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Science Practicum Activities through Freshwater Bioremediation Experiments on Biotechnology Materials in Junior High Schools

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Abstract: This research aims to design practical learning for bioremediation of aquatic ecosystems from dye waste by utilizing enzymes extracted from baglog waste. The topic chosen was bioremediation of water polluted by acid blue 113. Purpose of this practicum is to provide in learning science by conducting a practicum that supports environmental sustainability. Variables in this study were enzyme extracts taken from baglog waste of brown oyster mushroom and the percentage of textile dyedecolorization. As a results showed that the percentage of decolorization of wastewater polluted by acid blue dye 113 was as follows: concentrations of 30 ppm (45.41%), 45 ppm (49.7%), 60 ppm (35.08%), and 75 ppm (23.09%). The enzyme extract of mushroom baglog waste can effectively degrade the content of acid blue 113 textile dye which is dissolved in water. This study is the first to be conducted in Indonesia which can be done easly and using available materials.

Keywords: bioremediation, decolorization, baglog waste, mushroom.

Abstrak: *Penelitian ini bertujuan merancang pembelajaran praktikum tentang bioremediasi ekosistem perairan dari limbah pewarna dengan memanfaatkan enzim yang diekstrak dari limbah baglog. Topik praktikum yang dipilih adalah bioremediasi air yang tercemar pewarna tekstil acid blue 113. Tujuan praktikum ini adalah untuk memberikan bekal dalam pembelajaran IPA dengan melakukan praktikum yang mendukung kelestarian lingkungan. Variabel dalam penelitian ini adalah ekstrak enzim yang diambil dari limbah baglog jamur tiram coklat dan persentase dekolorisasi zat warna tekstil. Hasil penelitian menunjukkan bahwa persentase dekolorisasi air limbah tercemar zat warna asam biru 113 adalah sebagai berikut: konsentrasi 30 ppm (45,41%), 45 ppm (49,7%), 60 ppm (35,08%), dan 75 ppm (23,09%)). Hasilnya menunjukkan bahwa ekstrak enzim limbah baglog jamur secara efektif dapat mendegradasi zat pewarna tekstil acid blue 113 yang terlarut dalam air. Studi ini merupakan yang pertama kali dilakukan di Indonesia yang dapat dilakukan dengan mudah dan menggunakan bahan-bahan yang tersedia.*

Kata kunci: *bioremediasi, dekolorisasi, limbah baglog, jamur.*

▪ INTRODUCTION

The implementation of the curriculum 2013 in Indonesia aims to the achievement of attitudes, knowledge and skills competencies of students (Rahayu, 2019). In the curriculum 2013 development guidelines it is mentioned that science learning at the junior high school level must emphasize on scientific approach. Because, in groundworking of learning science, it introduces students to the dimensions of the scientific process, not only memorizing concepts. Furthemore, junior high school science learning is developed as an subject of integrative science that is applicatively oriented, developing 21st century skills, building a concerning and responsibility towards the natural and social environment (Susilowati, 2015). Practicum and science learning are two things that cannot be separated, because practicum provides the basis

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for developing scientific process skills and attitudes (Subiantoro, 2010). Science practicum is a way for students to investigate natural phenomena to generate a product that is in accordance with the characteristics possessed by science. Science process skills and 21st century skills are two things that are a entity that can be developed through practicum (Duda, Susilo, & Newcombe, 2019). In one study, it was stated that science learning using the practicum method and discovery model could effectively develop students' science process skills (Oktafianto, 2014).

The development of environment-based practicum has often been studied. This was done to make it easier for teachers to implement the demands of the Curriculum 2013 with relevant criteria (Subamia, Wahyuni, & Widiasih, 2014). Determining the model in practicum can affect the achievement of the expected competency, one that is often used is the problem-based learning model (Fitriani, 2019). Basic competencies of science learning in ninth grade is applying the concept of biotechnology and its role in human life as an aspect of knowledge, as well as making one of the conventional biotechnology products in the surrounding environment as an aspect of skills. All this time, activities that focus on skills aspects have only been in the form of classroom practise that are confirmative of the knowledge being studied. In fact, if it is related to environmental and sustainability issues, practise on biotechnology materials can be carried out through more innovative experimental methods. Overcoming the most common environment and can be used as an alternative topic in junior high school practise is about pollution in the aquatic environment (Suryawati et al., 2020). Taking up an environmental issues in conducting practical learning can improve students' critical thinking skills effectively

Related to water pollution, the textile industry that uses textile dyes can be appointed as a major problem of environment. Because those industry produces waste in the form of liquid substances that can pollute rivers and waters in Indonesia. According to (Lellis et al., 2019) one of the causes is that synthetic dye waste, such as that used in the textile industry, will have detrimental effects if released into the aquatic environment as waste, thereby changing water quality, interfering with the photosynthesis of aquatic plants, inhibiting the growth of aquatic plants, and increasing toxicity, mutagenicity, and carcinogenicity. As a consequence, DO (Dissolve Oxygen) levels in aquatic ecosystems will decrease, and will impact on an increase in COD (Chemical Oxygen Demand). Through practicum learning students are directed to investigate chemicals that can cause pollution, then students are encouraged to provide ideas as a solution that is in accordance with the principles of green chemistry (Putri, 2019).

Relevant research to this article is about the practicum activity of phytoremediation of textile dyes which was carried out at the high school level. They believe that phytoremediation of dyes is suitable and easily understood by both college students and high school students (Ibbini, Davis, & Erickson, 2009). In the practicum we did, the material used for dye remediation came from baglog waste, thus providing a dual solution to two waste problems that occur in the surrounding environment. The experiments in the practicum that we carried out did not only use one dye variable, but there were 6 textile dyes so that students promoted more in-depth inquiry. Based on this situation, students were introduced to alternative solutions for overcoming with textile dye waste that had already been disposed of in the waters. According to Sen et al.

(2016) several types of fungi have been studied for their ability to decolorize dyes. In Indonesia, with abundant biodiversity, potential mushroom resources can be used as synthetic dye removal agents from the textile industry which are considered very effective, inexpensive and environmentally friendly. There are many agricultural agents who cultivate mushrooms for food purposes so that they require the main material in the form of a planting medium which is called "baglog". Because mushroom growing media has usage limits and will eventually become waste. So far, the use of baglog that has been carried out a lot includes only being used as organic fertilizer and making briquettes. From this situation, practical learning at the junior high school level on biotechnology material has the opportunity to become an innovation, namely a learning by developing practicum in the form of simple bioremediation activities on water polluted by textile dyes. So far, research on bioremediation learning has only been carried out at the high education level through problem-based learning (Wahyuni, Rahayu, & Indana, 2020).

▪ METHOD

Participans

This research was conducted as a trial phase in the development of a science practicum, aiming to facilitate its implementation among junior high school students. The activities were meticulously carried out in collaboration with pre-service students from the Science Education Department. Their role was to thoroughly assess the readability and practicality aspects of the procedures and practicum activities that were being developed. As a result, the focus of this study was not on determining a specific population or utilizing sampling techniques. Instead, the primary objective was to obtain valuable feedback and insights from the pre-service students, ensuring that the procedures and activities were refined and optimized for future implementation in junior high school settings.

Research Design

This research is the first practicum learning method development conducted on biotechnology materials for science learning at the junior high school level. The purpose of practicum development is to provide an alternative in learning science by conducting practicums that support environmental sustainability. Meanwhile, experimental method is used through practicum with the tools and materials which available in the school laboratory.

In this study, the research instrument used was field notes. Field notes were created to record and document direct observations made by the researcher during the process of designing a practical learning module on the bioremediation of aquatic ecosystems from dye waste using enzymes extracted from spent mushroom substrate. Observations also included the stages of enzyme extraction from the spent mushroom substrate and the process of adding the enzymes to the contaminated aquatic ecosystem.

The independent variables in this bioremediation experiment include is an enzyme extract from oyster mushroom waste which can be made simply in a school laboratory, while the dependent variable determined is the degree of color change due to the process of decolorizing water polluted by textile dyes namely Acid Blue 113 (Table 1) with varying concentrations specified dye. After conducting the practicum, the experimental results were subsequently presented in the classroom to observe the students' responses. After conducting the practicum, the experimental results were subsequently presented in the classroom to observe the students' responses.

Table 1. Tools and materials

Tools		Materials	
Name	**Qty**	**Name**	**Qty**
1. Blender	1	1. Brown Oyster Mushroom Baglog Waste (*Pleurotus cyctidiosus*)	200 grams
2. Centrifuge or Mesh Filter no. 200 & 400	2 4		
3. Beaker	2	2. Distilled water	1 litres
4. Glass Funnel	1	3. Textile dye solution of Acid Blue 113	500 ml
5. Digital Scale	8		10
6. Test Tube	2	4. Filter paper	sheets
7. Drop pipette	3		
8. Measuring Cup	2		
9. Spatulas	1		
10. TDS meter	1		
11. Spectrophoto meter)*			

Hazardous materials: acid blue 113 (irritant & environmental hazard)
)* = quantitative measurement tools that can be replaced with qualitative measurements that can be conducted at school.

Procedure

The first step in this practicum procedure is to weigh and mix as much as 100 grams of mushroom baglog waste with 400 milliliters of distilled water which is carried out at 4 degrees Celsius. The mixture of both ingredients was then crushed using a blender (table 1) for 10 minutes. The second step in extracting the enzyme is centrifuging the mashed mixture at 8000 rpm for 10 minutes to produce a supernatant, it some of crude enzyme extracts. For most schools that are not available centrifuge (Tabel 1). They can perform a simple technique, namely using a mesh filter with a size of 200 and 400 (Table 1). The result is a slightly clear crude enzyme but can still be used for the bioremediation process. We recommend that at least 200 milliliters of crude enzyme be available to meet the requirements for the bioremediation process Put the crude enzyme obtained into the beaker and then cover with aluminum foil. Furthermore, the crude enzyme must be stored in the refrigerator with a constant temperature of 4 degrees Celsius. It's done so maintain the quality of the enzyme so that it is maintained properly.

The second step in this practical lesson is to prepare samples of water contaminated with acid blue 113 textile dye (Table 1). Aquades water is prepared in 4 beakers of 50 milliliters each. Furthermore, in each beaker containing distilled water, acid blue 113 textile dye was added with a concentration range of 30, 45, 60 and 75 ppm. Then the water was measured temperature and pH. In determining the ppm level of the solution easier, schools can provide TDS meters (Table 1) which are sold at farm shops. Before use, the TDS meter must be calibrated with a solution that can also be purchased at the store.

The third step in this practicum learning design is to carry out the bioremediation process of water contaminated with textile dyes using enzyme extracts. The first thing to do in this last activity is to prepare 45 milliliters of a solution polluted by textile dye waste with various concentrations. The remaining 5 milliliters of solution is used as a control variable (P0) to be compared later. Textile dye solutions with varying concentrations were then determined to be dependent variables with the names P1 (30 ppm), P2 (45 ppm), P3 (60 ppm), P4 (75 ppm). Then each added 5 milliliters of enzyme extract. After that, let the dye solution that has been given enzyme extract stand and observe changes within 24 hours. Observations can be made in two methods, direct observation by comparing the color with the control solution or if equipment is available at the school, quantitative observations can be made using a spectrophotometer.

The last stage is implementation, where the researcher incorporates the findings of the practicum into the biotechnology science curriculum. During this stage, students are provided with the necessary tools and practicum materials. They are then instructed by the teacher to engage in practical activities. The outcomes of implementing these activities in the practicum are reflected in the students' responses to the learning process.

Data Analysis

After the data is collected, the data is then analyzed descriptively to describe the right formulation that can be used for science practicum activities at the junior high school level. Data analysis is described in tables and graphs to see baglog waste's effectiveness in reducing Acid Blue 113 textile dye levels. Data is entered according to the rubric grouping to determine the level of student response to the learning process, as shown in Table 2 (Arikunto, 2013).

Tabel 2. Criteria for interpreting student response scores

Percentage (%)	Category
0-20	Very weak
21-40	Weak
41-60	Quite Good
61-80	Good
81-100	Very Good

▪ RESULT AND DISSCUSSION

In this study, the discussion focuses on the design of a practical learning module on the bioremediation of aquatic ecosystems from dye waste using enzymes extracted from spent mushroom substrate. The objective of this module is to provide students with practical experience in environmental science education with a specific focus on sustainability.

The selected topic for the practical module is the bioremediation of water contaminated with acid blue 113 textile dye. In this module, the variable of interest is the enzyme extract obtained from spent mushroom substrate of the brown oyster mushroom, as well as the percentage of textile dye decolorization.

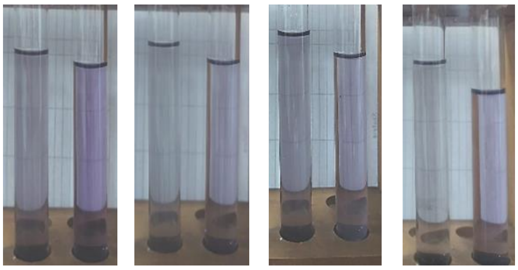
The research findings demonstrate that the enzyme extract from spent mushroom substrate effectively degrades the acid blue 113 textile dye dissolved in wastewater. The percentage of decolorization varies at different concentrations of the dye tested. At a concentration of 30 ppm, the decolorization percentage reached 45.41%, while at a concentration of 45 ppm, it reached 49.7%. Meanwhile, at concentrations of 60 ppm and 75 ppm, the respective decolorization percentages were 35.08% and 23.09%.

This research holds significant importance as it represents the first study conducted in Indonesia within this particular context, which can be easily implemented using readily available materials. Consequently, this practical module presents an attractive alternative to introduce students to the concepts of bioremediation and environmental sustainability through a hands-on approach in science education. The findings of this study contribute to the development of environmentally friendly bioremediation methods and have the potential for real-world applications in the preservation of aquatic ecosystem sustainability.

Utilization of lignocellulosic waste for the production of ligninolytic enzymes has been studied extensively. Research on the production of laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP) from various agro-industrial residues has been carried out by many researchers. Most of the production of ligninolytic enzymes from agro-industrial residues is related to fungi (Gassara et al., 2010). In this study, enzymes were extracted using conventional filtration methods using distilled water without a purification process (crude enzyme) so that students could easily work on them. The baglog waste (Figure 1) that we use has an average incubation period of 40 days because in previous studies it was stated that this time had the highest enzyme activity (Dimawarnita & Perwitasari, 2017).

Figure 1. Mushroom growing media called baglog

The bioremediation process was carried out at room temperature 25oC and the pH of the enzyme extract was 6.4. Based on the experimental results, water contaminated with textile dyes mixed with enzyme extracts and stored for 24 hours showed a color change at all concentrations of textile dyes (Figure 2).



30 ppm 45 ppm 60 ppm 75 ppm

Figure 2. The left tube is a textile dye of Acid Blue 113 that given enzyme extract and the right tube is a textile dye as a control solution after 24 hours.

By comparing the control and treatment solutions, it can be seen that the treatment solution has a clearer color than the control solution (figure 2). The purple color faded clearly in the control solution. This kind of qualitative analysis is carried out if a spectrophotometer is not available in the school as a more reliable quantitative measurement tool. Meanwhile, based on the results of absorbance measurements using a spectrophotometer, the following data are obtained (Table 3):

Table 3. Absorbance reading percentage using a spectrophotometer

Sample	Wavelenght (nm)	Result	
		24 Hours	Absorbance
Control 1	500	0.394835	45.41821267
Treatment 1		0.215508	
Control 2		0.292146	49.79291176
Treatment 2		0.146678	
Control 3		0.46351	35.08295398
Treatment 3		0.300897	
Control 4		0.410995	23.09176511
Treatment 4		0.316086	

Based on the Table 2, it shows that the waste water contaminated with acid blue 113 dye with a concentration of 30 ppm showed a decolorization of 45.41%, the wastewater sample with a concentration of 45 ppm showed an increase in decolorization of 49.7%, in the third sample the decolorization showed a decrease with a value of 35.08%, while for the last sample it was 23.09%. The percentage of decolorization should have an inverse relationship with the textile dye concentration. So that in order to increase the percentage of liquid waste decolorization, it is necessary to increase the concentration of enzymes to produce an optimal bioremediation process (Gahlout et al., 2013). In this study, there were inconsistent data on the decolorization of wastewater with a dye concentration of 45 ppm. This can occur due to a measurement error when determining the mass of the dye with an digital balance or an inaccurate total disolve solid (TDS) reading.

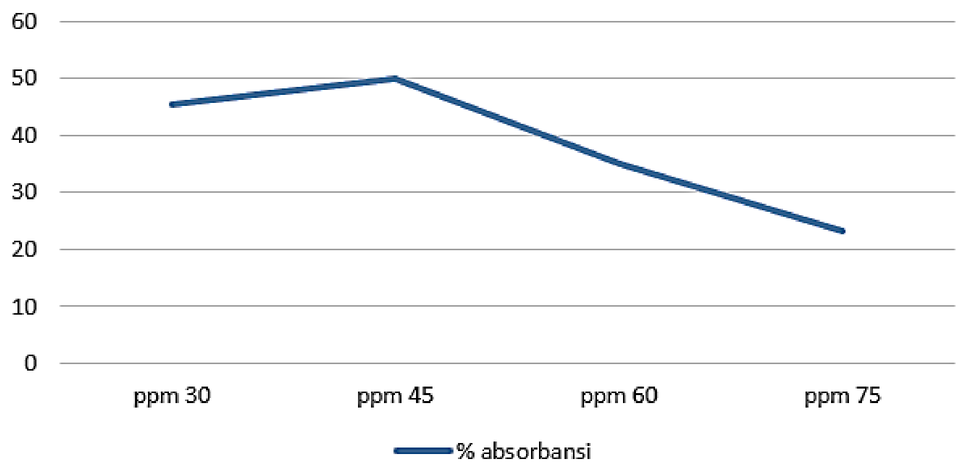


Figure 3. Decolorization percentage based on dye concentration level

The ability of lignolytic enzymes to degrade the Acid Blue 113 textile dye is due to the similarity in chemical structure between the dye compounds and the lignin found in wood/trees. Lignin as a natural wood-forming material in ecosystems is a substrate for decay fungi and then undergoes a decomposition process (Rekik et al., 2019). This similarity is the presence of an aromatic ring structure between groups of phenolic textile dyes and lignin which will react with a catalyst in the form of laccase and manganese peroxidase enzymes. Laccase is an oxidizing phenoxy containing copper (Cu) and using oxygen as its electron acceptor, then laccase will oxidize phenolic rings to become phenoxyl radicals. Laccase contains four or more copper atoms which are able to catalyze organic aromatic components and degrade molecular oxygen and convert it into water. In addition, laccase is also able to oxidize non-phenolic compounds under certain conditions, such as when a reaction is added with ABTS (Tuomela et al., 2000). Meanwhile, MnP has the ability to catalyze the oxidation of $Mn2+$ which is very reactive. This process is stabilized by organic compounds such as oxalic acid, resulting in $Mn2+$ bonding using oxygen to become $Mn3+$ which oxidizes phenolic substrates as non-specific oxidants that can diffuse freely (Hofrichter, 2002). MnP also has the ability to oxidize lignin and other phenolic compounds that depend on free manganese ions (Hofrichter, 2002).

Based on the results of student responses, it shows that most student responses are very positive with a percentage of 90% and quite good at 10%. This indicates that the bioremidiation practicum is well received by students. Discussion on innovations of practicum activities that are not widely carried out by researchers, especially the topic of how to overcome with environmental pollution. But as we know, sustainability education is very important to prepare students who will become environmental caretaker in the future (Vozzo & Smith, 2017). Bioremediation is very appropriate to be introduced to junior high school students. Freshwater waters such as rivers and lakes should be of primary concern because water is the source of life and builds a unique tropical ecosystem (Beeton, 2002). Learning to recover polluted water can be started by understanding how lignolytic enzymes work in degrading textile dyes. In Indonesia, students do not need to find it difficult to find textile dyes or baglog waste. Baglog waste, which so far has rarely been recycled or reused (Israilides & Philippoussis, 2003), is the main material brought to the laboratory. They can extract enzymes in a practical and simple way. Students can also experiment with various colors of textile dyes. The results would certainly be encouraging if this activity could become a more developed program, especially if students were involved in it.

▪ CONCLUSION

Biotechnology practicum with bioremediation experiments on polluted water can easily be carried out in school laboratories. Students can compare and analyze the factors that affect the work of enzymes and find a more optimal solution in the utilization of enzymes from baglog mushroom waste for the degradation process of textile dyes. After carrying out the bioremediation practicum in water polluted by textile waste, it can be concluded that the enzyme extract of baglog mushroom waste can effectively reduce or degrade the levels of Acid Blue 113 textile dye which is dissolved in water. The percentage of decolorization was 45.41% (30 ppm concentration), 49.7%

(45 ppm concentration), 35.08% (60 ppm concentration), 23.09% (75 ppm concentration).

In general, practicum activities in Indonesian schools that raise the topic of pollution do not try to take real action to reverse the effects of pollution. This article provides new ideas for practicum that encourage students to find solutions to environmental problems. The limitations of this research may only be applied to schools located around agricultural areas that provide baglog waste. So it is very possible difficult to do in urban schools.vThis classroom practicum has never been studied in a learning experiment, so it has a great opportunity to be developed by future researchers. They can also improvise variables by changing or increasing the number of colors of textile dyes, taking into account other influencing factors, using other types of mushrooms, and modifying enzyme extracts in a better way.

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