To investigate the properties of Titanium Dioxide Nanoparticles

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Abstract

Titanium Dioxide nanoparticles have been widely studied for their photocatalytic properties (Nasikhudin et al., 2018). This project uses titanium (IV) isopropoxide to synthesize titanium dioxide nanoparticles using phenolic content found in plants as capping agents. After calcination, the titanium dioxide nanoparticles are investigated for their photocatalytic properties in degrading dyes. The titanium dioxide nanoparticles will be added to the dye solution in order to test the effect of concentration, time, pH, UV and presence of antioxidants on the degradation of dyes by titanium dioxide nanoparticles. The antibacterial effect will also be tested by using 2 strains of bacteria, Lactobacillus Casei and Escherichia coli. 2 Methods were used to determine the antibacterial properties of titanium dioxide nanoparticles, namely Agar Well and Disk Diffusion. The ability to act as an adsorbent for heavy metal ions will also be investigated by adding the nanoparticles to a solution of Copper (II) ions, Zinc ions, and Iron (III) ions and the initial and final concentrations will be measured using a colourimeter. The results show that titanium dioxide nanoparticles can be synthesized from Titanium (IV) isopropoxide with an average diameter of 119.7 nm. The titanium dioxide nanoparticles dye degradation and heavy metal ion adsorption abilities increase with increasing concentrations, stirring times, and pH. Presence of UV will also increase the rate of dye degradation and heavy metal ions adsorption. Introduction of Antioxidants such as L-Ascorbic Acid greatly decreased the rate of dye degradation. Antibacterial testing shows that titanium dioxide nanoparticles have an average zone of inhibition of 9.3mm for Lactobacillus Casei and 10.5mm for Escherichia Coli. We therefore conclude that titanium dioxide nanoparticles have good photocatalytic properties and are effective adsorbents for heavy metal ions

1. Introduction

Titanium Dioxide nanoparticles have uses in many fields, and they can be synthesized using a one-step, green method. The method involves using phenolic content from plants to act as a capping agents. Capping agents are bulky molecules that are bonded to the surface of the nanoparticles to prevent aggregation. Capping agents in many other synthesis of such nanoparticles usually involve long-chain fatty acids which cannot be harvested sustainably. The synthesized nanoparticles can degrade dyes and adsorb heavy metal ions that can be found in some water sources. Water sources in certain countries can contain such pollutants, rendering the water not safe for use. Titanium dioxide nanoparticles are able to adsorb such pollutants and clean up the water. Additionally, bacteria contaminated water can also be cleaned up using titanium dioxide nanoparticles as they also possess antibacterial properties. Titanium dioxide nanoparticles are capable of generating hydroxyl radicals when in aqueous solution (Nasikhudin et al., 2018)

This project aims to solve such problems by synthesizing titanium dioxide nanoparticles using a cheap and eco-friendly method.

2. Objectives

The objectives of this project are:

- To find out which plant has the highest total phenolic content
- To synthesize titanium dioxide nanoparticles
- To find out the effectiveness of the antibacterial properties of titanium dioxide nanoparticles
- To find out the effectiveness of titanium dioxide nanoparticles for degradation of dyes
- To find out the effectiveness of the titanium dioxide nanoparticles as an adsorbent of heavy metal ions

3. Hypotheses

The hypothesis of this project are:

- Onion extract has the highest total phenolic content (Reis Giada, 2013)

- Titanium (IV) isopropoxide can be used as a starting material to synthesise titanium dioxide particles

- Titanium dioxide nanoparticles are an effective adsorbent for dyes
  - Titanium dioxide nanoparticles are more effective as adsorbents in alkaline conditions
  - Titanium dioxide nanoparticles are more effective as adsorbents in the presence of UV light

- Titanium dioxide nanoparticles are an effective adsorbent for heavy metal ions
  - Titanium dioxide nanoparticles are more effective as adsorbents in the presence of UV light
  - Titanium dioxide nanoparticles are more effective as adsorbents in alkaline conditions

- Titanium dioxide nanoparticles have antibacterial properties

4. Materials and Methods

4.1 Materials

Ethanol, Sodium Carbonate, Sodium Hydroxide, Hydrochloric Acid, Zinc Nitrate, Iron (III) chloride, Copper (II) Sulfate, L-Ascorbic Acid, and Bromothymol Blue were purchased from GCE chemicals. Titanium(IV) isopropoxide, Methylene Blue, Methyl Orange, Rhodamine B and Folin Ciocalteu's Phenol Reagent (FC reagent) were purchased from Sigma Aldrich. Parsley (Petroselinum crispum), Kale (Brassica oleracea var. Sabellica), Broccoli (Brassica oleracea var. Italica), Strawberries (Fragaria Ananassa) and Onions (Allium Cepa) were obtained from a local market.

4.2 Preparation of Plant extracts

Each individual plant was dried in an oven at around 90ºC and grinded. 2.5g of the powder obtained was heated under reflux in 100 ml of ethanol for 1 hour. The result was filtered and stored.

4.3 Total Phenolic Content

Total phenolic content was determined using the Folin Ciocalteu's assay using gallic acid as the standard (Maurya & Singh, 2010). Concentrations of 20, 40, 60, 80, and 100 ppm of gallic acid were prepared in order to plot the calibration curve of Gallic acid. 0.3 ml of each solution was added to 1.5 ml of a 10 fold dilute FC reagent and 1.2 ml of 7.5% Sodium Carbonate. The solution was mixed and allowed to stand for 30 minutes. The FC reagent was reduced by the gallic acid and produces a blue colour. This colour was measured spectrometrically by reading the absorbance at 765 nm.

4.4 Synthesis of Titanium Dioxide nanoparticles

Titanium Dioxide nanoparticles were synthesized using Titanium Isopropoxide. 6.8 ml of Titanium Isopropoxide was added to a 250 ml round bottom flask, followed by 40 ml of the extract. The proposed reaction mechanism is as follows (Mustapha et al., 2019):

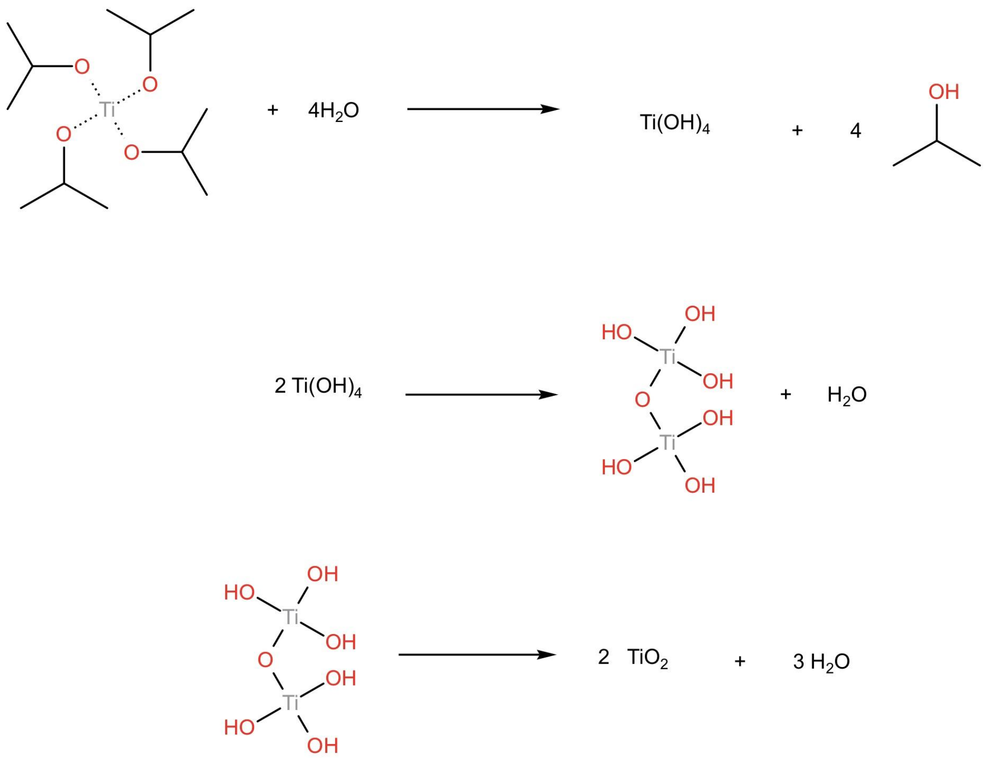


Figure 1: Proposed Mechanism of Reaction

The Titanium Dioxide nanoparticles precipitated out of solution when the Titanium(IV) Isopropoxide was added to the ethanolic plant extract. The reaction mixture was stirred and heated under reflux for 2 to 3 hours. The resulting mixture was centrifuged at 10000 rpm for 15 minutes, and the supernatant was collected. The Titanium Dioxide nanoparticles were then washed with 95% Ethanol and centrifuge again at 10000 rpm for 10 minutes. (Sundrarajan & Gowri, 2011). The result was calcined at 500℃ for 3 hours

4.5 Dye Degradation Properties of Titanium Dioxide nanoparticles

This Stage of the project is split into 5 parts, where the effect of concentration of nanoparticles, time of reaction, pH of reaction and presence of UV will be investigated. All tests were done in triplicate

4.5.1 Effect of Concentration on Dye degradation

50 ml of dye solution was added to 10mg, 20mg, 50mg and 100mg of nanoparticles respectively at the original pH. The suspension of Titanium Dioxide nanoparticles was stirred continuously for 30 minutes. The resulting suspension of nanoparticles was centrifuged at 6500 rpm and its absorbance was measured using a UV-vis spectrophotometer at the respective wavelength.

4.5.2 Effect of Time on Dye degradation

50 ml of dye solution was added to 20mg of nanoparticles respectively at their original pH. The suspension of Titanium Dioxide nanoparticles was stirred continuously for 10, 30, 60 minutes and overnight. The resulting suspension of nanoparticles was centrifuged at 6500 rpm for 5 minutes and its absorbance was measured using a UV-vis spectrophotometer at the respective wavelength

4.5.3 Effect of pH

pH studies were performed on Methylene Blue and Bromothymol blue as they showed significant absorption in the previous 2 parts. The pH values chosen were 2, 9 and 12. 20mg of nanoparticles were added to the dye solution of respective pH and the suspension of Titanium Dioxide nanoparticles was stirred continuously for 10 minutes (due to time constraints.) The resulting suspension of nanoparticles were centrifuged at 6500 rpm for 5 minutes and the supernatant for bromothymol blue was acidified with hydrochloric acid (For pH 9 and 11) and the absorbance was measured using an UV-vis spectrophotometer at the respective wavelength.

4.5.4 Effect of UV

UV studies were performed on Methylene Blue and Bromothymol blue. 20 mg of the titanium dioxide nanoparticles were added to the dye solution and was placed in the dark, and under UV and the suspension of Titanium dioxide nanoparticles was stirred continuously for 10 minutes. The resulting suspension of nanoparticles were centrifuged at 6500 rpm for 5 minutes and the absorbance of the supernatant was measured using an UV-vis spectrophotometer at the respective wavelength.

4.5.5 Effect of Antioxidants

Antioxidants are able to consume reactive oxygen species such as the hydroxyl radical generated by the titanium dioxide nanoparticles. This study was only performed on Methylene Blue dye. A 500 ppm solution of L-Ascorbic acid was prepared by dissolving 25 mg of L-Ascorbic Acid in 50 ml of water. 5 ml of the Ascorbic acid solution was added to 10mg of titanium dioxide nanoparticles, followed by 50 ml of dye solution. A negative control was set-up by replacing the Ascorbic Acid solution with DI water. The mixture was stirred for 10.minutes and 1 ml of Ascorbic Acid was added in intervals of 2 minutes to ensure that the hydroxyl radicals are continuously consumed. The mixture was then centrifuged at 6500 rpm for 5 minutes. the absorbance at 664 nm was read to determine the concentration of the final solution.

4.6 Antibacterial properties

The antibacterial properties of Titanium Dioxide nanoparticles were investigated using Lactobacillus Casei (Gram positive) and Escherichia coli (Gram negative). A broth solution was prepared by dissolving MRS broth and LB broth in Deionized water for Lactobacillus Casei and Escherichia coli respectively. The broth solution was autoclaved at 121℃ for 60 minutes. MRS and LB agar were also prepared by dissolving MRS and LB agar respectively in deionized water and autoclaved. Lactobacillus Casei and Escherichia Coli bacteria were spread on the MRS and LB agar plates respectively. 2 Methods were used to determine the antibacterial properties of titanium dioxide nanoparticles, namely Agar Well and Disk Diffusion. For the Agar Well method, 3 wells were dug in the agar plates and was filled with 0.01% Hydrogen Peroxide, bleach, and Titanium Dioxide nanoparticles suspension in 0.01% Hydrogen Peroxide. The prepared plates were incubated overnight. The zone of inhibition of the nanoparticles were then measured using a ruler.

For the Disk diffusion method, Disks were soaked in sterile water, 10% bleach solution and 2 mg/ml suspension of Titanium Dioxide nanoparticles for 15 minutes. The soaked disks were placed on the agar plate and incubated overnight. The zone of inhibition of the nanoparticles was then measured using a ruler.

4.7 Adsorption of heavy metal ions

This stage of the project aims to investigate the effectiveness of Titanium Dioxide nanoparticles as adsorbents for Heavy Metal Ions. Heavy Metal Ions that were investigated include Zinc ion, Copper (II) ion and Iron (III) ion. The effects of Time and UV were investigated. 50 ppm solutions of Iron (III) chloride, Copper (II) Sulfate and Zinc Nitrate were prepared as an stock solution. A concentration of 20 mg of nanoparticles for 50 ml of solution was used throughout the experiment. All tests were done in triplicate.

4.7.1 Effect of Time

20 mg of nanoparticles were added to 50 ml of each solution and was stirred continuously for 10 minutes, 30 minutes, 60 minutes and overnight. The suspension of nanoparticles was then centrifuged at 6500 rpm for 5 minutes. The concentration of heavy metal ions were then analyzed using a colorimeter.

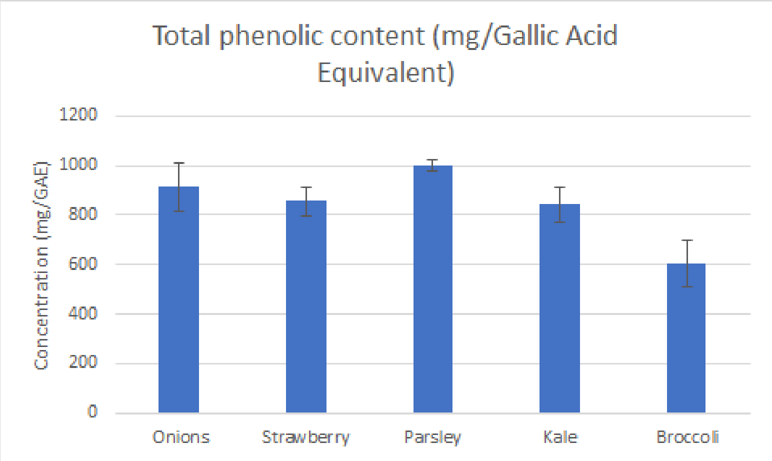
4.7.2 Effect of UV

20 mg of nanoparticles were added to 50 ml of each solution and was stirred continuously for 10 minutes. The experiments were repeated in the absence of light and in the presence of UV light. The suspension of nanoparticles was then centrifuged at 6500 rpm for 5 minutes. The concentration of heavy metal ions were then analyzed using a colorimeter.

5 Results and discussion

5.1 Total phenolic content

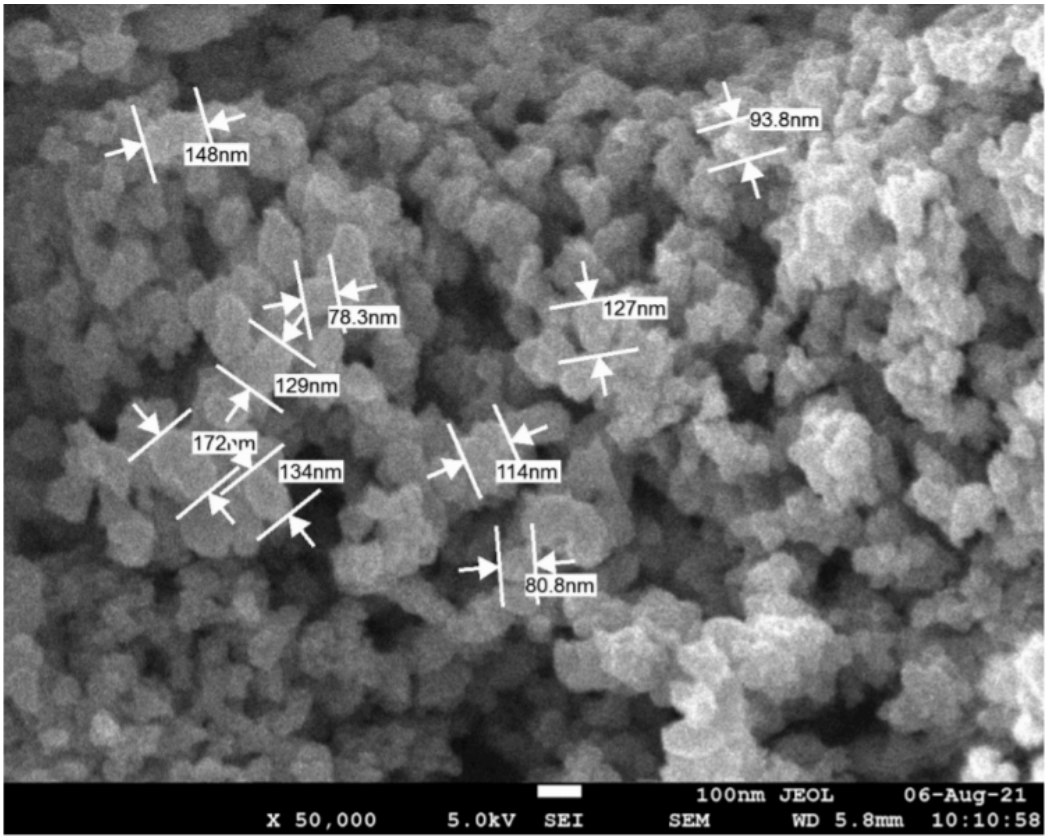
The concentration of the total phenolic content was expressed as milligrams of gallic acid equivalent per gram (mg GAE g^{-1}). The sample was diluted 10x in order to ensure that the total phenolic content fitted the calibration curve of Gallic acid. The following graph represents the results:



Therefore, parsley leaf extract was chosen to synthesize the Titanium dioxide nanoparticles.

5.2 Characterization of Titanium dioxide nanoparticle

5.2.1 SEM images



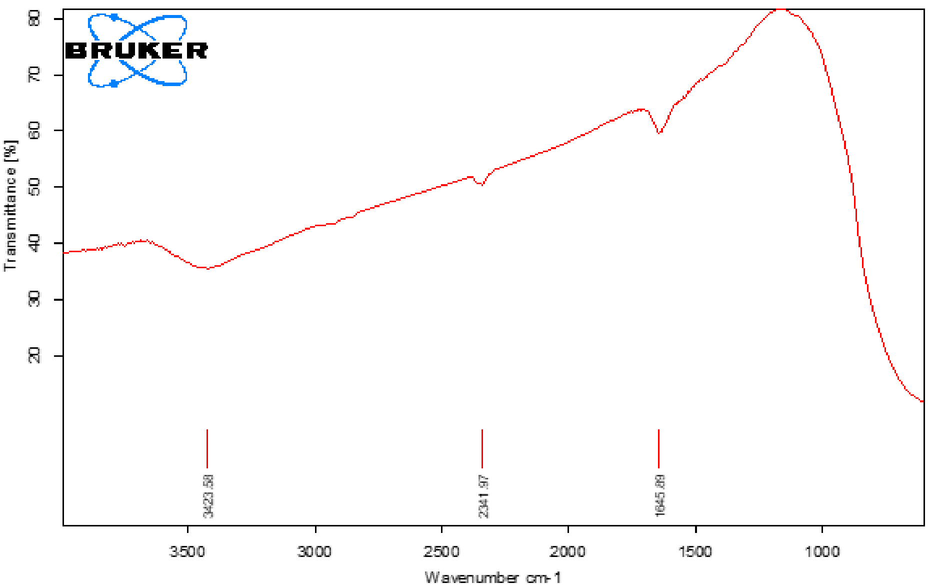
(Figure 2: SEM image of Titanium dioxide nanoparticles)

The SEM images of the titanium dioxide nanoparticles show that they have an average diameter of 119.7 nm. (As Shown in Figure 3) This shows that nanoparticles have been synthesized. The nanoparticles synthesized also have an overall circular shape.

5.2.2 UV-vis spectroscopy

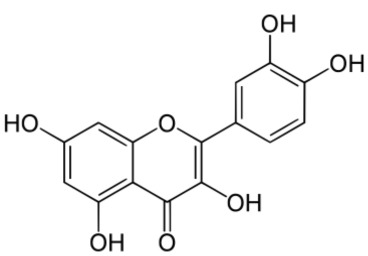
The UV spectra of Titanium Dioxide nanoparticles exhibited a peak at 292 nm. The peak was confirmed to be due to the nanoparticles due to the absence of such a peak in the plant extract used to synthesize the nanoparticles.

5.2.3 IR spectroscopy



(Figure 3: FTIR Spectra of TiO_2 nanoparticles)

FTIR spectra of the Titanium Dioxide Nanoparticles were obtained. The Sample to KBr mass ratio is 1:200. The FTIR was not repeated due to time constraints. The transmission peak is the same as reported by Priyono et al., 2015. The peak at 3423 cm^{-1} wavenumbers show O-H stretching, and the peak at 1645 cm^{-1} wavenumbers shows symmetric Ti-O stretching (Priyono et al., 2015). With reference to the structure of quercetin as an example, the FTIR spectra shows that Flavonols acted as the capping agent in the synthesis of titanium dioxide nanoparticles likely through carbonyl functionality of the flavonols due to the absence of a strong peak around 1600 wave numbers. The peak at 2341 wavenumbers is likely due to interference from atmospheric Carbon Dioxide which has a peak at 2349 wave numbers.

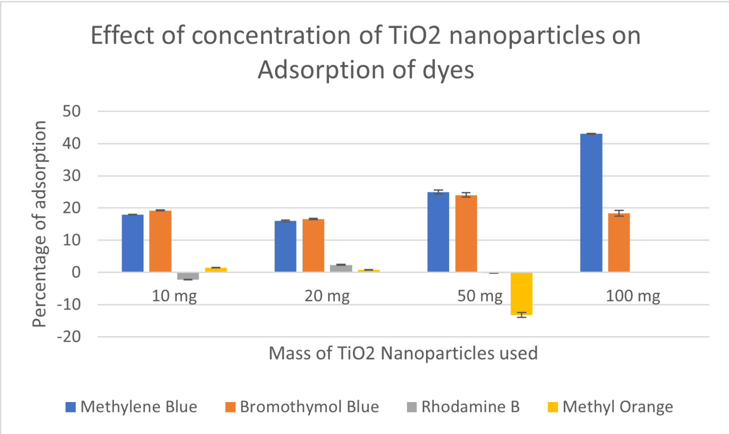


(Figure 4: Structure of quercetin)

5.3 Dye degradation

5.3.1 Effect of Concentration

We observed that generally, as the concentration of Titanium dioxide nanoparticles (TiO2 NPs) increased, the amount of dye degraded increased, which can be observed from the results below.

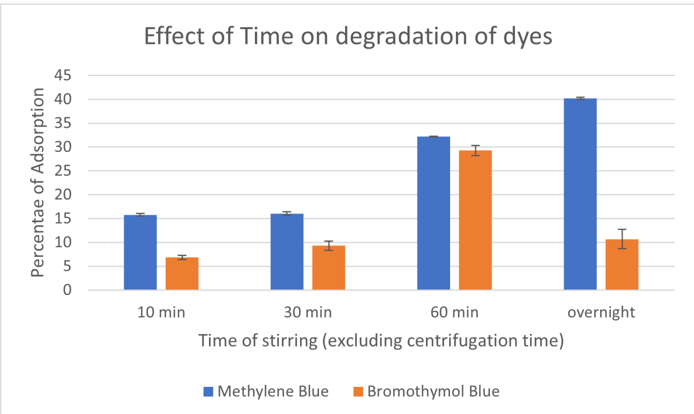


(Figure 5: Effect of concentration on degradation of dyes)

Methyl Orange and Rhodamine B did not have any significant adsorption. These 2 dyes were not included in further studies for this reason and also due to time constraints. Reasons for any negative results are unknown. The set-up was covered to prevent the evaporation of water but there was no significant effect on the adsorption.

5.3.2 Effect of Time

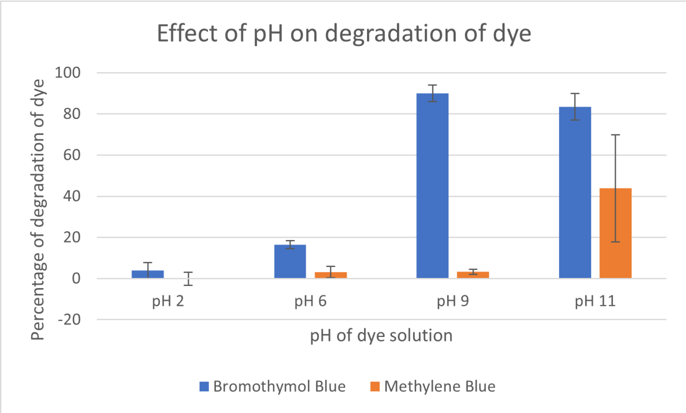
We observed that as the time we left the mixture to stir increased, the amount of dye degraded increased. This can also be observed from the graph below.



The result for the adsorption of Bromothymol Blue done overnight is an erratic result that can be due to loss of volume leading to increased concentrations. However, the experiment cannot be repeated due to time constraints.

5.3.3 Effect of pH

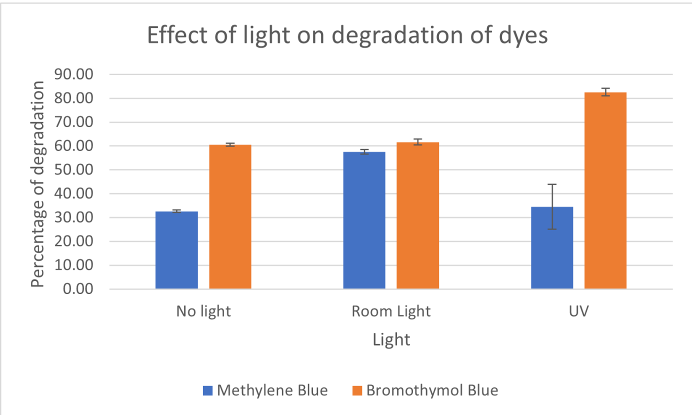
The effect of pH was rather significant in the adsorption of Dyes. Results show that TiO_2 nanoparticles generally degrade more dye at higher pH due to the increase in ease of generating hydroxyl radicals (Nasikhudin et al., 2018), which is apparent in the following graph.



The large error bar is likely due to improper dilutions. The results clearly indicate that higher pH allows for better degradation of dyes

5.3.4 Effect of UV

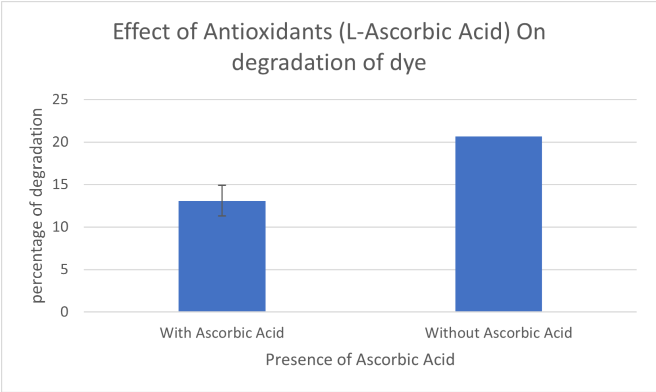
The effect of light was not very significant but still affected the results as shown below:



Light, especially UV light, likely promoted the degradation of dyes by Titanium dioxide nanoparticles and had a significantly higher effect on the degradation of bromothymol blue. Results shows that light is able to promote degradation of dyes, showing that Titanium Dioxide nanoparticles might act as photocatalysts. The dye removal in the absence of light is likely not due to photocatalytic activity, but due to adsorption of the dye by the nanoparticles (Nasikhudin et al., 2018). However, there is an abnormal result for Methylene Blue and the experiment can't be repeated due to time constraints

5.3.5 Effect of antioxidants

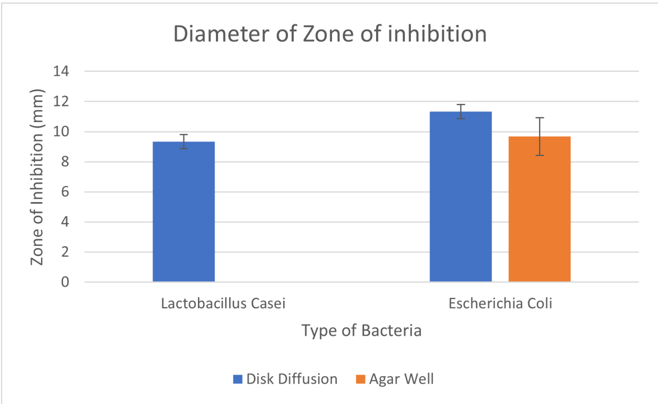
The effect of antioxidants was quite significant, as shown in the following graph:



The presence of L-Ascorbic Acid greatly lowered the percentage of dye degradation as it is able to consume the hydroxyl radicals generated by the titanium dioxide nanoparticles.

5.4 Antibacterial Properties

Titanium dioxide nanoparticles are effective against both gram positive and gram negative bacteria, which is shown in the graph below:

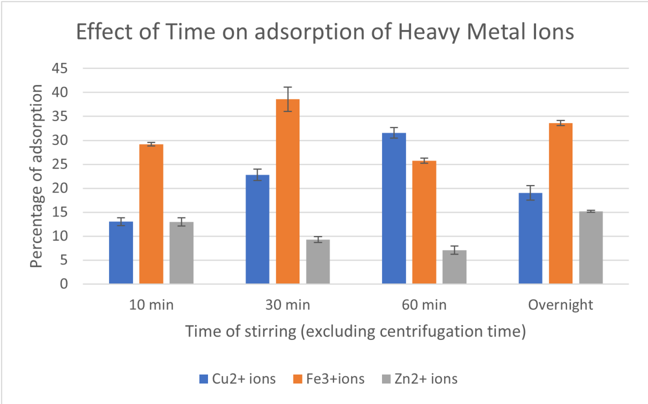


Hydroxyl radicals generated by Titanium Dioxide nanoparticles were likely able to oxidize sulfhydryl groups and double bonds in proteins, lipids, and membrane surfaces (Kobayashi et al., 2014) and cause to cell to be unable to function properly and eventually die out. The result for Agar Well method for Lactobacillus casei was invalid and could not be repeated due to time constraints.

5.5 Heavy Metal ion removal

5.5.1 Effect of Time

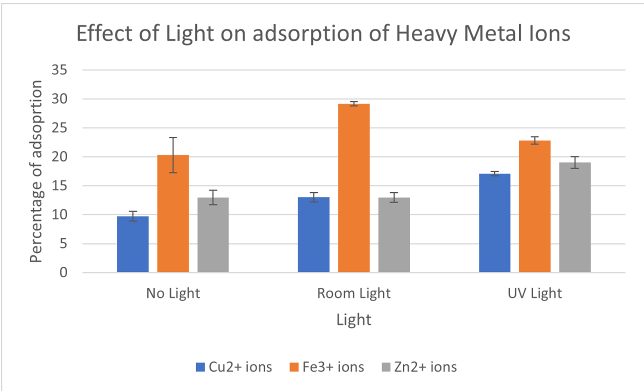
Time of stirring was an important factor in the adsorption of heavy metal ions, as shown in the following graph:



The graph shows that the adsorption of metal ions generally increases as the time of stirring increases. However, there was no significant adsorption of Zinc ions after leaving the stirring on overnight.

5.5.2 Effect of UV

The effect of light was not significant on the adsorption of heavy metal ions but presence of light increases the adsorption of heavy metal ions, as shown in the following graph:



This is likely due to the mechanism of adsorption being independent from radical generation, which is promoted by light.

6 Conclusion

Titanium Dioxide nanoparticles have been synthesized using ethanolic onion extract in a one-step precipitation reaction. Titanium Dioxide Nanoparticles are effective in dye degradation according to the experiment results. It can also be used as a heavy metal ion absorbent, which can be used for water treatment. The results also show that Titanium Dioxide Nanoparticles also have antibacterial properties against both gram positive and gram negative bacteria. The eco-friendly one-step synthesis of titanium dioxide nanoparticles enables it to be used in many fields such as wastewater treatment and in the medical field.

7 Future Work

The mechanism of degradation of dyes can be investigated. Titanium dioxide is a photocatalyst, as it is able to generate hydroxyl radicals with irradiation of UV. The presence of hydroxyl radicals can be investigated by using terephthalic acid. The effect of removing such radicals using Ascorbic acid can also be investigated. The effect of light on the antibacterial properties is a potential area of study. Titanium dioxide nanoparticles can also be attached to easily recoverable calcium alginate beads for more versatile use of the nanoparticles. Finally, the effectiveness of Titanium Dioxide Nanoparticles against cancer cells can also be investigated.

8 References

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