An investigation into the effects of varying pyrolysis temperatures and chitosan modification on the effectiveness of a banana peel biochar in adsorbing tetracycline hydrochloride from a synthetic solution

Wee Yi De John-Paul, Jaswanth Anand
Raffles Institution, Singapore, abigayle.ng@ri.edu.sg

Abstract

The widespread use of tetracycline in animal livestock and human disease treatment has caused a sharp rise in tetracycline pollution of water. This can lead to toxicity in humans and antibiotic resistance. Our research aims to investigate if banana peels, a common waste material, can be repurposed into biochar to effectively remove tetracycline and whether chitosan-modification and pyrolysis temperature variations will improve its efficacy. We hypothesise that high-temperature pyrolysis and chitosan modification are expected to increase the rate of adsorption and the total amount adsorbed by the biochar. 4 variants of biochar were prepared – pure banana peel and chitosan-modified biochar which were pyrolysed separately at 220°C and 600°C. The biochar samples are added to individual tetracycline hydrochloride solutions which are left to sit in a shaking apparatus for 24 hours. The concentration of tetracycline in the solution is determined by spectrophotometry. Results show that all 4 types of biochar reached the same maximum amount of adsorption within 30 minutes. A higher pyrolysis temperature causes the biochar to have a faster rate of adsorption within the first 30 minutes. Chitosan modification decreased the rate of adsorption in biochar pyrolysed at 220°C had no effect on the biochar pyrolysed at 600°C.

Keywords

Tetracycline, Biochar, Chitosan Modification, Adsorption behaviour

1 Introduction

Tetracycline is a commonly-used antibiotic in livestock and the pharmaceutical industry and often ends up in wastewater due to human and animal faeces. If wastewater management proves inadequate, tetracycline is likely to end up as run-off into nearby water bodies, such as rivers. Tetracycline concentration in wastewater in the USA was measured to be between 170 and 850 ng L^{-1} [1], while the concentration in other countries like Zimbabwe reached as high as 50 μg L^{-1} [2]. The high concentration of tetracycline can lead to bacteria developing resistance against it, which is particularly concerning given how much tetracycline is used in livestock and pharmaceuticals. Studies have shown that in European countries, "66.9% of *E. coli* and 44.9% of *Klebsiella* species were resistant against TC (tetracycline)" [3]. The development of antibiotic resistance among bacteria could leave humans more vulnerable to severe complications due to bacterial infections. Current methods for removing tetracycline include adsorption, membrane filtration, advanced oxidation processes, flocculation and coagulation and reverse osmosis [4]. Our study places emphasis on adsorption as a means of tetracycline removal due to the method's simplicity, cost-effectiveness and safe means of implementation.

1.1 The purpose of investigation

Raw biochar has been proven through many studies to be able to adsorb pollutants including tetracycline from contaminated water. However, raw biochar can only remove a limited amount of tetracycline molecules from water because of its undeveloped porous structure and scarce activated sites [5]. This experiment attempts to investigate the effectiveness of high-temperature biochar, high-temperature chitosan-modified biochar, low-temperature biochar and low-temperature chitosan-modified biochar in adsorbing tetracycline hydrochloride from an aqueous solution.

1.2 Literature review

To the best of the author's knowledge there seem to be no studies on examining the effect of only chitosan modification on the adsorption of tetracycline by biochar. However, a study has been conducted in 2019 studying the effects of chitosan, iron and sulfur modification on the performance of biochar [6]. The results of this study reveal that chitosan, iron and sulfur modification leads to a greater rate and equilibrium amount of tetracycline adsorption. As both the pure chitosan modification we intend to study and chitosan, iron and sulfur modification this paper studies involve the usage of chitosan, it is likely they have somewhat similar effects on adsorption. Thus, we hypothesise that chitosan modification leads to biochar absorbing a larger amount of tetracycline at a faster rate.

This is also supported by studies conducted on chitosan-modified biochar's adsorbency of other substances such as methyl orange and lead (Pb^{2+}) ions. Hydrophobic interactions, pore filling, electrostatic interactions, π-π stacking and hydrogen bonding are some of the mechanisms involved in the adsorption of tetracycline by a biochar particle [7]. Hence, if chitosan modification increases the number of functional groups on the biochar particles that lead to these interactions forming, then the adsorption of tetracycline will increase.

Both Dewage et al. [8] and Loc et al. [9] mention that chitosan modification leads to the formation of more $-NH_2$ groups on biochar particles. The study by Dewage et al. proves this through transmission electron microscopy by showing that while non-modified biochar had no nitrogen, chitosan-modified biochar had substantial surface regions of nitrogen. Therefore, these highly polar $-NH_2$ groups on the biochar can form hydrogen bonds with polar groups on tetracycline molecules. The study by Loc et al. [10] also reveals that chitosan-modified biochar has more polar hydroxyl groups which also form hydrogen bonds. Additionally, these $-NH_2$ groups will undergo protonation

depending on the pH of the solution to form $-NH^{3+}$ groups. The electric charge on this functional group means that these groups can form electrostatic attractions. Finally, a greater amount of, $-NH_2$ and $-OH$ groups caused by chitosan modification also lead to greater levels of surface complexation between biochar particles and tetracycline molecules. In conclusion, a greater amount of chemical interactions between chitosan-modified biochar particles and tetracycline molecules would likely lead to greater amounts of adsorption in practice.

According to studies done by Zhang et al. [11] and Li et al. [12] , pyrolysis temperature does have a significant impact on the adsorption capabilities of biochar. The study done by Li et al. [13] shows that biochar that was pyrolysed at higher temperatures had a higher ash content and C content while having lower O and H content than lower-temperature pyrolysis samples. More importantly, higher pyrolysis temperature results in biochar having a significantly higher surface area and micropore volume. Higher temperatures also resulted in greater surface rupturing and smaller pore diameter. All of these factors contributed to higher-temperature pyrolysis biochars being more adsorbent. The most likely mechanisms of adsorption are via "pores filling, π-π EDA interactions, hydrogen bonding, and electrostatic interactions" [14]. This is also corroborated by the study done by Li et al. [15], which also shows that higher pyrolysis temperatures resulted in much higher adsorption capacity. Images taken from an electron microscope in this experiment also support work done by Zhang et al. [16] as it shows a much higher number of pores at higher pyrolysis temperatures.

2 Methodology

2.1 Preparation of various biochar samples

14 bunches of Sumifru Philippines Bananas were bought from an NTUC supermarket. The banana peels were removed from the bananas. These peels were then washed with water and cut into small pieces and dried overnight at 100°C. The resultant dried banana peels weighed 264 g and were subsequently pyrolyzed at 220°C for 90 minutes. The pyrolyzed banana peels weighed 214 g. Using a grinder, the pyrolyzed peels were then ground into a powder. This powder was sieved to isolate biochar particles of grain size 250 μm to 2000 μm to form low-temperature-pyrolysis biochar (220BC). About 100 g of biochar met the required grain size after sieving was completed.

From the obtained 220BC, 4.0 g was treated to form chitosan-modified biochar. The method of preparation of chitosan-modified biochar is adapted from a previously conducted study [18] . 2.0 g of chitosan was dissolved in 100ml of 3% acetic acid solution with continuous stirring. 4.0 g of biochar was added to this mixture which is then constantly stirred for 60 minutes. Subsequently, the mixture was put into a water bath of 40C for 30 mins and 4 ml of 1% glutaraldehyde was injected into the mixture to form a gel. Sodium hydroxide solution (1 mol/dm3) was then added to the mixture to adjust its pH to 8.0 -10.0. The resulting precipitate (Chitosan-modified biochar) was then washed until its pH was about 7 and was then dried at 60°C overnight. Then, the chitosan-modified biochar (220CBC) was then ground and sieved to obtain particles of grain size of 250 to 2000 μm .

Additionally, another 20 g of 220BC was removed for high-temperature pyrolysis at 600°C for 90 minutes. The resultant high-temperature biochar (600BC) had a mass of 8.7 g. The weight of 600BC was 4.7g, which was subsequently set aside while 4g of 600BC underwent chitosan modification (following the aforementioned procedure) to form 600CBC. Upon sieving the 5.2 g of 600CBC produced, there was a final mass of 3.8 g of CBC obtained.

The 4 samples, 220BC, 220CBC, 600BC, 600CBC were then set aside for use in future experiments.

2.2 Plotting of standard curve

Tetracycline Hydrochloride (TCH) solution was bought from Sigma Aldrich and was then made up to the following concentrations: 100mg/L, 80mg/L, 60mg/L, 40mg/L, 20mg/L, 0mg/L using appropriate mixtures of TCH solution and distilled water. Samples of each concentration were then transferred to cuvettes and spectrophotometry at a wavelength of 358nm (Vrakin et al., 2017) was conducted on the samples. The absorbency values were plotted against the concentration of TCH and a trend line was plotted as shown in Figure 1. The R^2 value being very close to 1 indicates a near-perfect positive correlation between the concentration of TCH and absorbency value.

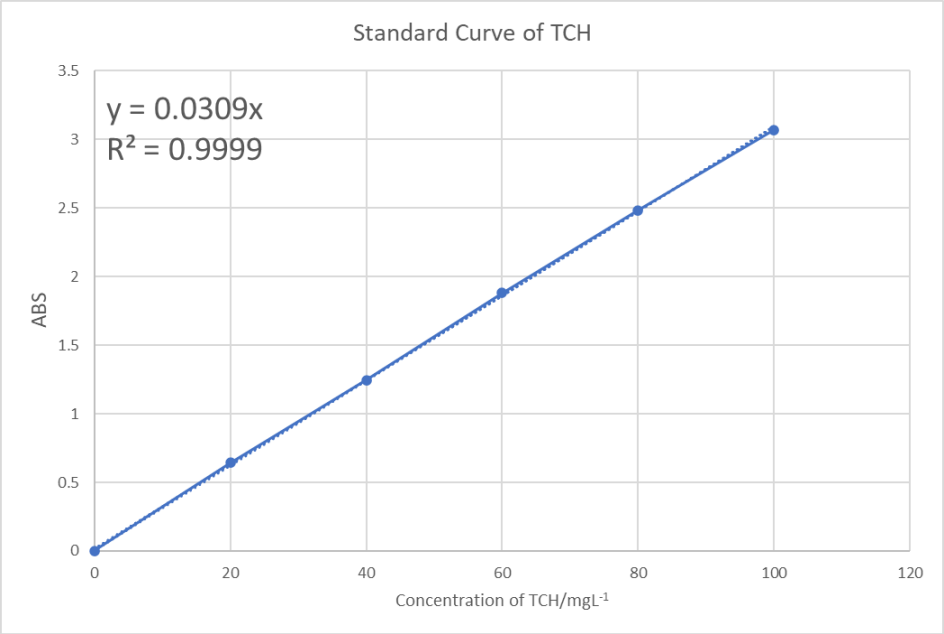

Figure 1. Standard curve of Tetracycline

2.3 Determining the effectiveness of biochar samples in removing tetracycline

For each of the BC and CBC samples, 100mg was extracted for subsequent experiments. The 100mg was added to a 200ml solution of 100mg/L tetracycline solution in a closed brown glass bottle. The bottles were then wrapped in aluminium foil to prevent degradation of light-sensitive tetracycline and were subsequently placed in a mechanical shaker at 360RPM. At various time points: 1 minute, 5 minutes, 15 minutes, 30 minutes, 60 minutes, 2 hours, 4 hours and 24 hours, the bottles were removed from the shaker. Approximately 1.5ml of tetracycline solution was extracted from each sample via micropipette and centrifuged at 10000RPM for 5 minutes to ensure any BC or CBC particles present accumulate at the bottom of the tube. The supernatant in the centrifuge tube is carefully poured into cuvettes. These efforts to ensure minimal to no particles of BC or CBC in the cuvette are taken as these particles may influence

spectrophotometry readings. Spectrophotometry was then conducted on samples at a wavelength of 358nm [19] to determine the concentration of tetracycline left in the mixture.

Based on the absorbency values, the adsorbency in mg/g of the BC or CBC was calculated based on the following formula:

$$Adsorbency\ (mg/g)\ = (20 - (\frac{ABS}{0.0309} \times 0.2)) \div 0.1$$

As ABS values are linearly related to concentration in mg/L via the standard curve,

$$\frac{ABS}{0.309} \times 0.2$$

Represents the concentration of tetracycline in (mg/L) multiplied by the volume of the solution (0.2L), giving the total amount of tetracycline present in the solution at that given time.

Since there was 20 mg of tetracycline in the beginning, the total amount of tetracycline absorbed by the BC or CBC (in mg) is given by:

$$20 - (\frac{ABS}{0.0309} \times 0.2)$$

Lastly, the total amount of tetracycline absorbed is divided by the mass of BC or CBC (0.1g) to give the final formula of adsorbency in mg/g.

3 Results of the experiment

3.1 Graph of experimental data

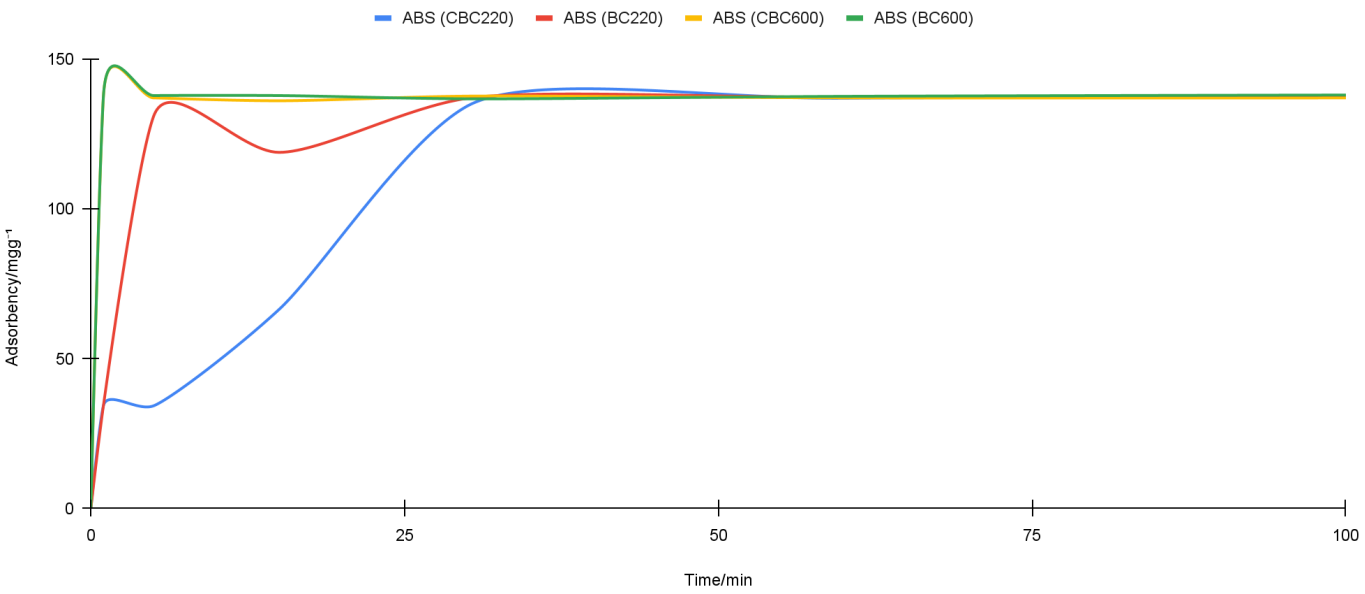

Figure 2: Adsorbency in mgg^{-1} of the four biochar variants

For all 4 variants of biochar, their amount of adsorbency rapidly increases before plateauing at about the 30 min mark at 140 mgg^{-1} (Figure 2). Adsorbency values recorded after 100 mins are not shown in the graph as the amount of TCH adsorbed by all 4 variants of biochar remained constant at approximately 140 mgg^{-1} from 30 mins to 1440 mins

Some adsorbents reached this maximum amount of TCH adsorption within shorter period of time than others. CBC600 and BC600 both took approximately 5 minutes while BC220 and CBC 220 took approximately 30 minutes to do so.

BC600 and CBC600 show very similar adsorbency characteristics however, on the other hand, there is significant variation in the adsorbency of BC220 and CBC220 during the time ranges of 0 to 30 mins. BC220 had a significantly greater amount of adsorbency than CBC220.

3.2 Data from scanning electron microscope

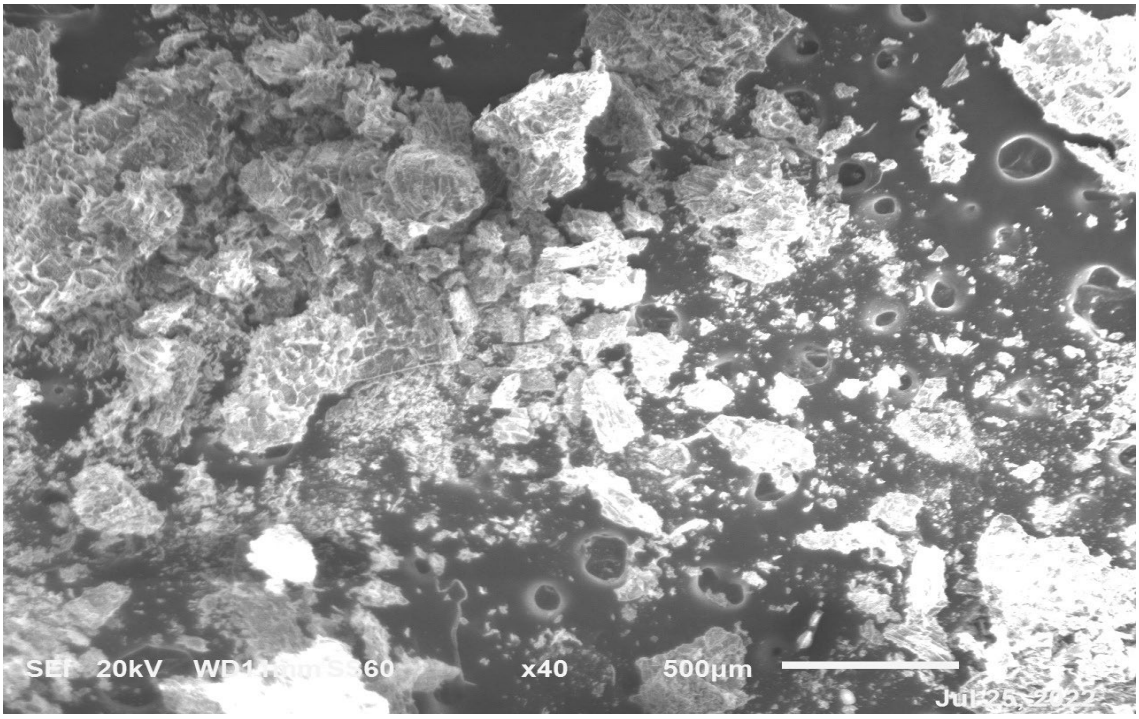

Figure 3: BC 600 at a magnification of 40 times under scanning electron microscope (SEM)

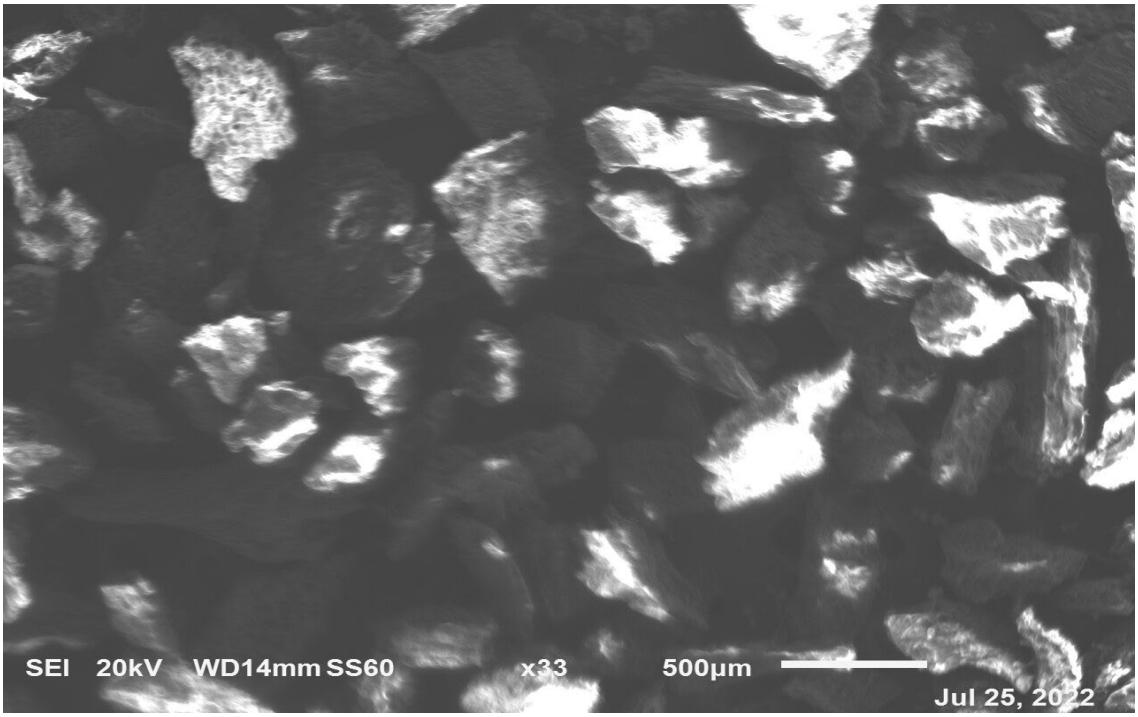

Figure 4: BC 220 at a magnification of 33 times under SEM

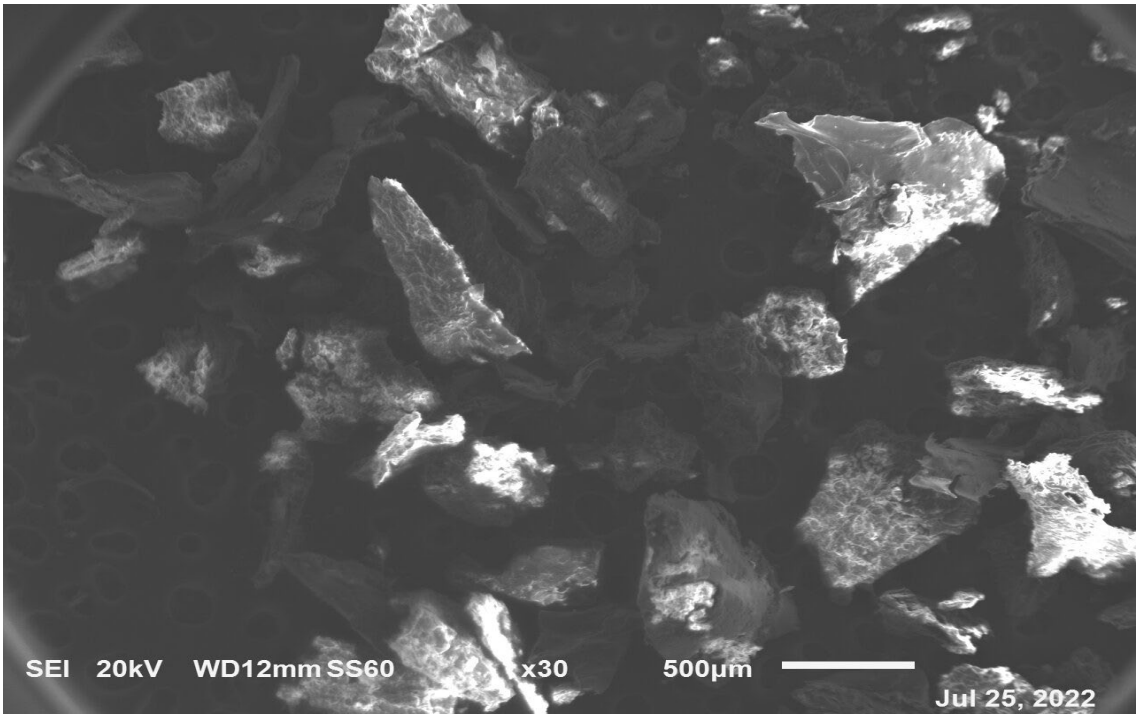

Figure 5: CBC220 at a magnification of 30 times under SEM

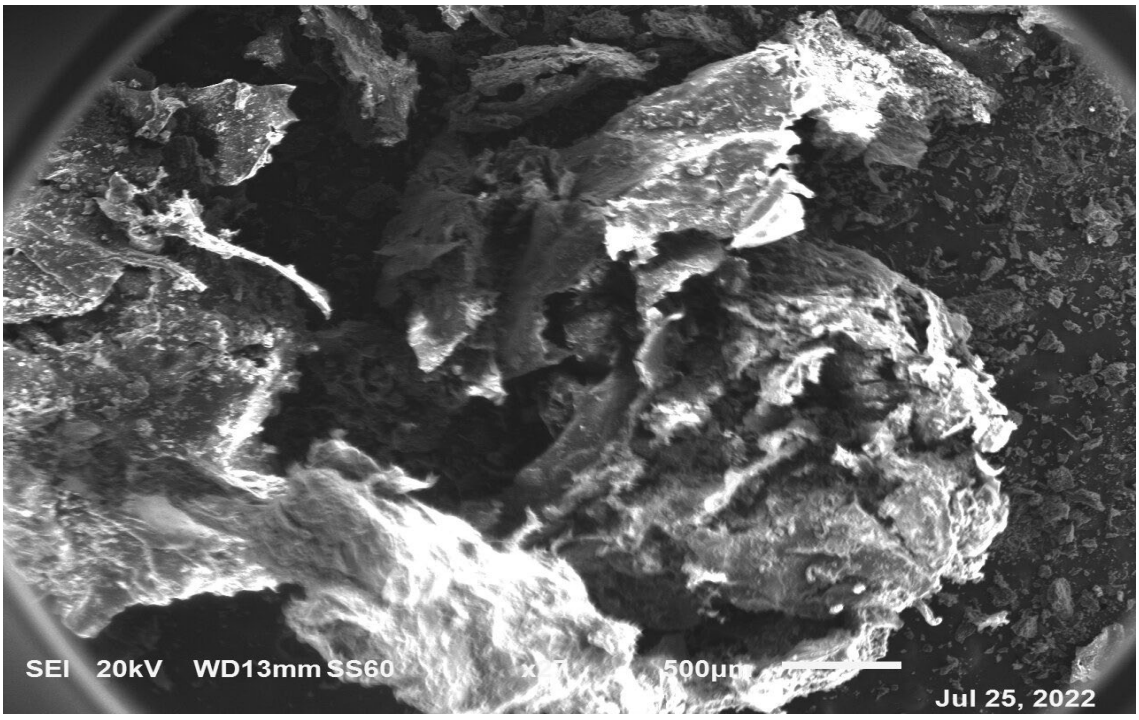

Figure 6: CBC600 at a magnification of 27 times under SEM

3.3 Discussion of results

The results reveal that all the adsorbence sites of all 4 types of adsorbent particles are occupied by tetracycline molecules 30 mins after the adsorbents are exposed to a tetracycline solution. Therefore, all the studied adsorbents only need to be placed in a tetracycline-contaminated solution for 30 minutes to achieve the maximum possible amount of adsorption. In the study by Liu et al. [20] both the unmodified and chitosan, iron and sulfur-modified biochar reached their respective maximum amount of adsorptions at approximately the same time after the experiment began. The same thing also occurred in our experimentation.

However, in Liu's study, both the unmodified and modified biochar samples took 300 minutes to reach their respective maximum amount of adsorption which is approximately 10 times the duration our biochar samples took to reach saturation. This suggests to us to reduce the dosage of biochar in each bottle containing tetracycline solution. This will allow us to better analyse the adsorption behaviour of the adsorbents over time.

Additionally, in Liu's study, the TCH adsorption of modified biochar plateaued at almost double the value it did for unmodified biochar. The TCH adsorption of modified biochar plateaued at a maximum of 185 mgg^{-1} while the TCH adsorption of unmodified biochar plateaued at a maximum of 85 mgg^{-1} . These findings contradict the findings of our study. The TCH adsorption of biochar pyrolysed at both 220 °C and 600 °C plateaued at the same TCH adsorption value of 140 mgg^{-1} after chitosan modification.

One of our findings seems to differ from the current literature. The rate of TCH adsorption of CBC220 is lower than that of BC220 from 0 to 30 mins. However, similar studies seem to suggest that chitosan modification increases the rate of TCH adsorption by biochar as chitosan increases the number of functional groups on biochar particles that facilitate interactions between tetracycline molecules and biochar particles [21] and [22]. The deviation in results could be due to differing methods of preparation of chitosan-modified biochar. For example, in the study by Liu et al. (2019), dried biomass was added to 2 M $ZnCl_2$ solution and magnetically stirred for 12 h as part of the methodology to produce biochar. However, our biochar preparation did not involve adding $ZnCl_2$ solution. Differences like this could alter the functional groups on individual biochar particles leading to different TCH adsorption characteristics.

Moreover, data from this study is supported by studies by Zhang et al.[23] and Li et al. [24], which indicate that biochar which has been pyrolysed at higher temperatures are more porous and have greater adsorption capability and capacity. The SEM images of CBC220 and CBC600 also seem to concur with this observation. CBC600 particles seem to have more pores on their surface which are seen through their rougher surface geometry in comparison to BC600 particles. The presence of more pores on the surface of biochar particles leads to biochar having a greater surface area in contact with the tetracycline solution with respect to volume. Indeed, this is evident in the data collected as CBC600 and BC600 had reached saturation at a higher rate than BC220 and CBC220, reflecting their better adsorption abilities due to the higher pyrolysis temperature.

3.4 Limitations

One key limitation of our study is that only one adsorption experiment for all four biochar variants was conducted due to time constraints. Thus, this reduces the reproducibility and reliability of our results as any human error that could have arisen would have an adverse effect on our results. This limitation can be relatively easily addressed by conducting at least 3 rounds of the same adsorption experiment.

4 Conclusion

With regards to real-world application, BC220 has an adsorbency rate only slightly less than the high adsorbency rates BC600 and CBC600. This presents a viable option for the removal of TCH, as production of BC220 is much less energy-intensive as 90 minutes of high-temperature pyrolysis is not required to produce it. This makes it a more cost-efficient alternative which is especially useful in developing countries like Zimbabwe where there are high amounts of tetracycline pollution. The production of BC220 is also more environmentally-friendly than the production of BC600 as it requires a lower use of resources like electricity. However, further research should be conducted on potential flow-through filtration prototypes which make use of BC220, as well as other modifications which can be made to the biochar, such as magnetic biochar. In particular, such flow-through studies prove particularly promising given the relatively quick removal rate of tetracycline in all the samples. Additionally, kinetics studies into the exact adsorption mechanism of tetracycline by biochar can be conducted.

Potential changes to be made in future related studies include using lower doses of biochar in each sample to prevent biochar saturation from being reached as quickly, allowing for a more in-depth comparison between samples.

References

[1] Batt, A. L., Snow, D. D., & Aga, D. S. (2006). Occurrence of sulfonamide antimicrobials in private water wells in Washington County, Idaho, USA. *Chemosphere*, *64*(11), 1963–1971. https://doi.org/10.1016/j.chemosphere.2006.01.029

[2 , 4] Gopal, G., Alex, S. A., Chandrasekaran, N., & Mukherjee, A. (2020). A review on tetracycline removal from aqueous systems by Advanced Treatment Techniques. *RSC Advances*, *10*(45), 27081–27095. https://doi.org/10.1039/d0ra04264a

[3] Grossman, T. H. (2016). Tetracycline antibiotics and resistance. *Cold Spring Harbor Perspectives in Medicine*, *6*(4). https://doi.org/10.1101/cshperspect.a025387

[5 , 6 , 20] Liu, J., Zhou, B., Zhang, H., Ma, J., Mu, B., & Zhang, W. (2019, December). A novel Biochar modified by Chitosan-Fe/S for tetracycline adsorption and studies on site energy distribution. *Bioresource Technology*, *294*, 122152. https://doi.org/10.1016/j.biortech.2019.122152

[7] Krasucka, P., Pan, B., Sik Ok, Y., Mohan, D., Sarkar, B., & Oleszczuk, P. (2021). Engineered biochar – a sustainable solution for the removal of antibiotics from water. *Chemical Engineering Journal*, *405*, 126926. https://doi.org/10.1016/j.cej.2020.126926

[8 , 21] Bombuwala Dewage, N., Fowler, R. E., Pittman, C. U., Mohan, D., & Mlsna, T. (2018). Lead (pb2+) sorptive removal using chitosan-modified biochar: Batch and fixed-bed studies. *RSC Advances*, *8*(45), 25368–25377. https://doi.org/10.1039/c8ra04600j

[9 , 10, 22] Loc, N. X., Tuyen, P. T., Mai, L. C., & Phuong, D. T. (2022). Chitosan-modified biochar and unmodified biochar for methyl orange: Adsorption characteristics and mechanism exploration. *Toxics*, *10*(9), 500. https://doi.org/10.3390/toxics10090500

[11 , 14 , 16 , 17 , 23]Zhang, P., Li, Y., Cao, Y., & Han, L. (2019). Characteristics of tetracycline adsorption by cow manure biochar prepared at different pyrolysis temperatures. *Bioresource Technology*, *285*, 121348. https://doi.org/10.1016/j.biortech.2019.121348

[12 , 13 , 15 , 24] Li, B., Zhang, Y., Xu, J., Fan, S., & Xu, H. (2022). Facile preparation of magnetic porous biochars from tea waste for the removal of tetracycline from aqueous solutions: Effect of pyrolysis temperature. *Chemosphere*, *291*, 132713. https://doi.org/10.1016/j.chemosphere.2021.132713

[18] Zhang, H., Xiao, R., Li, R., Ali, A., Chen, A., & Zhang, Z. (2020, December). Enhanced aqueous Cr(VI) removal using chitosan-modified magnetic biochars derived from bamboo residues. *Chemosphere*, *261*, 127694. https://doi.org/10.1016/j.chemosphere.2020.1276

[19] Vrakin, V., Savchenko, L., Materiienko, A., & Georgiyants, V. (2017, June 14). Development and validation of tetracycline hydrochloride assay procedure by spectrophotometry in compounded ointment. *Scripta Scientifica Pharmaceutica*, *4*(1), 35. https://doi.org/10.14748/ssp.v4i1.2117