Investigating the effect of *Alcaligenes eutrophus* on the removal of atmospheric sulfur dioxide

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Abstract

In recent years, the amount of sulfur dioxide gas being produced by countries all over the world is increasing due to industrialization, resulting in factories that produce mass amounts of sulfur dioxide gas. Sulfur dioxide gas is extremely harmful to the environment as well as the human body as shown in Nuntavarn, Nitaya *et al.* (2008) and currently, the methods used to remove sulfur dioxide gas from the atmosphere, through the use of calcium compounds, is non-renewable and costly. The bacteria, *Alcaligenes eutrophus*, has shown potential to effectively remove sulfur dioxide gas as during our research to test for the effectiveness of *Alcaligenes eutrophus* on the removal of sulfur dioxide gas, the bacteria showed clear signs that it was able to remove sulfur dioxide gas produced as there was a large gap in the lower absorbance values from the test as compared with our calibration graph. Since the potassium permanganate used to determine the amount of sulfur dioxide gas present as it is reduced by sulfur dioxide gas had a smaller difference between absorbance of unreduced potassium permanganate and potassium permanganate after our test than when the bacteria was absent, it shows that less potassium permanganate was reduced by sulfur dioxide gas when the bacteria was present, suggesting that the bacteria can remove sulfur dioxide gas.

Introduction

Research has revealed that the excess risk for non-accidental mortality was 1.3% per 10 microg/m(3) of PM(10), with higher excess risks for cardiovascular and above age 65

mortality of 1.9% and 1.5%, respectively (Nuntavarn *et al.*, 2008). Sulfur dioxide has been known to cause multiple respiratory problems such as asthma admissions in children shown in a study (Sunyer *et al.*, 2007). Sulfur dioxide also contributes to respiratory symptoms in both healthy patients and those with underlying pulmonary disease (Chen *et al.*, 2007). Controlled human exposure studies have demonstrated that experimental exposure causes changes in airway physiology, including increased airways resistance. As such sulfur dioxide has been recognised by as an ambient air pollutant (WHO, 2000).

Acidithiobacillus thiooxidans is a bacteria species that has presented evidence in utilising sulfur-containing compounds for growth as it undergoes anaerobic respiration via sulfur metabolism. The genus *Acidithiobacillus ferrooxidans* has been used industrially in bioleaching of copper ore to obtain copper, and it is shown to biologically oxidise reduced sulfur compounds to sulfuric acid which increases the solubilization of the Copper (II) cation to allow for more copper to be recovered using physico-chemical technologies such as solvent extraction and electroplating as shown in Valdes *et al.* (2009)

In addition *Acidithiobacillus thiooxidans* is also known to be able to fixate carbon dioxide via the Calvin-Benson-Bassham reductive pentose phosphate cycle (Calvin cycle) using energy and reducing power derived from the oxidation of iron or sulfur according to Valdes *et al.* (2009). *A.eutrophus* is thus able to utilise carbon dioxide, hydrogen, oxygen to give rise to water and hydroxybutyrate to synthesize polyhydroxybutyrate(PHB)s used in plastic bag production. Thus being able to remove two ambient air pollutants, carbon dioxide and sulfur dioxide at the same time.

The current solution to remove sulfur dioxide produced from industrial processes is via sulfur dioxide scrubbing, where sulfur dioxide in exhaust gases undergo dissolution in water and react with calcium compounds such as calcium carbonate in a neutralization reaction. The sulfur dioxide is transformed to sulphate and calcium sulphate and drained away in wet conditions as shown in Zagala & Abdelaal (2017). The use of calcium

compounds however, is not regenerative and thus costly and also uses significant amounts of resources.

It is believed that *A.eutrophus* is able to use atmospheric oxygen to reduce sulfate into sulfur which is then used for the bacteria's growth and maintenance as sulfur pathways are revealed to be present in a study conducted by Nicola, Günter and Dietrich (1997). Since sulfur dioxide is a sulfur compound, we believe that it might be able to reduce sulfur dioxide into sulfur by removing oxygen atoms and will then be able to utilise the elementary sulfur for its growth.

Objectives

The objective of this experiment was to investigate the effect of *A. eutrophus* on removing atmospheric sulfur dioxide.

Hypotheses

Our hypothesis was that we believed the bacteria, *A. eutrophus* would be able to effectively remove atmospheric sulfur dioxide and that the efficiency of sulfur dioxide removal increases with the increasing concentration of the gas.

Materials and Methods

Growth of *A. eutrophus*

A. eutrophus was inoculated into 10 ml sterile LB broth (BD Difco) and incubated in the shaking incubator at 35 degree Celsius for 48 hours. The concentration of bacteria was determined by measuring the absorbance at 600 nm using a UV-VIS spectrophotometer (Shimadzu, UV 1700). A standard curve was obtained by measuring bacterial culture with 1x to 100000x dilution factors.

Method for production of sulfur dioxide gas

100μl of 1.00 mol dm^{-3} hydrochloric acid was added to 0.12405g sodium thiosulfate in a 100ml round bottom flask. The flask was then placed on a mantle boiler and heated at 450ºC. The round bottom flask was connected to the airtight container via a rubber delivery tube. The container was sealed with a rubber stopper and parafilm at the exit point (refer to Fig. 1).

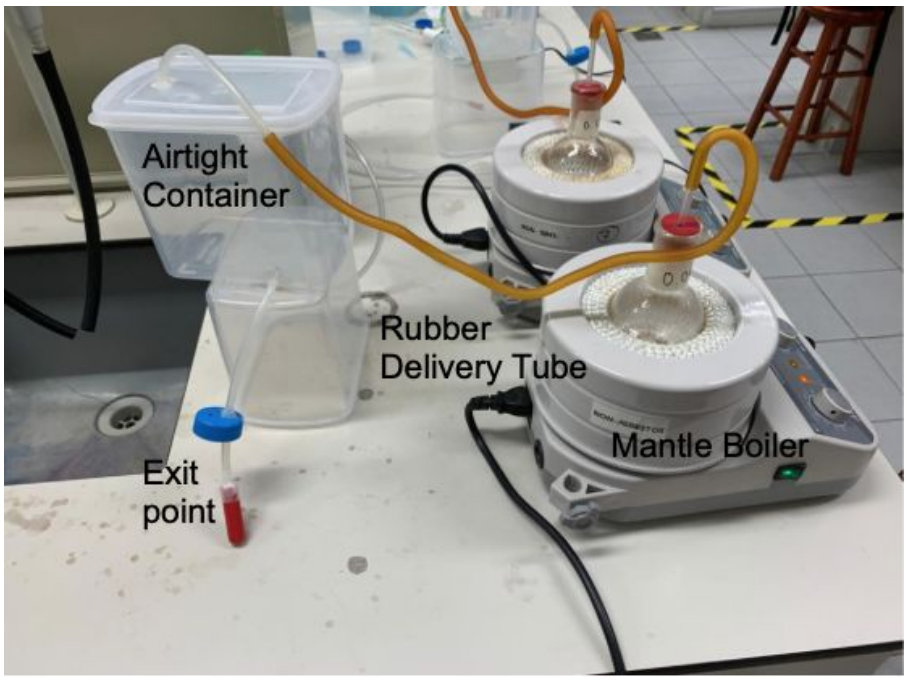

Fig. 1: Setup of sulfur dioxide production and container where petri-dish containing bacteria is placed in.

Description in detail of tests:

Plotting of calibration graph of amount of sulfur dioxide gas

The same set-up used to produce sulfur dioxide gas was used to plot the calibration graph. After 10 minutes of producing sulfur dioxide gas, the tube connected to the round-bottom flask was quickly connected to the air pump. The stopper at the exit point was removed and was placed inside a centrifuge tube containing 25cm^3 acidified potassium permanganate, The air pump was then turned on and the sulfur dioxide gas produced was bubbled into a centrifuge tube for 2 minutes. Solution in the centrifuge tube was then transferred to brown-glass bottles to prevent photodecomposition.

Absorbance of the solution was measured by a UV-vis spectrophotometer at 530nm (with water as the blank). Process was then repeated for 5 other different masses of sodium thiosulfate (0.0155g, 0.0248g, 0.0310g, 0.0414g, 0.0620g)

Testing the effectiveness of bacteria on the removal of excess sulfur dioxide

10ml of *A. eutrophus* in LB broth was pipetted into a petri dish with its cover removed and placed into the airtight container. The lid was then placed on sealing the setup and 0.1241g of sodium thiosulfate was used to produce sulfur dioxide gas via the gas production setup. The entire setup was left for 6 hours. 25ml of acidified potassium permanganate solution (48g/litre) was then pipetted into a 50ml centrifuge tube and connected to the exit point of the setup. An air pump (Hailea ACO-9905 400 litres/hour) was used to bubble the air in the setup through the potassium permanganate solution for 2 min. For the blank, water was used instead of potassium permanganate solution. The absorbance of the test permanganate were then measured against the blanks.

Risk and Safety

All bacteria used are biosafety level 1 microorganisms which may cause opportunistic infections. To avoid exposure to bacteria and corrosive chemicals, gloves and lab coats will be worn. Work involving bacteria is to be done in the biological safety cabinet. Bacterial cultures will be autoclaved at 15 psi for 15 min in biohazard bags before disposal.

Results

Calibration graph

To investigate the effect of sulphur dioxide on difference between absorbance of unreduced potassium manganate (VII) and potassium manganate (VII) after sulphur dioxide has been bubbled through, the sulfur dioxide gas produced using the setup shown in Fig 1. Was bubbled through 25ml of potassium permanganate. A change in colour of the potassium permanganate solution from purple to to colourless was looked out for as

confirmation that sulfur dioxide gas was being produced and could reduce the potassium permanganate.

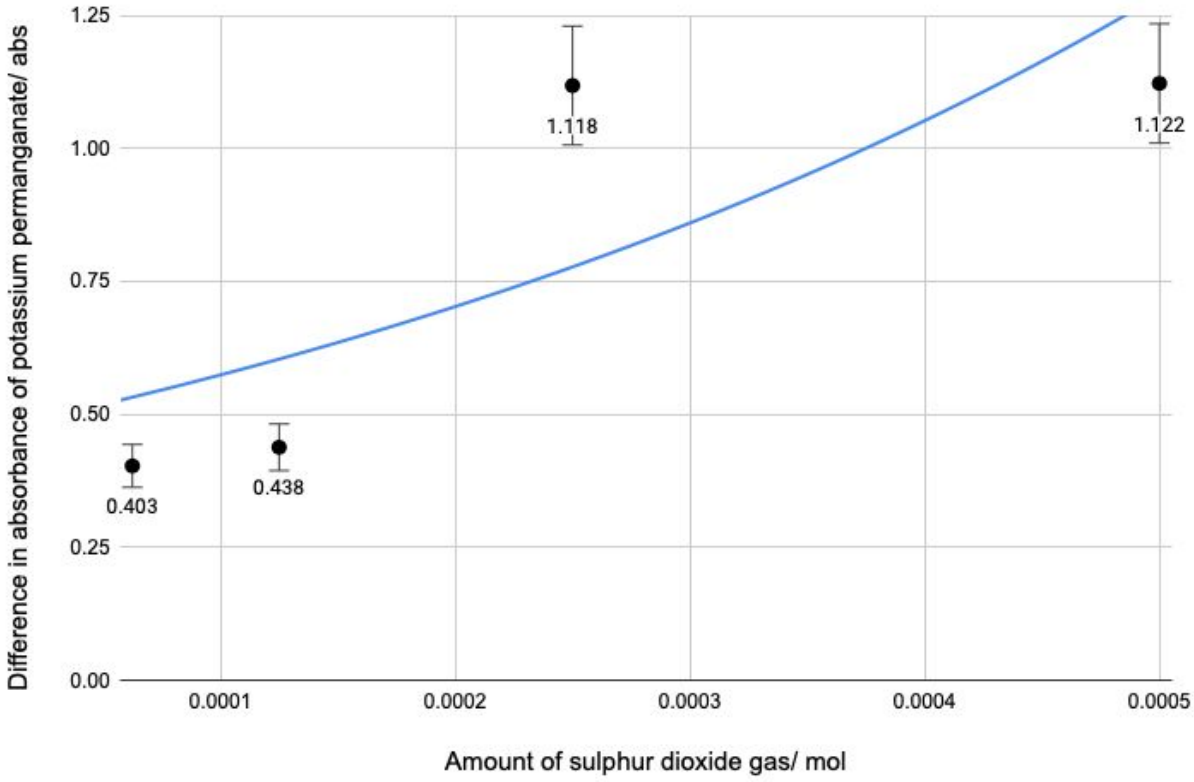

Fig. 2: Graph of effect of sulphur dioxide/ mol on difference between absorbance of unreduced potassium manganate (VII) and potassium manganate (VII) after sulphur dioxide has been bubbled through/ abs. 6 values of sodium dioxide were used (0.0000625, 0.000100, 0.000125, 0.000167, 0.000250, 0.000500) mol but 0.000100 mol and 0.000167 mol were eliminated as they gave outlier data points which could be due to the constant photodecomposition of potassium permanganate, however due to physical constraints this systematic error could not be eliminated.

As shown in Fig. 2, there is a general linear increasing trend in absorbance difference from the unreduced potassium permanganate, hence showing a relationship that as the amount of sulfur dioxide increases, the difference in absorbance of potassium permanganate increases as well.

Colour change of potassium permanganate solution due to reduction by sulfur dioxide.

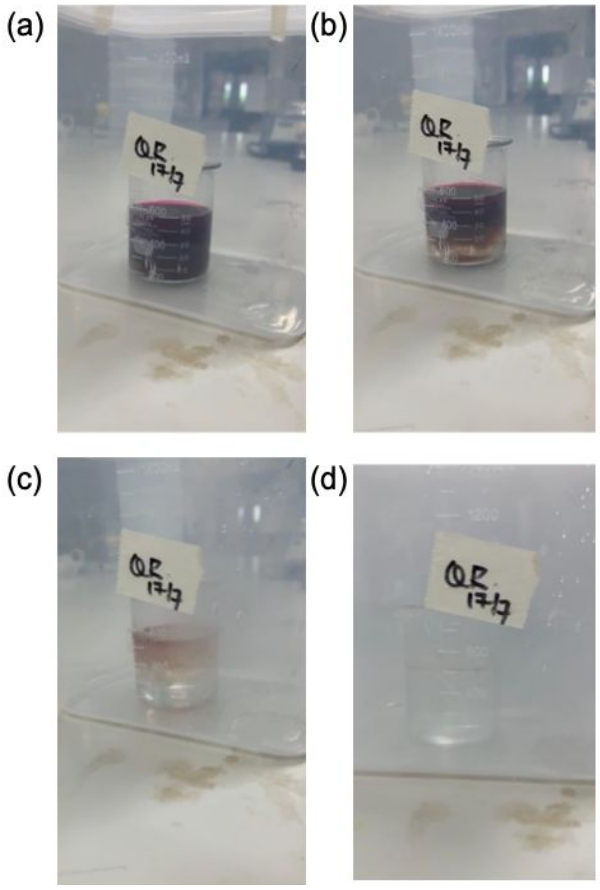

Fig 3: Colour Change of 50ml of potassium permanganate solution by sulfur dioxide. (a) Original potassium permanganate solution. (b) Potassium permanganate solution after 43 seconds. (c) Potassium permanganate solution after 69 seconds. (d) Potassium permanganate solution after 141 seconds.

As can be seen, potassium permanent solution turned from purple to colourless in the presence of sulfur dioxide. As potassium permanganate turns colourless when it's permanganate ion is reduced to the Mn^{+2} ion, this proves that potassium permanganate is reduced by sulfur dioxide produced by the method listed above.

Effect of *Alcaligenes eutrophus* on the removal of atmospheric sulfur dioxide

To investigate the effect of *Alcaligenes eutrophus* on the removal of atmospheric sulfur

dioxide, sulfur dioxide gas in the setup that was exposed to the bacteria broth was pumped out of the container with an air pump and bubbled through 25ml of potassium permanganate. Based on the calibration graph, a smaller difference between absorbance of unreduced potassium permanganate and potassium permanganate after gas in the container has been bubbled through was looked out for as confirmation that *Alcaligenes eutrophus* was effective on the removal of atmospheric sulfur dioxide.

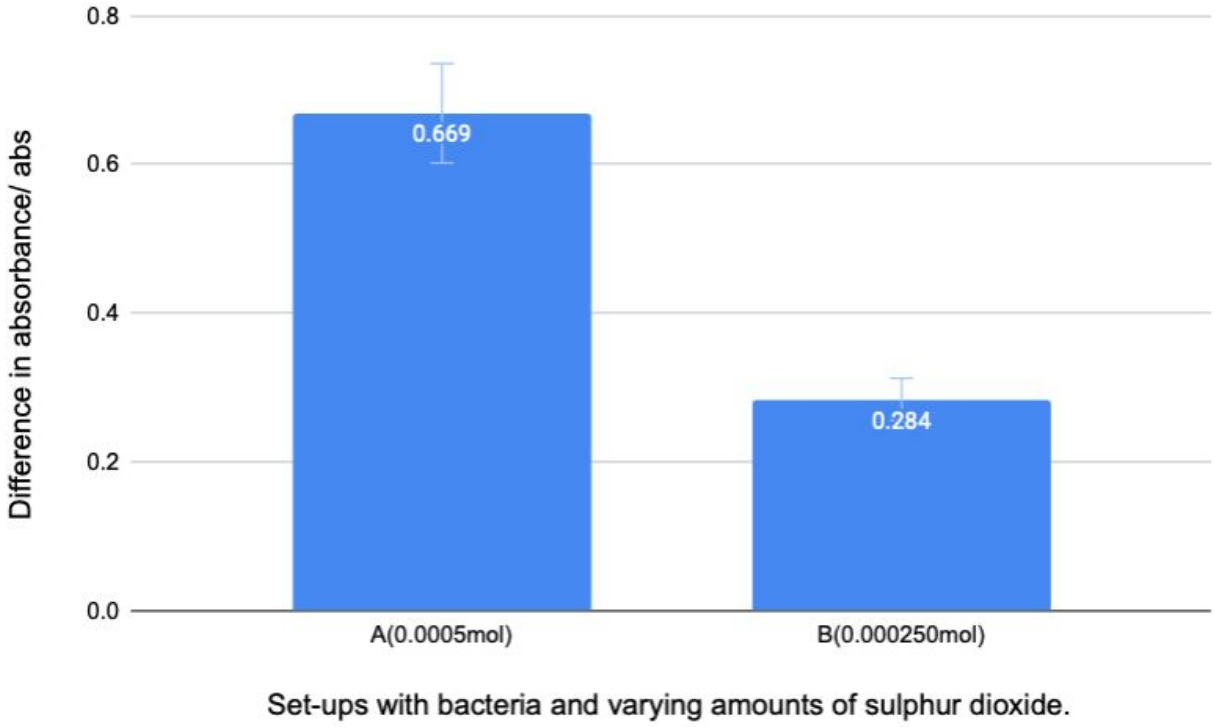

Fig. 4: Bar graphs showing difference in absorbance between unreduced potassium manganate (VII) and potassium manganate (VII) after test. Set ups A and B have 0.000500mol and 0.000250mol of sulfur dioxide respectively. 3 replicates were done for 0.000500mol of sulfur dioxide and 1 test was done for 0.000250mol of sulfur dioxide. Due to the size of the results and number of tests conducted there isn't enough data to perform a statistical test thus no *p*-value was generated.

As can be seen in A and B , there is a smaller difference of 0.699 and 0.284 in the absorbance of the test and original potassium permanganate for setup A and setup B respectively when compared to the expected values from the calibration graph, allowing us to conclude that the bacteria was able to remove sulfur dioxide gas.

Discussion

Previous research on *Alcaligenes eutrophus* revealed that it is able to remove sulfur for metabolism but did not specify sulfur dioxide gas (Peitzsch et al., 1997). Thus, our project has shown that *Alcaligenes eutrophus* has potential to remove atmospheric sulfur dioxide which does not have any prior experimental data that we know of. However, as our test results exhibit a constant change, we cannot be conclusive that *Alcaligenes eutrophus* is able to remove sulfur dioxide. This could be due to the fact that the potassium permanganate decomposes in light and this could be seen even when the potassium permanganate was in the spectrophotometer as the absorbance values constantly shifted down and as a result, this has affected our results obtained. This is further supported by an article by (Rideal and Norrish. 1923) which states that "while investigating the effect of light on the electrode potentials of solutions of various electrolytes a very marked and rapid change was observed in the case of potassium permanganate." While this problem might be able to be minimised by taking multiple readings and taking the average of those readings, the COVID-19 outbreak has resulted in a lack of time to perform multiple experiments. Furthermore, since this was the first time such an experiment had to be done within a school facility, a lot of time was spent planning and customising a set-up to contain the sulfur dioxide gas it was toxic. Hence, the already limited time was spent on trying to design a set-up suitable for our experiment leaving us with little time to conduct the actual experiment. Therefore originally proposed tests on investigating the effectiveness of *Alcaligenes eutrophus* in reducing atmospheric sulfur dioxide when used in a biofilm, investigating the reusability of *Alcaligenes eutrophus* on removing sulfur dioxide were cut out. However, even though we have few results, they appear to indicate that *Alcaligenes eutrophus* is able to remove atmospheric sulfur dioxide.

Conclusion

Our test conducted to determine whether *Alcaligenes eutrophus* is able to remove atmospheric sulfur dioxide revealed that it is likely able to remove sulfur dioxide from the atmosphere effectively.

However the calibration graph obtained for identifying the amount of sulphur dioxide based on absorbance of potassium permanganate may not be accurate as there are only 4 data points due to the outliers removed. We believe that given the large gap in the lower absorbance values from the test as compared with the calibration graph, it is still likely that *Alcaligenes eutrophus* is able to remove atmospheric sulfur dioxide.

Our project work can have industrial applications to remove harmful gas emissions such as in factories where the most amount of sulfur dioxide gas is being released if found to be effective in short time periods and having the capability to be used in a biofilm, both requirements for use in an effective filtration system as mentioned in Zagala & Abdelaal (2017) in terms of surface contact and flow volume. It could also potentially help to reduce the amount of sulfur dioxide gas in green spaces such as parks where more people spend their time outdoors by culturing the bacteria in water bodies such as ponds in such areas.

For future work, we intend to complete the originally proposed tests on investigating the effectiveness of *Alcaligenes eutrophus* in reducing atmospheric sulfur dioxide when used in a biofilm, investigating the reusability of *Alcaligenes eutrophus* on removing sulfur dioxide as well as investigate the effectiveness of *Alcaligenes eutrophus* in reducing

sulfur dioxide in short periods of times in order to simulate a situation similar to that of filtration of sulfur dioxide in factories.

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