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Approach of Aerob-Anaerob Biosystem Treatment On Biodegradation Of *Remazol Brilliant Blue.*

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ABSTRACT

The research about *Remazol brilliant blue* (RBB) wastewater treatment was conducted due to several issues related with dye pollutions in some rivers which surrounded by immersion and garment industries. The active suspension of microorganism inoculation into biosystem need to be advanced to enhance the effectivity of dye wastewater treatment. The soil resources which used to plant microorganisms take an important role in defining the ability of active suspension. This research was intended to obtain the finest active suspension that planted from several rivers sediment, moreover, to determine the effectivity and treatment capacity of vertical flow biosystem. The specific method was applied to specify the best active suspension by planting soil samples (purposive) inside selective media which contained RBB substances. Biomass growth was calculated by determining the *Mixed Liquor Volatile Suspended Solid* (MLVSS), and then the finest suspension was acquired from the fastest biomass growth. Furthermore, the finest active suspension was inoculated into vertical plant biosystem. The consortium of microorganisms in active suspension was built up in form of biofilm by immobilize it into volcanic rocks (biosystem) during 7 days before applied to treat RBB wastewater. Thus, the effectivity and treatment capacity of the biosystem was defined by calculating the concentration of RBB *artificial* wastewater from vertical biosystem and identified the bacteria species which involved in this treatment. The result showed that the best active suspension was obtained from soil sediment of river around the industries, the highest *Mixed Liquor Volatile Suspended Solid* (MLVSS) concentration was 1750 mg/L during 48 hour breeding time. In the biosystem, RBB removal occurred in the 6-hours treatment to the 114-hours, from 200 mg/L decline into 19.6211 mg/L. Then, it rose again into 19.8209 mg/L in the 120-hours. It figured the removal efficiency about 90.19 % in 114 hours treatment, whereas the treatment capacity was 1.6525 x 10^{-4} mg/gram along 114 hours. The specific bacteria which took the main role were *Pseudomonas sp.*, *Aeromonas sp,* and *Plesiomonas sp*. However, *Pseudomonas* sp. was the most prominent one who generated the tremendous initial colony amount of 7.2 x 10^{4} CFU/gram before degradation process and 2.6 x 10^{3} CFU/gram after degradation.

Keywords: Biodegradation, active suspension, *Remazol Brilliant Blue*, Inoculum, Vertical Biosystem.

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INTRODUCTION

Textile industries generate an excessive amount of wastewater contains extremely high organic concentration and high colour intensity. Those conditions can inflict the declining of environmental aesthetic value, interfere aquatic ecosystem, and affect human healthiness (Sastrawidana et al., 2010). Remazol Briliant Blue is one of the dyeing substances that mostly used by immersion and textile industries. It was known as an hazardous chemical and very dangerous if it is discharged to the environment and may create troublesome especially health issues due to contains main Azo dyeing chemicals that hard to degrade and stable. Furthermore, degradation of Azo dyeing chemicals aerobically in the bottom of water bodies may generate aromatic amine chemicals which possibly more toxic than the Azo chemicals itself(Van der Zee, 2002). Azo chemicals can be defined as aromatic chemicals or aliphatic. The aromatic Azo is stable and visualize bright colour, and its existence in the water may inhibit the penetration of sun rays into the water bodies, as the result, microbial photosynthesis activities will be interfered (Bollag, W. B and Bollag, J. M, 1992).

The alternative method which can be applied to solve this problem is the utilization of microorganisms to treat the textile wastewater. This method is called biodegradation that can be potentially developed to treat any kind of textile wastewater. The textile wastewater has a great amount of organic material and able to be digested directly or indirectly by microorganisms as nutrition resources. Otherwise, there are only several specific microorganisms which able to degrade textile dyeing chemicals, as the result, only some kind of bacteria and fungi that can be potentially observed as the treatment precursor. The advantage of using bacteria as main role of this treatment due to the ability of bacteria to endeavour specific substrate so that it literally works in specific spectrum (Budiarsa, S. and W. Dwijani, 2015).

Alternative method that was offered need to be scientifically observe especially on its effectivity. Because of that, this research was purposed to determine the effectivity and capacity of the biosystem which inoculated with active suspension in degrading Remazol Briliant Blue chemicals.

MATERIALS AND METHODS

Sediment Sampling and Materials Preparation

Sediment samples were taken from water bodies in several points of Sungai Mati River which located in Imam Bonjol street Denpasar in specific depth ± 10-15 cm from the sediment surface with the weight of 100 gram samples using plastic spoon. After that, the samples were put into plastic pockets and then labeled before stored in ice box. Liquid media composition consist of 2,0 gram glucose (KH) ; 0,1 gram K_2HPO_4 ; 0,1 gram KH_2PO_4 ; 0,1 gram $(NH_4)_2[Fe(SO_4)_2].6H_2O$; 0,02 gram $MgSO_4$; 0,02 gram $FeSO_4$; 0,02 gram yeast extract and 2 mg remazol brilliant blue then they were dissolved with aquadest (Suyasa, W.B.2015). Next, those mixes solution were stirred until reach homogenous solution and was put into volumetric flask 2 L. The solution then diluted using aquadest till reaches the border line. The optimum wavelength was determined by creating various series of standard solutions 20, 40, 80, 120, 160 and 200 ppm respectively. Hence, as much as 2, 4, 8, 12, 16 and 20 mL Remazol Brilliant Blue 1000 ppm were piped and put into 100 mL volumetric flask, then aquadest needed to be poured until reached border line. The solution was examined in UV-Vis Spectrophotometry with wavelength around 500 - 650 nm.

Active Suspension Establishment

Breeding were carried out by preparing 3 tubes of Erlenmeyer 500 mL with clean conditions. Each flask was filled by 250 mL liquid media and then labeled as Erlenmeyer A, B and C. After that, sediment samples from several sampling points in Sungai Mati River Denpasar were put into different Erlenmeyer flasks with 5 grams mass in each tube. Thence, the media were aerated using aerator equipped with plastic pipe and placed in the bottom of Erlenmeyer flasks. Erlenmeyer flasks were closed by cotton during aeration process and incubated for 3 days. Microbial growth was observed by determining MLVSS concentration. VSS concentration measurement was carried out by doing some iteration for each sediment sample every 1 day until it showed inclining MLVSS concentration. After acquired the VSS data, then a plot was made amidst MLVSS concentration against microbial growth time. Based on that growing curve, microbial growing time was determined as reach exponential phase. Observation was conducted on those three sediments. The sediment which contains bacteria that able to grow faster till reach exponential phase and obtain the highest MLVSS

concentration was defined as the best sediment seedling to be used in wastewater treatment (Suyasa, W.B. 2015). In order to determine MLVSS concentration, samples from three beaker glass were taken. Next, 3 porcelain cups were prepared and dried out in 100^0C oven then cooled away, and weighed until the mass was constant. Each of cup was filled by 25 mL sample from those three Erlenmeyer flasks, then heated on the oven with temperature 105^0C until the water vaporized. Cups which contain residual solids substances were chilled in desiccator and then weighed until the mass constant. The residual solids were heated on furnace (600^0C) for an hour. After heating process, cups with residual solids were detached from furnace and chilled in desiccator for 15 minutes and weighed until get constant mass (Sri Dian Meitasari, Suyasa, W.B. dan Mahardika, G,2016). MLVSS was calculated with the equation below :

$$VSS = \frac{a-b}{c} \times 10^6 \text{ mg/L}$$

Where: a = cup and residue weight before combustion/heating 600°C (gram)
b = cup and residue weight after combustion/heating 600°C (gram)
c = mL sample

Effectivity Determination and Biosystem Capacity

Biosystem was constructed in plastic tube ø 12 cm and 60 cm depth with sampling port in the bottom of the tube. Bacteria inoculum was immobilized according to the method that conducted by Castila, et al. (2003) as follows, as much as 800 - 1000 g sterile volcanic rocks were put into biosystem, and then sequentially the active suspension was added into the tube. Next, as much as 25 gram sediment was mixed with 1 L liquid media and combined with NPK until reach 2 L volume. Hence, the solution was stirred until reach homogenous phase and poured into bioreactor that had filled by volcanic rocks. The solution was discharged until whole volcanic rocks were submerged. When the seedling was submerged, formations of filaments in the volcanic rocks were observed. After 7 days of submersion was completed, the liquid media residuals need to be taken out slowly through water tap. Then, the amount microorganisms and their characteristics were distinguished in Microbiology Laboratory. On the bioreactor which contained rocks full filed by biofilm filament, Remazol Briliant Blue solution was added as much as 1000 mL with concentration of 200 ppm, thus the tap on the bottom of reactor was opened every 6 hours. The treated solution then stored in dark bottle 30 mL. The stored solution was observed to figure out the declining of its concentration using UV-Vis Spectrophotometry with maximum wavelength. After that, the same step and measurement was repeated on interval every 6 hours.

Next, the sum of microorganism's colonies and its characteristics were determined in Microbiology Laboratory. Vertical biofiltration capacity in removing dyeing chemicals was defined by the amount of circulation needed until the biosystem – which inoculated by active suspension- unable to reduce the concentration of Remazol Briliant Blue chemicals anymore, then it was drawn by curve. Moreover, % removal effectivity was calculated in order to acquire biosystem - which inoculated by active suspension in degrading Remazol Briliant Blue concentration – efficiency.

The collected data were drawn as samples behaving table and graph about the declining concentration of Remazol Briliant Blue. From the every 6 hours observation, the reducing concentration could be determined by calculating % effectivity in case to understand the effectivity of Inoculated Biosystem using Active Suspension in removing Remazol Briliant Blue chemicals. The equation to calculate % effectivity shown as follows :

$$\% \text{ effectivity} = \frac{(A-B)}{A} \times 100\ \%$$

Where: A = Initial concentration of Remazol Briliant Blue (ppm)
B = concentration of Remazol Briliant Blue after treated in biosystem((ppm)

Otherwise, to calculate the biosystem with inoculated active suspension capacity, the equation below was used:

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$$\text{Capacity} = \frac{(A-B)x\ Wastewater\ volume}{1000\ x\ rocks\ weight\ with\ biofilm\ in\ the\ surface}\ \text{mg/gram}$$

Where: A = Initial concentration of Remazol Briliant Blue (ppm)
B = concentration of Remazol Briliant Blue after treated in biosystem((ppm)
Wastewater volume = Remazol Briliant Blue artificial wastewater (L)
Rocks weight = rocks weight with biofilm (gram)

RESULT AND DISCUSSION

Finest Active Suspension

The result of MLVSS calculation (Figure 3.1) from those three sediments showed that Tukad Mati River's sediment emerged the best MLVSS concentration. Maximum microbial growth from the sediment attained 17200 mg/L in 48-hours seedling, while the minimum growth was up to 11200 mg/L which represented by sediment from Tukad Badung estuary. The differences amidst MLVSS value from each sediment was caused by behavior and alteration abilities of microorganisms to survive in selective media and also the ability to replicate and create consortium, so as the result, the biomass production in each sediment was completely different.

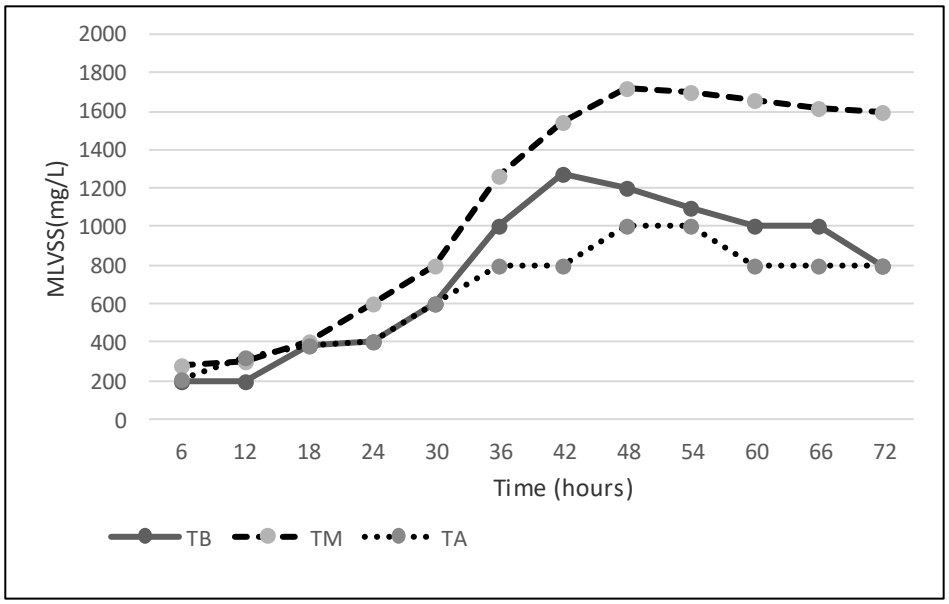

Figure 3.1 Active Suspension growth based on depth of sediment

where : TB = <5m, TM = 10 cm, TA = >20 cm

Standard curve for RBB measurement using Spectrophotometry Method

The wavelength that used to analyze was the wavelength where chemicals might provide the highest absorption of the light emitted by specific spectrum (based on the wavelength) then it called maximum wavelength (λ max). To obtain maximum wavelength, several standard solution of Remazol Briliant Blue was prepared in various concentration of 20, 40, 80, 120, 160, and 200 ppm. Standard solution 40 ppm was calibrated in Spectrophotometer UV-1800 Shimadzu using approximated wavelength around 500-700 nm. From observation, maximum wavelength was acquired in 591, 0 nm with absorbance 0.3608.

After that, calibration curve was made by aligning the absorbance value with several RBB standard solutions in exact wavelength 591, 0 nm.

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Table 3.2 RBB Standard curve

RBB standard concentration (ppm)	Absorbance
20.00	0.2019
40.00	0.3608
80.00	0.7036
120.00	1.0463
160.00	1.3766
200.00	1.6922

RBB treatment in Biosystem

RBB concentration determination in biofiltration system was conducted by utilizing immobilized active suspension using volcanic rocks to treat 200 mg/L RBB artificial wastewater. Soil sediment which generating the highest VSS value was used to treat RBB in biofiltration system that constructed by Paralon pipe ø 12 cm and 60 cm depth. Table 3.3 show the result of RBB treatment using active suspension in biofiltration system.

Table 3.3 Result of RBB treatment using Biofiltration

No	Time (hours)	Average RBB concentration (mg/L)
1	0	200 ± 0
2	6	160.7842 ± 0.03597
3	12	128.2822 ± 0.03018
4	18	104.4492 ± 0.02496
5	24	78.2062 ± 0.01199
6	30	66.9072 ± 0.01385
7	36	55.1846 ± 0.01199
8	42	46.6994 ± 0.01832
9	48	33.1223 ± 0.02398
10	54	32.5723 ± 0.02496
11	60	30.4181 ± 0.01832
12	66	30.3685 ± 0.02496
13	72	27.5028 ± 0.01832
14	78	25.4804 ± 0.01832
15	84	24.6970 ± 0.01832
16	90	23.5099 ± 0.02496
17	96	22.8953 ± 0.01832

Based on the treatment results using biosystem with inoculated bacteria which immobilized in volcanic rocks, It was found the decreasing concentration of artificial RBB wastewater in the first 6 hours about 160.7842 mg/L from the initial concentration of 200 mg/L. The slight removal was occurred since the treatment time within 54-hours from the concentration of RBB was 32.6 mg/L to 22.9 mg/L. As the result, rapid RBB removal was happened after 6-hours treatment until 48 hours. This showed that microorganism's consortiums on the biosystem accomplished the peak of growing rate in 40-hours. Microorganism ability with existing co-substrate as electron donor on organic chain structure of dyeing chemicals, affiliated with reductase enzymes cause the dismemberment of chemicals bounds, as the result, creating aromatic amines. Moreover, break over of Azo substances in this attachment growth system can be occurred with redox reactions catalyzed by enzymes. In glycolysis process, Co-enzyme nicotinamide adenine dinucleotide (NAD+) was released aided by dehydrogenase enzyme which acted as electron conveyor and involved on enzymatic reactions. When no oxygen was available, NADH would be oxidized resulting NAD^+ while Azo dyeing chemicals was reduced into appropriate aromatics amines. Breakdown of Azo dyeing chemicals boundaries caused its color become inconspicuous gradually. Yet, if the oxygen was existed, the Azo chemicals and oxygen would appear to compete each other to be the electron acceptor from NADH. Hydrogen Ion on NADH tends to be exchanged with oxygen than Azo chemicals instead, through chain electron transfer. Therefore, under aerobic conditions, Azo chemicals will be more difficult to be reduced, as the result, the color will remain the same. Moreover, anaerobic zone might be occurred in this biosystem (Van der Zee (2002).

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The Effectivity of the Inoculated Active-Suspension Biosytem in Degrading RBB

Biosystem effectivity in percentage was determined by comparing the highest concentration from RBB declining concentration result with the decreasing concentration of RBB artificial until the observed removal time. The effectivity of biosystem with Sungai Mati microorganism's inoculums against the RBB artificial removal was shown in Table 3.4 as follows :

Tabel 3.4 The biosystem effectivity in declining of RBB concentration

Time (hour)	Initial Concentration (mg/L)	Average effluent concentration (mg/L)	Effectivity (%)
0	200	200 ± 0	0 ± 0
6	200	160.7842 ± 0.03597	19.61 ± 0.0179
12	200	128.2822 ± 0.03018	35.86 ± 0.0151
18	200	104.4492 ± 0.02496	47.77 ± 0.0125
24	200	78.2062 ± 0.01199	60.90 ± 0.0060
30	200	66.9072 ± 0.01385	66.55 ± 0.0069
36	200	55.1846 ± 0.01199	72.41 ± 0.0060
42	200	46.6994 ± 0.01832	76.65 ± 0.0092
48	200	33.1223 ± 0.02398	83.94 ± 0.0120
54	200	32.5723 ± 0.02496	84.21 ± 0.0125
60	200	30.4181 ± 0.01832	85.29 ± 0.0092
66	200	30.3685 ± 0.02496	85.82 ± 0.0125
72	200	27.5028 ± 0.01832	86.25 ± 0.0092
78	200	25.4804 ± 0.01832	87.26 ± 0.0092
84	200	24.6970 ± 0.01832	87.65 ± 0.0092
90	200	23.5099 ± 0.02496	88.24 ± 0.0125
96	200	22.8953 ± 0.01832	88.55 ± 0.0092

Active suspension biosystem effectivity in treating RBB concentration reached the best performance in 48-hours treatment with removal percentage attained 83.94%. The biosystem capabilities in this research accomplished removal percentage up to 88.55% in 96-hours treatment. After 48-hours, the deficit of removal effectivity in each removal time was gradually reducing. It was caused by the decreasing endurance of bacteria in digesting RBB chemicals due to the beginning of death phase. According to the identification test, the amount of bacteria and total count of microorganisms were obtained in Table 3.5 as follow:

Tabel 3.5 Bacteria Identification in Biosystem

Sample Code	**Coliform (MPN/100gr)**	***E.coli* (MPN/100 gr)**	***Pseudomonas* sp. (CFU/gr)**	***Aeromonas* sp. (CFU/gr)**	***Vibrio* sp. (CFU/gr)**	***Plesiomonas* sp. (CFU/gr)**
A	95	95	7.2×10^4	6.3×10^3	0	1.4×10^3
B	76	76	2.6×10^3	2.0×10^2	0	8.0×10^2

Where:
A : Filament sample before the biosystem was filled by wastewater
B : Filament sample after the biosystem was filled by wastewater

From Table 3.5, It is found that the species of bacteria which presented in biosystem were Coliform, *E.coli*, *Pseudomonas* sp, *Aeromonas* sp., dan *Plesiomonas* sp., whereas *Vibrio* sp. was not found in the biosystem. Those bacteria's capability to remove RBB concentration substantiated by the results from the total colony survived of each species bacteria. Bacteria which created the most enormous amounts of colony was *Pseudomonas* sp. that showed the counts of colony before degradation process up to 7.2 x 10^4 CFU/gr and after degradation up to 2.6 x 10^3 CFU/gr. The second one was *Aeromonas* sp. which pointed the amount of

colony up to 6.3 x 10^3 CFU/gr and after degradation was about 2.0 x 10^3 CFU/gr. The third bacteria was *Plesiomonas* sp. which conducted the amount of colony up to 1.4 x 10^3 CFU/gr and after degradation was about 8.0 x 10^2 CFU/gr. Eventually, this research proved that *Pseudomonas* sp., *Aeromonas* sp. dan *Plesiomonas* sp. bacteria afford to demolish and survive the contamination of Remazol Briliant Blue chemicals.

CONCLUSION

- The finest effectivity of active suspension-inoculated biosystem in vanishing Remazol Briliant Blue chemicals was obtained in 48-hours treatment time with removal percentage up to 83.94%. After 48-hours, the removing rate was slightly decreasing until 96-hours treatment and reach total removal percentage up to 88.55 %.
- Microorganism that involved in Remazol Briliant Blue removal consisted of *Pseudomonas* sp., *Aeromonas* sp. dan *Plesiomonas* sp. *Pseudomonas* sp. took as the most dominant bacteria with the biggest amount of colony existed until the end of treatment about 2.6 x $\mathbf{10^3}$ CFU/gr.

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