Investigating the adsorption and antibacterial properties of a composite made up of biochar from pomelo peels and titanium dioxide nanoparticles encapsulated in calcium alginate beads

Koo Zhi Xian (3i319), Hagan Koh (3i318), Melvern Ng (3i119)
Group 01-10

Abstract

Water pollution is a serious problem faced by the world today. Industrial wastewater contains pollutants which consist of mostly heavy metal ions and bacteria, which cause diseases and pose serious threats to aquatic life. In this project, we have investigated the effectiveness of a composite made up of biochar from pomelo peels and titanium dioxide nanoparticles encapsulated in calcium alginate beads for water purification, by evaluating its adsorption capability in the adsorption of Zn^{2+}, Cu^{2+} and Fe^{3+} ions, and investigating its antibacterial properties.The composite was shown to have a higher adsorption capability of these ions than pomelo peel powder and pomelo peel biochar. Antibacterial tests against *E.coli* and *S.epidermidis* were conducted, and the composite was found to display antibacterial effects on both bacteria by reducing the absorbance of the bacterial cultures by an average of 53.2% and 74.0% respectively. This makes the composite a versatile multi-purpose adsorbent, and its adsorption and antibacterial properties makes it a cheaper alternative option for wastewater treatment.

1. Introduction

Water will always play an essential part in all life. However, water pollution has been a problem for mankind. Pollutants include heavy metal ions, with sources including agricultural, industrial, geogenic, pharmaceutical etc. Environmental pollution is also very prominent, due to mining, foundries, smelters, and other metal-based industrial businesses (Tchounwou et al., 2012). WHO estimates that occupational exposure to harmful metal ions account for an estimated 1.3 million deaths per year (WHO, 2013).

Consumption or usage of polluted water presents many risks, like transmittable diseases including cholera, diarrhea, dysentery, typhoid, and polio. It is estimated to cause 485 000 diarrheal deaths a year. (WHO, 2019). Heavy metal ions in water also pose a big risk to people globally, causing serious medical diseases and conditions resulting from exposure. For example, lead (II) is the most toxic heavy metal ion in the environment and exposure can result in decreased cognitive performance in adults, and behavioral problems, lowered IQ and learning deficits in children. (Rubin & Strayer, 2008, as cited by Wani et al., 2015).

Current methods of heavy metal ion removal from wastewater include ion-exchange, chemical precipitation, adsorption, membrane filtration, coagulation-flocculation, flotation and electrochemical methods (Fu & Wang, 2012). However, most of these treatment methods are costly and unsuitable for smaller communities (Truong & Hart, 2001). Adsorption via biochar is not only power efficient but also inexpensive. Biochar is a porous carbonaceous material obtained during the pyrolysis of biomass derived from different kinds of feedstock (Zhao et al., 2019). Biochar is especially effective at the removal of heavy metals from wastewater due to highly specific surface area, high density of negative surface charges, porous structure and surface functional groups (Gai et al., 2014). In short, biochar is a low-cost adsorbent for treating heavy metals in wastewater, with multiple studies exhibiting effective removal of heavy metal ions, occasionally proving dominance over activated carbon (Inyang et al., 2015).

Microbes such as bacteria are commonly found in lakes, rivers, streams and large bodies of water. Although some are harmless, certain bacteria pose a threat to humans, causing sickness and diseases, such as the commonly found *Escherichia coli* (abbreviated *E. coli*). *E. coli* are a bacteria found in the environment, food and intestines of people and animals (USGS, 2018). Although some *E. coli* are harmless, others are the leading causes of moderate-to-severe diarrhea and mortality in low income countries. (Daneshmand et al., 2018)

In the recent decade, titanium dioxide nanoparticles have been emerging as an enticing antimicrobial compound. Furthermore, nanoscale compounds have gained traction due to their properties like a large surface-area-to-volume ratio. Titanium dioxide nanoparticles have a large surface area, are non-toxic in nature, have photocatalytic antimicrobial activity, and exerts excellent bio-related activity against bacterial contamination (Dicastillo et al., 2020).

Hence, our project aims to synthesise a composite made out of biochar from pomelo peels, a waste product from a fruit commonly consumed during the Lunar New Year and titanium dioxide nanoparticles, which has antibacterial properties, encapsulated in calcium alginate beads, which allows for the combination of the titanium dioxide nanoparticles and biochar, making it a versatile multipurpose adsorbent. It has uses in purification of water from heavy metal ions and bacteria, and is especially useful in developing countries due to its low cost efficiency.

2. Objectives and and hypotheses

Objectives

1. Synthesise a composite made up of biochar from pomelo peels and titanium dioxide nanoparticles encapsulated in calcium alginate beads.
2. Investigate the adsorption and antibacterial properties of the composite to determine whether it can be potentially used as an alternative water purification technique that is cost effective

Hypotheses

1. The biochar from pomelo peels is able to adsorb at least 75% of heavy metal ions from a 50 ppm solution of heavy metal ions. (Zn^{2+}, Cu^{2+}, Fe^{3+})
2. The titanium dioxide nanoparticles exhibit antibacterial properties on the Gram-positive *Staphylococcus epidermidis* and Gram-negative *Escherichia coli* bacteria.
3. The composite comprising biochar from pomelo peels and titanium dioxide nanoparticles encapsulated in calcium alginate beads is able to adsorb at least 75% of heavy metal ions (Zn^{2+}, Cu^{2+}, Fe^{3+}) from a 50 ppm solution, and exhibit antibacterial properties on bacteria in water.

3. Methods and Materials

Materials

Materials			
Apparatus	**Chemicals/material**	**Equipment**	**Bacteria**
1. Pasteur pipette 2. 500cm^3 beaker 3. Funnel 4. Round bottom flask 5. Filter paper 6. 50cm^3 plastic vial tubes 7. Swab stick 8. Weighing balance	1. Titanium isopropoxide 2. Sodium alginate 3. Hydrochloric acid 4. Calcium chloride 5. Pomelo peels 6. Luria Bertani broth 7. Deionised water 8. Zinc sulfate 9. Copper(II) sulfate 10. Iron(III) nitrate	1. Ashing furnace 2. Dry heat oven 3. Blender 4. Magnetic stirrer 5. UV-vis Spectrophotometer 6. Orbital Shaker 7. Colorimeter 8.Centrifuge 9. Incubator 10. FTIR Spectrometer 11. Reflux set-up*	1. *Escherichia coli.* ATCC 25922 2. *Staphylococcus epidermidis* ATCC 12228

Variables

Experiment 1: Adsorption ability of pomelo peel powder

Independent variable	Type of heavy metal ions in set-up (Zn^{2+}, Cu^{2+}, Fe^{3+})
Dependent variable	Percentage removal of heavy metal ions from solution
Controlled variables	Mass of pomelo peel powder used, initial concentration of metal ions in water, volume of water, duration of adsorption, temperature in which reaction is carried out

Experiment 2: Adsorption ability of pomelo peel biochar

Independent variable	Type of heavy metal ions in set-up (Zn^{2+}, Cu^{2+}, Fe^{3+})
Dependent variable	Percentage removal of heavy metal ions from solution
Controlled variables	Mass of pomelo peel biochar used, initial concentration of metal ions in water, volume of water, duration of adsorption, temperature in which reaction is carried out.

Experiment 3: Adsorption ability of TiO_2-pomelo peel biochar composite encapsulated in calcium alginate beads

Independent variable	Type of heavy metal ions in set-up (Zn^{2+}, Cu^{2+}, Fe^{3+})
Dependent variable	Percentage removal of heavy metal ions from solution
Controlled variables	Mass of pomelo TiO_2-pomelo peel biochar composite used, initial concentration of metal ions in water, volume of water, duration of adsorption, temperature in which reaction is carried out

Experiment 4: Antibacterial ability of Composite

Independent variable	Type of bacteria tested on (*E.coli* or *S.epidermidis*)
Dependent variable	Absorbance of bacteria culture after composite beads were added
Controlled variables	Mass of TiO_2-pomelo peel biochar composite used, amount of bacteria cultured, duration of absorption

Preparation of pomelo peel biochar

Pomelos were bought, peeled and the pomelo peels were oven-dried for one hour before they were washed with deionised water.The dried pomelo peels were dried again in an oven before they were crushed into small pieces using a laboratory blender and put into an ashing furnace at a temperature of 400°C for 1 hour. The obtained pomelo peel biochar was allowed to cool down for 30 minutes before it was taken out of the furnace and ground into fine powder using a mortar and pestle.

Preparation of TiO_2 nanoparticles

10.0cm^3 of titanium isopropoxide was mixed with 20.0cm^3 of 0.2 mol dm^{-3} hydrochloric acid in a fume hood. Precipitation occurred immediately. The mixture was maintained under magnetic stirring for 8 hours at 60°C in a reflux system, resulting in a homogenous, white dispersion. The white dispersion was then diluted to form 90cm^3 of titanium dioxide solution, which was centrifuged at a speed of 7000 RPM for a duration of 4 minutes. The resulting white solid was heated in a dry heat oven at 80°C for 1 hour. The dried Titanium dioxide solid was crushed into fine powder using mortar and pestle.

Synthesis of composite made up of pomelo peel biochar and TiO_2 nanoparticles encapsulated in calcium alginate beads

2g of sodium alginate was added to 100cm^3 of water in order to form a 2% w/v sodium alginate solution. 2g of calcium chloride was added to 100ml of water to form a 2% w/v calcium chloride solution. Then, 1.5g of titanium dioxide and 0.5g of the produced pomelo biochar was added to the calcium chloride solution. Both solutions were mixed separately using a magnetic stirrer (with hot plate) at 200 rpm for 1 minute. After mixing, the sodium alginate solution was dropped slowly into the calcium chloride solution with dispersed titanium dioxide and biochar particles, using a pasteur pipette, forming beads. The resulting beads were soaked in calcium chloride solution overnight. The following day, the pomelo peel biochar-titanium dioxide nanoparticles-calcium alginate composite beads were filtered and rinsed in deionised water repeatedly until pH neutral. The beads were then oven-dried for 3 hours. Calcium alginate beads, with titanium dioxide and pomelo peel biochar solution encapsulated inside, were thus formed.

Preparation of spiked water

0.2198g of zinc sulfate, 0.1902g of copper(II) sulfate and 0.3620g of iron(III) nitrate were added separately to deionised water to obtain solutions with 50ppm of metal ions.

Characterisation of TiO_2 nanoparticles

Fourier-transform infrared (FTIR) spectroscopy was used to locate the Ti-O stretch of the TiO_2 NPs through the KBr pellet method.

Characterisation of pomelo peels

Fourier-transform infrared (FTIR) spectroscopy was used to analyse the different functional groups of the pomelo peels through the KBr pellet method.

Adsorption of heavy metal ions

0.5g of the composite made up of biochar from pomelo peels and Titanium dioxide nanoparticles encapsulated in calcium alginate beads was put into a plastic vial containing 25cm^3 of 50ppm Zn^{2+} solution. 5 replicates were set-up and the same procedures were repeated for Cu^{2+} and Fe^{3+}. All 5 tubes were placed in an orbital shaker at 200 rpm for 4 hours. The concentration of metal ions remaining in the solution of each tube after centrifugation at 8000 rpm was measured with a colorimeter.
The percentage removal of heavy metal ions could be calculated using the formula:

$$Percentage\ removal\ of\ heavy\ metal\ ions = \frac{Initial\ conc. (50ppm) - Final\ conc.}{Initial\ conc. (50ppm)} \times 100\%$$

Antibacterial test

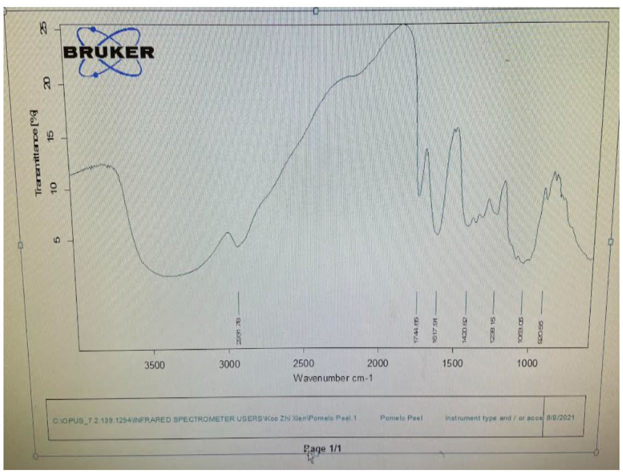
E.coli was cultured in Luria-Bertani broth. 0.5g of the composite made up of pomelo peel biochar and titanium dioxide nanoparticles encapsulated in calcium alginate beads was added to 100 µl of *E.coli* in a test tube and was shaken overnight. The absorbance of the bacterial culture was measured using a UV-spectrophotometer at 600 nm the next day and compared with that of a control set-up. The same procedures were repeated for *S.epidermidis*, with triplicates carried out per constituent.

The percentage absorbance of bacteria by composite could be calculated using the formula:

$$Percentage\ absorbance\ of\ bacteria\ by\ composite = \frac{Initial\ abs. - Final\ abs.}{Initial\ abs.} \times 100\%$$

4. Results and Discussion

Characterisation of pomelo peels and TiO_2 nanoparticles by FTIR spectroscopy



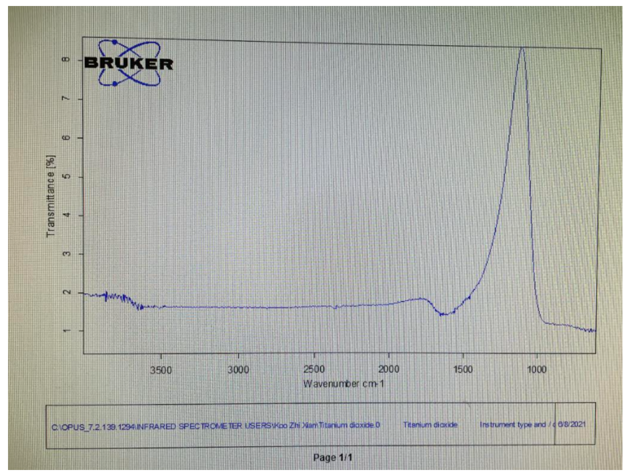


Fig. 1a. FTIR spectrum for pomelo peels

Fig 1b. FTIR spectrum for TiO_2 nanoparticles

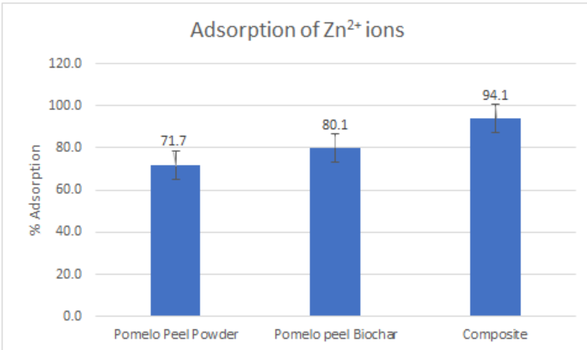
Analysis of FTIR spectrum for pomelo peels

The peak at at 2932 cm^{-1} was assigned to C-H stretching in methyl and methylene groups vibrations. The peak at 1745 cm^{-1} was assigned to the stretching vibrations of carbonyl bond due to the presence of non-ionic groups of carboxylic acids or pectin esters and the peak at 1063 cm^{-1} was assigned to the stretching vibration of C-OH of alcoholic groups and carboxylic acids, which corresponds to past findings (Ibragić, Smječanin, Milušić, & Nuhanović, 2021).

Analysis of FTIR spectrum for TiO_2 nanoparticles

TiO_2 powder showed a similar absorption broadband in the region from 1500 cm^{-1} to 1000 cm^{-1} with peaks at 1250 cm^{-1} characteristic for Ti-O bonds. The sample contained water, as the spectrum exhibited specific O-H absorption at 3600 cm^{-1}. (Molea, Popescu, & Rowson, 2011)

Results from adsorption tests



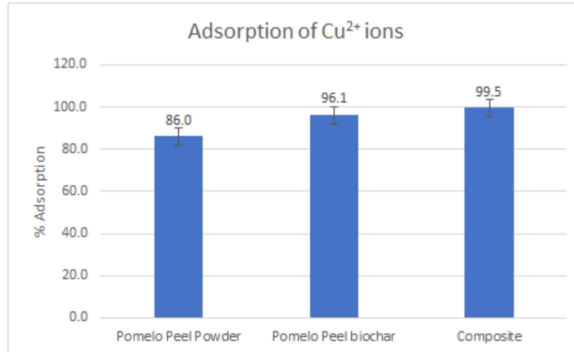


Fig 2a. Adsorbents' effectiveness on Zn^{2+} ions Fig 2b. Adsorbents' effectiveness on Cu^{2+} ions

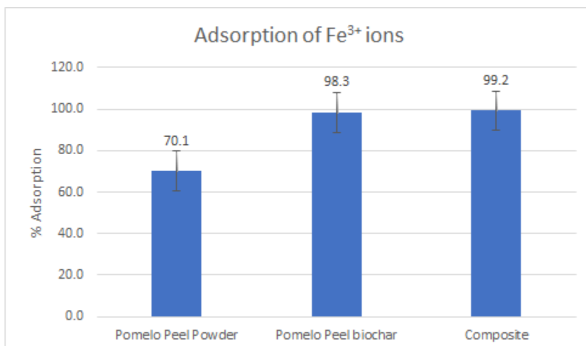


Fig. 2c: Adsorbents' effectiveness on Fe^{3+} ions

Discussion of results

The respective data sets were compared and analysed using the Mann-Whitney statistical tests to conclude whether a significant difference exists between the adsorption capabilities of the composite beads and pomelo peel powder. The composite has a higher adsorption capability than pomelo peel powder for all three ions. It has a percentage adsorption of 94.14%, 99.48% and 99.24% for Zn^{2+}, Cu^{2+} and Fe^{3+} ions respectively.

Results of Mann-Whitney U Test

Adsorption using composite against adsorption using pomelo peel powder

Metal ion	Zn^{2+}	Cu^{2+}	Fe^{3+}
p-value	0.012	0.012	0.012
Significant difference	Yes (Composite)	Yes (Composite)	Yes (Composite)

Fig 3a. Results of Mann-Whitney U Test for TiO_2-pomelo peel biochar-calcium alginate composite against pomelo peel powder

Adsorption using composite against adsorption using pomelo peel biochar

Metal ion	Zn^{2+}	Cu^{2+}	Fe^{3+}
p-value	0.012	0.012	0.296
Significant difference	Yes (Composite)	Yes (Composite)	No

Fig 3b. Results of Mann-Whitney U Test for TiO_2-pomelo peel biochar-calcium alginate composite against pomelo peel biochar

From Figure 3a, there was a significant difference between the adsorption of TiO_2-pomelo peel biochar-calcium alginate composite and pomelo peel powder of all three ions (Zn^{2+}, Cu^{2+}, Fe^{3+}), with p-values of 0.012 each, at a 5% significance level. Hence, the composite had significantly higher absorbance than the pomelo peel powder.

From Figure 3b, there was no significant difference between the adsorption of TiO_2-pomelo peel biochar-calcium alginate composite and pomelo peel powder for Fe^{3+} ions, with a p-value of 0.296 at a 5% significance level. There was a significant difference between the adsorption of Zn^{2+} and Cu^{2+} ions, where the composite had significantly higher absorbance than the pomelo peel biochar, with both having a p-value of 0.012 at a 5% significance level. We believe that this was due to the properties of pomelo peel biochar, which is an effective material for the sorption of heavy metals due to the abundance of functional groups on its surface such as phenolic, hydroxyl, and carboxyl groups, and due to its porous structure and its large surface area (Zhao et al., 2020). Calcium alginate in composite beads could also play a role in its adsorption ability.

Overall, the composite has high adsorption capabilities for all 3 heavy metal ions.

Antibacterial Properties Test Results

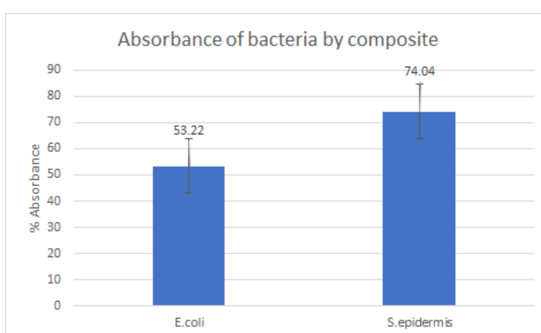


Fig 4. Absorbance of bacteria by composite

From Figure 4, the composite exhibits antibacterial properties on both *E.coli* and *S.epidermidis.* However, the percentage absorbance of *S.epidermidis* by the composite is 74.04%, which is greater than that of *E.coli*, which is 53.22%, showing that the composite exhibits greater antibacterial activity on the gram-positive *S.epidermidis* than the gram-negative *E.coli*.

5. Conclusion and recommendations for future work

Conclusion

A composite made up of biochar from pomelo peels and titanium dioxide nanoparticles encapsulated in calcium alginate beads was synthesised and a comparative analysis of its adsorption properties to Zn^{2+}, Cu^{2+} and Fe^{3+} ions was conducted. The composite was found to have significantly better adsorption of all three ions than pomelo peel powder. When compared to pomelo peel biochar, the composite was significantly better for adsorption of Zn^{2+} and Cu^{2+} ions. The TiO_2-pomelo peel biochar-calcium alginate composite has also demonstrated antibacterial properties on both gram-positive and gram-negative bacteria. The experimental results generally correspond to our hypotheses. As such, with pomelo peel biochar, titanium dioxide and calcium alginate as its constituents, a cheap and versatile composite which is suitable for use in wastewater treatment has been achieved. This composite can thus serve as an alternative technique of water purification that is cost-effective and versatile, allowing its use in less developed countries.

Limitations and future work

Due to time constraints, characterisation of TiO_2 NPs and pomelo peel powder using SEM cannot be carried out. In future, further reusability tests could be carried out on our composite, and the composite could be tested on its effectiveness on other pollutants such as ammonia and lead, as well as dyes which are commonly found in industrial waste. Moreover, a comparison between the adsorption capabilities of the composite and calcium alginate could be done to find out whether calcium alginate plays a significant role in the removal of heavy metal ions in water. The adsorption capacities of the composite can also be compared to commercial options for water purification such as activated carbon.

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Appendix

1. Preparation of pomelo peel biochar



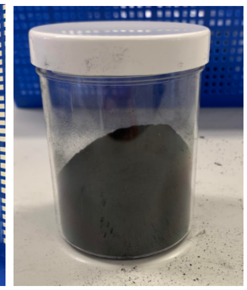


Fig 1a. Dried pomelo peels Fig 1b. Pomelo peel biochar Fig. 1c. Biochar powder

2. Preparation of TiO_2 nanoparticles

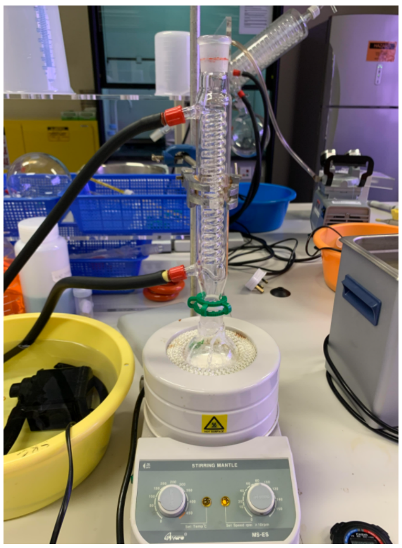



Fig 2a. Reflux process Fig 2b. Synthesised TiO_2

*Reflux set-up consists of a heating mantle, round-bottom flask, condenser, magnetic stirrer and clamp

3. Synthesis of composite

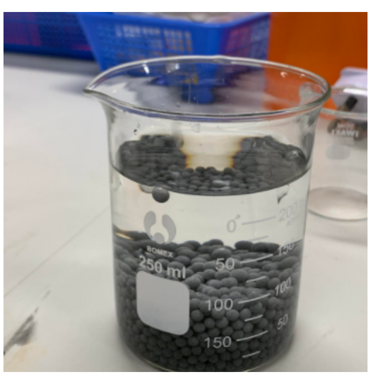
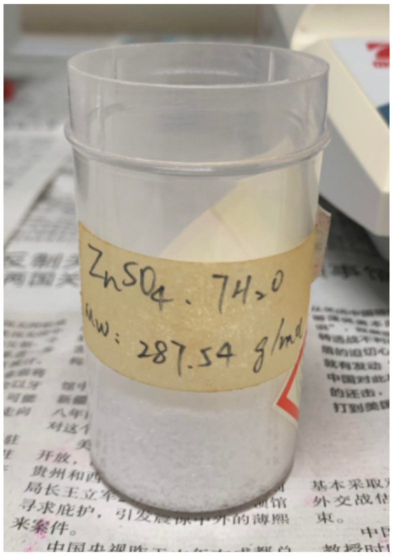


Fig 3. Process of synthesising composite beads

4. Preparation of spiked water



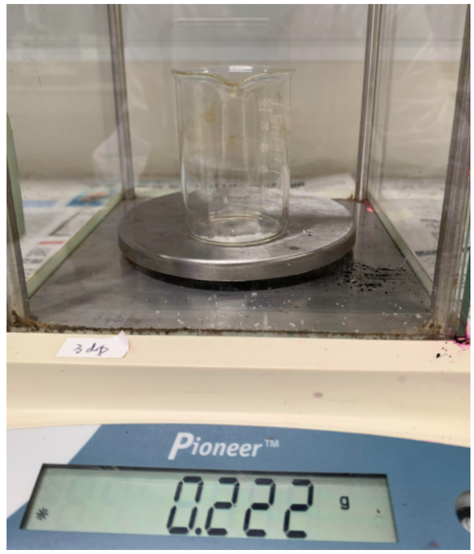


Fig 4a. Zinc sulfate　　Fig 4b. Process of preparing spiked water

5. Adsorption test

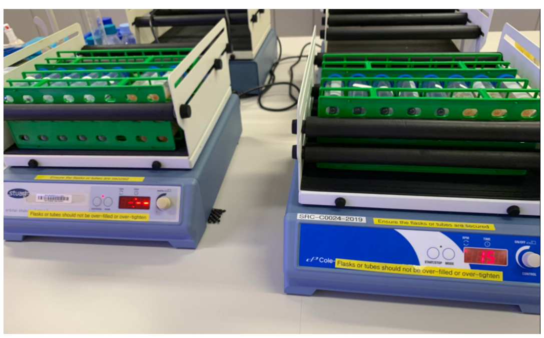


Fig 5a. Samples in orbital shaker　　Fig 5b. Process of adsorption tests

6. Data collected from adsorption tests

Adsorption of Zn^{2+} ions			
Reading	Pomelo Peel Powder	Pomelo peel Biochar	Composite
1	75.0	84.0	88.5
2	67.5	82.0	96.2
3	69.5	70.0	95.5
4	78.0	84.0	93.0
5	68.5	80.5	97.5
Mean	71.7	80.1	94.1
Std Dev	4.56	5.84	3.55
Std Error	2.04	2.61	1.59

Fig 6a. Table showing the adsorption of Zn^{2+} ions

Adsorption of Cu^{2+} ions			
Reading	Pomelo Peel Powder	Pomelo Peel biochar	Composite
1	85.2	96.4	99.0
2	85.8	93.6	99.6
3	86.0	96.8	99.0
4	86.0	96.6	99.8
5	87.0	97.2	100.0
Mean	86.0	96.1	99.5
Std Dev	0.648	1.44	0.460
Std Error	0.290	0.644	0.206

Fig 6b. Table showing the adsorption of Cu^{2+} ions

Adsorption of Fe^{3+} ions			
Reading	Pomelo Peel Powder	Pomelo Peel biochar	Composite
1	71.8	99.4	100
2	57.2	99.2	99.6
3	86.2	99.4	99.4
4	72.8	97.2	98.4
5	62.6	96.4	98.8
Mean	70.1	98.3	99.2
Std Dev	11.1	1.42	0.639
Std Error	4.96	0.634	0.286

Fig 6c. Table showing the adsorption of Fe^{3+} ions

7. Antibacterial test

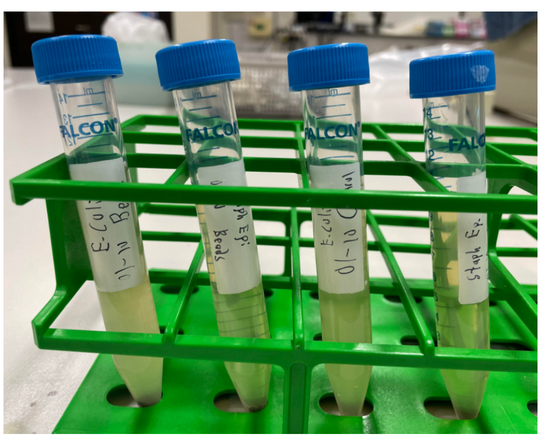


Fig 6a. Preparation of bacterial cultures

Fig 6b. Absorbance shown by UV-vis