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Study of N, P, K, and C on Degradation of Indigosol Batik Dye Effluent by *Aspergillus* sp. GPN

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ABSTRACT

Indigosol dye batik effluent is toxic since it excesses contain decreasing agent sodium hydrosulfite that is oxidized to alkali and alkaline earth metal sulfate (SO_4^{2-}), sulfite (SO_3^{2-}), and thiosulfate ($S_2O_3^{2-}$) that very corrosive. Therefore, the role of environmental biotechnology in the technology for restoring environmental functions is needed efforts to treat batik effluent have been carried out biologically by using microorganism agents. It can be used *Aspergillus* sp. that can reduce industrial dyes. From the previous research, the product of Indigosol Blue batik effluent degradation that produced by *Aspergillus* sp. GPN did not cause any toxicity in plants even better grew than giving watering with irrigation water. Content in the results of degradation can affect the plants, so the element analysis needs to be performed. It's must include the substance nitrogen (N) in the form of organic compounds that easily absorbed by plants, do not leave residual organic acids in the soil, has high carbon (C) content, Phosphorus (P), and Potassium (K). This study aimed to analyze the content of N, P, K, organic carbon, and ratio of organic carbon to total nitrogen (C: N) in degradation product of Indigosol Blue dye batik effluent produced by *Aspergillus* sp. GPN. The methods used were spectrophotometry for organic carbon and P_2O_5, Kjeldahl for total N, spectrophotometry, and AAS for K_2O. The result of the assay was the product degradation of indigo sol blue batik effluent had a total nitrogen content of 0.11%, K_2O of 0.01%, organic carbon of 0.24%, C/N ratio of 10.13%, while for phosphorus content of 0.01%. Fermentation of degradation products causes 0% frequency of wilted and dead plants, better when compared to water (40% frequency of wilted plants) and effluent (40% frequency of wilted and dead plants).

Keywords: *Aspergillus sp., Batik effluent, Elements.*

1. INTRODUCTION

Most of the textile wastewater, especially batik, contains dye residues that have the potential to pollute the environment. It is extremely dangerous because it carries chemical substances which are dangerous to the environment. The effluent has a detrimental effect on the content of organic matter, suspended solids, oil, or fat, and the content of dangerous heavy metals such as Zn, Cd, Cu, Cr, and Pb [1]. Generally, batik industrial effluent is discharged directly into water bodies or rivers without being treated first [2]. One type of synthetic batik dye is Indigosol Blue that one of the synthetic dyes of Anthraquinone, and has molecular bonds of -NH and C=C. This synthetic dye is not easily damaged by chemical or photolytic treatment. This dye waste has a strong chemical bond structure and is classified as non-biodegradable waste [3]. Indigosol wastewater is toxic because it carries extra reducing agent sodium hydrosulfite that is oxidized to alkali and alkaline earth metal sulfate (SO_4^2), sulfite (SO_3^{2-}), and thiosulfate ($S_2O_3^{2-}$) hat very corrosive. Thus, the formed sulfate deposits can create toxic hydrogen sulfide (HS) ions [4].

Efforts to treat batik effluent have been carried out biologically by using microorganism agents. The microorganism agent that can be used is Aspergillus sp. that can reduce industrial waste dyes. *Aspergillus* sp. 3 strain GPN is the superior isolate to decolorize the batik effluent [5]. Aspergillus species 3 nearly completely

(99.9%) decolorized the effluent within the liquid medium after a three-day incubation. *Aspergillus* sp. 3 can remove effluent as a single carbon and nitrogen source dye [6].

The previous study finds that effluent batik dye degradation by *Aspergillus* sp. 3 did now no longer motive any toxicity in plants. Plumule and radicle length of *Zea mays* and *Vigna radiata* has grown at the decolorized effluent being longer than withinside the untreated effluent [5]. The percentage of corn and mung bean seed germination on decolorized effluent turned better than on untreated effluent. Even, it's better growth than giving watering with irrigation water. Content in the results of degradation can affect the plants, so the element analysis needs to be performed. Elements that can fertilize plants are substance nitrogen (N) in the form of organic compounds that are easily absorbed by plants, do not leave residual organic acids in the soil, has high carbon (C) content, and have Phosphorus (P) and Potassium (K).

Substance N in the form of organic compounds that are easily absorbed by plants does not leave residual organic acids in the soil and has high organic C content such as charcoal hydrates. Nutrients required of the plant are not sufficiently available in the soil because the nutrients contained in the soil are relatively little, the number of nutrients cannot suffice certain plants, so it is necessary to add nutrients. The role of potassium in plants is to form protein and carbohydrates, harden straw and the bottom of the wood, increase plant retention against disease and improve seed or fruit quality [7]. Carbon is a food supply for soil microorganisms, so the presence of organic C withinside the soil will stimulate the activity of microorganisms [8]. The increase in C-Organic caused by carbon (C) with the application of elements on the soil will increase the organic substances contained in the soil [9]. This study aimed to analyze the content of N, P, K, organic carbon, and ratio of organic carbon to total nitrogen (C: N) in degradation product of Indigosol Blue dye batik effluent produced by *Aspergillus* sp. GPN.

2. METHODOLOGY

2.1. Reculture Aspergillus sp. GPN

Materials and tools are prepared, alcohol sprayed on palms and tables. The Bunsen fire is light. The wrapping paper of the petri dish is removed. The mouth of the petri dish is heated. A hole in the PDA was made using a straw. A piece of PDA took using a loop needle. Inoculated on new PDA media, placed in the middle of the PDA. The petri dish is covered, heated then wrapped in plastic wrap. Then incubated at room temperature for 3-7 days.

2.2. Preparation of Aspergillus sp. *GPN inoculum*

Materials and tools are prepared, alcohol sprayed on palms and tables. The Bunsen fire is light. The wrapping paper on the Erlenmeyer flask is removed. The mouth of the Erlenmeyer flask is heated. A hole in the PDA was made using a straw. A piece of PDA took using a loop needle. Inoculated on PDB media. The Erlenmeyer flask is covered, heated then wrapped in plastic wrap. Then incubated on shaking incubator at room temperature for 3-7 days.

2.3. Efluent Degradation

Indigosol was added on *Aspergillus* sp. 3 preparation then incubated on a shaking incubator at room temperature. Observed in each time interval. The degradation result is filtered then used for Macro Nutrient Assay.

2.4. Macro Nutrient Assay

2.4.1. Assay of K Nutrient

The testing used a Flame photometer. The 100 mL sample solution was added in a 100 mL volumetric flask. Diluted to the limit. The standard series of samples were made of 4 mL, 6 mL, and 8 mL. Each sample was diluted into a 100 mL volumetric flask. The absorbance was measured using a flame photometer.

2.4.2. Assay of C Nutrient

The C test used the walker and black methods. A sample of 100 mL was added with 5 mL of $K_2Cr_2O_7$ 2 N solution and then shake. The H_2SO_4 pa. 98% of 7 mL is added then shaken. The solution is left for 30 minutes then shake. Standard solution C of 5 ml is pipetted into a 100 ml volume measuring flask. H_2SO_4 of 5 mL and 7 ml of $K_2Cr_2O_7$ 2 N solution were added and then shaken. The solution is left for 30 minutes then shake. Blanko as standard 0 ppm C were prepared by diluting the sample with ion-free water. After cooling, the volume is adjusted to the mark of 100 ml. Then shaken back until homogeneous and overnight left. Then measured with a spectrophotometer at a wavelength of 651 nm.

2.4.3. Assay of P Nutrient

Analyzing the P content by inserting the waste sample in a cuvette before measuring it in a UV-Vis

spectrophotometer. Blanks using distilled water were included in the cuvette. Then the absorbance value was measured using a UV-Vis spectrophotometer with a wavelength of 650-750 mn.

2.4.4. Assay of N Nutrient

The sample for the analysis of the N content used the Kjeldahl micro method. There are three stages in the Kjeldahl micro method, namely digestion, distillation, and titration which function to obtain the values of N-organic, N-NH_4, and N-NO_3. The N-organic test begins to make titrant A as a sample, and A1 as a blank. The titran A and A1 were prepared by adding 0.25 g of selenium mixture to which 3 mL of H_2S_4 pa were added in a Kjeldahl flask, then shaken until evenly distributed, and then left for 2 hours. Gradual digestion with a temperature of 150˚C to a maximum temperature of 350˚C for three hours and clear liquid. Cool then dilute with a touch of distilled water. The solution is transferred to a distillation boiling flask and then added a little boiling stone and distilled water until it reaches the volume of the flask. Then 10 mL of 40% NaOH were added. The destination container includes 10 mL of 1% boric acid in Erlenmeyer and 3 drops of Conway indicator liquid are added until the Erlenmeyer reaches 75 mL. Continue the distillate titration for A1 with 0.05 N H_2SO_4 to the endpoint when the color changes from green to pink.

The N-NH_4 test was initiated using titrant B as the sample and B1 as the blank. Sample 1 g in a boiling distillation flask plus a little boiling rock, 0.5 mL of liquid paraffin, and 10 mL of distilled water for B while 100 mL for B1. Then add 10 mL of 1% boric acid, 3 drops of Conway indicator, and 10 mL of 40% NaOH and then distill it until it reaches 75 mL. The distillate was titrated with 0.05 N H_2SO_4 until the color of the solution changed from green to pink.

Testing for N-NO_3 begins with titrant C as the sample and C1 as a blank with the same procedure. The remainder of the N-NH_4 determination of the sample could cool then be added with distilled water to the original volume. The distillate is entered by adding 10 mL of 1% boric acid in 100 mL Erlenmeyer plus 3 drops of Conway indicator. Then distill it by adding 2 g of devarda alloy. Distillation is started indefinitely so that the foam does not overflow after the foam is almost exhausted starting from low temperature after boiling the temperature is increased to a normal. Distillation to 75 mL of liquid. Then titrated with 0.05 N H_2SO_4 until the color of the solution changes from green to pink. The results of N-organic, N-NH_4, and N-NO_3. Were calculated using the formula then totaled.

2.5. Plant Assay

The degradation products obtained were then tested on plants. This experiment was compared with the degradation product which was mixed with a small amount of goat urine and then fermented using EM 4 (Effective Microorganism-4). Then the spinach plants were watered and compared with irrigation water and batik waste. Observations were made on the frequency of wilting and the frequency of death.

3. RESULTS

In this research assay of N, P, K, and C element content derived from the degradation process of Indigosol Blue batik effluent was carried out. The test results for the assay of nutrients were presented in Table 1. Table 1 showed results of total nitrogen content in degradation product which has a value of 0.11%, while for phosphorus it has a value of 0.01%. Because it is possible to fertilize plants, it is compared with the content in liquid organic fertilizers (LOF). According to Minister of Agriculture Regulation No. 70/Permentan/SR.140/10/2011 [10], the minimum quality standard for nitrogen and phosphorus content of LOF is 3-6%. The results degradation product of this research was not reached quality standards for nitrogen and phosphorus content has a value of <1%.

Table 1. The Results for Assay nutrient from Degradation Indigosol Blue Batik Effluent

No	Parameter	Method	%
1	N-Total	Kjeldahl	0.11
2	P_2O_2	Spekrofotometri	0.01
3	C-organic	Spectrophotometric	0.24
4	K_2O	Spectrophotometric, AAS	0.01
5	C/N Ratio	-	10.13

Minister of Agriculture Regulation [10] contains standards for LOF, but types of samples obtained in this research cannot be categorized as LOF. The nitrogen content obtained is 0.11% and Phosphorus is 0.01%. According to its regulation, the minimum quality standard for nitrogen and phosphorus content of LOF is 3-6%. The fermented cassava wastewater with the addition of cow urine, and foot of cow washing water contained 5.11% Nitrogen and 3.08% Phosphorus [11].

Plants need one of the macronutrients Nitrogen for protein formation and vegetative growth (Sarief, 1986). Nitrogen deficiency will cause plants to grow stunted, leaves turn yellow, and reduce growth activity [12]. The following is a nitrogen formation reaction [13]:

Protein Organic Ingredients $\xrightarrow{\text{Aminization Reaction}}$ Amino acid

Amino acid $\xrightarrow{\text{Ammonification Reaction}}$ Ammonia (NH_3) & Ammonium NH_4^+

Ammonia $\xrightarrow{\text{Reaction of nitrification by Nitrococcus bacteria and Nitrosomonas}}$ Nitrate (NO_3)

The nitrogen contained in degradation product with indigo sol blue effluent does not reach quality standards to Minister of Agriculture Regulation [10], the minimum quality standard for nitrogen is 3-6%. That factor causing the decrease in N content is due to limited O_2 causing NH_3 cannot be converted into NO_3 and N, which causes it to evaporate into the air in the form of NH_3 [14].

Phosphorus is a macronutrient that is needed by plants, there are no other elements that can replace its function in plants. Plants must get or contain enough P for normal growth [15]. Phosphorus in plants has an important function, namely in the process of cell enlargement, division, respiration, and photosynthesis. Phosphorus can improve the quality of fruits, vegetables, grains, and is important in the formation of seeds. Phosphorus can help in the process of accelerating root development and germination, can increase water use efficiency, increase resistance to disease which ultimately improves crop quality. If there is a lack of phosphorus, the plant will show symptoms of growth such as slow growth, stunted growth, and stunted root development. Symptoms that will arise on leaves are diverse, some plants show an abnormal glossy dark green color, fruit maturation is inhibited, development of fruit shape, color, and seeds develop abnormally [16].

The Phosphorus contained in the degradation product with indigo sol blue effluent does not reach quality standards to Minister of Agriculture Regulation [10], the minimum quality standard for phosphorus content of LOF is 3-6%. In the substrate, there is a relationship between phosphorus and nitrogen content. the greater the nitrogen contained, the greater the number of microorganisms that break down phosphorus [17]. Phosphorus on the substrate will be used by certain microorganisms for cell growth and development.

The nitrogen content and phosphorus content were obtained from the structure of Indigosol Blue which contained anthraquinones and had ─NH and C=C molecular bonds. This dye waste has a strong chemical bond structure and is classified as non-biodegradable waste [3]. Indigosol Blue degradation products were identified as simple compounds called aliphatic compounds containing C=C, C-O, and -OH bonds [18].

The results research use of degradation product from tofu industrial waste gave results that affected changes in weight of cucumber plants. The treatment of changes in fruit weight of cucumber plants with a LOF dose from tofu waste of 25 ml showed the best results. That application of LOF from tofu industrial waste gives results that have less effect on plant length, number of leaves, leaf area, fruit length, number of fruits, and fruit circumference on cucumber plants [19].

In this research use of degradation products is derived from the degradation process of indigo sol blue batik effluent. The results showed a total nitrogen content of 0.11% and phosphorus of 0.01%. That indigo pasta batik natural dye waste for the manufacture of LOF with bio activator EM-4 has nitrogen elements with an anaerobic process of 0.11% and an aerobic process of 0.16%. Phosphorus element with the anaerobic process is 8.47% and aerobic is 8.49% [20].

The results of the degradation of batik effluent obtained potassium and carbon content. The carbon (C-organic) content was obtained from the structure of Indigosol Blue. Indigosol Blue contains Anthraquinone ($C_{14}H_8O_2$) and has molecular bonds of -NH and C=C. After degradation by *Aspergillus* sp. 3, the Indigosol Blue changed to a simpler structure which is an aliphatic compound [3]. The aliphatic compound contained bonds of C-O, C=C, and -OH that were identified by [18].

The potassium (K_2O) content result was fewer than carbon content. Indigosol Blue does not contain K_2O [3]. The potassium was found because the potatoes were contained in PBD media. Potatoes have high K_2O. The value of the potassium content of potatoes is 510 milligrams in a cup medium [21].

The result of assay potassium (K_2O) and C-organic content in degradation indigo sol effluent on Table 3.1. showed assay the content of K_2O by spectrophotometry and AAS methods was 0.01%. The Carbon (C-organic) by spectrophotometric methods obtained 0.24%. The results were not reaching the minimum standard of the previously mentioned Agriculture Minister Regulation [10], namely, the minimum quality requirement for LOF is 3-6% of K_2O and a minimum of 6% C-organic. Besides the degradation of indigo sol blue effluent, other batik dyes effluent also has the potential as LOF. Industrial waste for making batik natural dyes that are no longer used in the process, in the form of indigo paste (*Strobilanthes cuisine*), can be reused as raw materials for other industries, such as for LOF with the composting process [20]. Nitrogen (N) content anaerobically and aerobically has almost the same value, namely 0.11 percent and 0.16 percent, while the value of Phosphorus (P_2O_2) anaerobically and aerobically also has almost the same value, which is 4.87 ppm and 4.86 ppm, and Potassium (K_2O) in liquid organic fertilizer (compost) 2137.53 mg per kg for the

aerobic process and 556.30 mg per kg for the anaerobic process.

The degradation result may be used as LOF if added with have high nutritional content by others fermentation. Nutrients that can be added are obtained from fermenting banana peels or fermenting cow urine. Fermented content of banana peel waste added EM4 has K_2O 5.80% and C-organic 13.40% [22]. The content of potassium and carbon reached the minimum standard of LOF production. While fermented cow urine of Desa Dhusun Thekelan added EM4 in 6 days contains 7.80% organic C-content and 1.18% potassium [23]. The content of potassium was low, but the C-content was reaching the minimum standard of LOF production.

Using wastewater as fertilizer develops environmentally friendly, chemical-free processes that allow the recovered salt to be converted directly into organic nutrients for food crops [24]. Wastewater, sewage sludge, and liquid manure are sources of fertilizer that can be used for food production. The process that occurs is electrochemical which does the precipitation of struvite (magnesium ammonium phosphate), through electrolysis of a solution containing phosphorus and nitrogen. This process does not require the addition of a synthetic base or salt. In this electrolysis event, water molecules break down into negatively charged hydroxyl ions at the cathode. Oxidation then occurs at the anode to form struvite, as magnesium ions migrate through the water and react with the phosphate and ammonium molecules in solution. The struvite is then precipitated from the process water in the form of small crystals that can be directly used as fertilizer, without further processing. This process benefits because it saves energy and is free from chemical processes.

Nevertheless, it has the potential as LOF, therefore the test was carried out on plants. The plant used is spinach. Spinach was watered regularly with degradation products, fermented degradation products, and then observed the frequency of wilting and death (Table 2).

Table 2. The Results of Treatment in Plants

Treatment	Frequency of wilted plants (%)	Frequency of dead plants (%)
Water	40%	0%
Degradation product	0%	0%
Degradation Fermentation	0%	0%
Effluent	0%	40%

The results of fermentation of degradation products caused 0% wilting frequency and plant death as well as degradation products when compared with treatments using water (40% plant wilt frequency) and effluent (40% plant mortality frequency). This shows that the degradation product of batik liquid waste has the potential as a LOF but must be redeveloped with certain treatments to achieve the required composition as fertilizer.

The occurrence of death in the treatment of giving waste could be due to lack of macronutrients such as N. Nitrogen (N) as the main nutrient for plants is generally indispensable for the formation or growth of vegetative parts of plants, such as leaves and roots. Deficiency can result in severely impaired growth rate, causes the leaves to turn yellow or experience chlorosis, and a strong deficiency causes browning and death [25].

The product degradation of indigo sol blue batik effluent had a total nitrogen content of 0.11%, K_2O of 0.01%, organic carbon of 0.24%, C/N ratio of 10.13%, while for phosphorus content of 0.01%. Fermentation of degradation products causes 0% frequency of wilted and dead plants, better when compared to water (40% frequency of wilted plants) and effluent (40% frequency of wilted and dead plants).

AUTHORS' CONTRIBUTIONS

RSD came up with the presented idea and supervised the findings of this work. MK assists in project work and makes analytical methods. All authors contributed to processing the research data, writing the manuscript, and playing a role in working on the final manuscript.

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ORIGINALITY REPORT

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