Investigating the antibacterial and adsorption effects of Magnetic *Ananas Comosus*-Silver Nanoparticle Composite for water purification

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Abstract

Our project aimed to investigate the antibacterial effects and adsorption abilities of Magnetic *Ananas Comosus*-Silver Nanoparticle composite for water purification. Our composite was synthesised via coprecipitation to obtain a magnetite *Ananas Comosus* composite before silver nanoparticles were synthesised on the surface of Magnetite *Ananas Comosus* composite via reduction of silver nitrate, thus obtaining the final Magnetic *Ananas Comosus*-Silver Nanoparticle composite. The antibacterial tests involved conducting tests on 2 species of bacteria, *Staphylococcus epidermidis* ATCC 12228 and *Escherichia coli* ATCC 25922. Our composite was added to the respective species of bacteria and left to incubate overnight before conducting a colony count to test for its antibacterial properties. Our composite showed stronger antibacterial properties against *E. coli*, which is a gram negative bacteria. The adsorption tests involved conducting tests on 3 types of dyes and 1 type of heavy metal ion, namely Methylene Blue, Brilliant Green, Methyl Orange and Zinc (II) ions respectively. Our composite was added to the respective dyes and $ZnSO_4$ solutions and shaken before determining the remaining dye or metal ion concentration and calculating the percentage reduction. Out of the 3 dyes and 1 heavy metal ion tested, our composite had an affinity towards adsorbing Methyl Blue and Brilliant Green, which are cationic dyes as compared to Methyl Orange, which is an anionic dye. Our composite also showed effective adsorption abilities towards Zinc (II) ions.

1. Introduction

The recent decade has seen increased industrial and economic development worldwide. However, with economic development comes various side effects. The waste and sludge from factories and manufacturing industries contaminate the water which will lead to around 135 million people dying by 2020 (Gleick, 2002). As such, there has been an increasing amount of research done regarding water purification. There have also been growing interests and investments in the research of *Ananas comosus* and silver nanoparticles, between 1 to 100 nm and are widely known as a catalyst for the oxidation of methanol to formaldehyde and ethylene to ethylene oxide (Lin, Sharma & Yngard, 2009).

Silver is renowned for its antibacterial properties and has been used to treat chronic wounds, burns and infections for centuries. With the recent development of nanoparticles as an exciting new branch of materials that offers new scientific solutions and methods to be discovered, many intensive studies have been conducted on using the antibacterial properties of silver nanoparticles (AgNPs) in the disinfection of water. According to Hoek and Marambio-Jones (2010), AgNPs have been observed to possess strong antimicrobial activity against bacteria, viruses and fungi. They are also relatively non-toxic to healthy human cells in the suitable concentrations of 2-4 ppm (Okafor et al., 2013), hence their usage in water disinfection warrants further study.

However, it is still necessary to remove the AgNPs from the water after it has been disinfected due to a few reasons. Due to silver's tendency to aggregate in water, as a result its ability to disinfect water may be reduced or diminished (Fisher, Furlan, Furlan, Melcer & Warren, 2017). Furthermore, high concentrations of AgNPs (≥26 ppm) may cause harm to mammalian cells (Ivask *et al.,* 2014).

Ananas Comosus is of interest because of their variety of beneficial applications including: The production of Bromelain, the enzyme-complex of *Ananas Comosus* and adsorption of dyes . Bromelain was found to have properties such as interference with growth of malignant cells, inhibition of platelet aggregation, fibrinolytic activity, anti-inflammatory action and skin debridement properties (Batkin & Taussig, 1988). Silver nanoparticles is of similar interest amongst researchers as they have beneficial applications including: catalytic reduction and its antibacterial effects. Choi, Jang, Kim, Park and Shin (2009) recorded that silver nanoparticles could be exploited as solid phase catalysts for the reduction of 4-nitrophenol to 4-aminophenol in the presence of NaBH4.

Important applications of *Ananas Comosus* and silver nanoparticles are its adsorption abilities and antibacterial effects respectively. Patil, Patel and Renukdas (2012) found that *Ananas Comosus* could be used for the adsorption of Methylene Blue. According to Fisher, Furlan, Furlan, Melcer and Warren (2017), silver nanoparticles have been used in burn creams, food packaging and in odorless clothing and socks for their antibacterial characteristics.

Hence, this study aims to investigate the antibacterial effects and adsorption abilities of a *Ananas comosus*-magnetic silver nanoparticle composite for water purification and compare them with the antibacterial effects and adsorption abilities of *Ananas comosus* and the pineapple-magnetite compound individually.

2. Objectives and Hypotheses

Objectives

1. To find a cost effective yet efficient alternative to existing water purification methods.
2. To investigate the antibacterial effects of Magnetic *Ananas Comosus*-Silver Nanoparticle Composite on *Staphylococcus epidermidis a*nd *Escherichia coli*.
3. To investigate the adsorption abilities of Magnetic *Ananas Comosus*-Silver Nanoparticle Composite on various dyes (Methylene Blue, Brilliant Green and Methyl Orange) and Zinc (II) ions in water.

Hypotheses

1. Magnetic *Ananas Comosus*-Silver Nanoparticle Composite has potent antibacterial effects against the strains of bacteria such as *Staphylococcus epidermidis a*nd *Escherichia coli*.
2. Magnetic *Ananas Comosus*-Silver Nanoparticle Composite has potent adsorption abilities on Methylene Blue, Brilliant Green, Methyl Orange as well as Zinc (II) ions.

3. Methods and Materials

Methodology

Part I: Processing of Pineapple Waste

Fresh pineapples were obtained from various wet markets in Singapore. Pineapple peel was obtained and thoroughly washed with deionised water to remove dirt and blended until a consistent texture is observed. It was then grinded into fine pieces and dried in an oven at 80 degrees Celsius to constant mass. The resultant product was then stored and used for further

testing.

Fig. 1: Pineapple peel extract

Part II: Synthesis of Magnetic *Ananas-Comosus*-Silver Nanoparticle Composite

Part II(a): Synthesis of Magnetic *Ananas Comosus* composite via coprecipitation (Fisher, Furlan, Furlan, Melcer & Warren, 2017)

4.0mL of 1.0M $FeCl_3$ and 1.0mL of 2.0M $FeSO_4 . 7H_2O$ was added to 0.1g of *Ananas Comosus* extract as e as obtained earlier. The mixture was then stirred vigorously with a glass rod in a 50mL beaker. While stirring, 25 mL of 1.4M Aqueous NH_3 was added to the mixture. The magnetic *Ananas Comosus* composite solution was vacuum filtered with a Buchner funnel. The residue is thoroughly washed with DI water and left to dry in the oven at 50 °C for a period of 4 hours. This procedure was scaled up by factors of 10 and 5 to obtain greater amounts of the composite successfully.

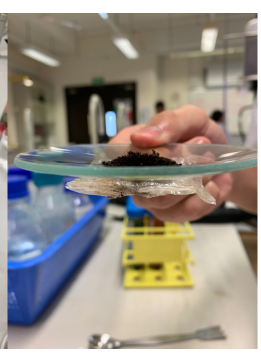

Fig. 2 (left): Vacuum filtration of the magnetite - Ananas Comosus composite

Fig. 3: Retrieval of the magnetite - Ananas Comosus composite

Part II(b): Synthesis of Silver Nanoparticles on the surface of the Magnetic *Ananas Comosus* Composite

0.1g of Magnetic *Ananas Comosus* composite was vigorously stirred with 30ml of 2.0mM chilled $NaBH_4$ solution in an ice bath for 5 minutes in a

150ml beaker to fully coat the Magnetic-*Ananas Comosus* Composite surface with it. 2ml of 1.0mM $AgNO_3$ solution was dripped into the mixture while it was being stirred. After a stable yellow colloidal solution was formed, a magnet was placed under the beaker to allow the solution and the composite to rest. With the magnet still underneath, the clear liquid was decanted off, and the residue was washed thoroughly with DI water. The Magnetic-*Ananas Comosus*-silver nanoparticle Composite (Pineapple-MAgNP) was then placed in the oven to dry over a period of 4 hours at 50 °C.

Part III: Testing the adsorption abilities of Magnetic *Ananas Comosus*-Silver Nanoparticle Composite on its removal of dyes

Pineapple-MAgNp composite was added to 50mL of 30ppm **Methylene Blue solution.** The tubes were shaken in orbital shaker for 4 hours at 200rpm, and were centrifuged at 9500rpm for 15 minutes to obtain product water after shaking. The product water was placed into the UV-Vis spectrophotometer to obtain remaining dye concentration.

The process was simultaneously conducted with control setups, which comprised of pure *Ananas Comosus* extract and *Ananas Comosus* - Magnetite composite individually. This entire process was repeated further with 2 other dyes, **Brilliant Green** and **Methyl Orange**. 5 replicates were conducted for each test.

Part IV: Testing the adsorption abilities of Magnetic *Ananas Comosus-Silver* Nanoparticle Composite on its removal of Zinc (II) ions in water

Similar to the procedure in part (IV), the composite was added to 50mL of 10ppm $ZnSO_4$ solution, shaken and centrifuged for the same duration as stated above. A colorimeter was used to determine the remaining concentration of Zinc (II) Ions. Once again, control tests were conducted with the same control setups and with 5 replicates for each test.

Part V: Testing of the antibacterial effects of Magnetic *Ananas Comosus-Silver* Nanoparticle Composite

The composite was tested against 2 species of bacteria, *Staphylococcus epidermidis* ATCC 12228 and *Escherichia coli* ATCC 25922. 0.1g of the Pineapple-MAgNp composite and 0.5mL of each strain of bacteria was added to 9.5mL of LB broth and shaken overnight. 5 replicates of this test were created. This was repeated simultaneously with 1 replicate each of 0.1g of pure *Ananas Comosus* as well as the *Ananas Comosus* - Magnetite composite individually. For each replicate tested, a control sample was created without any sample added. The tubes were shaken overnight once again. Serial dilution was performed 5 times for each sample, and 6 times for the control test (without any samples). The bacteria was then distributed into agar plates and left in the incubator overnight. The number of bacterial colonies was counted the next day.

4. **Results and Discussion**

FTIR Scan

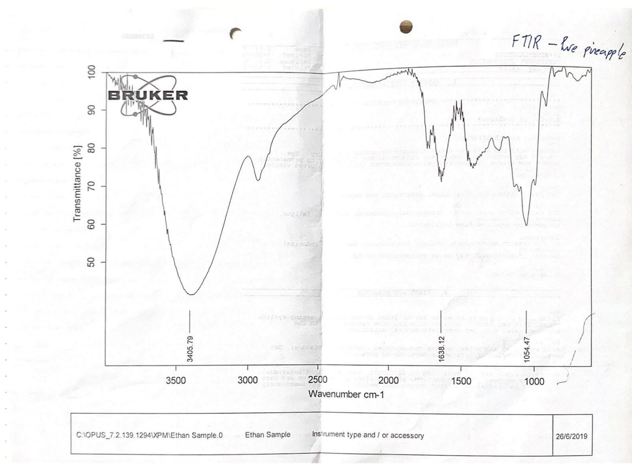

Fig 4: FTIR Scan for Pure Pineapple

The FTIR scan for pure pineapple shows the presence of O-H bond stretch from the cellulosic wastes of the pineapple peel which are possibly responsible for the adsorption of dyes and heavy metal ions in water.

Scanning Electron Microscopy (SEM)

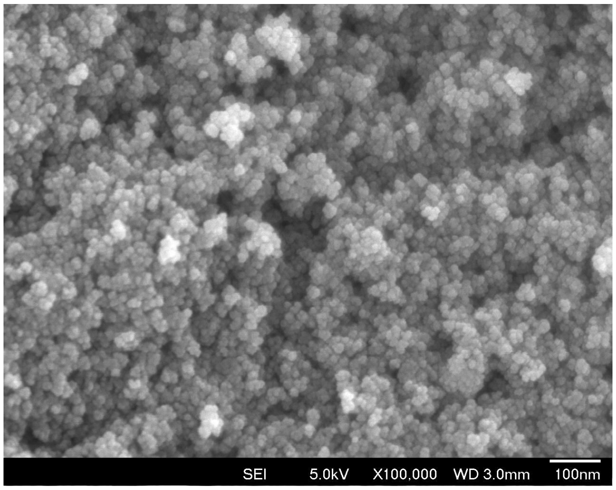

Fig 5: SEM of AgNP Composite (100nm)

Surface morphology of the composite shows the presence of nanoparticles. Hence, there is a large exposed surface area for adsorption of dyes and heavy metal ions.

Adsorption of Dyes and Metal Ions

The adsorption abilities of our composite were tested on 3 dyes, Methylene Blue, Methyl Orange and Brilliant Green using the procedure as described above.

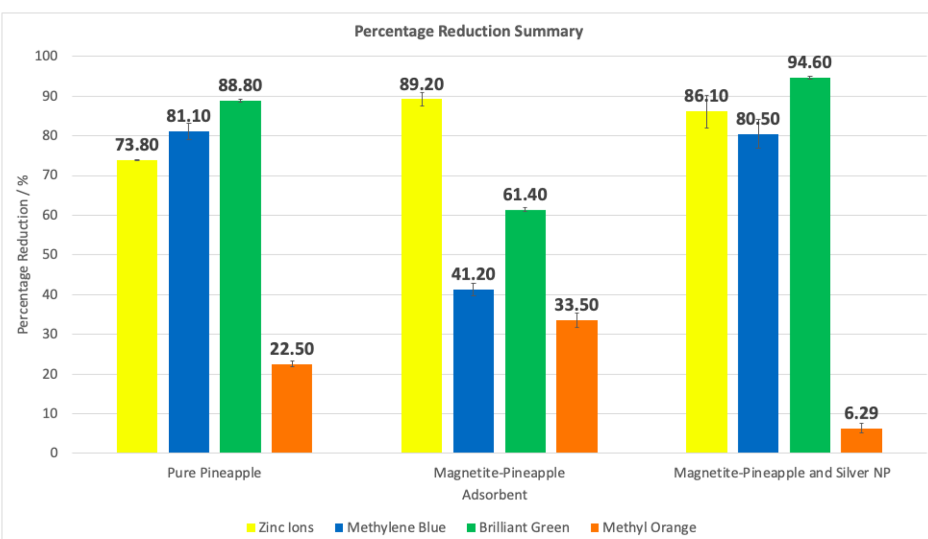

Fig. 9: Summary of Percentage Reduction in Dyes

The percentage adsorption of the dyes by the various adsorbents were then compared and analysed via the

Mann-Whitney U Test to obtain the respective p-values to investigate if there was any significant difference between the adsorption potential of the respective adsorbents. The results are compiled below.

Table 1: Mann-Whitney U Test on Dyes and Zinc (II) Ion

Sample Comparison	**Dye/Metal ion tested**	**p-value**	**Conclusion**
MAgNP composite against pure *Ananas Comosus*	Methylene Blue	0.583 (>0.05)	**No significant difference**
	Brilliant Green	0.006 (<0.05)	**Significant difference**
	Methyl Orange	0.997 (>0.05)	**No significant difference**
	Zinc (II) Ions	0.006 (<0.05)	**Significant difference**
MAgNP composite against Magnetic Ananas Comosus composite	Methylene Blue	0.006 (<0.05)	**Significant difference**
	Brilliant Green	0.006 (<0.05)	**Significant difference**
	Methyl Orange	0.997 (>0.05)	**No significant difference**
	Zinc (II) Ions	0.970 (>0.05)	**No significant difference**

Colony count test

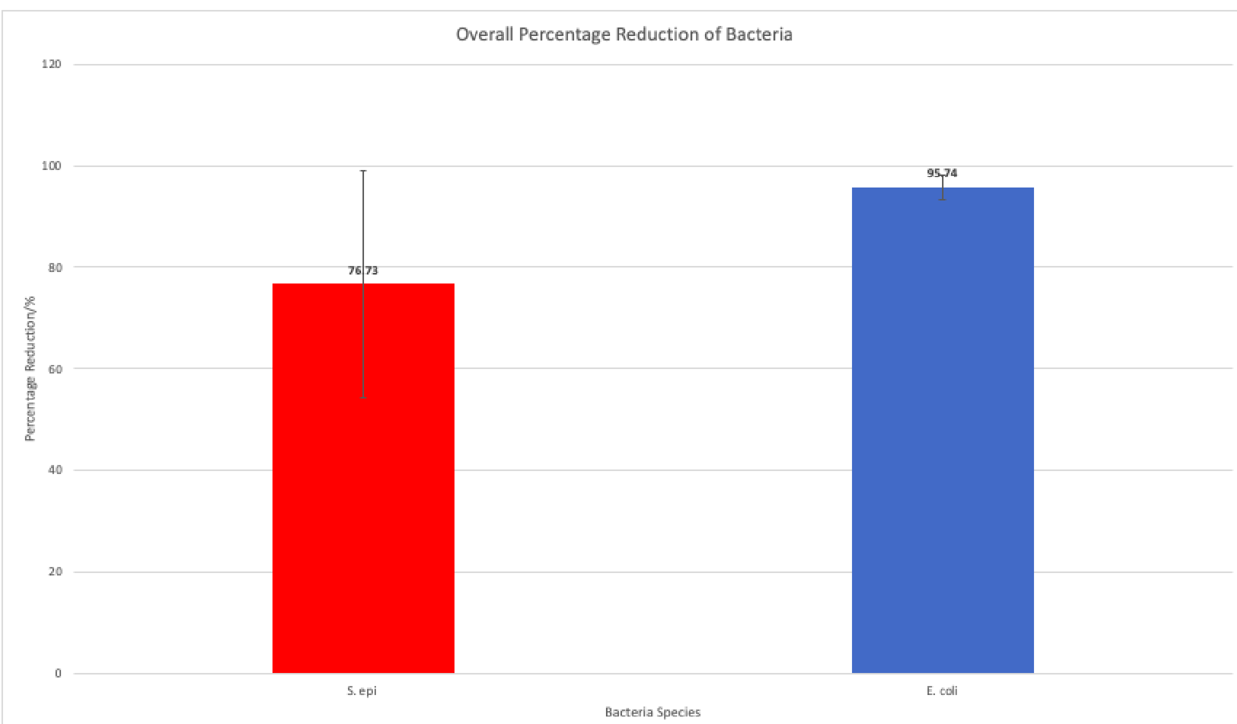

Fig. 10: Summary of Percentage Reduction of Bacteria

The antibacterial effects of our composite was tested on 2 species of bacteria, *Staphylococcus epidermidis a*nd *Escherichia coli*, which are gram-positive and gram-negative bacteria respectively. The overall average percentage reduction of each bacteria was calculated by finding the average of the individual percentage reductions for each of the 5 replicates that we tested. The graph above shows the overall average percentage reduction for the samples containing the Pineapple-MAgNP composite. The samples containing pure *Ananas Comosus* as well as the Pineapple-Magnetite composite have been omitted as they did not show any antibacterial properties at all.

Discussion

For the adsorption tests on dyes, our composite showed the best results when adsorbing cationic dyes such as Brilliant Green (94.6%) followed by Methylene Blue (80.50%) as compared to anionic dyes such as Methyl Orange (6.29%) (see Fig. 8). Our composite showed significant differences against pure *Ananas Comosus* as well as Pineapple-Magnetite against the cationic Brilliant Green dye (p-values both 0.006), the adsorption of which was the best performing. Against the anionic Methyl Orange dye, the composite performed poorly against the other control setups (p-values both 0.997), and lastly the composite showed significant difference in adsorption of Methylene Blue (p-value 0.006) against pure *Ananas Comosus* while it did not against the Pineapple-Magnetite composite (p-value 0.583), although the difference was shown to be very little.

Our composite showed an affinity towards cationic dyes as compared to anionic dyes. We hypothesize that this is due to the major compositions of pineapple peel being cellulose (70–80%), lignin (5–12%) and hemicelluloses. In an aqueous solution, cellulose and lignin in the pineapple release H^+ ions which result in the formation of a surface area with a negative net charge and since cationic dyes have a positive net charge, opposite charges between the cationic dyes and the pineapple will result in electrostatic attraction, and adsorption of the dye as a result. Anionic dyes on the other hand, have a negative net charge, thus electrostatic attraction is much weaker, resulting in poor adsorption rates. (Kamaru, Malek, Sani, 2015)

For the adsorption test on heavy metal ions, our composite showed potent adsorption abilities when adsorbing Zinc (II) ions (86.1%), as it showed significant difference in adsorption abilities against the Pineapple-Magnetite composite (p-value 0.006) but not against pure *Ananas Comosus* (p-value 0.970). We hypothesize is that the potent adsorption abilities of Zinc (II) ions are due to the formation of dative bonds with lone pairs from the oxygen atoms present in cellulose and Bromelain compounds present in the pineapple.

5. Conclusion and Recommendations for Future Work

Conclusion

Magnetite and silver nanoparticles were successfully synthesised to form a composite with pineapple. The composite had magnetic properties and showed effective adsorption abilities towards cationic dyes such as Brilliant Green and Methylene Blue and heavy metal ions such as Zinc (II) ions. Furthermore, the composite possessed antibacterial properties and was effective against both gram positive and gram negative bacteria tested.

Recommendations for Future Work

Further work can be done by using isotherms to calculate the maximum adsorption capacity of our composite and its rate of adsorption on various dyes and heavy metal ions over time. A greener method may also be identified to synthesise silver nanoparticles on the composite by avoiding the use of chemicals such as $NaBH_4$, where their production methods result in harmful impacts on the environment.

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