Multipurpose cellulose-based biodegradable organohydrogel from sugarcane bagasse for adsorption of heavy metal ions and antimicrobial properties for wound dressing.

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Abstract

Sugarcane bagasse is the fibrous matter that remains after sugarcane has been crushed to extract its juices during the production of sugarcane juice. Currently, food wastes such as sugarcane bagasse are converted into biofuels to be used for renewable energy. However, there has been recent discoveries of cellulose being treated and used as a wound dressing. In this project, we aim to investigate the simple application of the cellulose-based hydrogel through a wound dressing capable of removing bacteria and disinfecting wounds A cellulose-based wound dressing was synthesised from sugarcane bagasse via the extraction of cellulose after treating it with sodium hydroxide and hydrogen peroxide. The cellulose was then treated with sodium carboxymethyl cellulose (CMCNa) and hydroxyethyl cellulose (HEC) such that the cellulose takes a gel form, and citric acid was added later to finalise the crosslinking reaction to form a solid hydrogel. The hydrogel was then immersed in 0.03 mol dm^{-3} of zinc nitrate solution for 24h then 0.2 mol dm^{-3} of sodium hydroxide to coat it with zinc oxide nanoparticles. The adsorption properties of the hydrogel against of heavy metal cations (Cu^{2+} and Fe^{3+}) were investigated using a colorimeter while the antibacterial properties were investigated through spectrophotometer inspections and via the zone of inhibition. Colorimeter tests have shown that the hydrogel is efficient at the adsorption of heavy metal cations, with a maximum adsorption of 83.20% for Fe^{3+} and 38.96% for Cu^{2+} while the antibacterial tests have been inconclusive as of this stage.

1. Introduction

1.1. Literature Review

According to the World Bank Organisation, the world generates 2.01 billion tonnes of municipal solid waste annually, with at least 33 percent of that—extremely conservatively—not managed in an environmentally safe manner. According to Singapore’s National Environment Agency’s website, in 2019, Singapore generated 744 000 tons of food waste. However, out of the 744 000 tons, only 136 000 tons gets recycled, which is a mere 18%. With the onset of food wastage, there has been growing interest and investments in the research on sugarcane bagasse recently in order to make full use of its untapped potential

In recent years, the quest for materials-based hydrogels with potent functionalities including antimicrobial potentialities has revitalized the field of biomaterials (Iqbal, 2018). Hence, much focus has been placed on the production of novel hydrogels made of natural polysaccharides such as cellulose, starch and chitosan due to its biodegradability, availability, renewability and low cost of these materials (Demitri, Madaghiele, Grazia Raucci, Sannino & Ambrosio, 2019). Sugarcane bagasse is made up of cellulose (33–36%), hemicellulose (28–30%), and lignin (17–24%) (Sabiha-Hanim & Asyikin Abd Halim, 2019). This gives great accessibility for us to extract the cellulose, and ensures its viability, and due to its abundance.

There are many different uses for hydrogels, from wound dressing, implant coating to infection treatment with physiological conditions, however, hydrogels favour bacterial growth due to its moist environment. Hence, bacterial infection becomes a huge health challenge for hydrogel application, attracting research focus (Cui et al., 2021). Microbes such as bacteria are commonly found in all around us, from tabletops, floor surface to lakes, rivers etc. One such microbe is *Escherichia coli* (*E. coli*). *E. coli* are Gram-negatives which cause human infections, and its pathogenic strains cause many different intestinal or extra-intestinal infections, such as urinary tract, intra-abdominal and soft tissue, sepsis, neonatal meningitis, gastrointestinal infection and pneumonia which ultimately leads to bacteremia (Kim, 2012; Wasiński, 2019 as cited in Kumar et al., 2020). It is well-known that zinc oxide (ZnO) nanoparticles possess antibacterial activity, and it is currently used in many cosmetic materials and food packaging applications. The antimicrobial activity of ZnO nanoparticles may be related to the induction of oxidative stress due to generation of reactive oxygen species, which may cause the degradation of the membrane structure of the cell (P.J.P. Espitia et.al, 2012). ZnO has been used as an antimicrobial agent since 1995. Recently, more and more researchers have embarked on the fundamental studies on the antibacterial activities of ZnO (J.Sawai et.al, 1998).

Water pollution has dampened our clean supply of water and poses a problem to all life around us. Industrialisation has been mainly responsible for water pollution especially industrial activities such as smelting, refining, energy generation, to name some examples (Escudero-Oñate, Fiol, Poch & Villaescusa, 2017). As a result, lakes and rivers are being overwhelmed with large amounts of toxic substances. One issue that requires our attention is that heavy metal ions in water are reaching hazardous levels as compared to other toxic substances, such as dyes. Some examples of toxic heavy metal ions that are of concern are chromium (Cr^{3+}), Lead (Pb^{2+}) zinc (Zn^{2+}), copper (Cu^{2+}) to just name a few. Due to these metal ions being non-degradable, they accumulate over time in living organisms after consumption, leading to diseases and disorders, threatening human life (Kanamarlapudi, Chintalpudi & Muddada, 2018).

One of the main waste water treatment methods includes reverse osmosis. It uses a high-pressure pump to increase the pressure on the waste water in order to push it through a semi-permeable membrane. While reverse osmosis is one of the most well-known and reliable methods for waste water treatment, one major downside to this method is the high cost of production due to the great amount of electricity to push water through the filter, as well as the constant need to replace the filters used in reverse osmosis. Hence, reverse osmosis is extremely expensive and difficult to implement. Other methods of waste water treatment are ion-exchange, chemical precipitation, membrane filtration, coagulation-flocculation, flotation and electrochemical methods, for the removal of heavy method cations (Mishra, Saini & Singh, 2021). However, it is widely known that these techniques require heavy initial investment and are expensive.

On the contrary, adsorption separation method is an attractive process because it can be easily applied to waste water treatment, which includes efficiency and flexibility. When it is compared with other treatment methods, it appears superior than others (Ince M & Ince OK, 2019). Hydrogels are highly hydrophilic due to the presence of hydrophilic groups such as $-NH_2$, -COOH, -OH, $-CONH_2$, - CONH -, and $-SO_3H$, allowing it to remove heavy metal cations from wastewater through electrostatic attraction with the heavy metal ions (Rehman et al., 2019). This is due to the presence polar bonds with the partial positive charges in the cations and partial negative charges in the hydrophilic groups. Thus, the easy availability, low cost, biodegradability and effectiveness of heavy metal ions removal of such materials thus makes it promising alternative in the adsorption of heavy metal cations (Ahmad & Zaidi, 2020).

Hence, this study aims to explore the possibilities of using sugarcane bagasse in synthesizing a multipurpose cellulose-based organohydrogel for adsorption of heavy metal ions and antimicrobial properties. Furthermore, using sugarcane bagasse which are generally disposed after milling of juices will help boost the environmental factor of this study. With its ability to adsorb heavy metal cations coupled with its antimicrobial properties and reusability, the hydrogel compound will be a reusable and multi-purposed tool to be used in the medical industry. While sugarcane bagasse has been successfully synthesised to form hydrogels for different purposes, there has not been any studies, to the best of our knowledge, which have synthesised hydrogels that have multiple different functions, retaining the originality of this study.

1.2. Objectives and hypotheses

The objective of the study is to synthesise cellulose-based hydrogel for the adsorption of heavy metal cations (Cu^{2+} and Fe^{3+}) using delignified cellulose fibres from sugarcane bagasse, investigating the effectiveness of cellulose-based hydrogel in adsorption of heavy metal cations (Cu^{2+} and Fe^{3+}) and lastly to test the antimicrobial properties of cellulose-based hydrogel against *S. Epi* and *E. Coli*

It was hypothesised that the cellulose-based hydrogel can be synthesized, and that the organohydrogel would exhibit a strong ability with 65% removal of heavy metal ions (Cu^{2+} and Fe^{3+}), as well as exhibit antimicrobial properties against *S. Epi* and *E. Coli.*

2. Methods and Materials

2.1. Materials

Citric acid, hydrogen peroxide, copper(II)sulfate, iron(III) nitrate, Hydrochloric Acid and Sodium Hydroxide were purchased from GCE chemicals.
10% Bleach, Sterile Water. Escherichia coli and Staphylococcus epidermidis were obtained from the Biology Laboratory.
Sugarcane bagasse was obtained from drink stalls.

Constant Variables	Dependent Variables	Independent Variables
• Contact time, pH and temperature of the solutions • Mass of the hydrogel used • Temperature for culture of bacteria • Mass of reused hydrogel • Concentration of • HCl or NaOH	• Percentage of heavy metal cations removed • Size of zone of inhibition (Antibacterial tests)	• Type of hydrogel • Type of heavy metal cation • Immersion times of hydrogels • Amount of yeast phenolics in hydrogel • Types of hydrogel Types of bacteria used • Number of cycles of adsorption and desorption • Types of hydrogel

2.2. Extraction of Cellulose

Sugarcane bagasse strips were cut into small pieces. They were then treated in 700 cm^3 of 5 % sodium hydroxide (NaOH) solution at 80 ºC for 3 h. It was then removed and washed with deionised water (DI water) until pH 7. It was then soaked in hydrogen peroxide for 2 h and then removed and washed again until pH 7. Finally, the cellulose was then dried in an oven at 80 ºC for 48 h before it was blended and stored for further use.

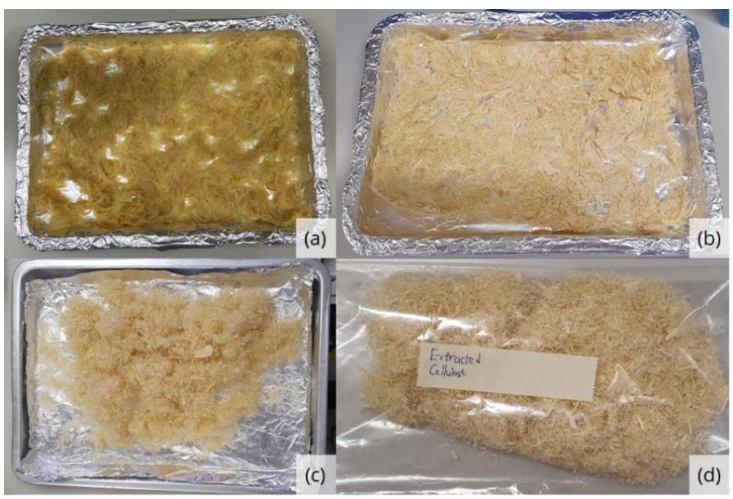


Figure 1:

(a) Sugarcane bagasse treated in 700 ml of 5 % sodium hydroxide (NaOH) solution at 80ºC for 3 h, then soaked in hydrogen peroxide for 2 h

(b), (c) and (d) Dried and blended cellulose

2.3. Synthesis of Hydrogel

Add total polymer concentration of 2% by weight of water, using a mixture of CMCNa and HEC, with weight ratio equal to 3/1 was dissolved in distilled water by stirring gently at room temperature until a clear solution was obtained. 3.75% of citric acid was added to the mixture to crosslink the hydrogels. 40 cm^3 of the solution was then transferred into separate petri dishes. All samples were first pre-dried at 30°C for 24 h to remove absorbed water and then kept at 80°C for the crosslinking reaction (24 h with intermediate control)

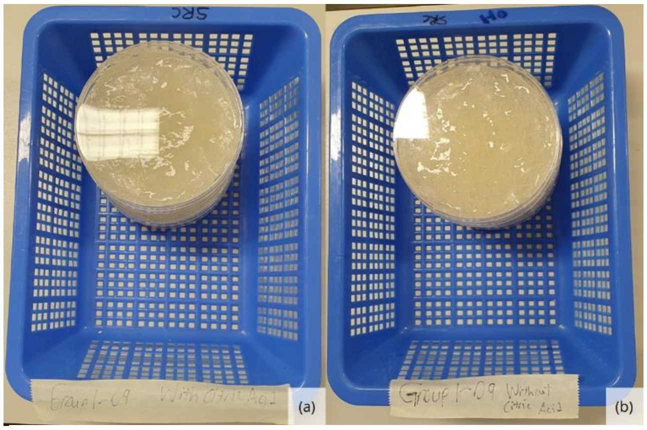


Figure 2:

(a) Hydrogel with citric acid in petri dish

(b) Hydrogel without citric acid in petri dish

2.4. Creation of Hydrogel Compound

Typically, 0.6 g of dried CMC hydrogel was immersed in zinc nitrate solutions with concentration 0.030 mol dm^{-3} for 24 h. The Zn^{2+} ion-loaded hydrogels were washed with distilled water to remove Zn^{2+} ions attached the hydrogel surface. Following cleaning, these hydrogels were placed in 100 cm^3 of 0.2 mol dm^{-3} NaOH solution for 24h. After the oxidation of the bound Zn^{2+} ions, the hydrogels were washed with distilled water and finally dried in an oven at 50 ºC for 24 h.

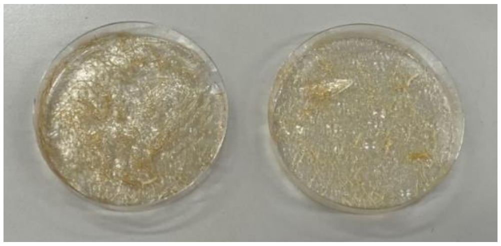


Figure 3:

Hydrogel samples immersed in zinc nitrate

2.5. Testing for adsorption of heavy metal cations

0.197g of Copper (II) sulfate and 0.3620g of Iron (III) nitrate were added separately into 1L of deionized water through pouring into the volumetric flask to obtain solutions with 50ppm of metal ions. Then 0.1g of the hydrogel were added to each sample. The contact time, pH and temperature of the solutions were kept constant throughout the experiment. The mixtures were then left on a orbital shaker at 200 rpm using centrifuge tubes for 4h. After which, the centrifuge tubes were centrifuged at 800 rpm for 5 minutes The initial and final concentrations of the heavy metal ions were analysed using a colorimeter with (HACH PR/ 890) model used. The formula used for the calculations of percentage adsorbed is:

$$Percentage\ adsorption\ of\ heavy\ metal\ cations\ by\ hydrogel = \frac{initial\ conc.-final\ conc.}{intial\ conc.} \times 100\%$$

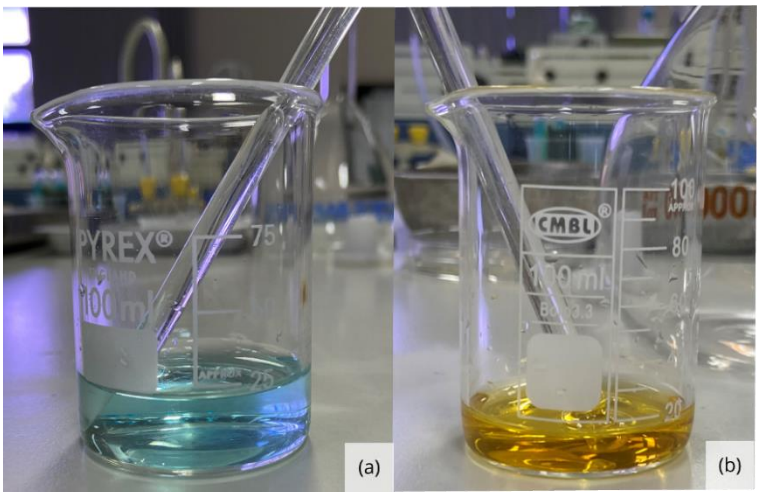


Figure 4:

(a) Solution of Copper (II) sulfate

(b) Solution of Iron (III) nitrate

2.6. Testing for antibacterial properties of hydrogel compound

S. Epi and *E. Coli* were cultured in LB broth and left in an orbital shaker overnight. The bacteria culture was split into 2 equal volumes of samples of 5.0cm^3 each the next day. One of the samples were used for for experimentation, while the other was used as a control. The OD of first sample the bacteria culture was measured using a spectrophotometer for samples of *E. coli* and *S. Epi* respectively. This formed the control set up. 0.12g of Hydrogel with Zinc Oxide Nanoparticles was added to 5.0cm^3 of half of the bacteria culture and left in a shaker overnight. The initial and final absorbance was measured using spectrophotometer (Shimadzu UV-1800) at OD_{600}. The absorbance was then calculated using the formula below:

$$Percentage\ absorbance\ of\ bacteria\ by\ hydrogel = \frac{initial\ abs - final\ abs}{intial\ abs} \times 100\%$$

S. Epi and E. Coli were also swabbed onto surface of 2 MHA agar plates prepared previously, and 3 wells were created. 0.06g of solid hydrogel samples were added to 10 ml of deionised water and left in a sonicator for 30 minutes at 40°C to form a liquid sample. A negative set-up of 0.080 ml of sterile water, a positive set up of 0.080ml of 8% Bleach and 0.080ml of liquid sample was added to the respective wells. The plates were then left in an incubator for 5 days.

3. Results and Discussions

3.1. Adsorptions of Cations

	Concentration of heay metal cations adsorbed by hydrogel synthesised with Citric Acid		Concetration of heay metal cations adsorbed by hydrogel synthesised without Citric Acid		Concetration of heay metal cations adsorped by sugarcane cellulose	
	Cu^{2+} ions	Fe^{3+} ions	Cu^{2+} ions	Fe^{3+} ions	Cu^{2+} ions	Fe^{3+} ions
Test 1	20.2	42.6	7.00	13.8	17.4	41.6
Test 2	20.2	43.0	4.6	24.6	15.8	44.2
Test 3	19.0	40.00	4.8	19.6	15.6	44.0
Test 4	17.8	41.2	6.00	19.3	13.6	43.4
Test 5	20.2	41.2	8.8	19.4	17	42.6
Average	19.5	41.6	6.24	19.3	15.9	43.2
Standard Deviation	1.07	1.21	1.73	3.82	1.49	1.07
Standard Error	0.48	0.54	0.77	1.71	0.67	0.48

Figure 5: Data on the adsorption of Cations

3.1.1. Test on Adsorption of Cu^{2+}

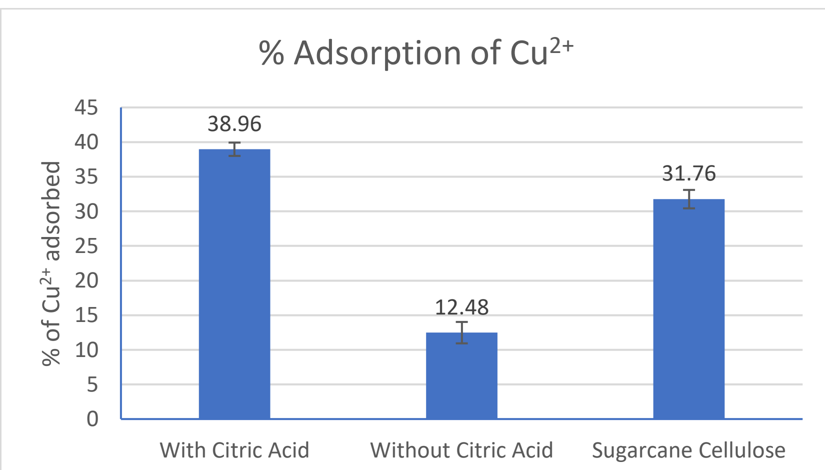


Figure 6: Data on the adsorption of Cu^{2+}

From the results of the adsoprtion tests for Cu^{2+}, it can be seen that the hydrogel synthesized with Citric acid showed a greater ability to adsorb Cu^{2+} ions compared to sugarcane cellulose and hydrogel synthesized without citric acid.

Mann Whitney U Test

Between hydrogel synthesised with citric acid and sugarcane cellulose

p value obtained from statistics test (Cu^{2+}): 0.011159

As the p value is less than 0.05, the null hypothesis is rejected as there is **significant difference** between the hydrogel synthesised with citric acid and the sugarcane cellulose in terms of adsorption of Cu^{2+} ions.

Between hydrogel synthesised without citric acid and sugarcane cellulose

p value obtained from statistics test (Cu^{2+}): 0.007937

As the p value is less than 0.05, the null hypothesis is rejected as there is **significant difference** between the hydrogel synthesised with citric acid and the hydrogel synthesised without citric acid in terms of adsorption of Cu^{2+} ions.

Between hydrogel synthesised with citric acid and hydrogel synthesised without citric acid

p value obtained from statistics test (Cu^{2+}): 0.011159

As the p value is less than 0.05, the null hypothesis is rejected as there is **significant difference** between the hydrogel synthesised with citric acid and the hydrogel synthesised without citric acid in terms of adsorption of Cu^{2+} ions.

3.1.2. Test on Adsorption of Fe^{3+}

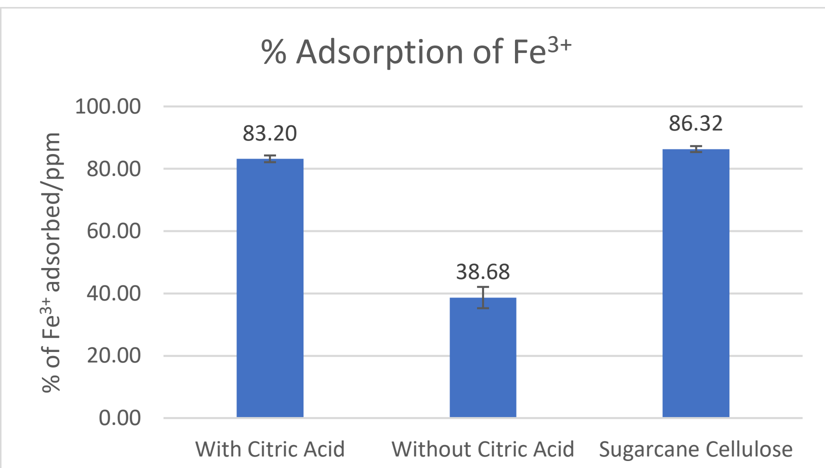


Figure 6: Data on the adsorption of Fe^{3+}

From the results of the adsoprtion tests for Zn^{3+} it can be seen that the hydrogel synthesised with citric acid and sugarcane cellulose showed a similar ability to adsorb Fe^{3+} ions, while being able to adsorb a greater amount of Fe^{3+} ions compared to hydrogel synthesised without citric acid.

Mann Whitney U Test

Between hydrogel synthesised with citric acid and sugarcane cellulose

p value obtained from statistics test (Fe^{3+}): 0.074026

As the p value is more than 0.05, the null hypothesis is not rejected as there is **no significant difference** between the hydrogel synthesised with citric acid and the sugarcane cellulose in terms of adsorption of Fe^{3+} ions.

Between hydrogel synthesised without citric acid and sugarcane cellulose

p value obtained from statistics test (Fe^{3+}): 0.007937

As the p value is less than 0.05, the null hypothesis is rejected as there is **significant difference** between the hydrogel synthesised with citric acid and the hydrogel synthesised without citric acid in terms of adsorption of Fe^{3+} ions.

Between hydrogel synthesised with citric acid and hydrogel synthesised without citric acid

p value obtained from statistics test (Fe^{3+}): 0.011925

As the p value is less than 0.05, the null hypothesis is rejected as there is **significant difference** between the hydrogel synthesised with citric acid and the hydrogel synthesised without citric acid in terms of adsorption of Fe^{3+} ions.

3.1.3. Summary

As seen from the results above, the hydrogel has proved that it has a higher efficacy of adsorbing Cu^{2+} than Fe^{3+} cations. In addition, it has been found that hydrogels synthesised with citric acid are more able to adsorb a higher concentration of heavy metal cations compared hydrogels synthesised without citric acid. Lastly, the hydrogels synthesised with citric acid are able to adsorb a high concentration of Cu^{2+} ions, while being able to adsorb a similar concentration of Fe^{3+} ions compared to sugarcane cellulose, which is due to the fact that sugarcane is highly porous and able to adsorb a large concentration of metal cations.

3.2. Antibacterial Properties of Hydrogel Compound

There were no conclusive data yet at this stage, to show the antibacterial properties of the hydrogel compound. For the first test done to determine the antibacterial properties, the initial absorbance for *S. Epi* and final absorbance is 1.268 and 1.642 respectively, while for *E.coli* initial and final absorbance is 1.516 and 1.896 respectively. However, these results are insignificant as the hydrogel dissolved in the sample, causing a pure liquid sample to be unable to obtain, affecting the absorbance calculation.

On another hand, there was no zone of inhibition shown via zone of inhibition. As seen in Fig 5a and Fig 5b, there is no zone of inhibition observed, hence antibacterial properties of the hydrogel compound is inconclusive.

There are two possibilities that caused this result to occur. Firstly, too little or no zinc oxide nanoparticles were synthesised to coat the hydrogel compound. Secondly, the concentration of zinc oxide nanoparticles in liquid form was not sufficient to display antibacterial properties.

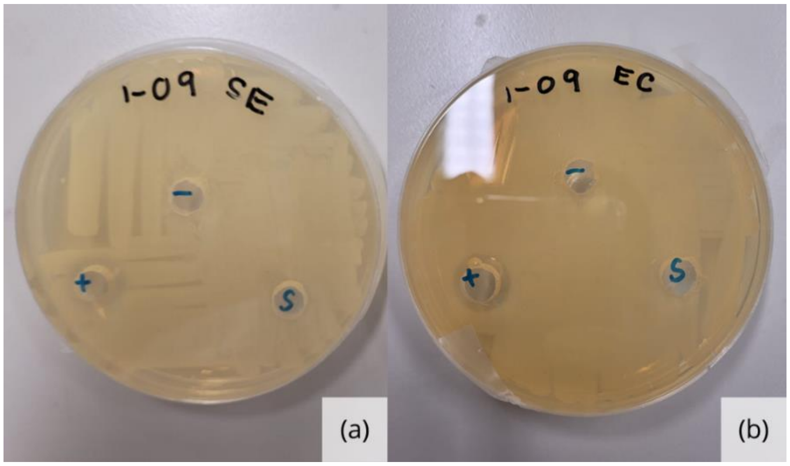


Figure 5

(a) Sample of *S.Epi* with sample after incubating for 5 days

(b) Sample of *E.Coli* with sample after incubating for 5 days

4. Conclusion and Recommendations for future work

4.1. Conclusion

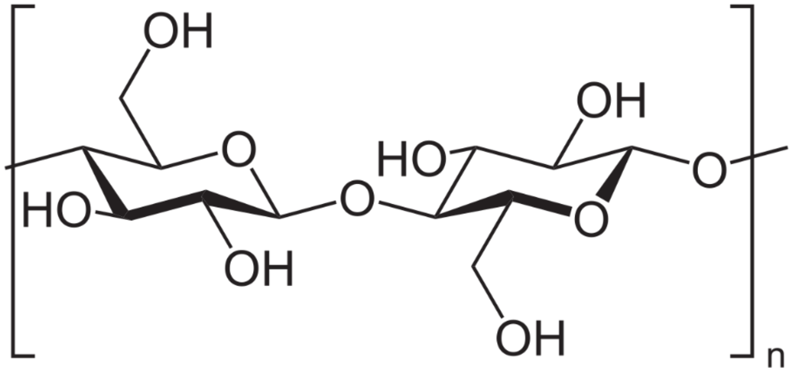
This written report has proved that it is viable for a hydrogel to be synthesized from cellulose extracted from sugarcane bagasse and the procedures are feasible to be produced on a commercial scale due to the relatively affordable price of the treatment of sugarcane bagasse as well as the synthesizing of hydrogel. Additionally, the hydrogel is partially applicable for usage as a wound dressing as the cations test has proved that the hydrogel is able to absorb a high concentration of heavy metal cations, with a maximum of 83.20% Fe^{3+} and 38.96% for Cu^{2+}. The results are exceeded the hypotheses' predictions, set at 65% and not met for Cu^{2+}. This proves that the experiments are successful in removing heavy metal cations. However, as for the antibacterial tests, the results are inconclusive as of this stage.

4.2. Recommendation for future work

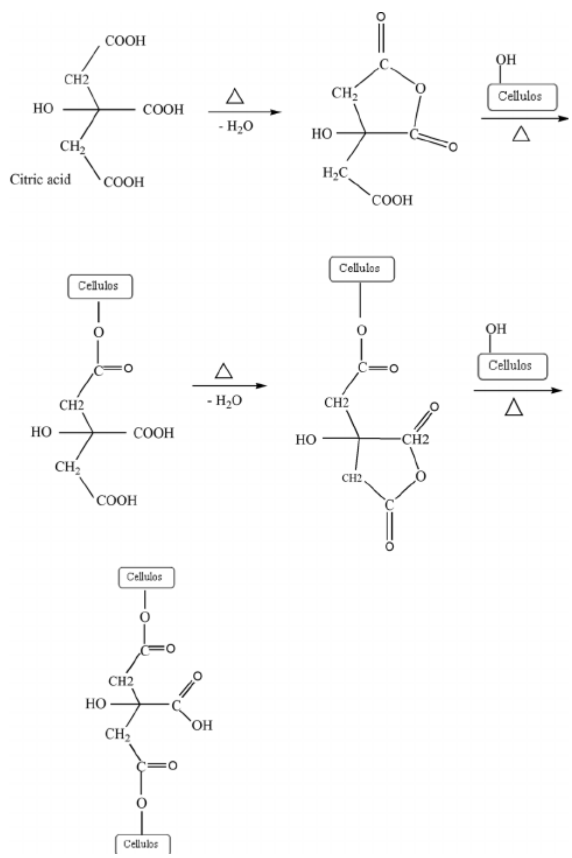
Given more time, the hydrogel that has been immersed in zinc oxide nanoparticles should be characterised by FTIR Spectroscopy and Scanning Electron Microscope, before being used in the antibacterial test using colony count method. More samples of hydrogel should be prepared in the beginning of the experiments to ensure sufficient samples in case of failure and external factors.

Appendix

Appendix A: Structure of cellulose



Appendix B: Reaction mechanism for cellulose-based hydrogel crosslinked with citric acid



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