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# Ethidium Bromide Waste Treatment with Activated Charcoal

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## Abstract

Ethidium bromide (EtBr) is a chemical agent commonly used to identify and visualize the bonding of DNA and RNA in molecular biology research. However, ethidium bromide itself is mutagenic and toxic. Ethidium bromide also has carcinogenic properties to human cells, teratogenic, and difficult to decompose. Therefore, a research for the treatment of EtBr waste is very important. There are several materials that can be used to reduce EtBr waste, but there is also needed a material which is cheap and widely available in the market. One of the methods to treat ethidium bromide waste is using activated charcoal. Activated charcoal has strong adsorption capacity due to its high surface area and porous structure. Another advantage of activated charcoal as an adsorbent is economical, effective, and environmentally friendly. In this study, EtBr waste was mixed with activated charcoal then homogenized for one hour. The mixing of EtBr waste (0.5 mg/mL concentration of EtBr) and activated charcoal was varied with a ratios weight by volume of 1:1, 1:2, and 2:1. The mixture of the solution was filtered using Whatmann filter paper No.1 to take the filtrate. The filtrate was further analyzed. The more activated charcoal was added, the more EtBr molecules were adsorbed. The pH in the filtration results for each variation was pH 7.0 - 8.0, dissolved oxygen with range 5.0 - 9.0 mg/L, biochemical oxygen demand (BOD) with range 25.06 - 78.2 mg/L, and chemical oxygen demand (COD) with range 302.56 - 310.98 mg/L. The concentration range was obtained as follows, for a 1:1 ratio of 0.045 - 0.060 mg/mL, for a 1:2 ratio of 0.028 - 0.032 mg/mL, while a 2:1 ratio of 0.081 - 0.090 mg/mL. In addition, the results showed that activated charcoal was able to bind Ethidium bromide due to bond interactions that occur between the EtBr molecule and activated charcoal.

***Keywords:*** Ethidium bromide; Chemical waste; Activated charcoal; Waste treatment; Adsorption

## 1. Introduction

Ethidium bromide has been widely used as a dye in molecular biology research. Both nucleic acids and proteins will be able to fluoresce under ultraviolet light. It will contribute to the identification and visualization of nucleic bands (Lalchhandama, 2016). Ethidium bromide contains tricyclic phenanthridine ring system that is able to interact with stacked base pairs of double stranded DNA. Ethidium is capable of forming close van der Walls contacts with the base pairs and due to that it can bind to the hydrophobic interior of the DNA molecule. Excessive use of ethidium bromide as a dye will cause negative effects. Ethidium bromide is a substance that is difficult to decompose and has high toxicity with $LD_{50}$ 0.075 mg/mL (Sukhumungoon *et al.,* 2013). Ethidium bromide also has negative effect to human cells such as carcinogenic, teratogenic, and mutagenic (Chang and Sarkar, 2020). Due to its negative impact, it is necessary to treat ethidium bromide waste. Activated charcoal could treat ethidium bromide. Activated charcoal is a porous material with a surface area of >1000 $m^2/g$. Its high surface area causes activated charcoal to have a strong adsorption capacity and widely used as an adsorbent. The advantages of adsorption using activated charcoal are simple, environmentally friendly, and inexpensive (Tadda *et al.,* 2018). Based on previous research, activated charcoal has been shown to be able to sorption ethidium bromide in solution (Quillardet and Hofnung, 1988), but not clear for adsorption mechanism. Several montmorillonites (Wang *et al.,* 2020) and mix layers illite and montmorillonite (Li *et al.,* 2020) were evaluated for ethidium bromide removal capacity, showed that both external and internal surfaces were effective sorption, but not clear for water quality. However, there has been no research related to the adsorption process of ethidium bromide by activated charcoal. Therefore, it is necessary to do further research related to the process of absorption of ethidium bromide waste by activated charcoal. So the purpose of this study was to determine the approximate mechanism of ethidium bromide adsorption on activated charcoal. As well as in this research will also be investigated related to water quality such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), concentration of EtBr after tratment and the pH of the filtrate treated with activated charcoal. This research is very important so that the waters around laboratories or institutions that use EtBr are not contaminated if there are problems in waste treatment. And this research can provide an alternative to laboratories or institutions that do not have private waste treatment.

## 2. Materials and Methods

### *2.1 Materials*

Powder Activated charcoal Lot # BCBV5687, $MnSO_4.H_2O$, alkali-iodide-azide reagent, sulfuric acid, and sodium thiosulfate, $Ag_2SO_4$, amilum indicator, $K_2Cr_2O_7$, ferroin indicator, ferro ammonium sulfate, ethidium bromide, and potassium hydrogen phthalate purchased from Sigma Aldrich. Filter papers 150 mm (diameter) from Whatman®. Ethidium Bromide wastes are collected from laboratories involved in research in the biological and biotechnological sciences at Institute of Tropical Disease.

### *2.2 Ethidium Bromide Treatment with Activated Charcoal*

To observe the level of EtBr adsorption by activated charcoal, it was carried out in 3 variations in the addition of EtBr with concentration 0.5 mg/mL and activated charcoal, including 1:1; 1:2; and 2:1. For the 1:1 variation, the ratio of EtBr and activated charcoal was 50 mL: 50 mg, 100 mL: 100 mg, and 150 mL: 150 mg. For a 1:2 ratio, 50 mL: 100 mg, 100 mL: 200 mg, and 150 mL: 300 mg. As for the ratio of 2:1, is 100 mL: 50 mg, 200 mL: 100 mg, and 300 mL: 150 mg.

Activated charcoal was weighed using an analytical scale according to the variation used and then placed into a beaker. Then, the EtBr solution was poured into the beaker, which was then allowed to stand for 1 hour. As long as it is allowed to stand for 1 hour, every 15 minutes the solution is stirred for 5 minutes to homogenize the solution. After 1 hour, the solution was filtered using Whatmann paper with the filtrate accommodated in an Erlenmeyer (Moradi *et al.,* 2014). After all the solutions were filtered and no filtrate remained, the precipitate on the filter paper was discarded while the filtrate was stored for further testing of pH with pH meter Horiba D-52 and concentration of EtBr after treatment. The filter paper is then placed in a solid waste reservoir and then burned at a temperature of 800 °C in the incenerator.

### *2.3 Determine of Ethidium Bromide Concentration after Treatment*

The concentration of EtBr after being treated with charcoal was determined using a UV-Vis Spectrophotometer (Shimadzu UV 1800). Standard curve was made with various concentrations 0.01 mg/mL, 0.02 mg/mL, 0.03 mg/mL, 0.04 mg/mL, and 0.05 mg/mL, also measured at a maximum wavelength of ($\lambda_{max}$) 210 nm (Merck, 2022).

*2.4 Dissolved Oxygen (DO)*

Ethidium bromide sample without bubbling in 200 ml glass bottle. Add 2 ml of $MnSO_4.H_2O$ solution interesting the tip of pipette tip into the sample because the drops of solution can allow inserting the oxygen into the solution. Add 2 ml of the alkali-iodide-azide reagent. Allow reacting the solutions with the oxygen present in the sample. When precipitates are settled down at the bottom add 2 ml of concentration sulfuric acid by placing the pipette tip very near to sample surface. Mix well to dissolve the precipitates.Take 50 ml of sample from in a flask. Titrate immediately with sodium thiosulfate solution using starch indicator until blue color disappears and note down the burette reading. Determine the burette reading for blank in the same manner.

The calculation of DO in mg/L = 8 x 100 x N/(Vxv) with V is volume of sample taken (ml), v is volume of used titrant (ml), N is normality of titranta, and 8 is the constant since 1 ml of 0,025N sodium thiosulphate solution is equivalent to 0.2 mg oxygen. The method used in the study is in accordance with previous research (Fadzry *et al.,* 2020) and refers to standard methods for the examination of water and wastewater 4500-O Oxygen (Dissolved) (Standard Methods Committee of the American Public Health Association, 2021).

*2.5 Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)*

Water quality analysis was carried out with chemical indicators such as BOD and COD. Determination of BOD and COD levels in the filtrate from treatment with activated charcoal refers to the previous research of Andika *et al.* (2020) and refers to standard methods for the examination of water and wastewater 5210 Biochemical Oxygen Demand (BOD) (Standard Methods Committee of the American Public Health Association, 2019), also 5220 Chemical Oxygen Demand (COD) (Standard Methods Committee of the American Public Health Association, 2022).

*2.6 Characterization of Activated Charcoal*

A scanning electron microscope (SEM) JSM-6510 Series was used to characterize the activated charcoal for morphological information after adsorption. Isothermal analysis in this study was used to determine the pore volume of activated charcoal after adsorption.

## 3. Results and Discussion

In this study, EtBr waste was mixed with activated charcoal and allowed to stand for one hour. For one hour every 15 minutes the solution is stirred. In this process, there is an adhesion force between the EtBr molecules on the surface of the charcoal. As a result, EtBr molecules are trapped on the surface of the charcoal through a process known as adsorption. The more activated charcoal added and the longer the adhesion time, the more EtBr molecules trapped on the charcoal surface (Radhi, 2020).

The result of the pH of filtrate of each variation of the ratio of EtBr with activated charcoal was pH 7.0 - 8.0 (detail of pH in the Table 1). The color of the EtBr waste solution before and after being filtered with activated charcoal was the same, which was colorless. The resulting filtrate volume at 1:1 variation was 49, 95, and 146 mL, respectively. The resulting filtrate volume at variation 1:2 was 83, 176, and 284 mL, respectively. The resulting filtrate volume at variation 2:1 is 46, 85, and 136 mL, respectively.

Based on the measurement of the concentration of EtBr waste after being treated with activated charcoal with a UV-Vis Spectrophotometer and standard curve in Figure 1, the concentration range is obtained as follows, for a 1:1 ratio of 0.045-0.060 mg/mL, for a 1:2 ratio of 0.028-0.032, while a 2:1 ratio of 0.081-0.090. In this study, it can be seen that there was a decrease in the concentration of EtBr from before treatment, which is 0.5 mg/mL.

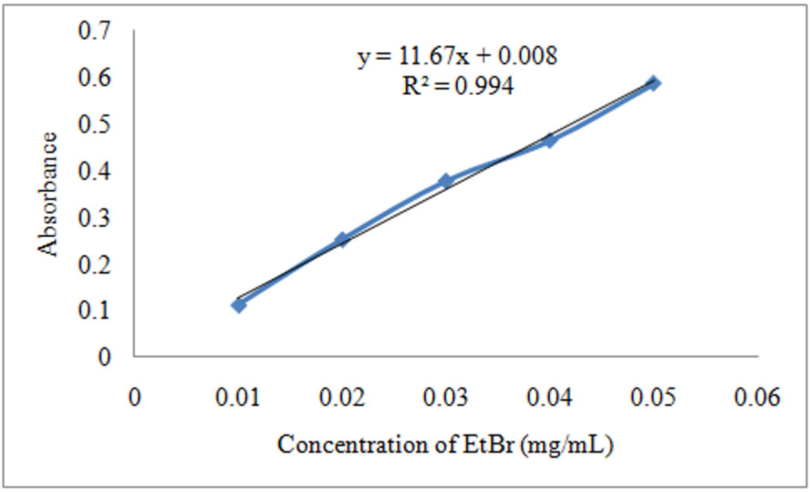


**Figure 1.** Standard curve of EtBr

**Table 1.** The pH of EtBr waste and concentration of EtBr waste after treatment

| Ratio of EtBr (mL) | Ratio of activated charcoal (mg) | pH Before treatment | pH After treatment | Concentration of EtBr after treatment (mg/mL) |
|---|---|---|---|---|
| 50 | 50 | 8.0 | 7.4 | 0.056 |
| 100 | 100 | 8.0 | 7.4 | 0.045 |
| 150 | 150 | 8.0 | 7.4 | 0.060 |
| 50 | 100 | 8.0 | 7.0 | 0.031 |
| 100 | 200 | 8.0 | 7.0 | 0.028 |
| 150 | 300 | 8.0 | 7.0 | 0.032 |
| 100 | 50 | 8.0 | 7.7 | 0.089 |
| 200 | 100 | 8.0 | 7.7 | 0.081 |
| 300 | 150 | 8.0 | 7.7 | 0.090 |

Dissolved Oxygen (DO) is needed by all living bodies for respiration, metabolic processes or subsequent exchange of substances produce energy for growth and reproduction. Besides that, oxygen required for the oxidation of organic and inorganic materials in aerobic processes. The main source of oxygen in waters comes from a diffusion process from the air free and the results of photosynthesis of organisms that live in these waters. In this study, the DO range in the EtBr sample after being treated with activated charcoal with several variations showed good results, namely 5.0 - 9.0 mg/L.

Biochemical Oxygen Demand (BOD) is the amount of dissolved oxygen needed by microorganisms to decompose organic matter under conditions aerobics. BOD value is not indicates the amount of organic matter actually, but only measures the amount oxygen needed to decompose the organic material. In this study, the BOD range of the filtrate from the treatment with activated charcoal was obtained at 25.06 - 78.2 mg/L. It still meets the criteria for wastewater in accordance with the Regulations Minister of Environment of the Republic of Indonesia No. 5 of 2014 concerning the waste water quality standard, which is 100.00 mg/L.

Chemical Oxygen Demand (COD) is the amount oxygen needed to oxidize organic matter present in water chemical. This matter because organic matter is broken down chemically using a strong oxidizing agent under conditions acid and heat with a catalyst. In this study, the BOD range of the filtrate from the treatment with activated charcoal was obtained at 302.56 - 310.98 mg/L, with the regulation 350.00 mg/L.

In addition, the ability of absorption by activated charcoal is determined based on the pore size and functional groups on the surface of the activated charcoal. The pore size of activated charcoal is divided into three types, namely: micropores (width < 2 nm), mesopores (width = 2 – 50 nm) and macropores (width > 50 nm) (Ilomuanya *et al.,* 2017). The wider pore size of activated charcoal will be more beneficial to improve the diffusion kinetics, while the smaller pore size will be an anabatic entry barrier for the absorption of large molecules. In previous studies, it was shown that micropores, especially ultramicropores, which have a pore size of about 0.7 nm, will determine the adsorption capacity of activated charcoal. In addition, the pore structure is also an important factor that determines the capacity and rate of activated charcoal adsorption (Li *et al.,* 2018).

Figure 2 show the scanning electron microscopy images (SEM) of the activated charcoal. Based on these texture characteristics explained above it is expected the activated charcoal would present higher sorption capacity for the EtBr adsorption. This is in accordance with the enlarged pore volume compared to before the adsorption was 2.76 x $10^2$ cc/g to 3.57 x $10^2$ cc/g with isothermal graph in Figure 3. Based on previous research, removal Ethidium Bromide with Carbon Nanotubes showed different solid morphology between before and after treatment. This is due to the presence of the EtBr molecule attached to the solid surface (Moradi *et al.,* 2014). Previous studies have also reported showing that after adsorption, the activated charcoal surface changes due to the presence of water molecules (Najafi *et al.,* 2013).

Functional groups on the surface of activated charcoal also determine the absorption ability of activated charcoal. The groups on the activated charcoal surface can be either acidic or basic (carbonyl, phenol, lactone, quinone, etc.) (Brennan *et al.,* 2002). The group will bind to the EtBr compound so that the EtBr compound will be bound to the activated charcoal. The ilustration of reaction mechanism for the binding of EtBr by activated charcoal is shown by Figure 4.

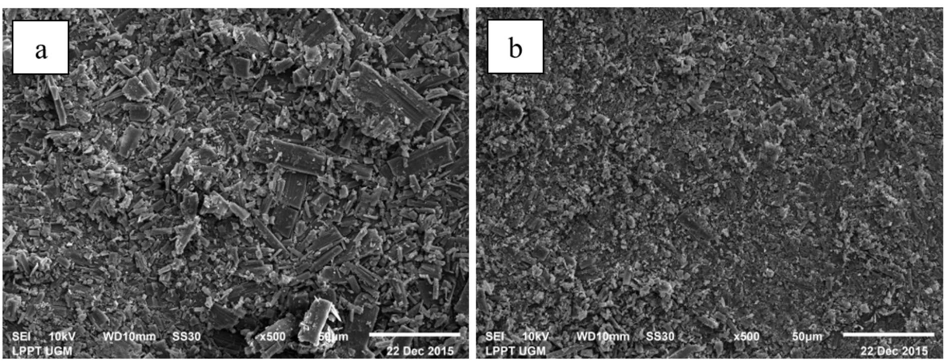


**Figure 2.** SEM images of activated charcoal (a) before tratment and (b) after adsorption with ethidium bromide

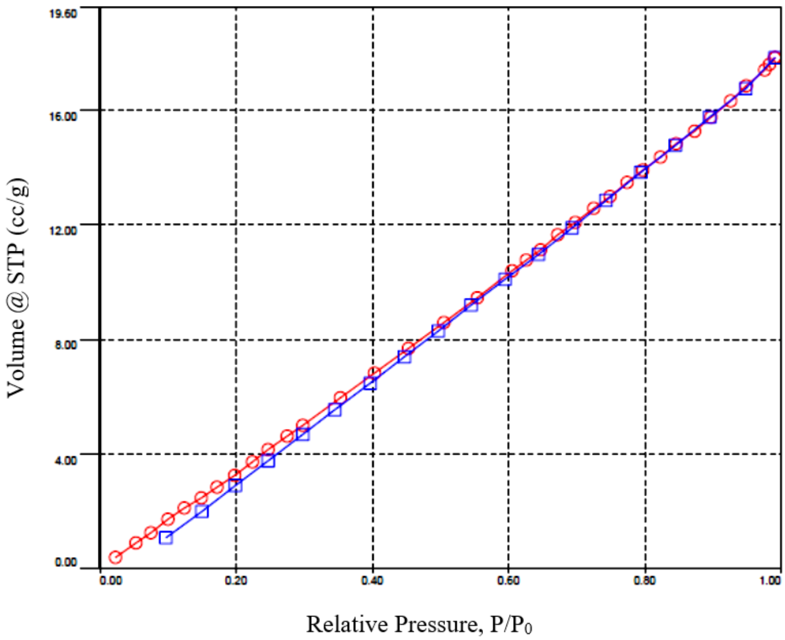


**Figure 3.** Isothermal graph of activated charcoal, with —○— adsorption and —□— desorption

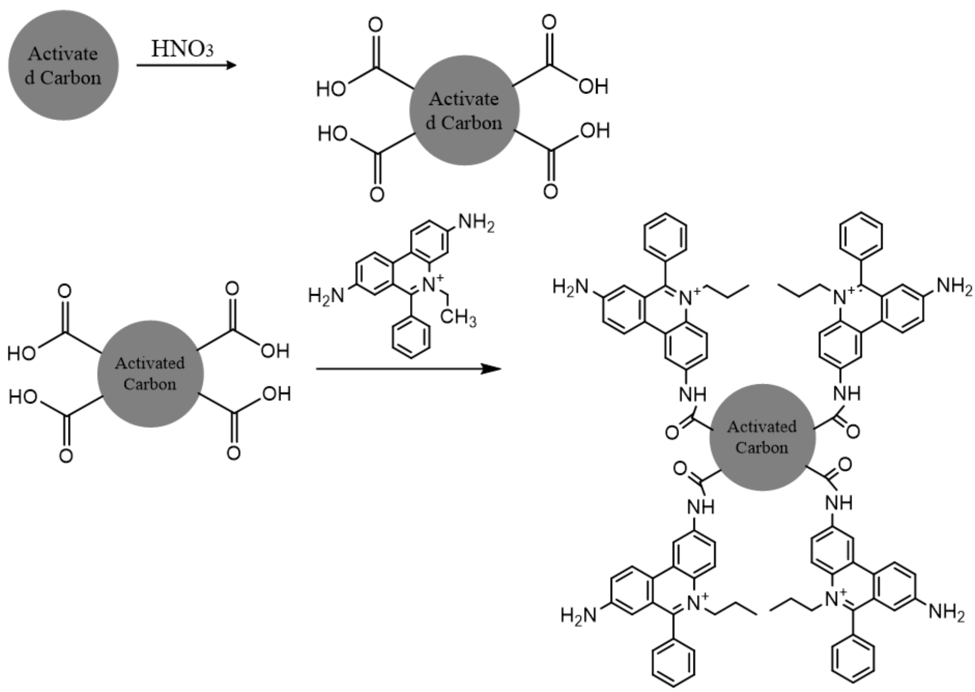


**Figure 4.** The reaction mechanism for the binding of EtBr by activated charcoal

Figure 4 shows that the EtBr molecules bind to the surface of the charcoal. Possible bond interactions that occur between the EtBr molecule and activated charcoal are London physical dispersion forces, classical electrostatic interactions, hydrogen bonds, functional group interactions, and chemisorption. The desorption of inorganic cations agreed with $Et^+$ adsorption, confirming the main mechanism of EtBr removal occurred through the exchange for $Et^+$ by activated charcoal. Adsorption of counterion $Br^-$ accompanying $Et^+$ suggested the formation of dimers of $Et^+$ on the external surface of activated charcoal at higher adsorption capacities.

This study provides good wastewater quality results based on the values of pH, DO, BOD, and COD after treatment with activated charcoal. Based on these 4 parameters, it is in accordance with the range of values of the Minister of Environment of the Republic of Indonesia regulation No. 5 of 2014 concerning the waste water quality standards. Thus, every laboratory or institution that uses EtBr can treat their own waste safely and safely when disposed of into the environment.

## 4. Conclusion

Activated charcoal has the ability to adsorb on EtBr compounds. This is due to several factors including the strong adhesion force possessed by activated charcoal. In addition, activated charcoal also has fairly large pores, and functional groups on the surface of activated charcoal that make activated charcoal bind EtBr compounds.

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