Biosorption of doxycycline using Carica papaya L. peels

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Abstract. This study focuses on the batch adsorption of doxycycline in simulated wastewater using Carica papaya L. (papaya) peels. Sorbate concentration (120-1,000 mg/L) and pH (3, 6, and 9) were varied while adsorbent dosage (0.1 g) and contact time (180 min) were fixed. Based on the observations done for both ripe and unripe papaya peels (PP), as the sorbate concentration increases while the pH decreases, the removal efficiency increases. The highest removal efficiency (87.97% for ripe PP and 87.52% for unripe PP) was achieved at 1,000 mg/L and pH=3. The adsorptive capacity for ripe and unripe PP was 215.09 and 213.99 mg/g adsorbent, respectively. Langmuir model fits the data for samples, which indicates a monolayer adsorption on surface of the adsorbent. The data also fits the pseudo-first order kinetic model which suggests that the reaction operates only at the monolayer of papaya peel. Overall, both ripe and unripe PP are capable of adsorbing DC in aqueous solution at 28°C, 3-hour contact time, 0.1 g of adsorbent dosage, and at pH 3 under acidic condition.

1. Introduction
Antibiotic pollutants in wastewater from hospitals and drug factories are considered threats to human and ecological health [1]. Doxycycline (DC), a type of Tetracycline which has an excellent therapeutic value against bacteria (i.e., gram-positive and gram-negative) and other infectious diseases, exhibits a high toxicity in ground and surface water because of its high solubility in water, and non-biodegradability [2,3]. It could cause various complications such as bacterial antibiotic resistance, discoloration of teeth, occurrence of the elimination of trophic levels of bacteria, change in microflora, and damage to aquatic life and plants [4,5].

The use of conventional methods for tetracycline removal from aqueous solution, such as electrocoagulation coupled electro-flotation [3], ozonation [6], photo-Fenton process [7], photo electro-catalytic degradation [8], and ion exchange [9], have high capital and operational costs. Thus, it is preferable to use a low-cost and effective process such as biosorption in the removal of antibiotics from aqueous solution [10]. Current studies in adsorption of cationic molecules in aqueous solution via ion-exchange mechanism have been inclined to the use of various low-cost adsorbents. Naushad et al [11] used amberlite Ira-938 resin to adsorb rose Bengal dye from aqueous solution. Furthermore, Pb²⁺ was effectively adsorbed in a nano-composite cation exchange material [12], whereas the adsorption of Coomassie brilliant blue R-250 was more effective in a nanohydrogel synthesized using starch and poly(alginic acid-cl-acrylamide) [13]. Although, these adsorbents are low-cost and efficient in removing pollutants in aqueous solution, synthetic processes are necessary to achieve the desired results. These processes are complex, tedious, and use synthetic chemicals that could contribute to the hazards of their preparation. Currently, agricultural wastes, such as rice straw [4], Moringa oleifera seed [14], spent black tea, and pomegranate peel wastes [15] have been used as biosorbent materials.

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for doxycycline due to their abundance in nature, availability, easy preparation, low-cost, and removal efficiency [16]. These sorbents were dried prior to the use.

Papaya peel (PP) is an agricultural waste that has shown a great potential as biosorbent for methylene blue, a cationic dye. It contains polyphenols (i.e. gallic acid, caffeic acid, p-coumaric acid, and ferulic acid) which serve as adsorption sites for the dye through the ion exchange mechanism [17-19]. Since DC is a cationic antibiotic, it was hypothesized that it could be adsorbed onto the PP via same mechanism [20].

The objectives of this research are: (1) to compare the adsorption capacity of ripe PP versus unripe PP in a simulated doxycycline-containing wastewater and (2) to determine the effects of pH and initial sorbate concentration on the adsorption of DC with ripe and unripe PP while maintaining the contact time and adsorbent dosage fixed. The study aims to produce an effective adsorbent for doxycycline with no chemical modification involved in its preparation and to provide comparable results to chemically synthesized adsorbents. The results of this study can serve as a benchmark for applying PP adsorbents to an industrial scale adsorption column for the purification of wastewater containing DC.

2. Methodology

2.1 Preparation of doxycycline solution
Doxycycline (100% purity) antibiotic as adsorbate was of laboratory-grade (Thomas Labs®). All reagents used were of analytical grade from research laboratory. A stock solution of 1,000 mg/L was prepared by dissolving 1,000 mg of DC to distilled water in a 500-mL volumetric flask. Different concentrations (20-1,000 mg/L) were prepared by serial dilution. The pH of the solution (3, 6, and 9) was adjusted by the addition of 0.1 M NaOH and 0.1 M HCl. The stock solution was placed in an amber bottle.

2.2 Preparation of papaya peel
Papaya was obtained from a Public Market in Bulacan and was authenticated by the Bureau of Plant Industry. The fruit was washed with distilled water to remove adhering dirt and soluble components such as tannins, resins, reducing sugar, and coloring agents. The peels were separated and air-dried at room temperature until the weight becomes constant. It was oven dried at 80°C to crisp, milled using Wiley Mill, and sieved on an 80-mesh screen of standard sieve shaker to obtain uniform particle size. The prepared material was placed in a 250-mL Erlenmeyer flask for future use and was capped tightly with an aluminum foil to avoid contamination and rapid decay.

2.3 Characterization of papaya peel
Fourier-transform infrared (FTIR) spectroscopy (Perkin Elmer FTIR SPECTRUM100) analysis was carried out to determine the functional groups present in papaya peels. To investigate the surface morphologies, before and after adsorption, scanning electron microscope (SEM, JEOL-JSM-5310) was used. Moreover, elemental analysis (ED-XRF Epsilon 5 PANalytical, USA) was performed to determine the elemental composition of the papaya peel.

2.4 Equilibrium studies
Batch adsorption was carried out in a 125-mL Erlenmeyer flask containing 25 mL aliquot of DC solution with concentrations ranging from 20 to 1,000 mg/L. 0.1 g of papaya peel was mixed with the prepared solution and the pH was adjusted at 3, 6, and 9 using a 0.1 M NaOH and a 0.1 M HCl. The prepared solutions (PP-DC) were placed in a bath shaker (Memmertson WNE14) at 150 rpm for 180 min and at a constant temperature of 28 ± 1°C. The concentrations of DC in the solution were examined using UV/VIS spectrophotometer (Perkin Elmer UV-VIS Lambda 35) at 275 nm.

2.5 Adsorption isotherm studies
The data were fitted to both Langmuir and Freundlich isotherms to determine which of the models
describes the adsorption process.

Langmuir adsorption isotherm is a quantitative description of the formation of a monolayer adsorbate on the outer surface of the adsorbent while no further adsorption takes place afterwards [20]. This isotherm is valid for monolayer adsorption onto a surface containing a finite number of identical sites. Also, a uniform energy of adsorption onto the surface will be assumed. On the other hand, the Freundlich adsorption isotherm describes the adsorption characteristics for heterogeneous surface.

2.6 Kinetic studies

The sorbent and the sorbate under the same condition as the batch adsorption were subjected to contact for 180 min. The concentration of the sorbates was determined at a 20-min interval and the resulting data were fitted to the linearized pseudo-first-order and pseudo-second-order models to identify the kinetics of adsorption.

3. Results and discussion

3.1 Characterization of biosorbents

The surface morphologies of ripe and unripe PP before and after adsorption were examined using scanning electron microscopy (SEM). Figure 1 shows that for the ripe and unripe PP, a rough cellular structure can be observed with an average pore size of 1.20 µm and 1.70 µm, respectively.

![SEM images of Raw Ripe PP a) before and b) after adsorption and Raw Unripe PP c) before and d) after adsorption.](image)

Figure 1. SEM images of Raw Ripe PP a) before and b) after adsorption and Raw Unripe PP c) before and d) after adsorption.

SEM images of ripe and unripe papaya peel after adsorption showed clumps of doxycycline
attached on the surface of papaya peel which filled up the pores of the adsorbent.

The comparison of Fourier Transform Infrared (FTIR) spectra of ripe PP and unripe PP before and after adsorption is shown in figure 2.

**Figure 2.** FFTIR Spectra of Raw Ripe PP before (Black Line) and after adsorption at 200 mg/L & pH 3 (Green Line) and Raw Unripe PP before (Blue Line) and after adsorption at 200 mg/L & pH 6 (Red Line).

Before the adsorption, both unripe and ripe PP have peaks at 1,029 cm\(^{-1}\), which can be attributed to C-N bond stretching. A broad signal at 1,618 cm\(^{-1}\) corresponding to the C=C bending and/or carboxyl C=O stretching has been seen from the spectrum. Apparently, the noticeable difference between the ripe and the unripe PP are the bands at 1,106 cm\(^{-1}\) for ripe PP and at 2,917 cm\(^{-1}\) for unripe PP. These two bands correspond to the C-O and O-H stretch, respectively. The peaks showed could be attributed to the polyphenols present at the surface of PP [15]. After the adsorption, the unripe PP has a noticeable change in peak at 2,200 and 1,250 cm\(^{-1}\) (red dashed lines). At pH 3 and 6, the O-H, C=O, and N-H bond of the tricarbonyl group and the O-H and C=O bond of phenolic diketone moiety are the active sites in DC, respectively [17]. The change in the spectra indicates the breakage of C=O bond at the phenolic diketone group due to the reaction with phenols found in the unripe papaya peel, thus, forming a bisphenol [18]. This is a clear indication that DC has adsorbed onto the unripe PP. As for ripe PP, there is a slight decrease in the transmission at peak 1400 cm\(^{-1}\) (black dashed lines), corresponding to the C=O bond. This loss of peak is associated with the phenols in polyphenols found in the ripe PP which reacts with protonated ketone (C=O bond) forming bisphenols [17]. This indicates that DC has adsorbed on ripe PP.

### 3.2 Equilibrium studies

Figure 3 shows the batch adsorption equilibrium data of DC adsorbed on ripe and unripe PP, measured at pH=3, 6, and 9. Results showed that a maximum adsorption capacity \(q_e\) of 215.085 mg g\(^{-1}\) (87.97% removal) and 213.915 mg g\(^{-1}\) (87.49% removal) for ripe and unripe PP, respectively, would
be achieved at an initial sorbate concentration \( (C_0) \) of 1000 mg/L at pH = 3.

![Graph](image)

**Figure 3.** \( C_e \) (equilibrium concentration) vs. \( q_e \) plot of (a) ripe and (b) unripe PP at \( C_0 = 200-1,000 \) mg/L and pH = 3, 6, and 9. Contact time = 180 min and agitation speed = 150 rpm.

The high adsorption of DC to ripe and unripe PP is due to its surface components. According to Salla *et al.* [15], papaya contains functional groups such as carboxyl and hydroxyl groups. The hydroxyl and carboxyl groups contain hydrogen which can act as either or both a hydrogen bond donor and an acceptor. The active compound of papaya peel is papain [21] which contains a carboxylic (electron acceptor) acid and a benzene ring (electron donor) groups that could serve as the active site for binding [22]. Also, the papaya peel contains Ca\(^{2+}\) and K\(^+\) based on the results of
elemental analysis that can be the cause of binding of DC onto PP through cation exchange.

3.3 Effects of pH

The influence of pH on the adsorption of DC with ripe and unripe PP was investigated at pH 3, 6, and 9. Figure 4 shows the % removal of DC with respect to pH change. Both ripe PP-DC and unripe PP-DC systems show that the % removal is increasing with decreasing pH, even though the % removal is high at any pH. This behavior indicates a high efficiency of PP with DC in neutral and alkaline conditions and most likely in acidic solution.

This observation was attributed to the molecular structure of DC, which has multiple ionizable functional groups, such as tricarbonyl group, phenolic diketone, and methyl amino group at pH 3, 6, and 9, respectively. The adsorption of DC strongly increases as pH decreases due to induction, electrostatic interactions, and H-bond formations between the functional groups of the DC and the PP
active sites. The presence of calcium ions strongly increases the adsorption of DC at pH > 3 due to the formation of ternary complexes by calcium-bridging [23]. The mechanism of adsorption at different pH are the same, but the active site varies.

At low pH, all the active adsorption sites of DC were all protonated, resulting to a net charge of +1 for the whole molecule [23]. The main active site for DC binding, in this case, is the dimethyl amine molecule whereas the active sites for PP binding are the polyphenols attached to the papain enzyme [20]. The mechanism of the adsorption is through cationic exchange. Other adsorption sites for PP, which could contribute to its high adsorption capacity, are the polyphenols and the minerals such as Ca\(^{2+}\) and K\(^{+}\) found on the surface of the peels. The dimethyl amine component of DC may bind to the negatively charge hydroxyl group of papaya peel. Since the oxygen atom of the hydroxyl group contains lone pairs, the hydrogen atom attached to the dimethyl amine has a partial positive charge due to the difference in electronegativity of nitrogen and hydrogen atom. Binding may occur due to the covalent bonding [24]. Furthermore, the minerals (Ca\(^{2+}\) and K\(^{+}\)) in the papaya peel may have an ion exchange with the acidic H\(^{+}\) ion of DC molecule. Hence, at pH 3, the % removal of both the ripe and unripe papaya peels was relatively high.

At pH 4.0-7.0, DC has no net charge because an enol within the molecule formed at this pH cancels out the charge of the dimethyl amine moiety. The enol at C3 region may bind to the carboxyl group of papain, with the same binding site on the PP. The active site of papain is at carboxylic acid, where the carbonyl group has partial positive charge thus it acts as an electron acceptor. Since, the DC molecule at this pH acts as a nucleophile, it attacks the carbonyl of carboxylic acid, which is an electrophile [19].

At pH > 8.0, the overall charge of DC molecule is -1 because two enols are formed within the molecule. The negative charge of the oxygen atom can be a nucleophile that may attack the electrophilic functional group of the papaya peel. Increasing the pH of the solution requires an addition of NaOH and low % removal was obtained at pH 9 compared to pH 3 and 6. The reduction in % removal is probably due to the repulsion forces between the DC molecule and the PP peel [24].

### 3.4 Isotherm study

The data in the batch adsorption show that DC removed using ripe and unripe PP best fit the linearized form of Langmuir isotherm, as shown in equation (1).

\[
\frac{q_{eq}}{C_{eq}} = b_L q_{max} - b_L q_{eq}
\]

where \(q_{eq}\) is the equilibrium concentration of DC per unit mass of adsorbent (mg/g), \(q_{max}\) is the maximum adsorption capacity (mg/g), \(C_{eq}\) is the equilibrium concentration of DC (mg/L), \(b_L\) is a Langmuir isotherm constant (L/mg), and \(q_{eq}\) is the amount of metal adsorbed per gram of the adsorbent at equilibrium (mg/g), agreed with the resulting \(q_{max}\) of the biosorbents. The \(q_{max}\) of the ripe and unripe PP at pH =3 is 215.08 mg/g (\(R^2 = 0.795\)) and 213.91 mg/g (\(R^2 = 0.582\)). These results showed that for both the ripe and unripe papaya peel, a monolayer adsorption of doxycycline is observed, wherein there is a monolayer adsorbate on the outer surface of the adsorbent and no further adsorption takes place afterwards [20].

Table 1 shows the comparison of the monolayer adsorption capacity of both the ripe and unripe papaya peel in doxycycline adsorption with other adsorbents. The data show that both the ripe and unripe PP have a comparable adsorption capacity with the NaY Zeolite from wheat straw and shows less efficiency as opposed to the MIL-53(Fe) and rice straw biochars. However, the PP used in this study is unmodified as compared to the other adsorbent used. It only means that the use of PP is more sustainable that there is no need to follow complex procedures and use of hazardous chemicals in the preparation of the adsorbent. When compared to unmodified adsorbents, such as spent black tea leaves and Pomegranate peel, it shows that both the ripe and unripe PPs are far more superior. This shows that PP is a compatible biosorbent to DC.
Table 1. Comparison of monolayer adsorption capacities of doxycycline in this study with other adsorbents.

| Adsorbent                        | Monolayer adsorption capacity (mg/g) | Reference |
|----------------------------------|--------------------------------------|-----------|
| Rice straw biochar               | 432.90                               | [4]       |
| Spent black tea leaves           | 3.365                                | [15]      |
| Pomegranate peel                 | 1.336                                | [15]      |
| NaY Zeolite from wheat straw     | 269.75                               | [25]      |
| MIL-53(Fe) (Metal organic framework) | 322.00                          | [26]      |
| Ripe Papaya Peel                 | 215.08                               | This Study|
| Unripe Papaya Peel               | 213.91                               | This Study|

![Figure 5. (a) Pseudo-first pseudo-second order kinetics for ripe PP-DC (black solid line) with its trend line (red dashed line) and unripe PP-DC (green solid line) with its trend line (violet dashed line) and (b) Pseudo-second order kinetics for ripe PP-DC (blue solid line) with its trend line (violet dashed line) and unripe PP-DC (black solid line) with its trend line (red dashed line) at 1,000 mg/L and pH 3.](image-url)
3.5 Kinetic studies

Two kinetic models are considered in this study: the pseudo-first order and the pseudo-second order. The comparison made by the researchers centered on the pseudo-first order and pseudo-second order since these two, according to several studies, are the best fit for adsorption. The adsorption kinetics is shown in figure 5.

Based on the results, the pseudo-second order ($R^2 = 0.9922$) has a better fit compared to the pseudo-first order ($R^2 = 0.9745$) because of a higher $R^2$. However, the results do not depart from each other much. Pseudo-first order coincides with the monolayer adsorption whereas the pseudo-second order coincides with the multilayer adsorption [4]. Since the nature of adsorption based on the isotherm studies represents a monolayer adsorption, pseudo-first order was the selected model for the adsorption of both papaya peels.

4. Conclusion

The biosorption equilibrium data reveal the adsorption capacity of ripe and unripe papaya peel in a simulated doxycycline-containing wastewater by varying the pH and initial sorbate concentration of the system in a batch process. For both ripe and unripe PP, the maximum adsorption capacities were 215.08 mg/g (87.97% removal) and 213.91 mg/g (87.52% removal), respectively. These results were obtained at an initial sorbate concentration of 1000 mg/L and at pH 3. Increasing the pH decreases the % removal of doxycycline by a minute amount whereas increasing the sorbate concentration results to a higher % removal and adsorption capacity. Freundlich and Langmuir isotherm models were both applicable for the biosorbent but the model that provided a better fit in the biosorption of doxycycline at the 1,000 mg/L sorbate concentration was the Langmuir model. Pseudo-first order kinetics provides a correlation of the biosorption data for both ripe and unripe papaya peels. It indicates that the adsorption between the sorbate and adsorbent operates only at the monolayer.

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