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Application of Sargassum Cinereum and Rhizobateria as Biosorbent Zn in Batik Wastewater

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Abstract: Heavy metals such as Zinc (Zn) in batik wastewater may be reduced with biosorption. *Sargassum cinereum* and rhizobacteria may be used as a biosorbent as they are cheap, abundant, and easily obtainable. The process of biosorption depends on a biosorbent surface area. A large biosorbent surface area can be made by modifying a tea bag. This study aims to get the mixture ratio of *S. cinereum* and rhizobacteria isolates (Rb2) to adsorb Zn in batik wastewater and to get rhizobacteria isolates that absorb the highest Zn in batik wastewater. This experimental study was conducted using a split plot design. The adsorption capacity data was analyzed using an F-test with a significance level of 95%. The result shows that the highest content of Zn adsorbed is 0.0595 mg/g^{-1}, on the mixed ratio of *S. cinereum* and rhizobacteria isolates 1:3 decreasing the efficiency by 84.421%. Isolate Rb2 is the best isolate to adsorb Zn in batik wastewater. A biosorbent mixture packed into modified tea bags effectively removes Zn in batik wastewater.

Keywords: algae, bacteria, biosorption, heavy metal, tea bag.

羊栖菜和根瘤菌作为锌吸附剂在蜡染废水中的应用

摘要：蜡染废水中的重金属，例如锌可以通过生物吸附而减少。灰藻和根瘤菌可以用作生物吸附剂，因为它们便宜，丰富并且易于获得。生物吸附的过程取决于生物吸附剂的表面积。可以通过修改茶袋来制造大的生物吸附剂表面积。这项研究的目的是获得灰霉菌和根瘤菌分离物（Rb2）的混合比，以吸收蜡染废水中的锌，并获得吸收蜡染废水中锌最高的根瘤菌。该实验研究是使用分割图设计进行的。吸附容量数据使用显着性水平为 95％的 F 检验进行了分析。结果表明，在灰霉菌和根瘤菌的 1：3 混合比例下，锌的最高吸附量为 0.0595 毫克/ g-1，效率降低了 84.421％。分离物 Rb2 是吸附蜡染废水中锌的最佳分离物。装在改良茶袋中的生物吸附剂混合物可有效去除蜡染废水中的锌。

关键词：藻类，细菌，生物吸附，重金属，茶袋。

1. Introduction

Indonesian batik is an important part of national culture with a high economic value. Industrial batik production has positive impacts on the economy but negative impacts on the environment. Many small and medium sized batik production facilities do not have adequate waste management installed; therefore, their wastewater is disposed of directly into the environment. Batik wastewater contains a lot of heavy metals, dyes, organic matter, and suspended solids. Synthetic dyes used in the batik industry contain highly toxic heavy metals, such as Zn. Zinc is a trace element that may be toxic when accumulated in the body, and continuous accumulation of Zn in tissue may be carcinogenic. Zinc may cause nausea, vomiting, diarrhea, and stomach disorders [1].

Zn in batik wastewater may be removed biologically through bioremediation, one form being biosorption. The process of biosorption is the binding of metals via adsorption to inactive or dead organisms

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[2–4]. Biosorption is effective at adsorbing heavy metals contained in wastewater since it is a relatively quick, selective process with a high rate of adsorption [5], [28]. A good biosorbent should be selective, cheap, and efficient [6]. A potential biosorbent which may adsorb Zn in batik liquid waste is a mixture of *Sargassum cinereum* and rhizobacteria.

Each type of biosorbent has different adsorption capacities. Combining two biosorbents leads to a higher adsorption capacity than individual applications. Much research has been conducted on mixtures of two biosorbents, such as: cellulose and microbes [7], apple seeds and *Aspergillus niger* [8], and *Annona squamosa* seed and *Aspergillus niger* [9]. However, no research on a mixture of *Sargassum cinereum* and rhizobacteria has been conducted to date. In this paper we present novel research on this mixture as a biosorbent.

Previous applications of biosorbents have been achieved by immobilizing the biosorbent on silica gel [10], dust [11], powder [12], pellets [12], and tea bags [13]. These forms are undesirable due to high costs, narrow surface areas, and coagulation. Our research packages the biosorbent mixture into a modified tea bag. The modification divides the tea bag into six small zones in order to prevent coagulation. The research scope is ecotoxicology. The research objective is to obtain a mixture ratio of *S. cinereum* and rhizobacteria isolate to adsorb Zn in batik liquid waste, and rhizobacteria isolates that absorb the highest Zn in batik wastewater.

2. Materials and Method

2.1. Research Method

Experimental research was arranged based on a completely randomized design (RAL) with a split plot design. The experiment involved the treatment of rhizobacteria isolate, which may potentially serve as the main plot, and a ratio of a mixture of S. cinereum and rhizobacteria as the subplot. The rhizobacteria isolate was from the collection of the Microbiology Laboratory and isolated from the root of Ipomoea batatas growing in an iron sand area. The result of isolate screening, which may potentially reduce Zn, consisted of Rb1, Rb2 and Rb3. The sample of S. cinereum was taken from Menganti Beach, Cilacap Regency. The ratios of the mixture of S. cinereum and rhizobacteria isolate consisted of 5 levels:

M1 = S. cinereum and rhizobacteria isolate with ratio of 1:0

M2 = S. cinereum and rhizobacteria isolate with ratio of 3:1

M3 = S. cinereum and rhizobacteria isolate with ratio of 1:1

M4 = S. cinereum and rhizobacteria isolate with ratio of 1:3

M5 = S. cinereum and rhizobacteria isolate with ratio of 0:1

The rhizobacteria isolate consisted of 3 levels: Rb1, Rb2, and Rb3 isolates.

All treatments were repeated 3 times for a total of 45 treatments.

The research was conducted from May–July 2019 at the Microbiology Laboratory of the Faculty of Biology, Unsoed. Zn was measured using AAS and biosorbency was characterized using SEM at the Integrated Research Laboratory, Jenderal Soedirman University.

2.2. Isolate Regeneration and Reculture

The rhizobacteria isolate was regenerated with nutrient agar (NA) medium using four lined methods for a pure culture of the isolate. Pure isolate was planted in nutrient broth (NB) medium as a stock. The isolate was cultured in NB medium and incubated for 48 fours at 370°C. The pure culture was ready for use.

2.3. Preparation of Rhizobacteria Biomass

Bacterial culture was grown in the Lauryl Broth (LB) medium and incubated for 2 x 24 hours. The culture was centrifuged at 4000 rpm for 5 minutes. Natan was obtained from incubation at 60^0C. The biomass obtained was then scraped and packaged in a tea bag.

2.4. Preparation of Biosorbent

The biosorbent comprised a mixture of *S. cinereum* and rhizobacteria. *S. cinereum* was submerged in distilled water for 24 hours and dried under the sun for 2 x 24 hours. Then, it was dried in an oven at 60^0C and collected at constant weight. The mixture of biosorbent obtained from the oven was blended. The biosorbent used was 250–425 μm, which passed sieve 60 and was suspended by sieve 40. The biosorbent was weighed for 300 mg and put in a tea bag.

2.5. Preparation of Modified Tea Bag

The tea bag was made of a 3 x 4 cm sheet of tea bag paper . It was divided into six zones to prevent the biosorbent from coagulating on coming in contact with waste. It was attached using plastic adhesive.

2.6. Biosorption Test

Batik wastewater was taken from the Batik Industry Center at Sokaraja Tengah Village, Sokaraja District, Banyumas Regency, Central Java. The pH of batik liquid waste was set to 8 [13] by adding NaOH 0.1 M. Erlenmeyer volume 250 mL was prepared for 45 units, each filled with 100 mL batik wastewater, one biosorbent added, packaged in a modified tea bag. The Erlenmeyer was then covered with cotton and aluminium foil and homogenized in a shaker incubator at 175 rpm at 25 ^{0}C for 1 hour [13].

2.7. Batik Wastewater Destruction

Batik wastewater resulting from biosorption was separated from biosorbent. 50 mL of wastewater was put into a 100 mL glass beaker. 5 mL of concentrated HNO3 and 2 mL HCl 20% were then added to the wastewater. The solution was heated using a hot plate at 180°C until 10-20 mL remained. The solution was filtered using Whatman paper No. 42. The filtrate obtained was diluted using distilled water in a measuring flask up to the 50 mL mark, and Zn absorbance was measured using AAS instrument at a wavelength of 213.9 nm.

2.8. Adsorption Capacity

The adsorption capacity of Zn was calculated using formula:

$$q=\frac{V(Co\text{-}Ceq)}{m} \quad (1)$$

where: q = adsorption capacity (mg/g); V = solution volume (L); m = biosorbent weight (g); C_o = metal initial concentration (mg/L); Ceq = metal final concentration (mg/L).

2.9. Zn Reduction pcercentage

The percentage of Zn reduction was calculated using the following formula:

$$\% Zn = \frac{Zn\ initial\ - Zn\ final}{Zn\ initial}\ x\ 100$$

2.10. Scanning Electron Microscopy

In a JEOL JED-2300 microscope, the samples were placed directly on metal studs and examined to obtain information on surface morphology. In the range of a few microns, the secondary electron images of the material represent estimated sizes.

2.11. Analysis Method

The data of Zn adsorption capacity was analyzed using F-test to examine any influence between treatments, followed by a Least Significance Different (LSD) test to examine the optimum mixture of *S. cinereum* and rhizobacteria to adsorb Zn. The data of surface morphology biosorbent was analyzed descriptively.

3. Result

The concentrations of Zn in batik wastewater were different before and after biosorption using a biosorbent packaged in a modified tea bag. The Zn adsorption capacity is influenced by the ratio of the mixture of the biosorbent and rhizobacteria isolate. The highest adsorption capacity was found for the mixture of S. cinereum isolate Rb2 with a 1:3 ratio of 0.02747 ± 0.0023 mg/g (Fig. 1) with an adsorption efficiency 84.42% from the initial concentration ranging from 0.0972 mg/L to 0.0102 mg/L. The lowest adsorption capacity of Zn was found in the mixture of S. cinereum and isolate Rb1 with a 1:0 ratio of 0.0117 ± 0.078 mg/g (Fig. 1) with an adsorption efficiency 41.14% from the initial concentration ranging from 0.1123 mg/L to 0.0638 mg/L.

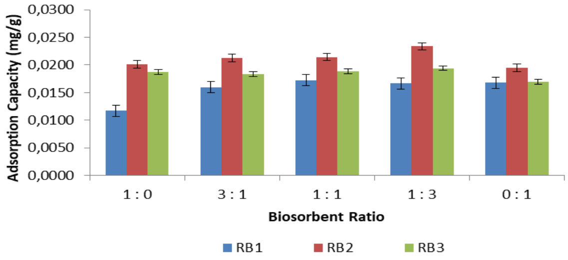

Fig. 1 Adsorption capacity of Zn by mixture S. cinereum and Rhizobacteria in the modified tea bag

Based on the ANOVA analysis, the treatment of the ratio of the mixture of the biosorbent and rhizobacteria isolate significantly influenced the adsorption capacity of Zn (Fcount>Ftable 0.05). The LSD result shows that the mixture of S. cinereum biosorbents and rhizobacteria isolate at a ratio of 1:1 and 1:3 is significantly different from that of the other treatment (Table 1). Based on the data shown in Table 2, the treatment of isolate Rb2 is significantly different from that of Rb3 and Rb1.

Table 1 Average of LSD on the ratio of biosorbent to adsorbed Zn

Ratio of biosorbents *S. cinereum* and rhizobacteria isolate	**Amount of adsorbed Zn (mg/g)**
Ratio 1:0	0.0506 c
Ratio 3:1	0.0556 b
Ratio 1:1	0.0576 a
Ratio 1:3	0.0595 a
Ratio 0:1	0.0533 b

Table 2 Average of LSD on rhizobacteria isolate on adsorbed Zn

Isolate	**Amount of adsorbed Zn (mg/g)**
Rb1	0.0784 c
Rb2	0.1057 a
Rb3	0.0924 b

The topography of the biosorbent surface is in the form of an irregularly-shaped powder. Empty pores are seen in the biosorbent morphology before the biosorption process (Fig. 2a); in contrast, after biosorption the pores were filled (Fig. 2b).

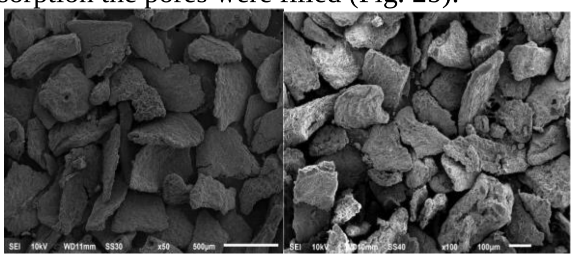

Fig. 2 Electron micrograph of biosorbent a) before biosorption (50x) and b) after biosorption (100x)

4. Discussion

As seen in Fig. 1, the adsorption capacity of the ratio of the mixture of S. cinereum and rhizobacteria isolate in the isolates of Rb1, Rb2, and Rb3 is higher than when the biosorbent is applied separately (1:0 and 0:1, respectively). This shows that the combination of two biosorbents has a higher adsorption capacity. The 3:1 ratio of the mixture of S. cinereum and P. ostreatus baglog waste [14], cellulose and microbes [15], Annona squamosa seed and Aspergillus niger [9], and apple seed and Aspergillus niger [9] are more effective when used together than when they are applied separately.

The 1:3 ratio has the highest adsorption capacity of 0.0595 mg/g (Table 1). This proves that the mixture of the biosorbent with a 1:3 ratio is capable of reducing the metallic content of Zn in batik wastewater. Isolate Rb2 has the highest adsorption capacity of Zn than any of the other treatments; its 0.1057 mg/g. Rb2 isolate is a potential isolate for the degradation of batik wastewater.

Biosorption is one of the passive mechanisms used to remove heavy metal ions [7, 15]. Heavy metal ions bind to the cell wall in two ways: through ion exchange and complex formation [16]. Heavy metal ion exchange replaces monovalent and divalent ions, such as Na, Mg, and Ca, in the heavy metal ion substituted cell wall. Complex formation occurs between heavy metal ions with functional formations, such as carbonyl, amino, hydroxyl, phosphate, and hydroxyl-carboxyl on the cell wall. The active process occurs simultaneously in line with the metal ion consumption and intracellular accumulation of metal ions.

The structural composition of the cell wall is complex, and it affects the adsorption capacity. S. cinereum contains large amounts of alginate and fucoidan [17] and the cell walls of G+ bacteria contain a thick peptidoglycan layer (90%) of teichoic acid. In contrast, G- bacteria have a very thin peptidoglycan layer (only 10 to 20%), and they do not contain teichoic acid [18]. The bacteria have an outer membrane that consists of phospholipids, lipoproteins, and lipopolysaccharides [19–21].

Biosorption is a process in which the cells of microorganisms passively absorb metals. This usually results in the formation of organic complexes between the metals and the constituent cell walls of the microorganisms, capsules, or extracellular polymers that are synthesized and excreted by these microorganisms [22]. Bacteria can bind to Zn through interactions with metals using cell membranes, cell walls, and cell wall-related material (polysaccharide capsules) [23]. The efficiency of heavy metal biosorption by microbial biomass is related to the structure of the cell walls of the microorganisms because it determines the natural interactions between the microorganisms and metal cations [24].

Acidity (pH) is a factor that has a significant impact on the process of adsorption of metal ions in solution, since H+ ions in solution will compete with the cation to bind with the active site. All the protonated binding sites, and Zn for complete desorption of the linked metal ions used for regeneration of biosorbents, are at a sufficiently low pH. Extreme pH values may damage the structure of the biosorbent [16]. The adsorption process reduces the pH of the batik wastewater. The average pH of the batik wastewater after biosorption is 5.8. S. cinereum contains alginate, which is very abundant in its cell walls. Alginate has two carboxyl groups of monomers, namely manuronic acid and guluronic acid, so the amount of Zn adsorbed can be influenced by the pH. The decrease in pH indicates that there is a reaction between the compounds in the solution and the biomass. This will cause the metal adsorption to increase because the acidic pH of heavy metals is in the form of free ions. In alkaline pH, heavy metals tend to precipitate. At low pH, the H+ ions surrounding the bacterial surface and Zn2+ are hydrolyzed to ZnOH+, preventing interaction between the Zn ions and the functional groups on the bacterial surface. The optimum pH for adsorption is 5.5 [25], since the amount of H+ ions on the bacterial surface starts to decrease. Thus, the competition with H+ decreases and the active bacterial surface tends to be ionized by releasing H+ ions causing the adsorbent surface to be negative.

As seen in Fig. 2, the pores on the surface of the biosorbent almost completely disappear after biosorption since they are covered by Zn. Very few pores are present on the surface of biosorbent that is bound to metal [26, 27]. This proves that Zn biosorption occurs on the biosorbent surface.

5. Conclusion

The optimum ratio of the biosorbent mixture containing S. cinereum and rhizobacteria needed to adsorb Zn is 3:1, and the optimum rhizobacteria isolate needed to adsorb Zn is Rb2. The adsorption capacity is higher for a mixture of two biosorbents that are packaged in a modified tea bag than when the biosorbents are applied separately. The results of the research can be applied by small-scale batik craftsmen. The advantages of this research are that the biosorbent is affordable, abundant, and is highly effective in terms of facilitating Zn adsorption.

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