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Treatment of Fabric Dye Wastewater from Household Industries Using Fixed Bed Reactor (FBR) With Local Microbial Isolates from Water Channel Sediment in Denpasar City, Bali Province, Indonesia

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Abstract

The research was intended to study the capability of the Fixed Bed Reactor (FBR) combined with volcanic rocks and local microbial isolates on treating fabric dye wastewater. The microbial isolates were cultivated from the sediment of water channel in Imam Bonjol area, Denpasar City and then attached onto the surface of volcanic rock which was taken from the slopes of Mount Batur. In addition to examine the treatment performance, this research also determined the profile of biomass growth related to the origin of sediment samples as the source of microorganisms, and explain the effect of various nutrient compositions on biofilm formation. Furthermore, this research determines the effectiveness of FBR in reducing color content, COD, and BOD_5 of fabric dye wastewater. The research workflow was divided into three stages. The first stage was cultivating microbial isolates with several various nutrients. Second stage was growing biofilm on rock with various media composition, and third was determining the ability of biofilm to reduce color, COD and BOD of dye industries wastewater. The results showed that the nutrient composition presented a significant effect on the growth of local cultures in the active suspension. Furthermore, the composition of media also showed notable effect on the formation of biofilm in FBR. FBR was able to reduce levels of color, COD, BOD_5 with successive effectiveness of 87.35% 87.25% and 93.11% in 8 hours of FBR treatment.

Keywords: BOD; COD; Color; FBR; Local culture; Volcanic rock

1. Introduction

The fabric dyeing cottage industry is likely to continue to develop along with the economic growth. It is undeniable that the coloring of the fabric is to increase the attractiveness and commercial value of the textile products produced. Dyes used in the textile industry are generally synthetic dyes that are not environmentally friendly. Dyes are widely used for dyeing cotton, wool, silk, rayon, and nylon fabrics (Rani, 2016). The impact of waste containing dyes can threaten environmental sustainability, especially waters and pose a health risk because of its toxic, carcinogenic and mutagenic properties (Asses *et al.,* 2018). Therefore, it is very important to treat dyestuff waste before being discharged into the environment.

Several microorganisms that have been reported to degrade textile dyes such as *Serratia nematodiphila, Morganella morganii, Lysinibacillus* sp. and *Bacillus* sp. which can degrade the azo group of dyes (Quan *et al.,* 2018), *Enterobacter* sp. which can degrade crystal violet dyes (Roy *et al.,* 2018) and *Klebsiella* can degrade brilliant green dyes (Zablocka-Godlewska, *et al.,* 2015).

The biodegradation of dyes using microorganisms involves a reduction-oxidation (redox) reaction with the help of an enzyme catalyst (Yoo, 2000). Dye-degrading microorganisms generally have the ability to secrete enzymes such as azoreductase, laccase, and peroxidase, which are enzymes that can remodel the chemical structure of dyes through certain mechanisms resulting in simpler and colorless molecules (Sudha *et al.,* 2018). The use of a consortium of microorganisms can be developed with an attached growth system or known as a biofilm. Biofilm is a layer of microorganisms attached to the surface of the media. The use of this biofilm is often called FBR, relatively easy to form, efficient in the use of nutrients, relatively cheap operating costs or economical and can be used in a sustainable manner (Sastrawidana *et al.,* 2012).

Based on the results of previous studies, it was shown that the treatment of dyestuff waste using FBR was quite competitive in the ability to reduce dye levels and pollutant parameters such as COD, BOD, TDS, TSS with treatment efficiency up to 68% - 97% (Francis and Sosamony, 2016). In this study, the development of dye waste treatment using FBR was conducted by generating an active suspension inoculum from seedlings of local sediments taken from the water channel that was close to the household dye industries. This study aims to determine the profile of biomass growth related to the origin of soil samples as a source of microbes, explain the effect of nutrient composition on biofilm formation, and at last determine the effectiveness of FBR in reducing the levels of color, COD and BOD_5.

2. Research Method

2.1 Sediment Samples Collection

Sediment samples were taken from the water channel around the Imam Bonjol street in Denpasar, Bali using the grab sampling method. Sediment was taken from 3 different sampling points within a depth of ± 10 cm from the surface of each channel and as much as 100 grams of sediment samples were taken. The sampling points can be seen in Figure 1. These sediment samples were the source of microorganism for the sludge activation process.

2.2 Sludge Activation as an Activator

In order to generate the microbial isolates, nutrients solution need to be prepared. Microorganisms' nutrients solution was prepared by weighing as much as 2.0 grams of glucose; 0.1gram K_2HPO_4; 0.1 gram KH_2PO_4; 0.1 gram $(NH_4)_2(Fe(SO_4)_2).6H_2O$; 0.02 gram $MgSO_4.7H_2O$; 0.02 gram $FeSO_4.7H_2O$; 0.02 grams of yeast extract and mixed it with distilled water until the volume reaches 2 L. The solution was sterilized for 15 minutes at

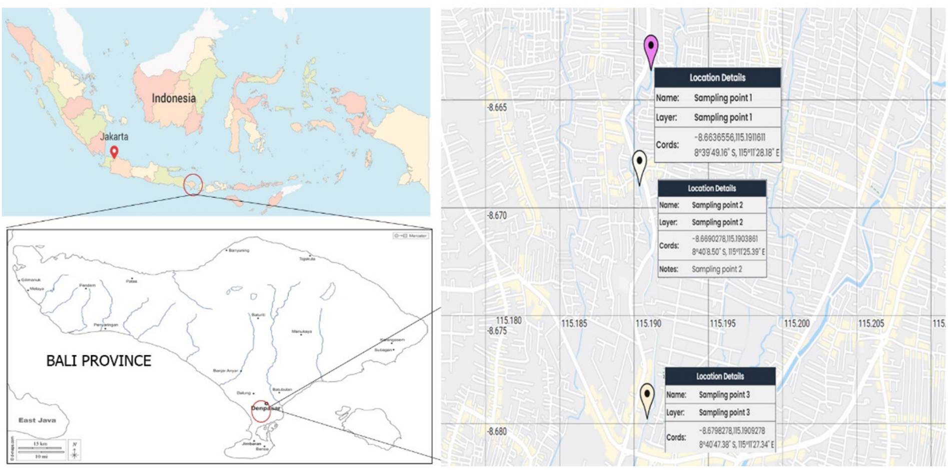


Figure 1. Sediment sampling points in Denpasar City, Bali Province, Indonesia.

a temperature of 121 °C using an autoclave, after which it was allowed to stand at 37 °C for 5 minutes, then the media, was stored in the refrigerator until it is needed (Kurniawan *et al.,* 2017). Activation was then carried out by taking ± 5 grams of each sediment samples (from sampling point 1, 2 and 3) and then each of them was put into a 500 mL Erlenmeyer and then filled with 300 mL of nutrients solution. Each solution was labelled as Nutrient 1 (S1), Nutrient 2 (S2), and Nutrient 3 (S3) according to the origin of the sediment samples respectively. After that, it was incubated at room temperature. The growth rate of active biomass was observed by measuring the Mixed Liquor Suspended Solid (MLSS).

2.3 Fixed Bed Reactor Preparation

Particular volcanic rocks obtained from Mount Batur (± 1 cm diameter) were prepared and sterilized. It was then arranged into a tub with dimensions of length x width x height for about 40 cm x 22.5 cm x 13 cm. In order to fill 75% of the tub space, 2.5 kg of sterilized corals were collected and arranged into a porous plastic framework. Three variations of the treatment were prepared for each tub with an effective volume of 10 L. It was filled with nutrients and active (selective) suspension with specification as follows: media I (K1) contained 30% nutrient (1% active suspension) and 70% dying wastewater; media II (K2) contained 40% nutrient and 60% wastewater; and media III (K3) contained 60% nutrient and 40% wastewater. The biofilm formation was identified by the type of microorganism and the number of colonies. Visual observation of the surface of the corals, before and after the biofilm was formed, was carried out using a Scanning Electron Microscope (SEM). The Fixed Bed Reactor scheme can be illustrated as in Figure 2.

2.4 Dye Textile Wastewater Treatment Using Fixed Bed Reactor

A total of 6 L of dye waste with a color concentration of 25 ppm was processed using a Fixed Bed Reactor system and observed at variations in treatment time of 0, 2, 4, 6, and 8 hours. Treatment observations were carried out on changes in the dyestuff content of COD, and BOD_5.

2.5 Calculating the Colour, COD and BOD_5 Removal Efficiency in Fixed Bed Reactor

The effectiveness of Fixed Bed Reactor treatment with active suspension on the surface of the coral was calculated according to the effectiveness of the process, namely the percentage reduction in color content, COD, and BOD_5 concentration levels during the processing process. The percentage of reduction in color content, COD, and BOD_5 respectively was determined based on the following equation :

$$\%\text{effectiveness} = \frac{C_a - C_t}{C_a} \times 100\%$$

Definition:
Ca = color parameter; COD; dan BOD initial (mg/L)
Ct = color parameter; COD; dan BOD (mg/L) (in particular time)

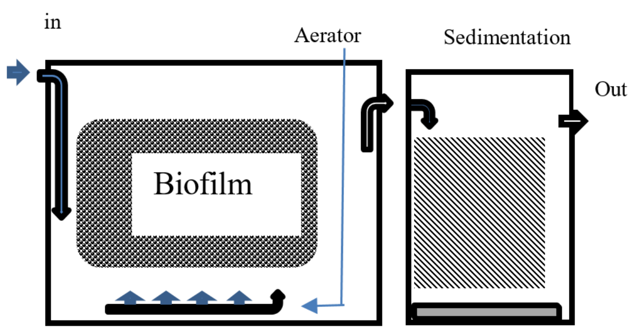


Figure 2. Fixed Bed Reactor Scheme

2.6 Data Analysis

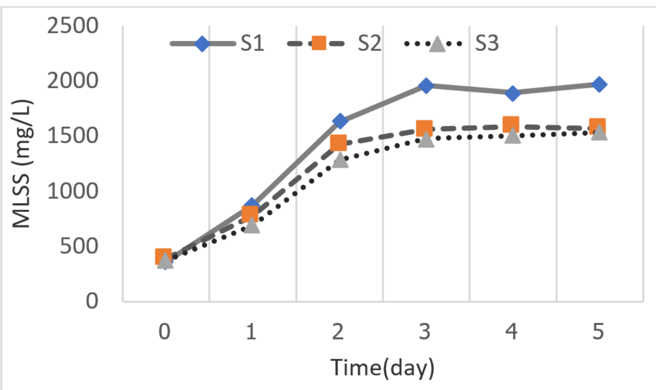
Data analysis was carried out qualitatively and quantitatively. Qualitative data analysis was conducted by describing the results of fabric dyeing waste treatment with a Fixed Bed Reactor, while quantitatively by calculating the levels of pollutants that can be degraded by Fixed Bed Reactors with active suspension. The data obtained were analyzed statistically in the form of covariance analysis and Least Significant Differences (LSD) using the SPPS 16.0 application.

3. Results and Discussion

3.1 Sludge Activation

The growth of biomass during the active suspension seeding process was observed through MLSS measurements. The results of the observations that showed the MLSS value during the active suspension seeding process from the three specified locations is presented in Figure 3.

There were significant differences in MLSS profiles on nutrient variations during the incubation process. The active suspension of nutrient 1 has the best growth profile when it is compared to nutrient 2 and 3. This happened because of the availability of macro and micro elements with better solubility properties. The increase in MLSS indicates an increase in biomass due to the activity of microorganisms. The higher the activity of microorganisms in breaking down organic matter, the higher the biomass produced (Kriswidatari *et al.,* 2017). The highest increment in biomass occurred in the exponential growth phase. In the exponential phase, microbes grow rapidly with cells producing the metabolites needed for their growth (Irdawati *et al.,* 2018). The maximum MLSS in the active suspension of S1 was 1893.00 mg/l, while for S2 and S3, 1587.43 mg/l and 1503.51 mg/l, respectively. The stagnant phase in the three treatments occurred after the fourth day, where there was a decrease in MLSS. The active suspension reaches the death phase, cell growth begins to stop and the bacteria have used up their reserve energy in term of Adenosine Triphosphate (ATP) for respiration (Madigan *et al.,* 2008). The different growth of each nutrient treatment was taken place due to the differences in microbial diversity and the ability to adapt to nutrients. S1 nutrient treatment was able to provide nutrients faster than S2 and S3. Previous research reported that the location of sediment sampling with a high level of organic pollution was able to produce optimal biomass growth (Widyasari *et al.,* 2017).



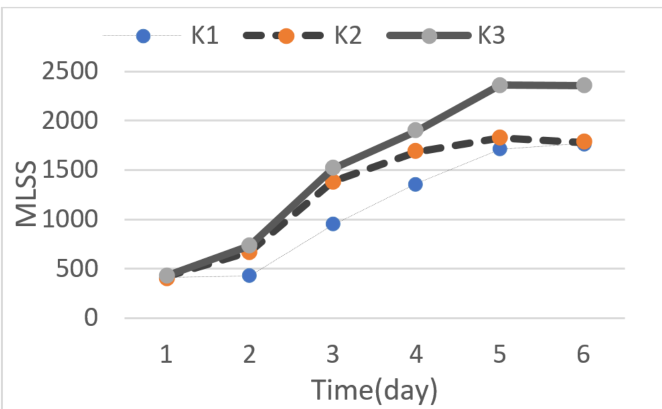
Annotation:
S1 = Nutrient 1
S2 = Nutrient 2
S3= Nutrient 3

Figure 3. Comparison of biomass growth in different nutrients.

Changes in pH during active suspension seeding showed that the average pH in the S1, S2, and S3 treatments were 7.23 - 7.83; 7.16 - 8.00; and 7.35 - 7.77. There was an increase in pH at optimum growth on the third day, and then decreased on the following day. It was caused by biochemical reactions, namely the process of decomposing organic substances and utilizing minerals contained in nutrients. Furthermore, there was also generation of CO_2, H_2O, energy and NH_3. Ammonia (NH_3) is a polar compound, which is alkaline, as a result of degradation of organic matter including dyes which dissolve easily in water. Meanwhile, the decrease in pH from the third day to the fifth day was caused by exothermic microbial activity resulted in a decrease in the solubility of ammonia (NH_3) in water. Ammonia concentration in water can vary depending on temperature, when the temperature is high the ammonia concentration will be lower than at low temperatures (Bitton, 2010). According to the results of the covariance analysis, it was discovered that the nutrient factor, seeding time and the interaction between times had a significant effect on the MLSS value. Further analysis of Least Significant Differences showed that nutrient II (S2) was not significantly different from nutrient I (S1), but was significantly different from nutrient III (S3).

3.2 Biofilm Formation

Biofilm formation is indicated by the growth of biomass on the surface of the media. The profile of biofilm growth on the composition of active suspension and liquid waste is shown in Figure 4. Figure 4 shows an increase in MLSS for each nutrient composition variation, which indicates biofilm formation. Biomass growth occurs from day one. The best growth rate occurs after three days. The peak of the biomass growth curve occurred on the fifth day. The highest biomass was achieved from the composition of media III (K3), reaching 1636.67 mg/l. Meanwhile, the composition of media II (K2) and media I(K1) respectively had a maximum biomass of 543.33 mg/l and 890.00 mg/l. After the fifth day, there was a decrease in biomass growth, a decrease in suspended biomass happened because the suspended solids or particles in the pores of the support medium and holding solids or particles, were not easily penetrating to the surface. This difference in biomass growth profile occurs depend on the availability of the consortium, acclimatization time and available nutrients. The availability of food reserves can increase the growth and activity of microorganisms (Minarti and Hermana, 2013).



Annotation:
K1 = 30 % active suspense and 70 % wastewater
K2 = 40 % % active suspense and 60 % wastewater
K3 = 60 % % active suspense and 40 % wastewater

Figure 4. Biofilm growth profile on different composition of active suspension and wastewater

The biofilm layer on K3 can be seen clearly in Figure 5, (a) the rock before being overgrown with biofilm and (b) the rock after being overgrown with biofilm. The SEM observation identified the type of biofilm-forming bacteria, namely *Klebsiella* sp. and *Bacillus* sp. The total number of bacterial colonies was 3.66 x 10^4 cfu/g of sediment. The number of colonies was sufficient enough for the dye waste treatment process. The results showed that the average pH of microbial growth during the biofilm formation process from nutrient composition I, II, and III had ranges of 7.13 - 7.63; 7.17 – 7.63; and 7.20 - 7.73 respectively. The pH value had increased from the first to the third day and the next day had decreased. There was an increase in the biological activity of microbes and the activity of overhauling the artificial dye waste contents. Meanwhile the decrease in pH happened due to a decrease in the solubility of ammonia that was caused by the exothermic activity of the microbes. The results of the covariance analysis showed that the nutrient composition and the interaction between time and nutrient composition had a significant effect on the MLSS value.

Meanwhile, biofilm formation time did not have a significant effect on the MLSS value. Further analysis of Least Significant Differences shows that the nutrient composition III (K3) is significantly different from the nutrient composition I (K1) and II (K2) on the MLSS value.

3.3 Biofilm Efficiency in Color Concentration Removal

Measurement of color content in the FBR system was carried out with a time span of 1 day. Furthermore, Figure 5 shows a significant decrease in colour contents (> 60% removal) at 2 hours of FBR treatment, from the colour levels of 136.80 Pt-Co unit to 52.60 Pt-Co unit with a removal efficiency of 61.54%. Furthermore, at the 8 hours of treatment, the colour content decreased to 17.30 mg/l with a removal efficiency of 87.35% (above 80%). The rapid decline in colour content in the first 2 hours indicated that the biofilm has been able to break down the dye into simpler compounds. The consortium in the active suspension that forms the biofilms was capable to reduce colour content by breaking the bonds of the dye. This was supported by the results of previous research reported by Abo-state *et al.* (2017) and Selva *et al.* (2012) that Bacillus and Klebsiella were able to degrade dye.

The reduction process used direct enzymatic reduction. In the process of bacterial cell metabolism, NADH (ncotinamide adenine dinucleotide) was produced which functions to transfer electrons to azo dyes catalyzed by azoreductase. The NADH produced would then undergo an oxidation reaction to produce NAD^+, while the azo dye undergoes a reduction to produce an aromatic amine compound which was indicated by

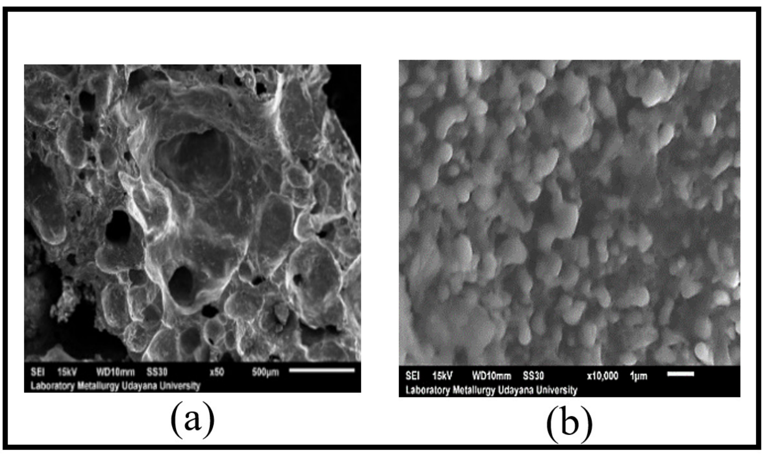


Figure 5. SEM observation (a) volcanic rock before biofilm formation; and (b) volcanic rock with biofilm formation on the surface

a change from colour to colourless. The aromatic amine that was formed then undergoes an oxidation process to form CO_2, H_2O and NH_3 compounds (Manurung, *et al.,* 2004). The ability of biofilm to reduce the color content was getting slower after 4 hours which indeed was caused by the declining source of nutrients and consequently leads to a decrease in the ability of breaking down the colour content substance. The decrease in activity indicates that the microbes are in a stationary state where the microbes start to run out of nutrients and there are no additional nutrients causing the microbes to be unable to experience growth so the process of renovation has decreased (Mau *et al.,* 2018).

3.4 COD and BOD_5 Removal

The ability of FBR to reduce COD and BOD_5 levels is shown in Figure 6. COD reduction from 2 to 8 hours of treatment occurred from 2145.60 mg/l to 273.60 mg/l with effectiveness reaching 87.25% and BOD_5 concentration from 1173.30 mg/l to 57.30 mg/l with effectiveness reaching 93.11%. The decrease in COD and BOD values was related to the activity of decomposing organic compounds by microbes as a carbon source. The breakdown of pollutants in biofilms was done by diffusion into the biological layer. At the same time, using dissolved oxygen in the compound wastewater, these pollutants would diffuse into the bacteria through the cell walls to be further broken down into simple molecules and excreted into the outside of the cell walls.

One of the products secreted by bacteria was an extracellular enzyme which purposed to break down large organic molecules into small molecules for digestion. Bacteria use small molecules to synthesize new molecules in their growth (Davies, 2005).

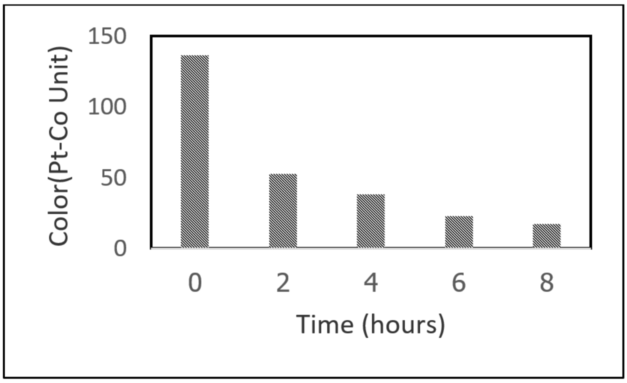


Figure 6. Color concentration removal in FBR treatment

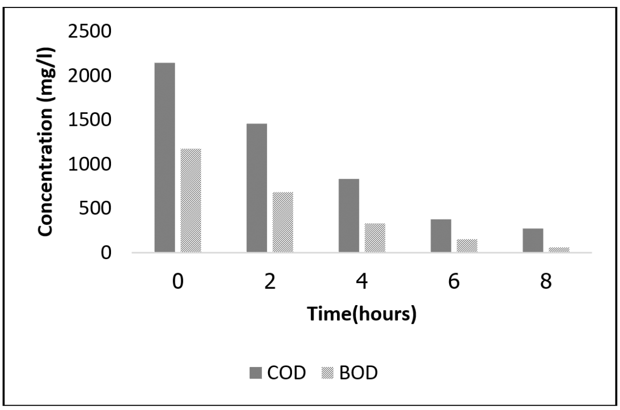


Figure 7. COD and BOD5 concentration during 4 days treatment

4. Conclusion

From this study, it can be concluded that the local microorganisms obtained and cultivated from sediment in water channel in Denpasar City, attached to FBR unit, had an outstanding capability in treating fabric dye wastewater. FBR was able to reduce levels of color, COD, BOD_5 with decent removal efficiency of 87.35%, 87.25% and 93.11% in 8 hours of FBR treatment. From the MLSS observation, the maximum MLSS in the active suspension was obtained by nutrient 1 (sampling point 1) about 1893.00 mg/l. While the best composition of media judged from its performance to generate microbial biomass was achieved by media III (K3) containing 60% nutrient and 40% wastewater with biomass concentration of 1636.67 mg/l.

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