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Characterization of Antibiotic Trimethoprim Adsorption by Granular Activated Carbon

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Abstract
The presence of antibiotics in aquatic environments has been known to lead to the development of bacteria with resistant genes. One type of antibiotic that has been found in the environment is trimethoprim, necessitating methods to remove this substance from the environment. Adsorption is a process that has been extensively studied in the field of water remediation. This study aims to investigate the adsorption of trimethoprim by granular activated carbon (GAC). Experiments were conducted by creating artificial wastewater containing distilled water and trimethoprim, which was then contacted with the adsorbent with variations in pH and trimethoprim concentration measured periodically up to the 3rd hour. Trimethoprim detection was performed using a UV-Vis spectrophotometer. The contact time required to reach equilibrium increased with the initial concentration of trimethoprim. The variations in initial trimethoprim concentrations of 5, 10, 20, 35, and 50 mg/L reached adsorption equilibrium after a contact time of 180 minutes with a GAC mass of 3 grams. All the percentages of trimethoprim removal after the contact time of 180 minutes at various concentrations used in this study were above 98%. The pH level has been proven to affect the trimethoprim removal process. The pseudo-second-order kinetic model has a high correlation with the adsorption process of trimethoprim by GAC. The isotherm model suitable for the interaction between trimethoprim and GAC was the Freundlich model.
Keywords: *trimethoprim, adsorption, GAC, adsorption kinetics, isotherm*

Abstrak
Keberadaan antibiotik di lingkungan perairan diketahui telah menyebabkan berkembangnya bakteri dengan gen resisten. Salah satu jenis antibiotik yang telah ditemukan di lingkungan adalah trimetoprim, sehingga dibutuhkan metode untuk menyisihkan bahan tersebut dari lingkungan. Adsorpsi merupakan proses yang telah banyak diteliti dalam bidang remediasi air. Penelitian ini bertujuan untuk menginvestigasi adsorpsi trimetoprim oleh karbon aktif dalam bentuk granular (GAC). Percobaan dilakukan dengan membuat air limbah artifisial yang mengandung air suling serta trimetoprim, dimana dikontakkan dengan adsorben dengan variasi pH dan konsentrasi trimetoprim diukur secara berkala hingga jam ke-3. Deteksi trimetoprim dilakukan dengan menggunakan spektrofotometer UV-Vis. Waktu kontak yang dibutuhkan untuk mencapai kesetimbangan meningkat seiring peningkatan konsentrasi awal trimetoprim. Variasi konsentrasi awal trimetoprim 5, 10, 20, 35, dan 50 mg/L telah mencapai kesetimbangan adsorpsi setelah waktu kontak 180 menit dengan massa GAC sebesar 3 gram. Seluruh persentase penyisihan trimetoprim setelah waktu kontak pada berbagai variasi konsentrasi yang digunakan pada penelitian ini berada di atas angka 98%. Kadar pH telah dibuktikan berpengaruh pada proses penyisihan trimetoprim. Model kinetika pseudo-orde kedua memiliki korelasi yang tinggi dengan proses adsorpsi trimetoprim oleh GAC. Model isoterm yang sesuai untuk interaksi antara trimetoprim dengan GAC adalah model Freundlich.
Kata Kunci: *trimetoprim, adsorpsi, GAC, kinetika adsorpsi, isoterm*

1. Introduction

Pharmaceutical products are produced with the aim of influencing the physiological functions of living organisms. Throughout the world, pharmaceutical products such as analgesics, anti-inflammatories, antibiotics, lipid regulators, β-blockers, and other types of drugs have become part of everyday life. Antibiotics are used to prevent the spread of infectious diseases in humans, poultry, aquatic organisms, and other animals by reducing or inhibiting the growth of microorganisms [1]. Due to the metabolism process of antibiotics, these types of drugs have been found in various environmental matrices worldwide. Antibiotics have been found in industrial wastewater, groundwater and rivers, hospital wastewater, sludge, soil, as well as terrestrial animals and plants [2]. Excessive global antibiotic use has increased the possibility

of detecting the presence of antibiotics in the environment, especially in various water bodies, increasing the antibiotic load in water and posing a potential threat to human health and environmental balance [3].

Trimethoprim (5-(3,4,5-trimethoxy-benzyl) pyrimidine-2,4-diamine) is a sulfonamide antibiotic, which is one of the most commonly prescribed antibiotics and has been widely used to treat diseases caused by bacteria [4]–[6]. Trimethoprim is known to work as an antibiotic by inhibiting DNA replication through reducing the activity of dihydrofolate reductase [7]. Trimethoprim in the treatment process is generally combined with sulfamethoxazole and used in human and animal treatment worldwide as an inhibitor in chemotherapy due to the antifolate effect provided through interaction with the coenzyme dihydrofolate [8]–[10]. In addition to this function, trimethoprim is also used as a feed additive in the livestock industry to accelerate the growth of livestock [11].

Several studies have reported that most trimethoprim cannot be metabolized by the human body, resulting in a large amount of trimethoprim entering the environment [12]. Because the average human and animal body can only metabolize about 20% of trimethoprim after consumption, a significant amount of this drug is excreted and can then be found in aquatic ecosystems [13], [14]. Worrisome levels of trimethoprim have been detected in water and wastewater, where these concentrations have been shown to result in the formation of bacteria with antibiotic-resistant genes in the environment [6]. Furthermore, it has been found that many bacteria resistant to trimethoprim can survive in aquatic environments for up to 10 years [15].

Several studies have concluded that trimethoprim has harmful effects on living organisms. Trimethoprim contributes to aspects of mortality, development, and reproduction in various non-target aquatic organisms including phytoplankton, zooplankton, fish, and others [16]–[18]. Because the use of antimicrobial agents such as antibiotic drugs cannot be stopped, preventive measures must be taken to prevent the continuous release of antibiotics into the environment [9]. Therefore, the need for effective and efficient methods to remove trimethoprim from water is becoming increasingly urgent [19]. This is also of utmost importance considering the increasing demand for clean water for human consumption and other uses such as agricultural and industrial needs [4].

Adsorption is a well-developed and efficient technology for wastewater remediation [20]. Among various types of different adsorbents, activated carbon is typically used to treat wastewater with relatively low pollutant concentrations. The porous structure developed during the activation process of carbon for adsorption processes is responsible for physical adsorption, while the chemical surface characteristics of the adsorbent allow specific interactions to occur between the adsorbate and the adsorbent in the system [21]. Since it is known that synthesizing activated carbon materials with controlled structures and chemical characteristics is relatively easy, its use for removing pharmaceuticals from wastewater enables the use of attractive and simple tools to address emerging environmental challenges [22]–[25]. Thus, this research was conducted to determine the adsorption kinetics model and isotherm model that best fit experimental data between activated carbon and trimethoprim.

2. Material and Methods

Artificial waste preparation

To create easily controllable conditions, artificial waste will be utilized in this research. The material used to produce artificial waste consists of a mixture of distilled water and pure trimethoprim. The trimethoprim used is of analytical grade (TLC with a minimum value of 98%). A stock solution of trimethoprim is prepared by mixing 400 mg of trimethoprim powder with 1 L of distilled water in a 1 L volumetric flask and then stirred on a magnetic stirrer for 2 hours. The trimethoprim concentrations used in this study are 5, 10, 20, 35, and 50 mg/L, prepared by diluting the stock solution of trimethoprim with distilled water.

Trimethoprim detection

A standard curve was established based on the trimethoprim detection method developed by Gupta & Shrivastava [26] to ensure the minimal concentration that can be accurately quantified and adheres to Beer's law. According to their method, trimethoprim can still be detected down to a concentration of 10 µg/L using an Orion AquaMate 8100 UV-Vis Spectrophotometer at a wavelength of 285 nm.

Procedure for adsorption experiments

In the experiments conducted in this study, the granular activated carbon (GAC) to be used for adsorption testing was washed with distilled water and dried in an oven at 105°C for 1 hour, then cooled in a desiccator for 15 minutes. Various concentrations of trimethoprim were poured into five 500 mL glass beakers and filled to a volume of 400 mL, with one beaker filled with 400 mL of water without trimethoprim serving as a blank. Subsequently, the adsorbents were added to the six glass beakers, which were then

stirred for a specific duration. The stirring process was carried out using a jar tester. Then, the water contained in the 500 mL glass beakers was filtered through Whatman GF/C filter paper, yielding 25 mL, using a vacuum pump, and transferred to 200 mL glass beakers. The concentration of trimethoprim was then measured using a UV-Vis spectrophotometer.

Determination of the optimum adsorbent dosage

Referring to the various experiments conducted before [8], [27]–[30], the variations in adsorbent mass using GAC were 1, 2, 3, 4, and 5 grams. The designated concentration was 25 mg/L. A concentration of 25 mg/L was established based on the measurement of trimethoprim levels obtained from wastewater samples from several hospitals in Indonesia. Samples were obtained from a large public hospital (class B general hospital), a small public hospital (class C general hospital), and a private hospital. In the wastewater from each of these three types of hospitals, trimethoprim concentrations were found to be 21.717 mg/L, 38.103 mg/L, and 11.812 mg/L, respectively. The set time for the adsorption process was 30 minutes, wherein the adsorbent mass capable of achieving the highest removal efficiency would be utilized for subsequent experiments.

Determination of contact time and pH influence

During the contact between the adsorbent and trimethoprim, samples are periodically taken. The adsorbent will be in contact with trimethoprim in artificial wastewater for 180 minutes. During this time range, samples will be taken at the 5th, 10th, 20th, 30th, 40th, 50th, 60th, 80th, 100th, 120th, 150th, and 180th minutes. This procedure aims to determine the time required by the adsorbent with trimethoprim to reach equilibrium conditions. The concentration data of each sample will be recorded. For all trimethoprim concentration variations and at each optimum adsorbent dosage, the pH level will be set to 4, 7, and 10. These three pH variations refer to research conducted by Berges [27] and Kim [8]. Samples will be measured once the time have reached 60 minutes.

Determination of adsorption kinetics

The data used to determine adsorption kinetics are samples with an initial trimethoprim concentration of 20 mg/L. The calculated adsorption kinetics models are the pseudo-first-order and pseudo-second-order models. The pseudo-first-order model is calculated using the formula: $\log(Q_e - Q_t) = \log Q_e - \frac{k_1}{2{,}303} t$. Meanwhile, the pseudo-second-order model is calculated using the formula: $\left[\frac{t}{Q_t}\right] = \frac{1}{k_2.(Q_e)^2} + \frac{1}{Q_e}.t$.

Determination of the isotherm model

The data used to determine the Isotherm model with high correlation between the adsorbent and trimethoprim is the trimethoprim concentration data when adsorption equilibrium has been reached. In this study, the Langmuir and Freundlich isotherm models are used to describe the adsorption equilibrium of trimethoprim. The Langmuir isotherm model is described by the equation: $\frac{C_e}{Q_e} = \frac{1}{Q_m b} + \frac{C_e}{Q_m}$. Meanwhile, the Freundlich isotherm model is described by the equation: $\ln Q_e = \ln k_f + \frac{1}{n} \ln C_e$.

3. Results and Discussion

Standard curve for trimethoprim

The result of the preparation of a standard curve for trimethoprim in distilled water is displayed in Fig**. 1**. Based on **Fig. 1**, it is found that the standard curve for trimethoprim has the equation $y = 0.0196x - 0.0236$ with an R^2 value (coefficient of correlation) of 0.9955. According to the standard curve creation rules, the closer the R^2 value is to 1, the stronger the correlation [31]. Although the R^2 value generated from the standard curve creation is very close to 1, it does not prove that the curve generated is linear. However, based on visual observation, the curve displayed in Fig. 1 is considered sufficiently linear, so the generated standard curve can be used to detect the concentration of trimethoprim in the subsequent experimental stages.

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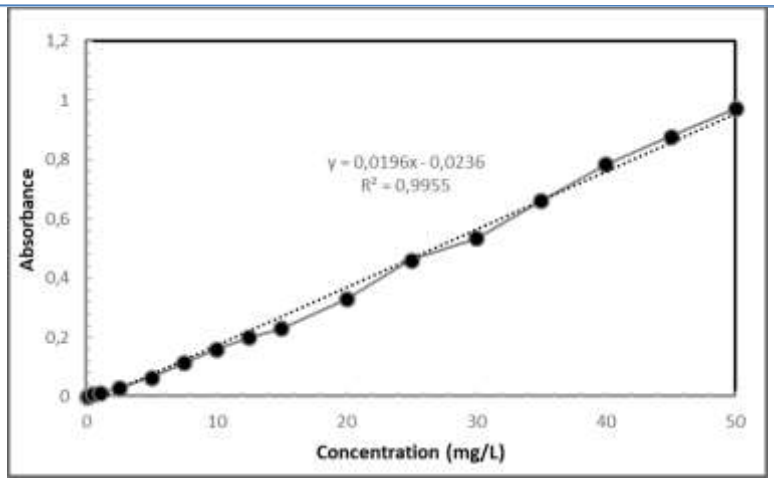

Fig. 1: Standard curve for trimethoprim

Optimum dosage of adsorbent

The data from the experiment determining the optimum adsorbent dosage is displayed in **Fig. 2** below. In the subsequent experiments, the adsorbent dosage to be used will be the dosage that yields the highest removal efficiency.

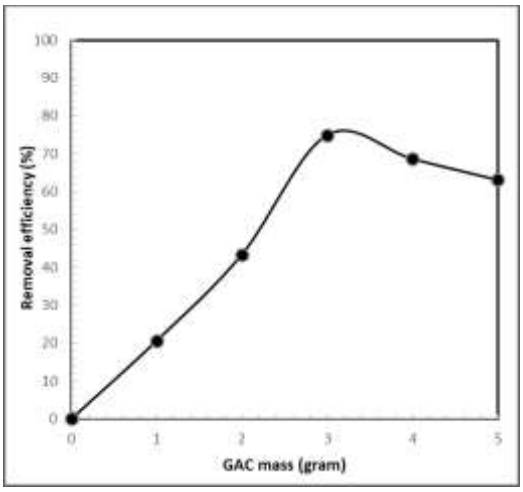

Fig. 2: Removal efficiency at various GAC masses

As shown in **Fig. 2**, it is found that the optimal dosage to be used is 3 grams for GAC. This value is not the highest mass tested, but it is chosen because it yields the lowest absorbance values in the spectrophotometer readings. This indicates that using a higher amount of adsorbent can lead to higher levels of absorbance reading in the water.

Influence of contact time

The experimental result depicting the comparison of the impact of contact time between activated carbon and trimethoprim based on variations in the initial trimethoprim concentrations is displayed in **Fig. 3** below. Based on observation of **Fig. 3**, trimethoprim has reached adsorption equilibrium or steady state conditions after a process duration of 20 minutes for the initial concentration of 5 mg/L, 50 minutes for the initial concentration of 10 mg/L, 60 minutes for the initial concentration of 20 mg/L, 80 minutes for the initial concentration of 35 mg/L, and 120 minutes for the initial concentration of 50 mg/L. In **Fig. 3**, there is a tendency where trimethoprim with lower initial concentrations tends to result in higher removal efficiencies.

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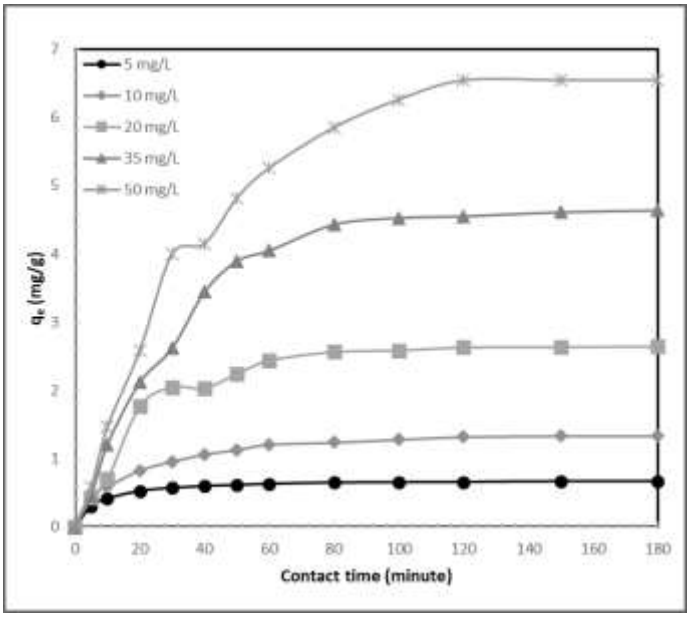

Fig. 3: Comparison of Q_e values and contact time between trimethoprim and GAC

However, there is a limitation in detecting the concentration of trimethoprim in the artificial wastewater created in this experiment. This limitation is due to the capability of the spectrophotometer used, where the UV-Vis spectrophotometer used can only detect the minimum concentration of trimethoprim up to 0.01±0.005 mg/L based on the measurements taken. Therefore, concentrations of trimethoprim that are very small and undetectable by the spectrophotometer are assumed to be in the range of 0.01 mg/L.

Based on **Fig. 3**, the adsorption pattern of trimethoprim on GAC has proven to be effective. In samples containing trimethoprim with lower concentrations (5-10 mg/L), minor adsorption processes are observed, while higher adsorption processes occur in solutions with higher trimethoprim concentrations. The results obtained in this experiment are in line and consistent with previous research by Berges [27] and Liu [29]. The low Q_e values for lower concentrations (5-10 mg/L) are due to the limited quantity of trimethoprim present in the artificial wastewater. Conversely, higher initial trimethoprim concentrations (20-50 mg/L) result in higher Q_e values. This indicates a direct impact of trimethoprim concentration on adsorption capacity, where linearity suggests that the higher the concentration of the target compound in a system, the higher the adsorption capacity by the adsorbent [32].

Antibiotic adsorption on activated carbon is determined by various factors, such as: (i) the ability of absorbed molecules to form π-π interactions between their aromatic rings and the activated carbon surface; (ii) formation of hydrogen bonds with electronegative atoms in the molecule; and (iii) electrostatic interactions between molecules and activated carbon [33], [34]. It is known that trimethoprim itself contains two aromatic rings in its structure. Therefore, theoretically, π-π interactions are considered the main influencing factor in the adsorption process of trimethorprim by activated carbon.

Influence of pH to the adsorption process

The result of the experiment on the impact of pH on the removal efficiency of trimethoprim is shown in **Fig. 4** below. According to **Fig. 4**, it can be concluded that GAC is able to adsorb trimethoprim with the highest efficiency when the pH value is 4. Fig. 4 also demonstrates that an acidic environment (pH 4) results in more efficient adsorption compared to a neutral environment (pH 7), while a neutral environment produces a better adsorption efficiency curve than the alkaline conditions (pH 10). However, there is an exception where pH 7 depicts a better scenario than pH 4 at the initial concentration of 35 mg/L. The results obtained from Fig. 4 are in line with the research conducted by Opanga [28] and Kim [8]. The decrease in adsorption efficiency at high pH (alkaline conditions) is due to the pH properties affecting the surface charge on the activated carbon. When the pH level is above 7, there is an increase in hydroxyl ions. This causes both the activated carbon and trimethoprim in the artificial solution to become negatively charged, thereby reducing the efficiency of the adsorption process through electrostatic repulsion [28].

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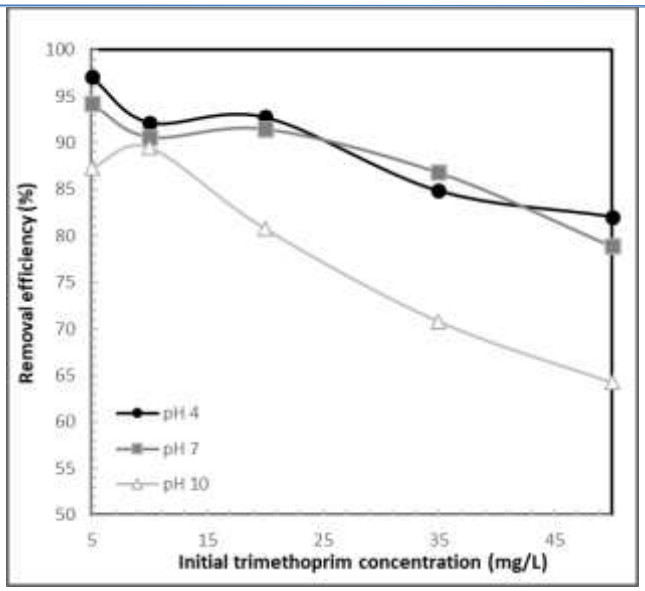

Fig. 4: The impact of pH on the removal efficiency of trimethoprim by GAC

However, these results differ from the research conducted by Berges [27], Guo [30], and Liu [29]. In these three studies, it was found that trimethoprim is adsorbed better at pH levels within the range of 8±0.5. The adsorption behavior of antibiotics by activated carbon at various pH levels is determined by the electrostatic interactions between activated carbon and trimethoprim. When $pH<pH_{pzc}$, the activated carbon surface is positively charged. When $pH>pH_{pzc}$, the adsorbent has a negative charge on its surface. The pH of the environment also affects the likelihood of charges appearing on the molecules under study (trimethoprim), leading to interactions of repulsion and attraction [35]. pH_{pzc} stands for pH of zero charge, referring to the pH of a solution where the surface of a material has a neutral charge or an average charge of zero. Molecules with pKa values similar to pH_{pzc} will exhibit poorer adsorption performance at pH values far from the zero charge point, due to reduced electrostatic repulsion for pH values close to pH_{pzc} [36].

It is known that the adsorption of trimethoprim by activated carbon involves strong interactions between trimethoprim species and the surface of the activated carbon. The properties of trimethoprim and the surface charge properties of activated carbon are highly sensitive to the pH of their environment [29]. This sensitivity is due to the nature of trimethoprim and the surface charge properties of activated carbon being highly susceptible to the pH of the solution, as the two amino groups in the trimethoprim molecule can be protonated by excess H^+ ions in an acidic environment. Additionally, the acidic and basic groups of activated carbon can also be deprotonated or protonated [29]. However, in this study, it can be assumed that the C π-electrons in the graphene structure and the amino groups of trimethoprim are not protonated, so trimethoprim retains its anionic properties at low pH levels. In contrast, at low pH levels, previous studies have indicated that activated carbon has a positive charge [8], [27].

Adsorption kinetics

The adsorption kinetics of trimethoprim by activated carbon are depicted as a function of contact time using a fixed initial concentration of trimethoprim. The experimental basis used is a trimethoprim concentration of 20 mg/L with a mass of 3 grams for GAC. In this study, the data were analyzed using the pseudo-first-order model and the pseudo-second-order model, with contact time starting from minute 0, followed by sequential measurements of trimethoprim concentration at minutes 5, 10, 20, 30, 40, 50, 60, 80, 100, 120, 150, and 180. The resulting model for GAC is shown in **Fig. 5** below.

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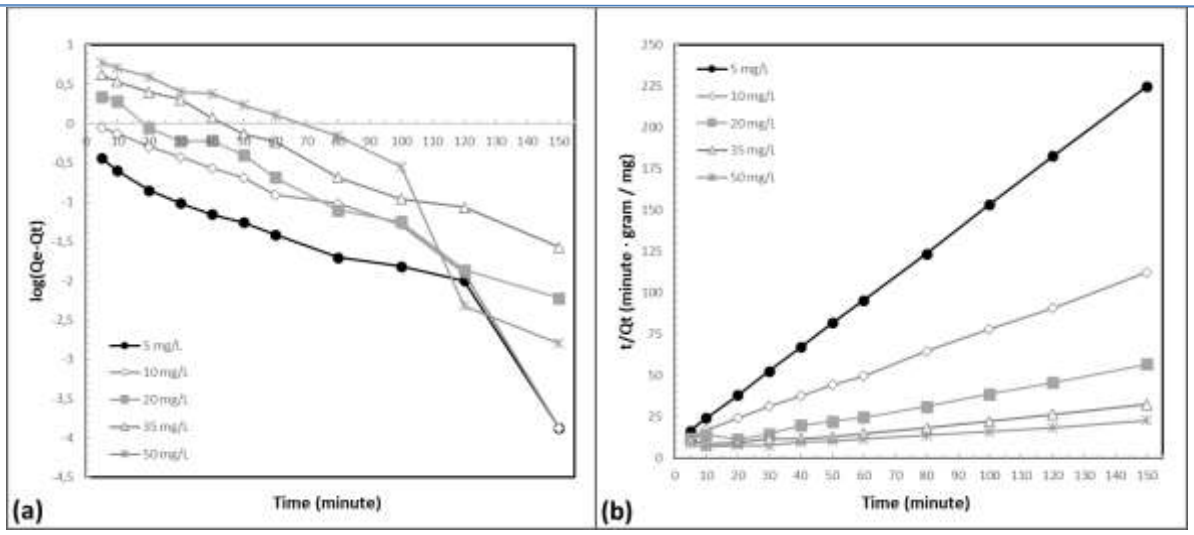

Fig. 5: (a) Pseudo-first-order and (b) pseudo-second order adsorption kinetics for trimethoprim using GAC

Meanwhile, the results of the calculations based on the model shown in the previous figure (Fig. 5) are presented in the **Table 1** below.

Table 1. Adsorption kinetics parameters of trimethoprim adsorption on GAC

Initial concentration (mg/L)	Qe (mg/g)	Pseudo first order			Pseudo second order		
		R^2	Theoretical Qe (mg/g)	k_1 (min^{-1})	R^2	Theoretical Qe (mg/g)	k_2 ($g.mg^{-1}.min^{-1}$)
5	0,6665	0,8765	0,4577	-0,04284	1	0,6949	0,2194
10	1,3332	0,8593	1,9266	-0,04951	0,9991	1,4639	0,0481
20	2,6457	0,9882	2,5592	-0,04099	0,9814	3,1635	0,0137
35	4,6411	0,9904	4,8629	-0,03547	0,9578	6,2151	0,0039
50	6,5491	0,8859	16,9161	-0,05573	0,9607	9,3721	0,0021

Based on the results obtained from the experiments, the adsorption process kinetics show a better correlation with the pseudo-second-order model, indicating that intraparticle diffusion is not the rate-limiting step in the adsorption process. The higher correlation is inferred by comparing the correlation coefficient (R^2) values between the pseudo-first-order and pseudo-second-order models. In **Table 1**, some R^2 values for the pseudo-first-order model is smaller than some of the R^2 values of the pseudo-second-order model. The R^2 values for the pseudo-second-order model are all above 0,95 which indicates that the adsorption of trimetoprim on the surface GAC involves both physical and chemical processes.

Adsorption kinetics are depicted with the aim of determining the reaction rate between the adsorbent and adsorbate [37]. Adsorption kinetics are also studied to identify the crucial factors influencing the speed of the adsorption process. According to the pseudo-first-order kinetics model, the adsorption rate itself is controlled by adsorbate diffusion, where the adsorption rate is directly proportional to the amount of adsorbate or target compound remaining in the solution [38]. According to the pseudo-first-order kinetics model, the adsorption rate is also directly proportional to the difference between the adsorption capacity at equilibrium and the adsorption capacity at any given time (time-t). Meanwhile, the pseudo-second-order kinetics model assumes that the chemical interaction process is the primary factor influencing the adsorption process, where the adsorption rate is directly proportional to the squared concentration of the pollutant [39].

The adsorption kinetics process obtained in this study is consistent with the literature previously reported for trimetoprim [8], [27], [29], [37]. The trends resulting from the experimental data suggest that in the adsorption process of trimetoprim by activated carbon, there is a chemical process involving valence forces through electron exchange between the adsorbent and adsorbate [40], [41].

Adsorption isotherm

The data when adsorption equilibrium on all initial concentration of trimethoprim was achieved (120 minutes) was calculated using the Langmuir and Freundlich isotherm equations to predict the adsorption mechanism. The adsorption isotherm curves for GAC are shown in **Fig. 6** below.

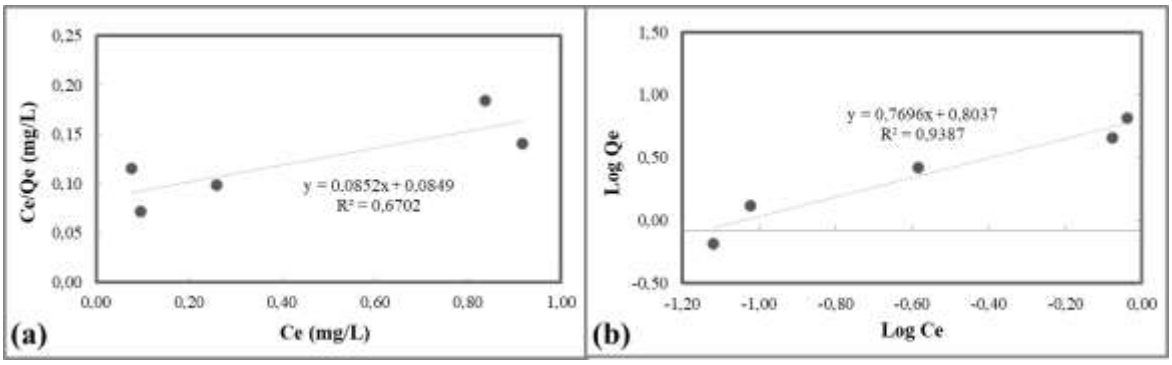

Fig. 6: (a) The Langmuir isotherm curve for GAC; (b) The Freundlich isotherm curve for GAC

Based on the results obtained from **Fig. 6**, several conclusions can be drawn. The experimental results indicate that the adsorption process of trimethoprim by GAC can be better described by the Freundlich model. This is because the R^2 value of the Freundlich curve in experiments using GAC is closer to 1 compared to the Langmuir curve. The data processing results from GAC correspond to the experiments conducted by Xue [37]. In GAC, the Freundlich model is more appropriate for describing the trimethoprim adsorption process. Adsorption of trimethoprim onto the surface of GAC is not monolayer (single layer) homogeneous [37] but there is heterogeneous distribution of active sites on the activated carbon surface, with the possibility of forming multilayer adsorption. The adsorption capacity of GAC is characterized by the parameter n in the Freundlich model, where n<0,5 indicates difficult adsorption and n>1 indicates easy adsorption [42]. Based on the calculation results for trimethoprim, the value of n from the Freundlich model is 1,29937. The value of n is indicating that GAC can efficiently adsorb trimethoprim involving both physical and chemical interactions [43], [44].

Based on the physical and chemical characteristics of activated carbon as an adsorbent for trimethoprim, there are three aspects to consider: (i) Based on the molecular geometry of trimethoprim which is sized 7.03×7.5×12.28 Å [29], the process of filling the micropores of activated carbon may trigger the adsorption process of trimethoprim into the porous activated carbon because the molecular size of trimethoprim approaches the micropore width of activated carbon which is only slightly larger [45]; (ii) Various chemical interactions can occur between trimethoprim and the activated carbon surface [29], including: π–π EDA interactions, cation–π bonds, Lewis acid–base interactions, electrostatic attractions, and hydrogen bonds. The three methoxy (–OCH_3) groups on the benzene ring and two amino groups (–NH2) on the pyrimidine ring of trimethoprim are strong electron donor groups, making the aromatic ring of trimethoprim π electron-rich [29].

The amino groups of trimethoprim can be positively charged under acidic conditions, allowing these groups to undergo electronic coupling (interaction between adjacent molecular electronic orbitals or involved in chemical reactions that affect molecular properties and behavior, including electron transfer, structural changes, or chemical reactions). Carboxylate and lactate groups of activated carbon can attract π-electron from the graphene layer of trimethoprim, causing π-electron-deficient structures. Therefore, the structure of the activated carbon can act as an effective π-electron acceptor to strongly interact with the electron-rich aromatic ring of trimethoprim via the π–π EDA interaction mechanism [29]; and (iii) There is a 'filtration' effect of the micropores on the activated carbon surface. This effect is called the size-exclusion effect and occurs when oxygen complexes are present on the surface of trimethoprim [46]. Several oxygen groups can be located on the external surface of activated carbon and hinder the adsorption process of trimethoprim [47]. These oxygen groups can absorb water molecules through hydrogen bonding and further form groups of water molecules. This phenomenon can prevent trimethoprim species from accessing some adsorption sites within the pores of activated carbon. In this study, these three factors are considered important for understanding the adsorption process between trimethoprim and activated carbon, thus resulting in the obtained data.

4. Conclusion

Based on the research findings regarding the adsorption process of trimethoprim by granular activated carbon (GAC), the following discoveries have been made: (i) The contact time required for the adsorption process by GAC to reach equilibrium is determined by the initial concentration of trimethoprim, where it takes longer to reach equilibrium if the trimethoprim were to be in a higher amount; (ii) The optimum dosage for the adsorption process of trimethoprim is 25 mg/L, which corresponds to 3 grams for GAC; (iii) All the percentages of trimethoprim removal after the contact time of 180 minutes at various concentrations used in this study were above 98%; (iv) pH influences the adsorption efficiency of trimethoprim by GAC, with the optimum pH being 4; (v) The adsorption process between trimethoprim and GAC involves chemical interactions, as evidenced by the high R^2 values for the second-order pseudo-kinetic model; and (vi) The appropriate isotherm model for the interaction between GAC and trimethoprim is the Freundlich model with an R^2 value of 0.9387.

Moving forward, it is recommended to use instruments capable of detecting trimethoprim concentrations at even lower levels. Additionally, further research is needed on the size-exclusion effect that inhibits the adsorption process of antibiotics, especially trimethoprim. To date, there has been no research on the antibiotic levels in various environmental matrices or in wastewater known to contain various active pharmaceutical compounds in Indonesia, highlighting the need for research in this area.

5. Acknowledgment

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