49.665 U/mg and 129.347 U/mg). The product of degradation was identified as a simple compound which was an aliphatic compound containing C=C, C-O and -OH bonds, so that it was predicted as a group of aliphatic alcohol compounds. This isolate can be used for further development of applications for environmentally friendly dye wastewater processing technology.

Keywords: *Aspergillus* sp.3, biosorption, degradation products, ligninolytic.

Introduction
Indigosol coloring is chosen than others because it has many superior characteristics. These dyes are fast, flat and bright. The dyes can be applied in dyeing and 'coletan' on batik industry[23]. Considering its fastness properties, it is good to be applied in dyeing. These dyes are characterized by fine dispersion through increasing rapid coloring and are able to be used for high speed in continuous dyeing techniques, brighter colors and better techniques than other dyes during bleaching. Indigosol dyes can be applied in dyeing at various types of fabrics such as cellulose fabric, t-shirts, silk, wool, cotton yarn and also applied to knitted goods[4].

Indigo dyes are acute toxic, irritating to the skin and eyes, toxic after repeated application, sensitive, mutagenic and carcinogens[17]. Indigosol wastewater is toxic since it contains excess reducing agent sodium hydrosulfite which is oxidized to alkali and alkaline earth metal sulfate (SO_4^{2-}), sulfite (SO_3^{2-}) and thiosulfate ($S_2O_3^{2-}$) affecting environmental damage. Wastewater containing sulfate⁄ high sulfite is very corrosive. Thus, the formed sulfate deposits can create toxic hydrogen sulfide (HS) ions[5]. Therefore, easy and inexpensive processing techniques are needed to save the environment.

Fungi isolated from dye industry waste are reported to be able to degrade various coloring wastewater[2,3]. A number of studies have reported that fungi can reduce the concentration of indigo dyes and can degrade indigo dye wastewater.[6,24]

The mechanism of degradation by fungi can be grouped into two processes namely biosorption and/or biodegradation (with ligninolytic enzyme activity). The connection of those two processes in the degradation of indigosol batik wastewater is unknown. Therefore, the decomposition mechanism that occurs in the role of the fungi needs to be investigated. The amount of ligninolytic enzyme activity that plays a role in the mechanism of degradation needs attention. A special discussion on testing specific activities of ligninolytic enzymes is needed to prove that the enzyme is a system with an important role in the process of degradation of batik waste.

Products resulted from degradation of batik waste are important to know. The purpose of this study was to determine the mechanism occurred when microscopic isolate of *Aspergillus* sp. 3 was isolated from the disposal of batik industrial waste in degrading the IB batik dye wastewater and to decide the degradation products been formed.

Material and Methods
Materials: *Aspergillus* sp. 3 was obtained from previous research results. It was isolated from batik wastewater taken

from the disposal location of the batik industry and then selected from others as superior isolate[12,13]. Batik wastewater used in this research was indigosol blue O4B (IB). It was obtained from the batik industry in Banyumas District, Central Java, Indonesia.

Methods

1. Preparation of isolate: Pure culture of *Aspergillus* sp. 3 in the slant media agar was rejuvenated on Potato Dextrose Agar media in Petri dishes, incubated for five days at room temperature and then used at the next stage.

2. Determination of the mechanism of degradation of IB batik wastewater by fungi *Aspergillus* sp. 3

a. Mechanism of biosorption: The growth medium used in the adsorption mechanism is the Potato Dextrose Broth cultivation medium supplemented with 0.8% diammonium hydrogen phosphate. 75 mL of medium in 250-mL Erlenmeyer flasks were inoculated with 3.5 x 10^7/ mL fungi spores. The inoculated medium was then incubated in a reciprocal shaker at 90 rpm for 96 hours. The fungal mycelium was harvested at the end of incubation by centrifugation using a refrigerated centrifuge and washed with distilled water. Dead biomass was made by autoclaving at 121° C for 15 minutes before harvesting the culture[11].

The adsorption mechanism step was carried out using 100 mL IB batik dye wastewater in 100-mL Erlenmeyer flask added with 0.25 g biomass fungi dry weight and then incubated for 24 hours above shaker at 90 rpm. After the end of incubation, the biomass was separated by centrifugation and the concentration of dyestuff in the supernatant was measured using spectrophotometry[11].

$$\%\ adsorption = \frac{Co - Ce}{Co} x\ 100\%$$

where Co = Initial concentration of solution and Ce = The solution concentration at equilibrium (mg / L).

The adsorption mechanism was known by the color change of the mycelium formed which was originally white to blue like the indigosol blue O4B dye. Color changes were seen as evidenced by microscopic shooting using a SEM microscope. The indication of adsorption is also known by the reduction in solution concentration after the decolorization process.

b. Mechanism of Biodegradation:

1) Test enzyme activity: The fungi of the selected isolates were grown in the PDB medium in the reciprocal shaker with a speed of 90 beats per minute for 96 hours. Mycelium grows to form pellets. The formed pellets are separated from the production medium using centrifuges. Pellets are used as an inoculum to decolorize IB batik wastewater for enzyme production. The supernatant obtained was a crude enzyme and was used to measure enzyme activity after decolorization. Crude enzyme obtained is then used in measuring enzyme activity. The enzyme activity measured was the enzyme lakasse, manganese peroxidase enzyme and lignin peroxidase enzyme activity.

Before the measurement of enzyme activity, crude enzyme production was carried out. The isolate of *Aspergillus* sp. 3 fungal inoculum was used to decolorize wastewater using PDB in room temperature shakers. After 4 days of cultivation, a small pellet is formed, this pellet is homogenized at 1500 rpm for 30 seconds. 8 ml of homogeneous pellet samples were used to inoculate the Erlenmeyer flask. IB batik dye wastewater was then added to the Erlenmeyer Flask. The culture of strain fungi was shaken out in a shaker using an Erlenmeyer flask with agitation of 90 rpm.

The sample was used to test the filtration enzyme activity. The sample was then tested for enzyme activity. The activity was measured by the enzyme is LiP, MnP, Lac.

Laccase activity was obtained by mixing 900 µl of filtrate culture with 750 µl of sodium acetate buffer 0.1 M and then added with 100 µl of syringaldazine in 0.5 mM at room temperature. Then the absorbance measurements were carried out at 525 nm. The absorbance reading for 1 minute was repeated several times until the data is good.

MnP activity was obtained by mixing 1000 µl of the filtrate culture with 1750 µl malanoic acid mM pH 4.5 and then adding 125 µl 2.6 DMP 20 mM and 125 µl manganese sulfate ($MnSO_4$) 20 mM and then adding also with 300 µl of 2 mM H_2O_2. Subsequent absorbance measurements were carried out at 470 nm. The absorbance reading for 1 minute was repeated several times until the data is good.

The LiP activity tested was obtained by mixing with 1000 µl of filtrate culture and 300 µl of 2mM H_2O_2, then adding 2000 µl of LiP buffer (Tween 80 0.5 g, veratyl alcohol 33.6 mg and 0,1 M buffer sodium tartrate solution 500 mL pH 2.9). Then the absorbance measurements were carried out at 310 nm. The absorbance reading for 1 minute was repeated several times until the data was good.

2) Enzyme Purification: Purification of the enzyme is called enzyme purification. This stage is through deposition of ammonium sulfate[22], dialysis[22] and gel filtration column chromatography.

Crude enzyme was produced then to be deposited using ammonium sulfate salt to obtain saturation with concentrations of 0-20%, 20-40%, 40-60% and 60-80%. Salt was added slowly in cold conditions by continuing to stir. The mixture obtained was then centrifuged for 20 minutes at a speed of 3580 g. Protein deposits were dissolved with 0.5 M pH acetate buffer 5. The deposition fraction of ammonium sulfate at each concentration was then tested for laccase enzyme activity, MnP and LiP and protein content to determine the best ammonium sulfate fraction.

Dialysis was carried out by cutting the dialysis membrane with 10 kD and washing it in running water[22]. The dialysis membrane was boiled in a mixture of 2% (b/v) Na-Carbonate and 0.05% (b/v) EDTA for 10 minutes. Dialysis membranes were boiled again in distilled water twice for 10 minutes each. The dialysis membrane was cooled and fastened to one end of the membrane. The fractionated ammonium sulfate extract with the highest specific activity was pipetted into the membrane. The membrane that has been fastened was immersed in acetate buffer 0.5 M, pH 5 with a ratio of 100 times the sample volume. The dialysis process was carried out at 4° C with stirring for 9 hours with three times the replacement of the soaking buffer. Dialysis enzymes were tested for laccase, MnP and LiP activity and protein content.

The dialysis results obtained were then purified using gel filtration column chromatography[18]. The Sephadex G100 gel matrix in the column is slowly flown with acetate buffer 0.5 M pH 5 for rinsing. The dialysis enzyme extract was passed into a column containing Sephadex G100 and eluted with an acetate buffer 0.5 M pH 5 with a flow rate of 1.66 x 10-5 L. s^{-1}. Each mL of the fraction formed was analyzed for the activity of lakase enzymes, MnP and LiP and their protein content.

3) Determination of specific activities of purified enzymes: Specific activity test aims to determine the size of laccase, MnP and LiP produced by isolate of *Aspergillus* sp. 3 fungi. The results are expressed by U/mg which proves that the *Aspergillus* sp. 3 produces an enzyme of a number of values obtained. These results also assume the extent of the role of the three enzymes in decolorizing IB batik wastewater.

Protein values were calculated using the Bradford method[7]. The test solution (enzyme fraction) of 5 µL was mixed with 100 µL of Bradford solution in cuvette. The mixture is homogenized for 10 seconds. The mixture was left to stand for 10 minutes and then measure its absorbance at a wavelength of 595 nm. The standard used is bovine serum albumin (BSA) with a concentration of 0-100 ppm.

3. Product Analysis results from degradation of IB batik wastewater by *Aspergillus* sp. 3 fungi: *Aspergillus* sp. 3 fungi are grown at optimum conditions. After incubation, filtration is then carried out. The solution obtained was accommodated in a sterile Erlenmmeyer flask and then used for identification using a UV-Vis spectrophotometer[8] and FTIR[19].

Identification of the products resulting from degradation using a UV-Vis spectrophotometer was carried out to see changes in the spectrum. Measure absorbance with a UV-Vis spectrophotometer at one minute intervals for 30 minutes. Absorption of the spectrum between 275 and 800 nm is recorded during decolorization.

FTIR identification is used to study changes in dye structure before and after degradation. The 50 ml solution of the degradation solution sample was characterized using a Fourier Transform Infrared Spectrometry (FTIR) spectrometer. The wavelength range used was 400-4600 / cm.

Results and Discussion

1. Determination of the degradation mechanism of IB batik wastewater by fungi *Aspergillus* sp. 3:

a. Mechanism of biosorption: The biomass of fungi was used to study their capacity to adsorb dye from its solution[11]. The presence of dead mycelium was used to prove the existence of decolorization mechanism through adsorption because with the death of the cell, there was no enzyme involvement in decolorization so that only adsorption event occurred.

The pellet surface observations of *Aspergillus* sp. 3 were incubated within 24, 48 and 72 hours and presented in figure 1. Pellets appear to be colored blue when compared to control isolates without the addition of IB batik wastewater. Since the incubation of the first day, the blue color has begun to stick both of pellet surface and cell wall. This proves that the adsorption mechanism occurs during incubation.

Evidence of adsorption was also shown in observations using SEM. SEM microscopy has been used as a tool for biosorbent characterization[21] because it can show the accumulation of dyes on the surface[11]. The optical microscopic image of *Aspergillus* sp. 3 was shown in figure 2. The SEM image supports the proof of the adsorption of IB batik wastewater. Before the treatment, it showed the morphology of mycelium surface which looked smooth; no particles were stuck and irregular with a large area for dye surface interactions. Significant changes occurred in the morphology of mycelium surface and became compact after adsorption of IB batik wastewater. SEM images showed colored molecules. IB batik wastewater was adsorbed in a completed form. The surface of the mycelium looked rough and there were particles stuck.

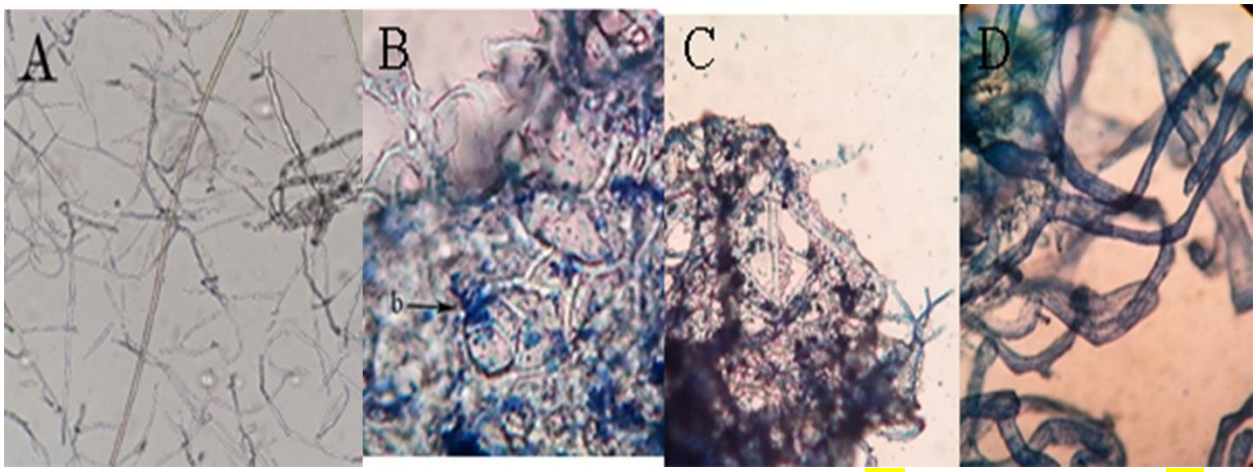


Figure 1: Pellet surface of *Aspergillus* sp. 3 on mycelium without treatment of (A) IB batik wastewater, (B) 24 hours, (C) 48 hours and (D) 72 hours incubation time.

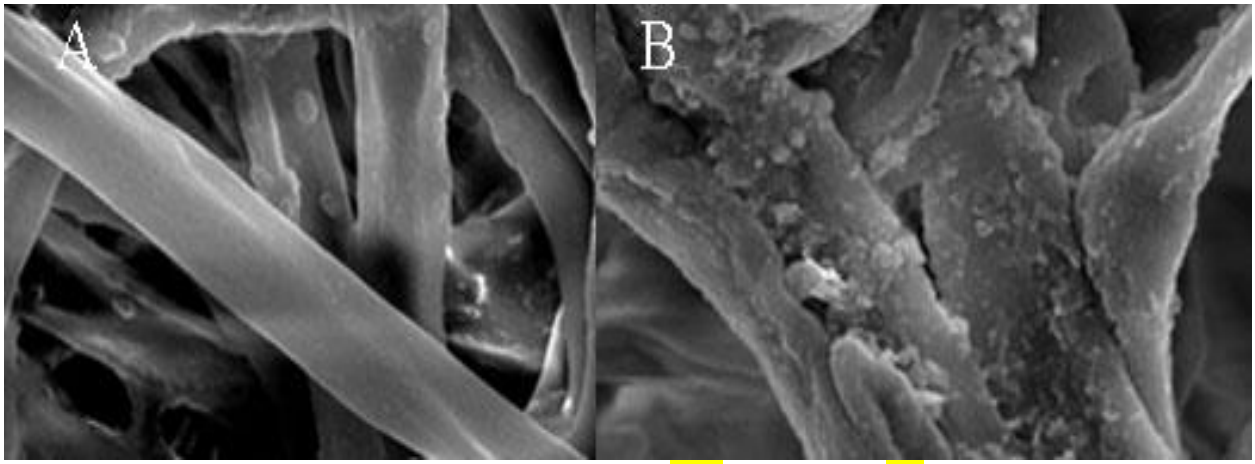


Figure 2: SEM of *Aspergillus* sp. 3. Isolate of *Aspergillus* sp. (A) 3 before and (B) after decolorization treatment at magnification 7500x.

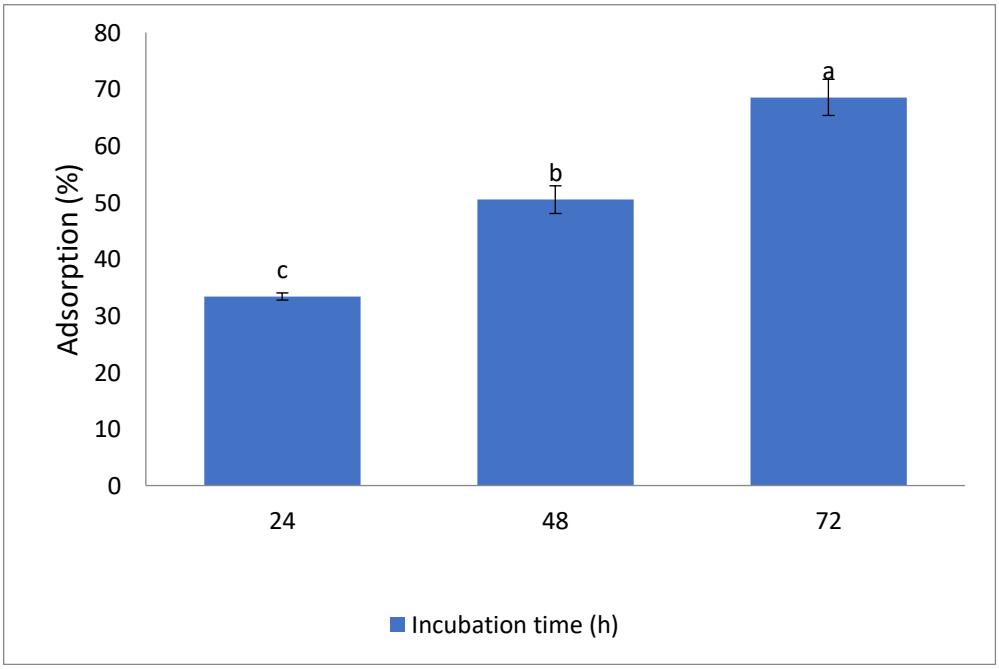


Figure 3: Percentage of *Aspergillus* sp. 3 adsorption at dead mycelium.

Figure 4: Dead mycelium after decolorization. The dye was adsorbed on (a) the cell surface, the dye accumulates in (b) the cytoplasm.

Proving the existence of an adsorption mechanism that occurs, out further treatment was carried out by calculating the percentage of dead mycelium adsorption so as not to be affected by cell metabolic activity. The results of the percentage of adsorption obtained were presented in figure 4. The percentage of adsorption increased with incubation time. The average value obtained was 33.39% on the first day, then 50.49% on the second day and 68.55% on the 3rd day.

The observation of dead mycelium surface was also done to ensure the mechanism occurred. Microscopic appearance of dead mycelium after decolorization could be seen in figure 4. The image provides information telling about the dead mycelium with presence of dyes that stick to the surface of the mycelium and also the presence of dyes entering the cytoplasm. This indicated that the mechanism occurred during degradation was a biosorption mechanism either adsorption or intracellular accumulation. Biosorption can be classified as adsorption/cell surface precipitation and intracellular accumulation depending on location of adsorbate[15]. The adsorption surface is generally assisted through ionic, chemical and physical interactions.

Figure 4 showed the mycelium after adsorption process. This showed that the dye molecule was adsorbed on the cell surface. The cell wall of fungi contains large sources of chitin, chitosan, â-1,3-D-glucan, â-1,6-D-glucan and mannoprotein on different functional groups such as carboxyl, amine, hydroxyl, phosphate and sulfonate[11]. The presence of amino groups, carboxyl, hydroxyl, phosphate and sulfonates in biomass can interact with dyes. Therefore, the initial attachment to the dye molecule with the cell surface follows a complex pattern.

The dye adsorption of batik IB wastewater on biomass occurs through (1) chemical interactions between colored molecules and fungi cell wall components, (2) electrostatic interactions between dye molecules and electron-rich sites on the cell surface and (3) weak physical forces such as hydrogen bonds and van der Cell walls interact between hydrophobic parts of colored molecules (for example, aromatic rings) and biomass polysaccharides.

During the incubation in this study, it can be observed that the color of IB batik wastewater changes from blue to a clear color. It relates to previous research that the color of the solution changes from blue to light blue to a clear color, as in controls without dyes because of the activity of laccase enzymes that is able to oxidize indigo dyes[24].

The mechanism of biodegradation is a breakdown of dyes which involve activity of various enzymes[1]. Measurements of enzyme activity in this study were carried out to prove the mechanism of biodegradation and to know the enzymes which play a role in the decolorization of IB batik wastewater. It is known that fungal enzymes such as laccase (Lac), manganese peroxidase (MnP), lignin peroxidase (LiP)

play a role to degrade dyes[20]. The activity of LiP, MnP and Lac enzymes assayed on crude enzyme can be seen in tables 1, 2 and 3.

Based on table 1, it can be seen that laccase activity in crude enzyme was 11,666.67 (U) where the specific activity was 58.39 (U/mg). Table 2 informed that the magnitude of MnP enzyme activity was 5,256.048 (U) with a specific activity of 26,307 (U/mg) and table 3 showed for LiP has an enzyme activity of 156,838.7(U) with specific activity 784,98 (U / mg).

Test results of specific activities of laccase, MnP and LiP enzymes were presented in table 1, 2 and 3. The data in the table proved that there was an increase in activity after enzyme purification from dialysis level to chromatographic column. The laccase enzyme specific activity increased from 58.39 to 148.39 and then to 1091.02 U/mg. Similarly, the peroxidase enzyme increased from 26.31 to 26.44 and 198.66 U/mg for MnP and 784.98 to 3682.4 and 5638.21 U/mg for LiP.

2. Product Analysis results from degradation of IB batik wastewater by *Aspergillus* sp. 3 fungi:

UV-VIS: The analysis of products from degradation using a UV-Vis spectrophotometer was carried out to see changes in the maximum absorption spectrum. Changes in peak spectrum occured during decolorization. The UV-Vis spectrophotometer showed a sharp new peak at different wavelengths than the wavelength before the degradation process.

Figure 5 presented graphical form at the visible wavelength. The results of UV-Vis analysis of IB batik wastewater samples before decolorization showed a peak detected at a wavelength of 604.5 nm (Figure 5a). The curve in figure 5a showed the maximum wavelength seen in IB batik wastewater but was not seen in the decolorization results. The peaks seen in IB batik wastewater were not seen in decolorization results with isolate of *Aspergillus* sp. 3. Figure 5b showed that the decolorization solution showing a peak that cannot be detected at the visual wavelength (400-750 nm). The peak was in the UV wave at a wavelength of 345 nm. This showed that there was a degradation characterized by the loss of peaks and the existence of other compound with the appearance of new peak.

Figure 5 indicated the degraded IB batik wastewater sample showing undetectable peaks at wavelengths of 400 and 800 nm. This proved that after the decolorization assay, there was a reading shift in the wavelength. The occurrence of a wavelength shift also showed degradation. This shift caused the structure of the IB batik wastewater changed and conjugated bond decreased, resulting in a shift in the wavelength toward a shorter wavelength[25].

Table 1
Stages of laccase enzyme purification from superior fungi isolates

Stage	Volume (ml)	enzyme Activity (U)	Protein (mg/ml)	total protein (mg)	Spesific Activity (U/ mg)	(%)	Purification factor
Crude enzyme	1000	11,666.67	0.19	199.8	58.39	100	1
Dialisis	25	1,316.24	0.36	8.87	148.39	11.28	2.54
Kromatografi kolom	7.5	255.77	0.01	0.23	1,091.02	2.19	18.68

Table 2
Stages of MnP enzyme purification from superior fungi isolates

Stage	Volume (ml)	enzyme Activity (U)	Protein (mg/ml)	Total protein (mg)	Spesific Activity (U/ mg)	(%)	Purification factor
Crude enzyme	1000	5,256.05	0.199	199.8	26.31	100	1
Dialisis	25	234.57	0.355	8.87	26.44	4.46	1.01
Kromatografi kolom	7,5	13.97	0.009	0.07	198.66	0.27	7.55

Table 3
Stages of LiP enzyme purification from superior fungi isolates

Stage	Volume (ml)	enzyme Activity (U)	Protein (mg/ml)	Total protein (mg)	Spesific Activity (U/ mg)	(%)	Purification factor
Crude enzyme	1000	156,838.70	0.19	199.8	784.98	100	1
Dialisis	25	32,662.90	0.36	8.87	3,682.40	20.83	4.69
Kromatografi kolom	7.5	396.53	0.01	0.07	5,638.21	0.25	7.18

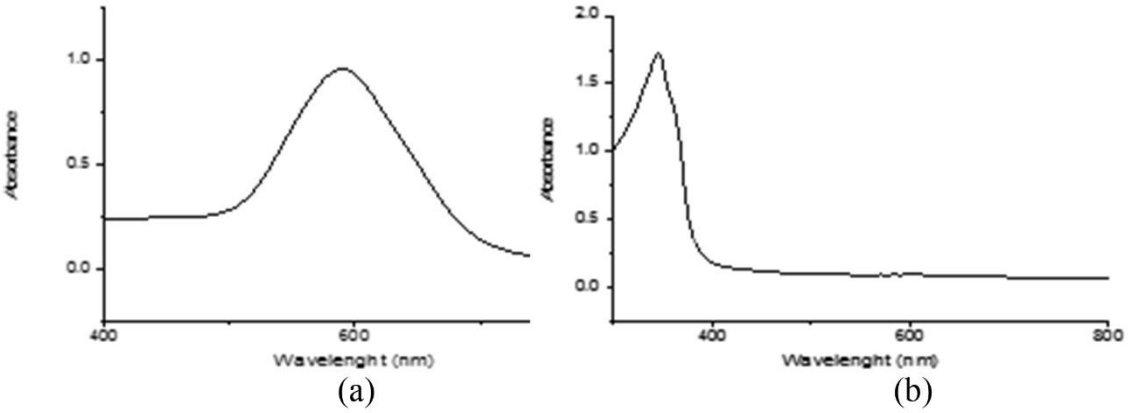


Figure 5: Spectra UV-Vis of IB batik wastewater (a) before and (b) after treatment using *Aspergillus* sp. 3.

Table 4
Spectra of FTIR IB batik wastewater before and after degradation using isolate of Aspergillus sp.3

Sample	Number of Waves (cm^{-1})	Functional groups
IB batik wastewater	501.49, 555.50, 594.08, 617.22	C-Br Association
	1519.91, 1543.05,1558.48	Aromatic and aliphatic groups (stretch C = C) and C = N–
	1697.36	Cluster N-H
	3734.19	Hydroxyl group (O–H).
Degradation products	1635.67	Aliphatic C=C
	2108.38	C–O stretch group
	3332.05	–OH stretch

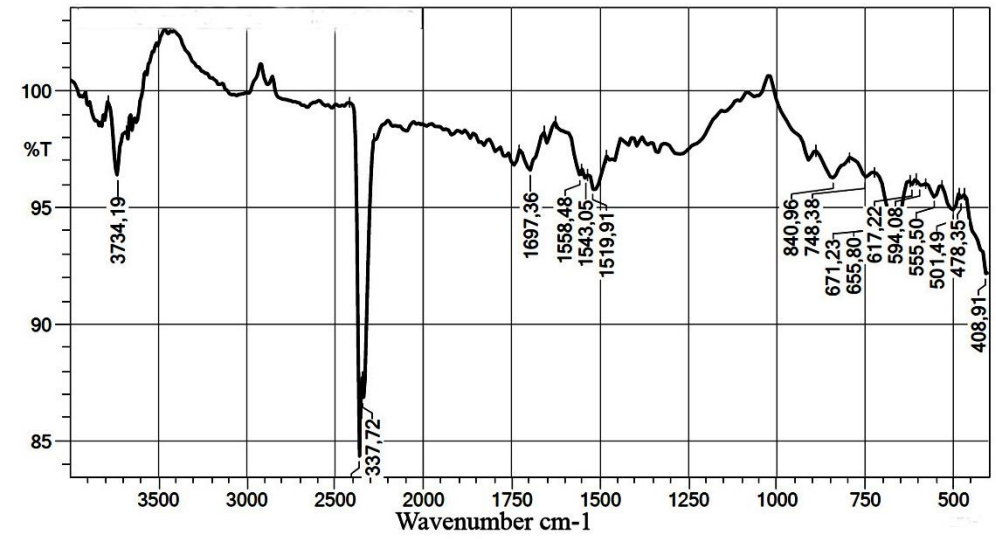
FTIR: FTIR was used to characterize and identify functional groups of IB batik wastewater before and after treatment using isolate of *Aspergillus* sp. 3. The FTIR spectrum in both samples was measured on absorption bands at 400-4000 cm^{-1}. Figure 6 and table 4 showed FTIR spectra of IB batik wastewater before and after degradation. FTIR spectra data in figure 6a for IB batik wastewater showed the presence of several absorption bands. Absorption bands that appear in IB batik wastewater samples were 501.49; 555.50; 594.08; 617.22, 1519.91; 1543.05: 1558.48, 1697.36, 3734.19 cm^{-1}.

Absorption band at wave number 501.49; 555.50; 594.08; 617.22 cm^{-1} indicates the presence of C-Br bonds. As also reported by researchers, alkyl halide is recognized by the appearance of absorption bands in the region of 200-750 cm^{-1}, especially in the area of 500-680 cm^{-1} for C-Br[23-26].

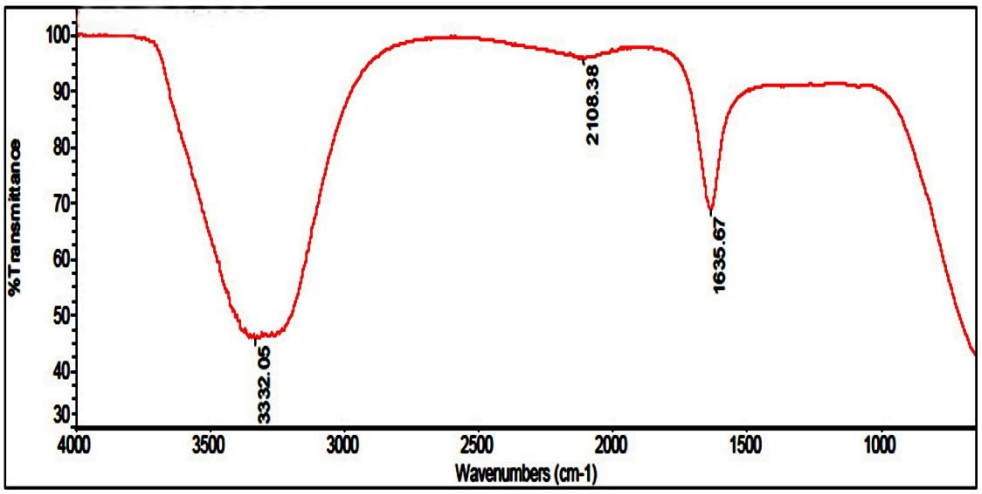
The analysis results in figure 6a also show the absorption bands of aromatic and aliphatic groups (strain C=C) and C=N– appearing at wave number 1519.91, 1543.05, 1558.48 cm^{-1}. According to Clifford et al[10], at wave number 1675-1500 cm^{-1} there is a C=C (aliphatic and aromatic) strain and a C=N– strain. The peak caused by stretching vibrations of C=C) and C=N– was located in the wave number 1690-1600 cm^{-1}. The aromatic ring shows peaks in the region of 1650-1450 cm^{-1} and with a low degree of substitution shows the peaks at 1600, 1580, 1500 and 1450 cm^{-1} [9,16,26,27,29].
The wave number of 1697.36 cm^{-1} showed the absorption of the N–H group which was a special structure of Indigosol. The absorption band at 3734.19 cm^{-1} showed the presence of the hydroxyl group (O-H). Amide bonds to a number of waves 1670-1700 cm^{-1} and the wavenumber 3750 - 3000 cm^{-1} show a stretch of –OH.[9,14,16, 26-29]

FTIR spectra data from degradation products which are IB batik waste after treatment using *Aspergillus* sp.3 were presented in figure 6b. The absorption bands that emerged from the degradation product were different than before treatment. These results indicated the loss of the functional groups of the dye structure. The degradation products showed that there were only three absorption bands remaining namely 1635.67, 2108.38 and 3332.05 cm^{-1}.

Aliphatic group or strain C=C is located in the frequency of the wave number 1675-1500 cm^{-1} [10]. Wave number 2108.38 cm^{-1} that appears with an intensity of 95.931% was a C–O stretching group. The C–O stretching group at the wave number 2108 cm^{-1} [30]. –OH stretching appeared to be read in the region of wave number 3332.05 cm^{-1} with an intensity of 45.806%. –OH stretching at absorption from 3750-3000 cm^{-1}.[10]



(a)



(b)

Figure 6: FTIR spectrum (a) before degradation and (b) degradation products IB batik wastewater using isolate of *Aspergillus* sp. 3.
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Based on the results of the FTIR analysis, it can be assumed that in the indigosol blue O4B colorant contained in the IB wastewater, there were aliphatic, aromatic and C–Br alkyl halide groups and NH groups while after the treatment there are only three functional groups namely C=C, C–O groups and –OH while the typical Indigosol groups namely aromatic, NH and Br did not appear. This indicated that there was a degradation process by *Aspergillus* sp. 3. This isolate breaks the aromatic bond, C–Br alkyl halogen and –NH into simpler compounds. The compound has a bond from the remaining three groups which are predicted as alkenes with hydroxyl groups to become alkenol (CH4=C–OH).

Conclusion

The mechanism of isolate of *Aspergillus* sp. 3 in degrading IB batik wastewater was by using biosorption (adsorption) and biodegradation (enzymatic). Decolorization value with the mechanism of degradation through adsorption at 24, 48, 72 hours was 33, 39%; 50.49%; and 68.55%. Extracellular enzymes that were predominantly involved in degradation were LiP, MnP and laccase.

Purified enzymes on column chromatography from isolate had specific activities of laccase, MnP, LiP up to 1,091.02, 198.66 and 5,638.21 U / mg. The product of degradation was identified as a simple compound named aliphatic compound containing C=C, C–O and –OH bonds predicted as aliphatic alcohol compound group.

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Mechanisms of ~~Indigosol Blue~~indigosol blue O4B batik dye wastewater degradation by *Aspergillus* sp. 3 and its product analysis

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ABSTRACT

The role of fungi in the treatment of dye wastewater has been widely investigated, but ~~not many~~few have reported ~~in full that~~ how the mechanisms and degradation products are formed. ~~-~~The purpose of this study was to determine the mechanism that occurs when microscopic ~~isolate of~~ *Aspergillus* sp. 3 ~~were~~are isolated from the disposal water of the batik ~~industrial waste~~industry in ~~degrading~~ the ~~Indigosol Blue~~degradation of indigosol blue O4B (IB) batik dye wastewater, and ~~decide~~to determine the degradation products that were formed. The ligninolytic test using ~~tanic~~tannic acid was ~~carried out for~~performed as a qualitative ~~tests. To proof the~~ assessment. The mechanism of the degradation of IB batik wastewater was ~~carried out by the~~ identified using a biosorption mechanism assay (~~with changes in~~ the color ~~of~~and adsorption % changes when mycelium is formed ~~and measurement of adsorption %)~~) and the biodegradation mechanism assays of both *crude* and *purified enzymes* (Laccase~~Lakase~~, MnP, LiP enzyme). The degradation products ~~of degradation~~ were identified using an UV-Vis spectrophotometer~~,~~ and FTIR absorption measurements. The isolate produced clear zones on agar media with the addition of tannic acid. The isolate mechanism was superior in degrading batik IB wastewater by biosorption (adsorption percentage of 33.39~~%;~~%, 50.49~~%;~~%, and 68.55% at 24, 48, and 72 hours) and enzymatic degradation (laccase enzyme specific activity, MnP, LiP ~~in the amount of~~at 75.087 U/mg~~;~~, 49.665 U/mg, and 129.347 U/mg). The ~~product of~~ degradation product was identified as a simple ~~compound which was an~~ aliphatic compound containing C=C, C-O, and -OH bonds, ~~so that it was predicted as~~and is predictive of a group of aliphatic alcohol compounds. This isolate can be used ~~for~~in further ~~development of~~ applications for an environmentally friendly dye wastewater processing technology.
Keywords: *Aspergillus* sp.3; biosorption; degradation products; ligninolytic

INTRODUCTION

Indigosol coloring ~~is chosen than others because it has many superior characteristics. These dyes are fast, flat, and bright. The dyes can be applied~~, used in dyeing and ~~“~~“coletan”~~”~~ ~~on~~in the batik industry[1]~~. Considering its~~, has many advantages including being fast, flat, and bright. Its fastness ~~properties, it is~~has good applicability to ~~be applied in~~ dyeing. These dyes are characterized by fine dispersion ~~through increasing~~with rapid coloring~~, able to~~ and can be used ~~for~~at high ~~speed~~speeds in continuous dyeing ~~techniques.~~, for brighter colors and better techniques ~~than~~compared to other dyes ~~during~~used with bleaching. Indigosol dyes can be ~~applied in dyeing at~~ used on various types of fabrics such as cellulose ~~fabric~~, t-shirts, silk, wool, cotton yarn, and ~~also applied to~~ knitted goods[2]. ~~Indigo~~However, indigo dyes ~~are~~have acute ~~toxic,~~ toxicity, are irritating to the skin and eyes, are toxic after repeated application, ~~sensitive~~are a sensitizer, mutagenic, and ~~carcinogens~~[3]~~.~~carcinogenic[3]. Indigosol wastewater is also toxic ~~since~~as it contains excess reducing agent, sodium hydrosulfite, which is further oxidized to alkali and alkaline earth metal sulfate (SO_4^{2-}), sulfite (SO_3^{2-}) and thiosulfate ($S_2O_3^{2-}$) ~~affect~~that cause environmental damage.

~~Wastewater~~Such wastewater containing sulfate~~,~~/high sulfite is also very corrosive~~. Thus,~~ and the formed sulfate deposits can create toxic hydrogen sulfide ~~(HS)~~ ions[4]. Therefore, easy and inexpensive processing techniques of indigo dyes are needed to ~~safe~~protect the environment. Fungi ~~that is~~ isolated from dye industry waste are reported to ~~be able to~~ degrade various coloring wastewater[5,6]. A number of studies ~~have~~ reported that fungi can reduce the concentration of ~~Indigo~~indigo dyes and degrade ~~Indigo~~indigo dye ~~wastewater~~[7]wastewater,[7,8] but ~~not many~~few have completely ~~report about how~~reported the mechanisms and degradation products are formed. The ~~mechanism of~~ degradation mechanisms by fungi can be grouped into two processes ~~namely:~~ biosorption and/or biodegradation (with ligninolytic enzyme activity). The ~~connection~~contributions of those two processes in the degradation of ~~Indigosol~~Indigosol batik wastewater is unknown~~.~~ ~~Therefore, the decomposition mechanism that occurs in the role of the fungi~~ and needs to be investigated. The amount of ligninolytic enzyme activity that plays a role in the ~~mechanism of~~ degradation mechanism also needs attention. A ~~special~~ discussion on ~~testing~~the specific activities of ligninolytic enzymes is needed to prove that the enzyme ~~is a system with~~plays an important role in the ~~process of~~ degradation process of batik waste.
~~Products resulted from~~ It is critical to identify the degradation products of batik waste ~~are important to know. There are rarely~~and few research reports ~~on~~ have identified the biodegradation products ~~or Indigo~~and indigo dye intermediates[7]. ~~Therefore, it is necessary to study the products they had produced.~~ The ~~purposes~~purpose of this study ~~were~~was to determine the mechanism ~~occured when~~of the microscopic isolate of *Aspergillus* sp. 3 ~~that was isolated~~ from ~~the disposal of~~ batik industrial waste disposal in ~~degrading~~ the degradation of indigosol blue O4B (IB~~-~~) batik dye wastewater, and to ~~decide~~identify the degradation products ~~been~~ formed.

METHODOLOGY

Materials

Aspergillus sp. 3 was obtained ~~from~~ according to methods described in previous research ~~results. It~~. The fungi was isolated from batik wastewater ~~that taken from the~~ disposal ~~location of the batik industry~~from the Banyumas District, Central Java, Indonesia, and ~~then had been selected from others as~~ superior isolate[9,10]. ~~Batik~~The batik wastewater used in this research was i~~I~~ndigosol b~~B~~lue O4B (IB). ~~It was obtained from the batik industry in Banyumas District, Central Java, Indonesia.~~

Methods

1. Preparation of isolate

~~Pure~~A pure culture of *Aspergillus* sp. 3 in ~~the~~ slant media agar was rejuvenated on ~~Potato Dextrose Agar~~potato dextrose agar media in ~~Petri~~petri dishes~~,~~ and incubated for five days at room temperature ~~then~~prior to ~~be used at the next stage~~use.

2. Determination of the ~~mechanism of~~ degradation mechanism of IB batik wastewater by fungi *Aspergillus* sp. 3

a. Mechanism of biosorption

The growth medium used in the adsorption mechanism ~~is the Potato Dextrose Broth~~was potato dextrose broth cultivation medium supplemented with 0.8% diammonium hydrogen phosphate. 75 mL of the growth medium ~~in 250-mL Erlenmeyer flasks~~ were inoculated with 3.5 ~~x~~ × 10^7/ mL fungi spores in 250-mL Erlenmeyer flasks. The inoculated medium was then incubated in a reciprocal shaker at 90 rpm for 96 hours. The fungal mycelium was harvested ~~at the end of incubation~~ by centrifugation using a refrigerated centrifuge and washed with distilled water. Dead biomass was ~~made~~created by autoclaving at 121° C for 15 minutes before harvesting the culture[11].
The adsorption mechanism step was ~~carried out using~~performed by mixing 100 mL IB batik dye wastewater ~~–~~in a 100-mL Erlenmeyer flask ~~added~~ with 0.25 g biomass fungi (dry weight) and then ~~incubated~~incubating for 24 hours ~~above~~in a reciprocal shaker at 90 rpm. After ~~the end of~~ incubation,

the biomass was separated by centrifugation, and the concentration of the dyestuff in the supernatant was measured using spectrophotometry[11].

$$\% \, adsorption = \frac{Co - Ce}{Co} x \, 100\%$$

Detail~~-~~:
Co = Initial solution concentration ~~of solution~~
Ce = The solution concentration at equilibrium (mg / L)

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The adsorption mechanism ~~was known by the color change~~ of the mycelium formed ~~which was originally~~was observed by the change in color from white to blue like the ~~Indigosol Blue O4B~~IB dye. ~~Color~~The color changes ~~that~~ were ~~seen as evidenced by microscopic shooting~~observed using a SEM ~~microscope~~. The ~~indication of~~ adsorption ~~is also known~~was identified by the reduction in the solution concentration after the decolorization process.
b. ~~Mechanism of~~ Biodegradation mechanism
1) Test enzyme activity
The fungi of the selected isolates were grown in ~~the~~ PDB medium in the reciprocal shaker with a speed of 90 beats per minute for 96 hours. Mycelium grows to form pellets. The formed pellets are separated from the production medium using centrifuges. Pellets are used as an inoculum to decolorize IB batik wastewater for enzyme production. The supernatant obtained was a crude enzyme and was used to measure the enzyme activity after decolorization. ~~Crude enzyme~~The obtained ~~is~~ crude enzyme was then used ~~in measuring~~to measure the enzyme activity. The ~~enzyme~~ activity was measured ~~was the enzyme~~ for lakasse, manganese peroxidase ~~enzyme,~~, and lignin peroxidase ~~enzyme activity~~.
~~Before~~Crude enzyme production was performed prior to the measurement of the enzyme activity~~, crude enzyme production was carried out~~. The isolate of *Aspergillus* sp. 3 fungal inoculum was used to decolorize wastewater using PDB in room temperature shakers. After 4 days of cultivation, a small pellet ~~is~~was formed~~, this~~ and the pellet ~~is~~was homogenized at 1500 rpm for 30 seconds. 8 ~~ml~~mL of homogeneous pellet samples were used to inoculate the Erlenmeyer flask. IB batik dye wastewater was then added to the ~~Erlenmeyer Flask.~~flask. The fungi strain culture ~~of strain fungi~~ was ~~shaken out in a shaker~~isolated using an Erlenmeyer flask with agitation of 90 rpm~~.~~
~~The~~ and the sample ~~will be~~was used to test the filtration enzyme activity. The sample was then tested for enzyme activity. The activity measured by the enzyme is LiP, MnP, and Lac. The method used to test the enzyme activity ~~is~~was:
~~Laccase~~The laccase activity was obtained by mixing 900 ~~µl~~µL of filtrate ~~culture~~ with 750 ~~µl~~µL of 0.1 M sodium acetate buffer ~~0.1 M,~~ then ~~added with~~ 100 ~~µl~~µL of 0.5 mM syringaldazine ~~in 0.5 mM~~was added at room temperature. ~~Then the~~The absorbance measurements were ~~carried out~~conducted at 525 nm~~. The absorbance reading~~ for 1 minute~~,~~. The measurements were repeated several times ~~the data~~ until the data ~~is~~was good.

The MnP activity was obtained by mixing 1000 ~~µl~~µL of the filtrate culture with 1750 ~~µl~~ µL of mM pH 4.5 malanoic acid ~~mM pH 4.5~~and then adding 125 ~~µl~~µL of 20 mM 2.6 DMP ~~20 mM, and~~, 125 ~~µl~~µL of 20 mM manganese sulfate ($MnSO_4$) ~~20 mM then added also with~~and 300 ~~µl~~µL of 2 mM H_2O_2. Subsequent absorbance measurements were ~~carried out~~conducted at 470 nm~~. The absorbance reading~~ for 1 minute~~.~~. The measurements were repeated several times ~~the data~~ until the data ~~is~~was good.

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The LiP activity tested was obtained by mixing with 1000 ~~µl~~µL of the filtrate culture and 300 ~~µl~~µL of 2 m~~2m~~M H_2O_2, ~~then added with~~followed by 2000 ~~µl~~µL of LiP buffer (Tween 80 0.5 g, veratyl

alcohol 33.6 mg and 0,1 M buffer sodium tartrate solution 500 mL pH 2.9). ~~Then the~~The absorbance measurements were ~~carried out~~conducted at 310 nm~~. The absorbance reading~~ for 1 minute~~,~~. The measurements were repeated several times ~~the data~~ until the data was good.

2) Enzyme Purification

~~Purification of the enzyme is called enzyme~~Enzyme purification~~. This stage is through~~ was conducted by deposition of ammonium sulfate[12], dialysis[12]~~,~~, and gel filtration column chromatography.

~~Crude~~The crude enzyme was ~~produced to then be~~ deposited using ammonium sulfate salt to ~~obtain~~ saturation ~~with concentrations of~~ at 0~~–~~–20%, 20~~–~~–40%, 40~~–~~–60%, and 60~~–~~–80%. Salt was added slowly in cold conditions ~~by continuing to stir.~~under continuous stirring. The mixture ~~obtained~~ was then centrifuged for 20 minutes at ~~a speed of~~ 3580 g. Protein deposits were dissolved with 0.5 M, pH 5 acetate buffer ~~5~~. The deposition fraction of the ammonium sulfate at each concentration was then tested for laccase enzyme activity, MnP and LiP, and protein content to determine the ~~best~~optimal ammonium sulfate fraction.

Dialysis was ~~carried out~~performed by cutting ~~the~~a 10 kD dialysis membrane ~~with 10 kD~~ and washing it in running water[12]. The dialysis membrane was boiled in a mixture of 2% (b/v) Na-~~Carbonate~~carbonate and 0.05% (b/v) EDTA for 10 minutes. ~~Dialysis~~The membranes were twice boiled ~~again~~ in distilled water ~~twice~~ for 10 minutes ~~each~~. The dialysis membrane was cooled and fastened to one end of the membrane. The fractionated ammonium sulfate extract with the highest specific activity was pipetted into the membrane. The fastened membrane ~~that has been fastened~~ was immersed in 0.5 M, pH 5 acetate buffer ~~0.5 M pH 5 with a ratio of~~ at 100 times the sample volume. The dialysis process was ~~carried out~~performed at 4° C ~~with~~under stirring for 9 hours with three ~~times~~ replacements of the ~~replacement of the soaking~~ buffer. ~~Dialysis enzymes were~~The solution was tested for laccase, MnP and LiP activity, and protein content.

The dialysis ~~results obtained~~solutions were then purified using gel filtration column chromatography[13]. ~~The~~A Sephadex G100 gel matrix in the column ~~is~~was slowly flowed with 0.5 M, pH 5 acetate buffer ~~0.5 M pH 5~~ for rinsing. The dialysis enzyme extract was passed into a column containing Sephadex G100 and eluted with an 0.5 M, pH 5 acetate buffer ~~0.5 M pH 5 with~~at a flow rate of 1.66 ~~x~~ × 10^{-5} L.s^{-1}. Each mL of the fraction formed was analyzed for the activity of ~~lakase~~laccase enzymes, MnP and LiP, and their protein content.

3) Determination of the specific activities of purified enzymes

~~Specific~~The specific activity test aims to determine the size of the laccase, MnP and LiP produced by ~~isolate of~~the *Aspergillus* sp. 3 fungi isolate. The results are expressed by U/mg, which proves that ~~the~~ *Aspergillus* sp. 3 produces ~~an~~ enzyme ~~of a number of values obtained~~. These results also assume the extent of the role of the three enzymes in decolorizing IB batik wastewater.

Protein values were calculated using the Bradford ~~-~~method[14]. The test solution (enzyme fraction) of 5 µL was mixed with 100 µL of the Bradford solution in a cuvette. The mixture ~~is~~was homogenized for 10 seconds~~. The mixture was~~ and left to stand for 10 minutes ~~then measured its~~. The solution absorbance was measured at ~~a wavelength of~~ 595 nm. The standard used ~~is~~was bovine serum albumin ~~(BSA)~~ with a concentration of 0~~–~~–100 ppm.

3. Product ~~Analysis~~analysis results from degradation of IB batik wastewater by *Aspergillus* sp. 3 fungi

Aspergillus sp. 3 fungi ~~are~~were grown at optimum conditions and ~~given~~ wastewater. After incubation, filtration ~~is then carried out. The~~was performed, and the solution ~~obtained was accommodated~~accumulated in a sterile ~~Erlenmmeyer~~Erlenmeyer flask ~~then used~~ for identification using a UV-Vis spectrophotometer[15]~~,~~ and FTIR[16].

Identification of the products resulting from degradation using aA UV-Vis spectrophotometer was carried outused to seeobserve changes in the spectrum. to identify the degradation products. Two mlmL of the solution was measuredused for absorbance measurements with a UV-Vis spectrophotometer, at one 1-minute intervals for 30 minutes. Absorption of the spectrum between 275 and 800 nm iswas recorded during decolorization.
FTIR identification is was used to study changes in the dye structure before and after degradation. TheA 50 ml solution of the degradation solution sample was characterized using a Fourier Transform Infrared Spectrometry (FTIR) spectrometer. The wavelength range used was 400 – 4600 / cm^{-1}.

RESULT AND DISCUSSION

1. **Determination of the degradation mechanism of IB batik wastewater by fungi *Aspergillus* sp. 3**

a. Mechanism of biosorption

The biomass of fungi werewas used to study their capacity to adsorb dye from itsa solution[11]. The presence of dead mycelium was used to prove the existence of decolorization mechanism through adsorption because with the death of the cell there wasis no enzyme involvement in decolorization so thatand only adsorption event occured.occurs.
The pellet surface observations of *Aspergillus* sp. 3 that were incubated withinfor 24, 48, and 72 hours wereare presented in Figure 1. Pellets appear to be coloredappeared blue when compared to control isolates without the addition of IB batik wastewater. SinceIn the 24 hour incubation of the first day the blue color has begun to stick both of , the pellet surface and cell wall, the more days more thickened were blue, which increased with longer incubations. This proves that the adsorption mechanism occurs during incubation.
Evidence of adsorption was also shown in observationsobserved using SEM. SEM microscopy, which has been used as a tool for biosorbent characterization[17] because it can show the accumulation of dyes on the surface[11]. The optical microscopicmicroscopy image of *Aspergillus* sp. 3 wasis shown in Figure 2. The SEM image supports the proof of the adsorption of IB batik wastewater. Before the treatment, it showed the morphology of the mycelium surface which looked smooth, with no particles were stuck and irregular with a large areaor irregularities for dye surface interactions. Significant changes occurred in the morphology of the mycelium surface and, which became compact after adsorption of the IB batik wastewater. SEM images showed adsorbed colored molecules of the IB batik wastewater adsorbed in a completed form.. The surface of the mycelium looked rough and there werewith particles stuckon the surface.

Figure 1. Pellet(A) The pellet surface of *Aspergillus* sp. 3 on mycelium without treatment of IB batik wastewater (A), and after (B) 24 hours (B),, (C) 48 hours (C), and (D) 72 hours (D) incubation time.

Figure 2. SEM of *Aspergillus* sp. 3. Isolateisolate of *Aspergillus* sp. 3 before (A) and after (B) decolorization treatment at magnification 7500x.

Figure 3. Percentage of *Aspergillus* sp. 3 adsorption at dead mycelium.

Figure 4. Dead mycelium after decolorization. The dye was (a) adsorbed on the cell surface (a), the dye accumulatesand (b) accumulated in the cytoplasm (b).

Proving After demonstration of the existence of an adsorption mechanism that occurs then carried out further treatment by calculating, the percentage of dead mycelium adsorption so as not to be affected by cell metabolic activity. The results of the percentage of adsorption obtained were presented was calculated, as shown in Figure 4. The percentage of adsorption increased with

incubation time. The, with an average value obtained wasof 33.39% on the first day, then 50.49% on the second, day and 68.55% on the 3rdthird day.
The observation of dead mycelium surface was also donanalyzed to ensure the mechanism occuredoccurred. Microscopic appearanceimages of the dead mycelium after decolorization could be seenis shown in Figure 4. The image provides information telling aboutillustrates the dead mycelium with its presence of dyes that stick toon the surface of the mycelium and also the presence of dyes enteringin the cytoplasm. This indicated that, indicating the mechanism occuredthat occurred during degradation was a biosorption mechanism eitherof adsorption noror intracellular accumulation. Biosorption can be classified as adsorption/cell surface precipitation and intracellular accumulation depending on the location of the adsorbate[18]. The adsorption surface is generally assisted through ionic, chemical, and physical interactions.
Figure 4 showedshows the mycelium after the adsorption process. This showed that, illustrating the dye molecule was adsorbed on the cell surface. The cell wall of fungi contains large sources of chitin, chitosan, â-1,3-D-glucan, â-1,6-D-glucan, and mannoprotein on different functional groups such as carboxyl, amine, hydroxyl, phosphate, and sulfonate[11]. The presence of amino groups, carboxyl, hydroxyl, phosphate, and sulfonates in the biomass can interact with dyes. Therefore, the initial attachment to the dye molecule withto the cell surface follows a complex pattern. The dye adsorption of batik IB wastewater on biomass occurs through (1) chemical interactions between colored molecules and fungi cell wall components, (2) electrostatic interactions between dye molecules and electron-rich sites on the cell surface, and (3) weak physical forces such as hydrogen bonds and van der Cell walls interactWaal's interactions between hydrophobic parts of colored molecules (for example, aromatic rings) and biomass polysaccharides. During the incubation in this study, it can be observed that, the color of IB batik wastewater changes from blue to a clear color whose the occurrenceas an indication of enzyme activity indication. It relates to , which confirms previous research that the color of the solution changes from blue to light blue to a clear color, as in compared to controls without dyes because of thelaccase enzyme activity of laccase enzymes that are able to oxidize Indigooxidizing indigo dyes[19].
The mechanism of biodegradation mechanism is a breakdown of dyes whichthat involve activity of various enzymes[20]. Measurements of the enzyme activity in this study were carried outperformed to prove the mechanism of biodegradation and to knowidentify the enzymes whichthat play a role in the decolorization of IB batik wastewater. It is known that fungalFungal enzymes, such as laccase (Lac), manganese peroxidase (MnP), lignin peroxidase (LiP)), play a role to degrade in the degradation of dyes[21]. The activity of LiP, MnP and Lac enzymes assayed on crude enzyme can be seenare shown in Tables 1, 2, and 3.
Based on Table 1, it can be seen that: the laccase activity in crude enzyme was 11,666.67 (U), where the) with a specific activity wasof 58.39 (U/mg). Table 2 informed that the magnitude of), the MnP enzyme activity was 5,256.048 (U) with a specific activity of 26,307 (U/mg), and Table 3 showed forthe LiP has an enzyme activity ofwas 156,838.7 (U) -with a specific activity of 784,98 (-U / mg).
Test results of specific activities of laccase, MnP, and LiP enzymes were presented in Table 1, 2 and 3. The data in the Table provedproves that there was an increase in activity after enzyme purification from dialysis level to the chromatographic column. The laccase enzyme specific activity increased from 58.39; to 148.39 and then to 1091.02 U/mg. Similarly, the peroxidase enzyme increased from 26.31 to 26.44 and 198.66 U/mg for MnP, and 784.98 to 3682.4 and 5638.21 U/mg for LiP.

2. **Product Analysisanalysis results from the degradation of IB batik wastewater by *Aspergillus* sp. 3 fungi**

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UV-VIS

The analysis of ~~products from~~ the degradation products using ~~a~~ UV-Vis ~~spectrophotometer~~spectrophotometry was ~~carried out~~performed to ~~see~~observe changes in the maximum absorption spectrum. Changes in the peak spectrum ~~occured~~occurred during decolorization. The UV-Vis spectrophotometer showed a sharp new peak at different wavelengths ~~than~~compared to the wavelength before the degradation process.

Figure 5 ~~presented~~presents the graphical form at the visible wavelength. The results of the UV-Vis analysis of the IB batik wastewater samples before decolorization showed a peak ~~detected at a wavelength of~~at 604.5 nm (Figure 5a). The curve in Figure 5a showed that ~~the~~a maximum wavelength ~~seen~~observed in the IB batik wastewater but ~~was~~ not ~~seen~~ in the decolorization results. The peaks ~~seen in~~from the IB batik wastewater were not ~~seen~~observed in the decolorization results with isolate of *Aspergillus* sp. 3. Figure 5b ~~showed~~shows that the decolorization solution ~~showing a peak that~~ cannot be detected at the visual wavelength (400~~–~~750 nm). The peak was in the UV ~~wave~~range at ~~a wavelength of~~ 345 nm~~. This showed~~, indicating that ~~there was a~~the degradation was characterized by the loss of peaks and ~~the existence of other~~another compound ~~with the appearance of~~ introduced a new peak.

Figure 5 ~~indicated~~indicates that the degraded IB batik wastewater sample ~~showing~~showed undetectable peaks at ~~wavelengths of~~ 400 and 800 nm~~. This proved~~, indicating that after the decolorization assay, there was a ~~reading~~ shift in the wavelength. The ~~occurrence of a~~ wavelength shift also showed degradation~~. This shift caused~~, causing the structure of the IB batik wastewater ~~changed~~to change and the conjugated bond ~~decreased, resulted~~to decrease, resulting in a shift ~~in the wavelength~~ toward a shorter wavelength[22].

Figure 5. Spectra UV-Vis of the IB batik wastewater (a) before ~~(a)~~ and (b) after ~~(b)~~ treatment using *Aspergillus* sp. 3.

FTIR

FTIR was used to characterize and identify functional groups of IB batik wastewater before and after treatment using isolate of *Aspergillus* sp. 3. The FTIR spectrum in both samples was measured ~~on absorption bands at~~over 400~~–~~4000 cm^{-1}. Figure 6 and Table 4 showed the FTIR spectra of IB batik wastewater before and after degradation.

FTIR spectra data in Figure 6a for IB batik wastewater showed the presence of several absorption bands. ~~Absorption~~The absorption bands ~~that appear~~ in the IB batik wastewater samples were 501.49~~;~~, 555.50~~;~~, 594.08~~;~~, 617.22, 1519.91~~;~~, 1543.05~~;~~, 1558.48, 1697.36, and 3734.19 cm^{-1}. Absorption ~~band~~bands at ~~wave number~~ 501.49~~;~~, 555.50~~;~~, 594.08~~;~~, and 617.22 cm^{-1} indicates the presence of C-Br bonds. As ~~also~~previously reported ~~by researchers that Alkyl~~, alkyl halide is recognized by the appearance of absorption bands in the region of 200~~–~~750 cm^{-1}, especially in the area of 500~~–~~680 cm^{-1} for C-Br[23,24,25,26].

The ~~analysis~~ results in Figure 6a also show the absorption bands of aromatic and aliphatic groups (strain C=C), and C=N~~–~~ ~~appearing~~ at ~~wave number~~ 1519.91, 1543.05, and 1558.48 cm^{-1}. According to Clifford et al. (1982), at ~~wave number~~ 1675~~–~~1500 cm^{-1} there is a C=C (aliphatic and aromatic) strain~~,~~ and a C=N~~–~~ ~~–~~strain. The ~~peak caused by~~ stretching vibrations of C=C~~),~~ and C=N~~–~~ ~~–~~were located ~~in the wave number~~ 1690~~–~~1600 cm^{-1}. The aromatic ring shows peaks in the region of 1650~~–~~1450 cm^{-1}, and ~~with~~ a low degree of substitution shows ~~the~~ peaks at 1600, 1580, 1500 and 1450 cm^{-1} [23,24,25,26,27].

The ~~waves number of~~peak at 1697.36 cm^{-1} was the absorption of the N–H group~~,~~ which was a special structure of Indigosol. The absorption band at 3734.19 cm^{-1} showed the presence of the

hydroxyl group (O-H). ~~Amida~~Amide bonds ~~to a number of waves~~are in 1670-–1700 cm^{-1} and the ~~wavenumber~~ 3750-–3000 cm^{-1} ~~show a~~range is stretch ~~of~~ –OH [23,24,25,26,27,28,29].
The FTIR spectra data ~~from~~of the degradation products ~~which are~~of IB batik waste after treatment using *Aspergillus* sp.3 ~~were~~are presented in Figure 6b. The absorption bands ~~that emerged~~ from the degradation product were different ~~than~~from before treatment~~. These results indicated~~, which indicate the loss of the functional groups of the dye structure. The degradation products showed that there were only three absorption bands remaining, ~~namely~~ 1635.67, 2108.38 and 3332.05 cm^{-1}.
The absorption band for aliphatic stretching C=C at ~~wave number~~ 1635.67 cm^{-1} ~~with~~has an intensity of 68.945%. ~~Aliphatic~~The aliphatic group or strain C=C is located ~~in the frequency of the wave number~~ at 1675-–1500 cm^{-1} [30]. ~~Wave number~~ 2108.38 cm^{-1} ~~that~~ appears with an intensity of 95.931% was a C–O stretching group. The C–O stretching group appeared at ~~the wave number~~ 2108 cm^{-1} [31].—–OH stretching appeared to be ~~read~~ in the region of ~~wave number~~ 3332.05 cm^{-1} with an intensity of 45.806%.—–OH stretching at absorption ~~from~~occurred at 3750-–3000 cm^{-1} [30].

Figure 6. FTIR spectrum (a) before degradation ~~(a)~~ and (b) degradation products ~~(b)~~of IB batik wastewater using isolate of *Aspergillus* sp. 3.

Based on the ~~results of the~~ FTIR analysis, ~~it can be assumed that in the Indigosol Blue O4B colorant contained in the IB wastewater~~ there were aliphatic, aromatic, and C–Br alkyl halide groups and–NH groups in the IB colorant of the wastewater while after the treatment there ~~are~~were only three functional groups ~~namely~~, C=C, C–O groups, and—–OH, ~~while~~and the typical ~~Indigosol~~Indigosol groups ~~namely~~of aromatic, NH and Br did not appear. This ~~indicated~~indicates that there was a degradation process by *Aspergillus* sp. ~~3. This~~3, wherein the isolate breaks the aromatic bond, C–Br alkyl halogen and—–NH into simpler compounds. The compound ~~was~~has a bond from the remaining three groups ~~which~~that are predicted as alkenes with hydroxyl groups to become alkenol (CH_4=C–OH).

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CONCLUSION

The mechanism of ~~isolate of~~the *Aspergillus* sp. 3 isolate in degrading IB batik wastewater ~~was~~occurs by ~~using~~ biosorption (adsorption) and biodegradation (enzymatic). ~~Decolorization~~The decolorization value ~~with the mechanism of~~ and degradation ~~through~~mechanism via adsorption at 24, 48, and 72 hours was 33, 39~~%;~~%, 50.49~~%;~~%, and 68.55%. ~~Extracellular~~The extracellular enzymes ~~that were~~ predominantly involved in the degradation were LiP, MnP, and laccase. Purified enzymes on column chromatography from the isolate had specific activities of laccase, MnP, LiP up to 1,091.02, 198.66, and 5,638.21 U / mg. The ~~product of~~ degradation product was identified as a simple ~~compound named~~ aliphatic compound containing C=C, C–O, and—–OH bonds and are predicted ~~as~~to be an aliphatic alcohol compound group.

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