Green synthesis of silica nanoparticles and its properties

Jiang Ruiyang(3i2), Huang Xiaotian(3i1)

Group 1-26

Abstract

Silica nanoparticles are recently under great interest as it is being evaluated in its use for many industries and applications. Therefore, this study aims to synthesise silica nanoparticles using sugarcane bagasse, an agricultural waste, in a relatively green method as compared to conventional ways, and test for its antioxidant or antibacterial properties. The result was a successful synthesis of silica nanoparticles which was characterised using Fourier Transform Infrared (FTIR) spectroscopy. Antioxidant property tests were conducted using ABTS assay which resulted in no significant antioxidant properties. Antibacterial property testes were carried out using the well diffusion method using Staphylococcus epidermidis and E. coli. No zone of inhibition was observed, but this does not void the biofilm inhibiting properties observed by other researchers.

1. Introduction

Nanotechnology has been a known field of study since the last century, which produced materials of various types at a nanoscale level. Nanoparticles (NPs) are particulate materials that have a dimension of less than 100 nm at least (Khan *et al*., 2019). Silica nanoparticles have been in great attention as it is currently being evaluated for being used in many industries and applications (Mohd *et al*., 2017). Silica nanoparticles are promising for biological applications owing to their excellent biocompatibility, low toxicity, thermal stability, facile synthetic route, and large-scale synthetic availability. The particle size, crystallinity, porosity, and shape can be precisely manipulated, enabling the silica nanoparticles for various applications. Moreover, numerous available surface modifications of silica nanoparticles permit their control of surface chemistry to achieve drug loading, good dispensability, and site-specific targeting. These properties, if combined and developed appropriately, make silica nanoparticles a platform for biomedical imaging, detecting, therapeutic delivery, monitoring, and ablative therapies.

However, the current methods of synthesizing, such as flame synthesis method, sol-gel method, microemulsion modified method, and Stöber method presents many disadvantages as high operation cost, difficulties in controlling the particle size, morphology, and phase composition as well as the requirement for additional step if surfactant is used in the synthesis procedure (Mohd *et al*., 2017). Ho research carried out by Mohd *et al*. (2017), a greener synthesis method was found.

This synthesis method involves sugarcane bagasse and presents many advantages such as less toxic chemical consumption, reduction in chemical amounts, economical and less involvement of high energy processes, such as calcination (Mohd *et al*., 2017). Indeed, 54 million tonnes of dry sugarcane bagasse are produced every year and the burning of them produces various gases which will contribute to the greenhouse effect (Mohd *et al*., 2017). By

utilizing these wastes for the synthesis of silica nanoparticles, it is extremely environmentally friendly and efficient.

Nanoparticles have a variety of properties and silica nanoparticles are no exception. The biofilm inhibiting property of silica nanoparticles has been studied by AL-Azawi *et al.* (2019) to a successful extent. Indeed, it is important to investigate many possible properties silica nanoparticles can potentially hold. Thus, besides investigating the green synthesis of silica nanoparticles, this study also aims to research silica nanoparticles' possible antibacterial and antioxidant properties.

2. Objectives and hypotheses

Objectives of this study:

1. To synthesize silica nanoparticles from sugarcane bagasse
2. To study the antibacterial properties of silica Np
3. To study the antioxidant properties of silica Np

Hypotheses:

1. Silica nanoparticles can be extracted from sugar cane
2. Silica nanoparticles have antibacterial properties
3. Silica nanoparticles have antioxidant properties

3. Methods and Materials

Materials

For the synthesis of silica nanoparticles: sodium hydroxide, ethanol, nitric acid, hydrochloric acid, sugarcane bagasse
For antibacterial properties study: E. coli culture, staphylococcus epidermidis culture, MG agar, sterilised water
For antioxidant properties study: ABTS assay
Deionised water was used throughout the study

Methods

Synthesis of silica nanoparticles: Sugarcane bagasse was first soaked in distilled water overnight and washed thoroughly with distilled water to remove dirt.

a) b)

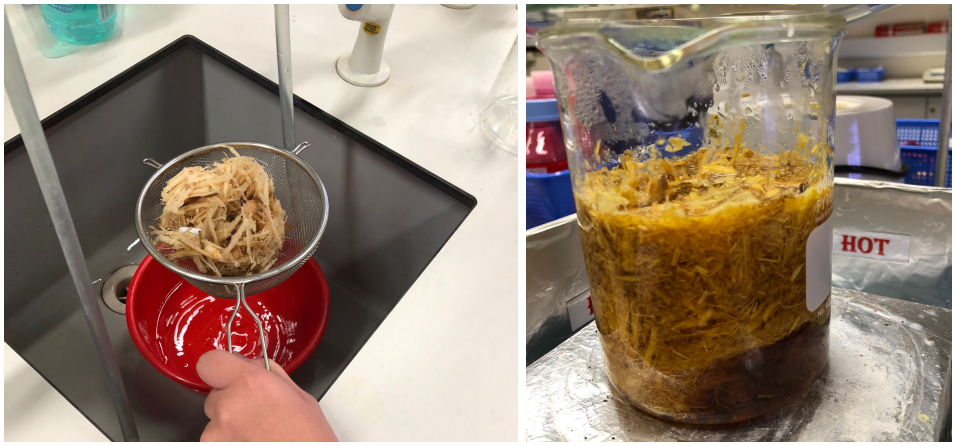

Figure 1. Washing of sugarcane bagasse a) and soaking of sugarcane bagasse in hydrochloric acid b)

The sugarcane bagasse was then dried at 90℃ in the furnace. The dried sugarcane bagasse was then divided equally into 2 beakers and covered with 1M of hydrochloric acid, then put on a hot plate to heat to a temperature of 90℃ and stirred for an hour to bleach out metal ions and other impurities.

a) b)

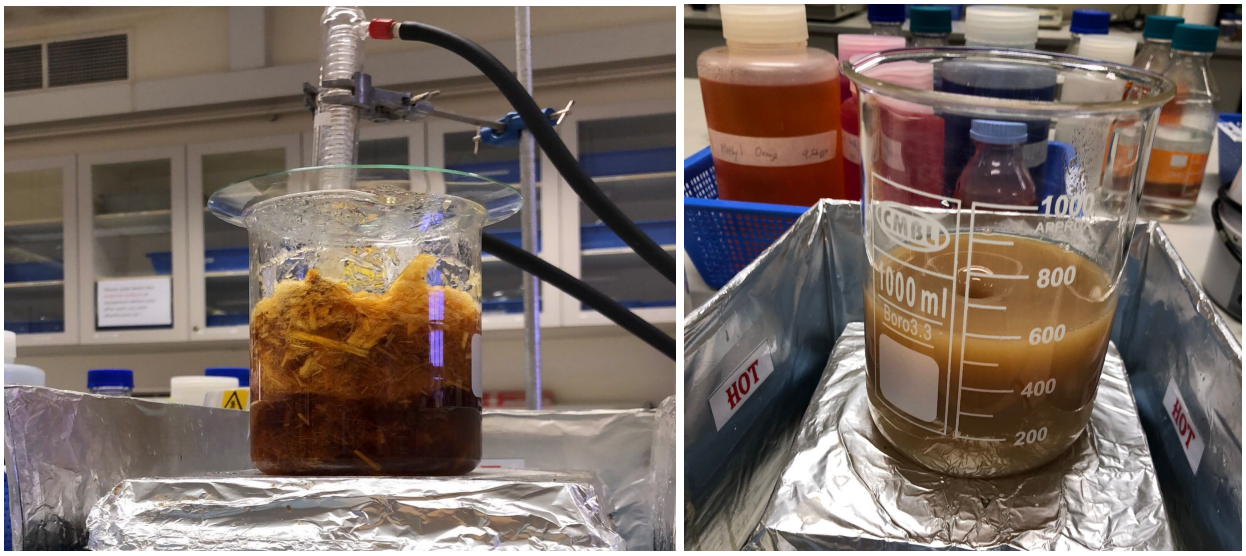

Figure 2. Covering sugarcane bagasse in sodium hydroxide solution a) and sodium silicate solution b).

The sugarcane bagasse was then sent to dry again in the 90℃ furnace. After that, the dried sugarcane bagasse (55.87g) was divided equally into 5 beakers and covered with 1M aqueous sodium hydroxide and then heated and stirred vigorously at a temperature of 90℃ for 1 hour, forming a sodium silicate solution. The mixture was filtered and the filtrate (sodium silicate) was collected

$$SiO_2 + 2NaOH \rightarrow Na_2SiO_3 + H_2O$$

a) b)

Figure 3. Precipitate in the sodium silicate solution a) and silica nanoparticles b)
The sodium silicate solution was then stirred vigorously and nitric acid was added dropwise into the sodium silicate solution until a pH of 8. The resulting suspension was then centrifuged at 4000 rpm for the precipitate formation and the resulting precipitate was washed, dried in a 90℃ furnace to obtain silica nanoparticles.

Test for antioxidant properties: 0.0013g of silica nanoparticles are dissolved in 10ml of deionised water, the resulting solution is taken as a sample. The US-VIS spectrophotometer is set to a wavelength of 734nm. 3ml of ABTS is added to 3µl of the sample. The reading was taken after 3 minutes and repeated 10 times. For the calibration curve, varying concentrations of ascorbic acid from 0.01g/100ml to 0.05g/100ml was used and the best fit line of % inhibition against concentration was plotted. The % inhibition was calculated using the formula:

$$\frac{A_{ini} - A_{final}}{A_{ini}} \times 100\%$$

Test for antibacterial properties: E. Coli and S. Epidermidis were inoculated separately on 2 separate MG agar plates. 3 holes of around 8mm in diameter are punched into the culture with a sterile cork borer. In the first well, 80µLof silica nanoparticles were introduced. In the second well, 80µL of bleach was added for a positive response. In the third well, 80µL of sterilised water was added for a negative response.

Another test where 3 MG agar plate is inoculated with E. coli and 3 MG agar plate is inoculated with Staphylococcus epidermidis. 4 holes of around 8mm in diameter are punched into the culture with a sterile cork borer. In the first well, introduce 60µL of silica nanoparticles and 20µL of 1mM of hydrogen peroxide. In the second well, add 80µL of bleach for a positive response. In the third well, add 80µL of sterilised water for a negative response. In the fourth well, add 80µL of 1mM hydrogen peroxide.

4. Results and Discussion

Synthesis of silica nanoparticles: The synthesis results were a white powder. The final yield was 0.055g. To characterise the silica nanoparticles, FTIR spectroscopy was carried out to determine the possible vibration of function groups present in the silica nanoparticles powder (Mohd *et al.* 2017). The FTIR spectra (fig 4) show essentially similar results with the one

reported by Mohd et al (fig 5)., although with one Si-O-Si bending dip missing beyond the range of our FTIR scanner.
In our results, The peak at 3454.75 cm-1 is due to O-H stretching. The peak at 1640.06 cm-1 was due to the O-H bending. It can be concluded that silica nanoparticles have been successfully synthesised.

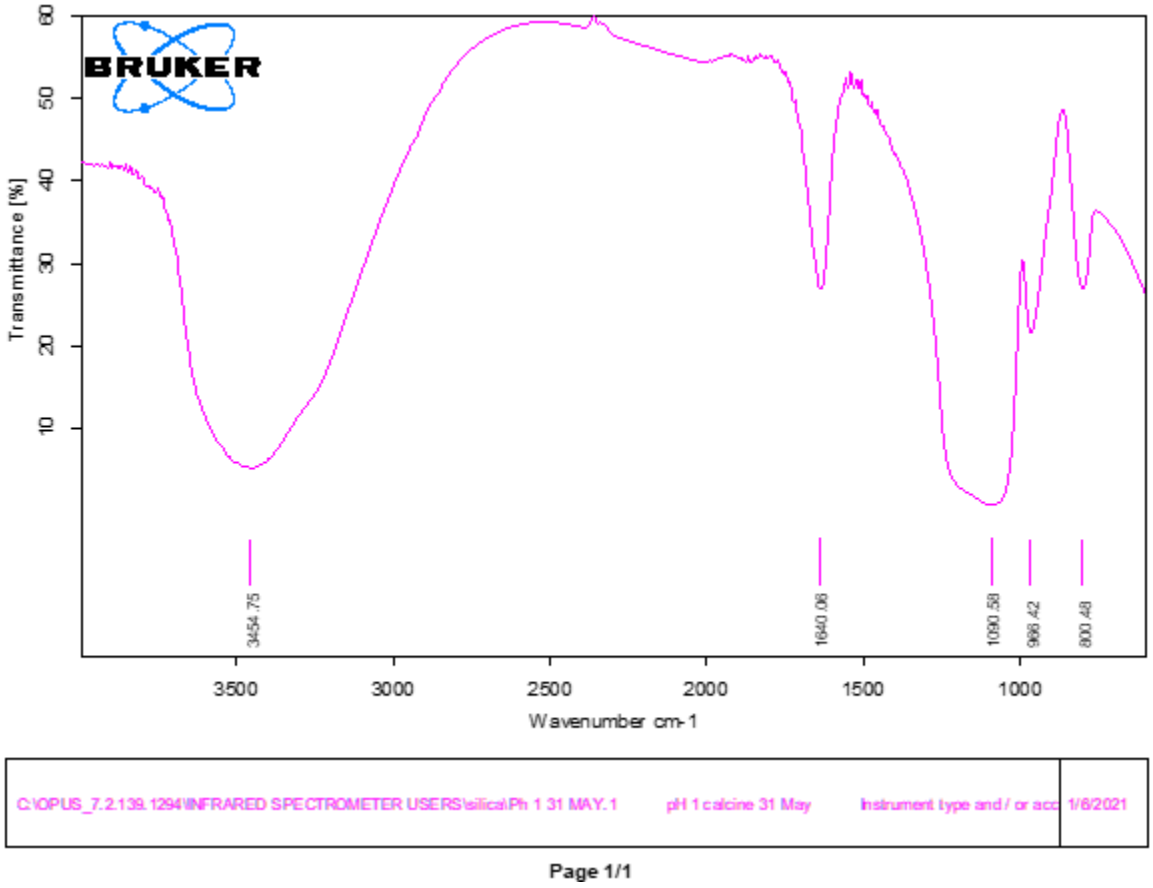

Figure 4. FTIR spectra obtained from our study

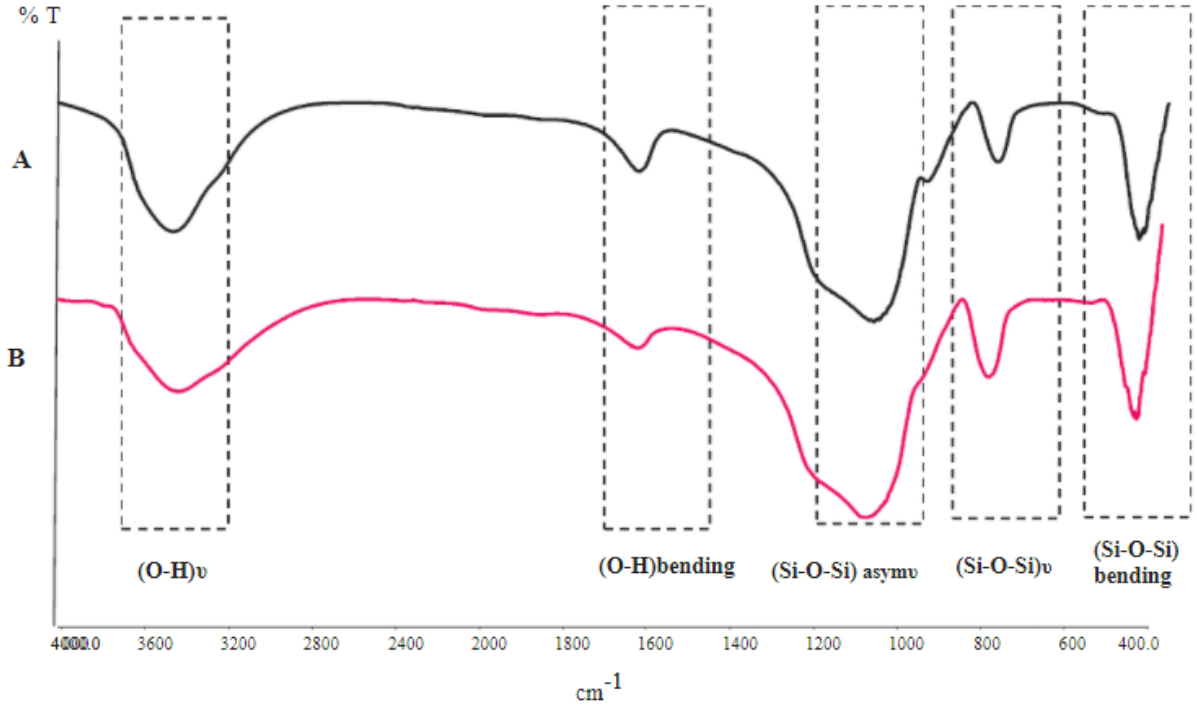

Figure 5. FTIR spectra from Mohd et al.

Test for antioxidant properties: After the test has been carried out 10 times, we picked the 3 best readings of 0.759 Abs, 0.757 Abs and 0.761 Abs, which averaged out to be 0.759 Abs. The result was a straight line with the equation y = 1878+1.2992 with an R^2 value of 0.9997. To see whether the silica nanoparticles demonstrate any significant antioxidant properties, raw % inhibition for the concentration was compared with the ideal % inhibition on the calibration curve. The raw % inhibition calculated was 0.78%, which was a far cry from the expected value of 25.7132% at 0.013g/100ml. Therefore, it is concluded that silica nanoparticles do not demonstrate significant antioxidant properties.

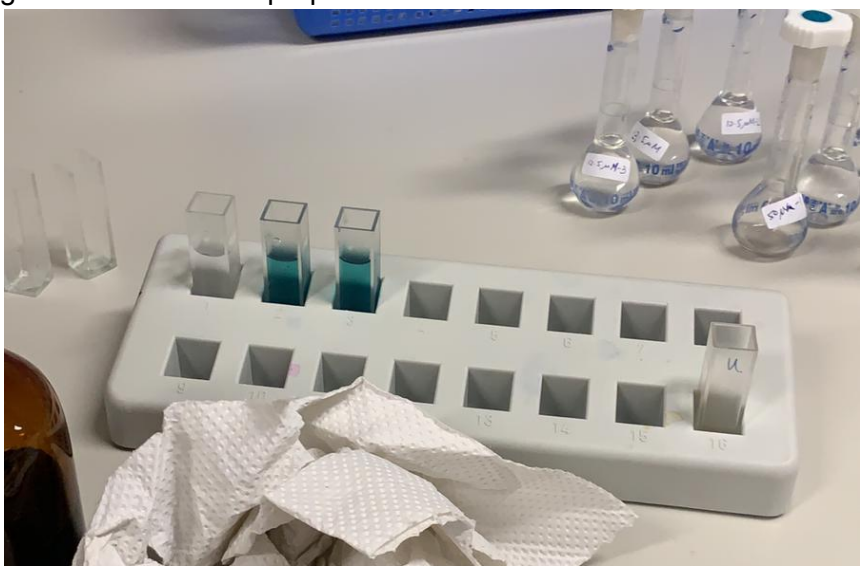

Figure 6. The turquoise solutions are the sample + ABTS assay

Concentration/ g 100ml-1	% inhibition
0.01	20.07874
0.02	38.84514
0.03	57.21785
0.04	77.29659
0.05	94.75066

Figure 7. Table of value for the calibration curve

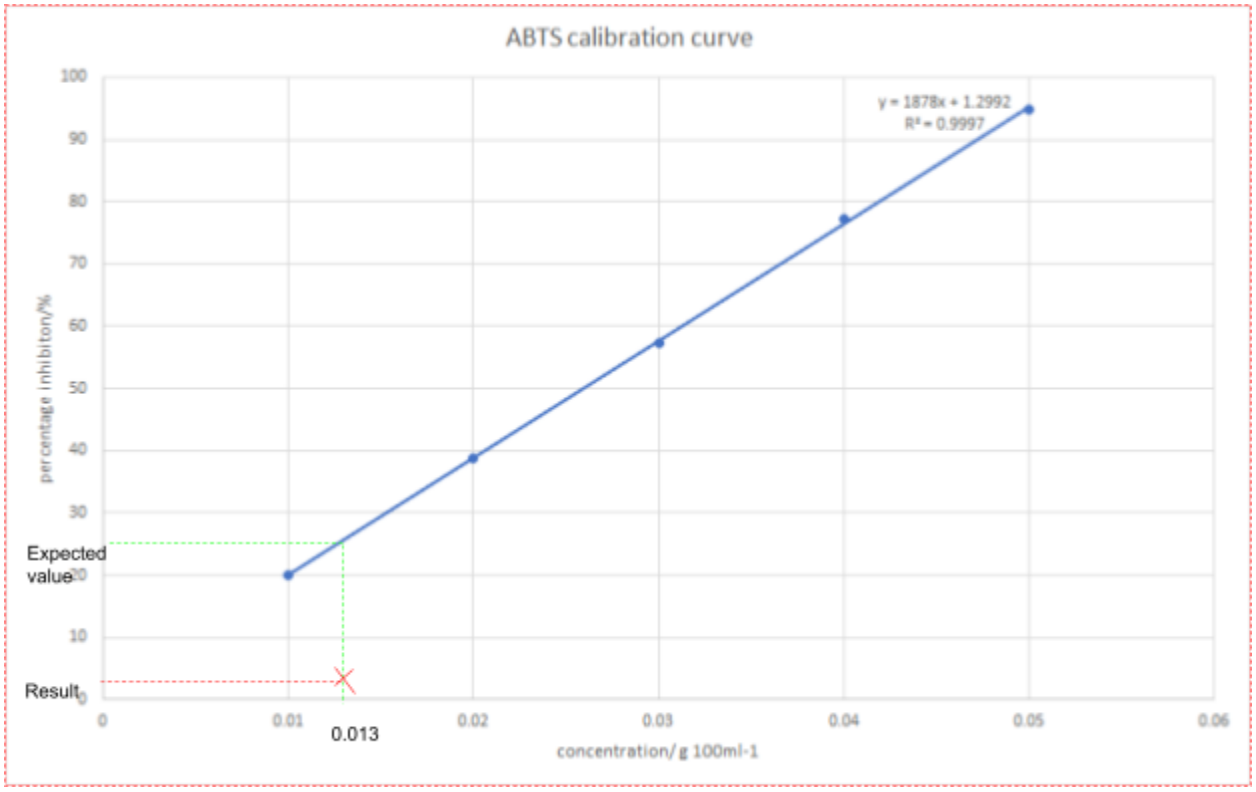

Figure 8. The ABTS calibration curve from ascorbic acid. The green interpolation represents the expected value while the red line represents the value we have gotten

Test for antibacterial properties:

Test 1

a) b)

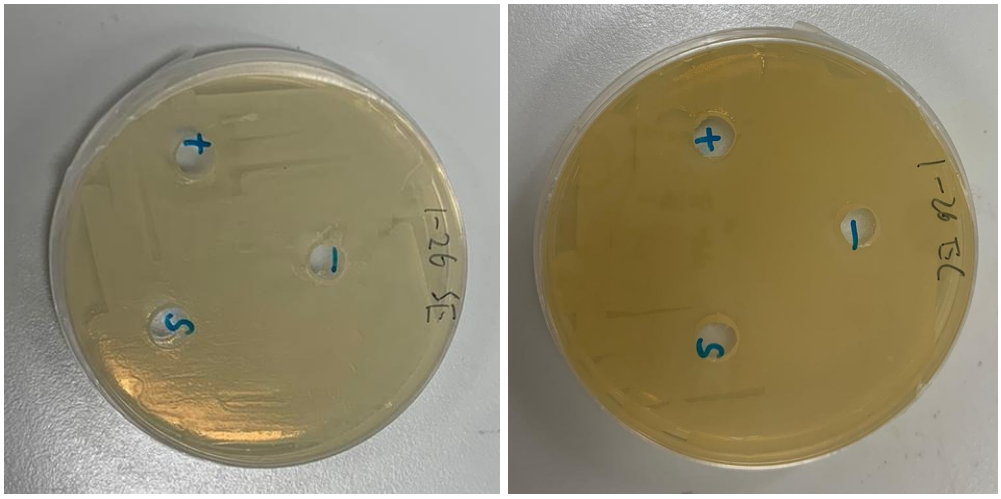

Figure 9. Results of antibacterial test for silica nanoparticles for Staphylococcus epidermidis a) and for E. coli b)

The silica nanoparticles did not show any significant antibacterial properties for both E. coli and Staphylococcus epidermidis. This can be seen in Figure 8, where bleach showed a zone of inhibition while there is no visible zone of inhibition for the silica nanoparticles. However, in a study carried out by Al-Azawi *et al*. (2019), antibacterial properties were discovered.

Test 2

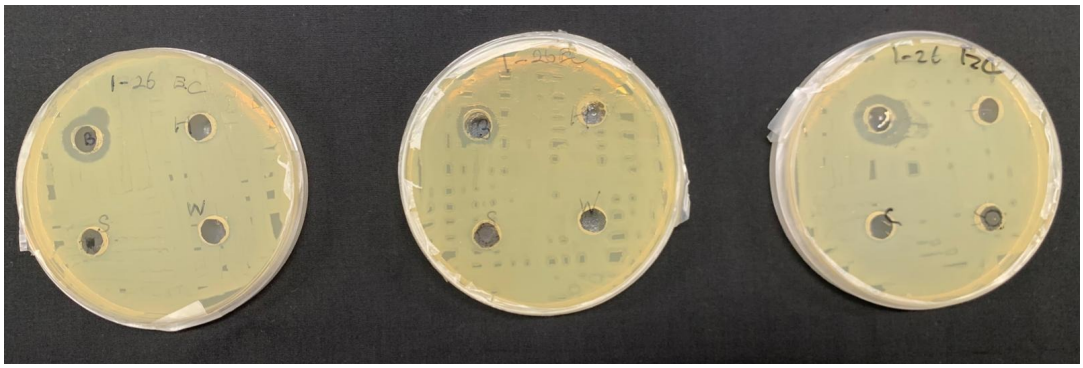

Figure 10. Results of antibacterial test of E. coli when the 60µl of sample is mixed with 20µl of hydrogen peroxide. B represents bleach, H represents hydrogen peroxide, S represents the sample and W represents sterile water.

Figure 11. Results of antibacterial test of Staphylococcus epidermidis when the 60µl of sample is mixed with 20µl of hydrogen peroxide. B represents bleach, H represents hydrogen peroxide, S represents the sample and W represents sterile water.

The silica nanoparticles were mixed with 1mM of hydrogen peroxide to test if its antibacterial properties only show in the presence of hydrogen peroxide. The silica nanoparticles did not show any antibacterial properties. This can be seen in Figure 11 and 10, where there is no observational zone of inhibition around the well which contains the sample whereas there is a significant zone of inhibition. Thus, it can be concluded that silica nanoparticles do not inhibit bacterial growth, even in the presence of hydrogen peroxide. However, the antibacterial properties mainly work in the area of biofilm inhibition, instead of zones of inhibition. In fact, the results are consistent with their findings as the findings from Al-Azawi *et a*l. (2019) also did not capture any zones of inhibition. Therefore, although silica nanoparticles do not produce zones of inhibition on bacterial agar plates, the antibacterial property may lie in the biofilm inhibition which cannot be captured by just looking out for zones of inhibition. Unfortunately, the biofilm inhibition properties could not be properly tested due to COVID-19 and related disruptions.

5. Conclusion and Recommendations for future work

It can be concluded that silica nanoparticles can be synthesized from sugarcane bagasse and it was confirmed by the FTIR analysis of the many vibration peaks present in the spectra. The silica nanoparticles did not display any antioxidant properties through comparisons with ascorbic acid, a known antioxidant material. Silica nanoparticles also did not show any antibacterial properties by themselves, which is confirmed by Al-Azawi *et al.* (2019), nor did it show any antibacterial properties when under the presence of hydrogen peroxide.

In future research, more can be done to characterize the silica nanoparticles, such as using an electron microscope or using an X-Ray Powder Diffraction (XRD) analyzer. Other properties of silica nanoparticles like their effect on biofilm formation, which was claimed by Al-Azawi *et al*. (2019), and UV blocking should also be studied. Batch absorption studies can also be carried out to test the ability of the silica nanoparticles to absorb heavy metals. The sodium silicate solution should also be centrifuged at a higher rpm and for longer periods of time so that more precipitate can be obtained. The precipitate obtained from the sodium silicate solution can also be heated in the furnace at a lower temperature to prevent the charring of the silica nanoparticles.

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