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Optimizing the Biosynthesis of Selenium Nanoparticles from Sodium Selenite via *Vibrio Natriegens* as a Microbial Factory to Solve Semiconductor Shortages

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ABSTRACT

This research project aims to optimize the biosynthesis of selenium nanoparticles (SeNPs) using *Vibrio natriegens* as a microbial factory. SeNPs have shown great potential in various applications, such as biomedicine, electronics, and environmental remediation. Selenium is an essential trace element that plays a vital role in many biological processes, including antioxidant defense and immune system function. Selenium deficiency has been associated with various health problems, including cancer, cardiovascular disease, and thyroid dysfunction. SeNPs have emerged as a promising candidate for selenium supplementation due to their enhanced bioavailability and antioxidant activity. The Biosynthesis of SeNPs using microorganisms is an eco-friendly and cost-effective method. In this study, *Vibrio natriegens*, a fast-growing marine bacterium, was utilized for the biosynthesis of SeNPs from sodium selenite (Na_2SeO_3). The primary parameter affecting SeNP biosynthesis, the concentration of Na_2SeO_3, was optimized to maximize Selenium production while minimizing Na_2SeO_3 toxicity. The biosynthesis of SeNPs was monitored using UV-Vis spectrophotometry. The optimized conditions for SeNP biosynthesis were found to be 60 mM Na_2SeO_3. The optimized biosynthesis method presented in this study offers a potential avenue for the large-scale production of SeNPs using *Vibrio natriegens* as a microbial factory. The eco-friendly nature and low cost of this biosynthesis method make it an attractive alternative to conventional chemical synthesis methods.

Keywords: Selenium Nanoparticles, Vibrio natriegens, Sodium Selenite, Semiconductor.

INTRODUCTION

Selenium is a trace element that is essential for the proper functioning of living organisms, including plants, animals, and humans. It is found in soil, water, and some foods, including nuts, seafood, and grains. In the human body, selenium plays a crucial role in many physiological processes, including the regulation of the

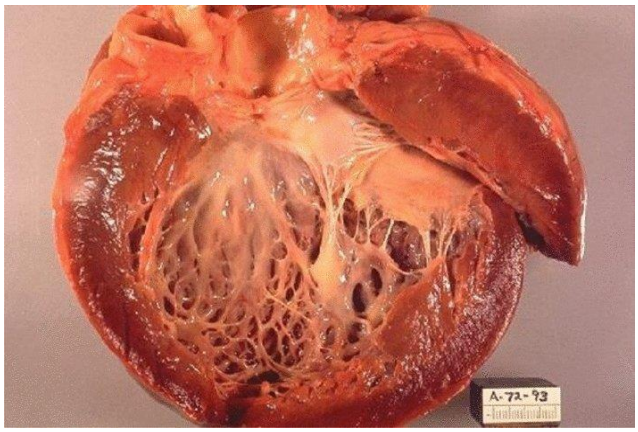

Figure 1: Keshan disease is endemic dilated cardiomyopathy (Petrović, 2021)

immune system, thyroid hormone metabolism, and DNA synthesis (National Institutes of Health, 2021). It also functions as an antioxidant, helping to protect cells from oxidative damage and reduce the risk of chronic diseases such as cancer, cardiovascular disease, and cognitive decline. Selenium deficiency is a significant public health concern, particularly in regions where soil and food are low in selenium. Deficiency can lead to a variety of health problems, including Keshan disease (Figure 1), which affects the heart, and Kashin-Beck disease, which affects the joints and bones. In addition, selenium deficiency has been linked to increased risk of certain cancers, cognitive decline, and compromised immune function. Selenium is also a semiconductor, meaning that it has unique electrical properties that make it useful in electronics and other applications (Santos, 2022). As a semiconduc-tor, selenium can be used in the manufacture of photoconductors, photovoltaic cells in solar panels, and other electronic compone-nts (Dodos, 2018). One of the most significant challenges facing the semiconductor industry is the availability and cost of materials. Traditional semiconductor materials, such as silicon, have become increasingly expensive, and there is a growing need to develop alternative materials that can offer compara-ble or better performance at a lower cost. Selenium is a promising candidate for this role, as it is more abundant and less expensive than many other semiconductors.

Vibrio natriegens (ATCC 14048) is a fast-growing bacterium that has gained attention as a potential host for industrial biotechnology and synthetic biology applications. Its rapid growth rate and short generation time of about 10 minutes make it easy to grow under simple environmental conditions (Tsang, 2022). Furthermore, *V. natriegens* has several other characteristics, including its ability to grow in high-salt environments, its tolerance to high temperatures, and its ability to efficiently incorporate non-natural amino acids into proteins. These features have led to the development of genetic tools and methods for *V. natriegens*, making it a promising model organism for research in synthetic biology and biotechnology.

Absorbance and transmittance are two important parameters used in spectroscopy to measure the amount of light absorbed or transmitted by a sample (Figure 2). Absorbance is a measure of the amount of light absorbed by a sample at a specific wavelength, and is usually denoted by the symbol “A”. It is related to the concentration of a substance in the sample, as well as the path length of the light through the sample, by the Beer-Lambert law, which states that the absorbance is proportional to the product of the concentration and the path length (Granite, 2022). The concentration selenium nanoparticles can be analyzed using a spectrometer, which measures the amount of light absorbed by a sample. Since selenium nanoparticles have a characteristic red color, the absorbance at a red wavelength can be used to quantify the selenium concentration according to the Beer-Lambert law. By measuring the absorbance at this wavelength, the concentration of selenium can be calculated based on the linear relationship between absorbance and concentration. A higher absorbance reading indicates a greater concentration of red selenium nanoparticles in the sample.

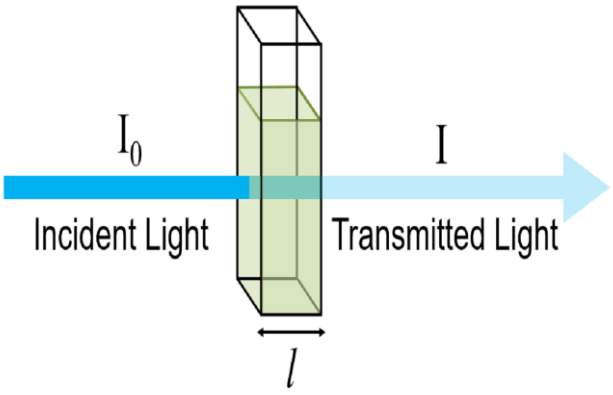

Figure 2 - Beer Lambert law: Transmittance & Absorbance. (Edinburgh Instruments, 2022)

While sodium selenite shows promise for cancer prevention and treatment, it can exhibit toxicity at higher doses. High levels of sodium selenite can induce oxidative stress and cytotoxicity in both cancerous and normal cells (Kieliszek et al. 2017). Selenite can cause cell death through mechanisms like oxidative DNA damage and disruption of redox homeostasis (Sanmartin et al. 2012). For example, in vitro studies show it can trigger apoptosis and necrosis in various cancer cell lines at concentrations above 50-100 μM (Xiang et al. 2009). However, cancer cells tend to be more vulnerable to selenite toxicity compared to normal cells when given similar doses.

The biosynthesis of SeNPs using microorganisms is a promising, eco-friendly, and cost-effective alternative to conventional chemical synthesis methods. However, there is still a need to optimize the biosynthesis process to achieve high yields, reduce the reaction time, and improve the stability and size distribution of the SeNPs produced. The aim of this study is to investigate one of the parameters affecting the biosynthesis of SeNPs from sodium selenite using *Vibrio Natriegens* as a microbial factory. Specifically, this study will focus on the concentration of sodium selenite as this factor will have the most significant impact on SeNP biosynthesis. The optimization of the biosynthesis process is essential for the large-scale production of SeNPs, which have shown great potential in various fields, such as biomedicine, electronics, and environmental remediation. SeNPs exhibit enhanced bioavailability and antioxidant activity compared to other forms of selenium, making them an attractive candidate for selenium supplementation. Therefore, optimizing the biosynthesis process aims to develop a sustainable, cost-effective, and scalable method for producing SeNPs with tailored properties, which can be used in various applications.

MATERIALS AND METHODS

1.5% NaCl solution was prepared by dissolving 1.5 g of NaCl in 100 mL of sterile distilled water. The bacterial cultures were inoculated by adding 1 mL of the bacterial suspension to each of the 7 10-mL beakers containing 9 mL of the sterile 1.5% NaCl solution. The beakers were then incubated at the appropriate temperature of 25°C.

After 24 hours of incubation, Sodium Selenite (Sigma Aldrich) was added to 6 beakers at the indicated concentration of 100 mM, 80 mM, 60 mM, 40 mM, 20 mM, and 10 mM (Figure 3). The last beaker was the control; therefore, no sodium selenite was added. Each beaker was labelled with their respective concentration. The bacterial cultures were incubated at the appropriate temperature and conditions required for the bacterial culture.

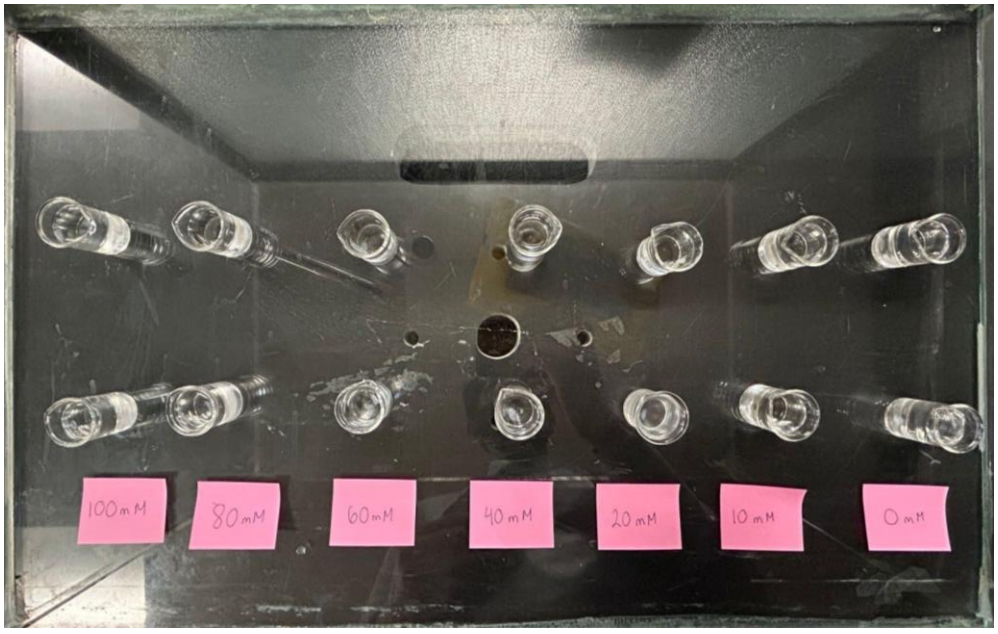

Figure 3 - Two trials with six beakers of varying concentration

A UV-Vis Pasco PS-2600 Spectrometer was calibrated; a light calibration was performed by placing a cuvette with distilled water in the spectrometer, and a dark calibration was performed. The spectrometer was then connected to the computer and the PASCO app was used for graphing. Each Selenite solution was poured into a separate cuvette, 1 cm in length, and placed in the Spectrometer to determine the wavelength with highest transmittance in each solution.

Each cuvette was then placed into a Vernier Go Direct Colorimeter, connected to the computer, and the wavelength found from the spectrometer was selected. The absorbance was measured for each solution and recorded.

The absorbance of each solution was determined using the colorimeter.

Beer-Lambert Law, $A = l\varepsilon c$, where A = absorbance, l = length of path of light, ε = molar absorptivity, and c = concentration (M), was rearranged to solve for concentration and was used to determine the concentration of Selenium in each solution.

The molar absorptivity of Selenium is 1.40×10^{4} L mol^{-1} cm^{-1} (Narayana et al., 2003). The length of a standard cuvette is 1 cm.

The determined concentration was then used to calculate the efficiency of the reaction for each solution by finding the ratio between product concentration and reactant concentration.

RESULTS

To determine the optimal wavelength for measuring the intensity of the color using a colorimeter, the absorbance spectra of the solutions were measured at different wavelengths. The wavelength with the highest transmittance was selected for further analysis.

The average spectrometer graph for all 7 solutions identified that the color with the highest transmittance was red (Figure 6).

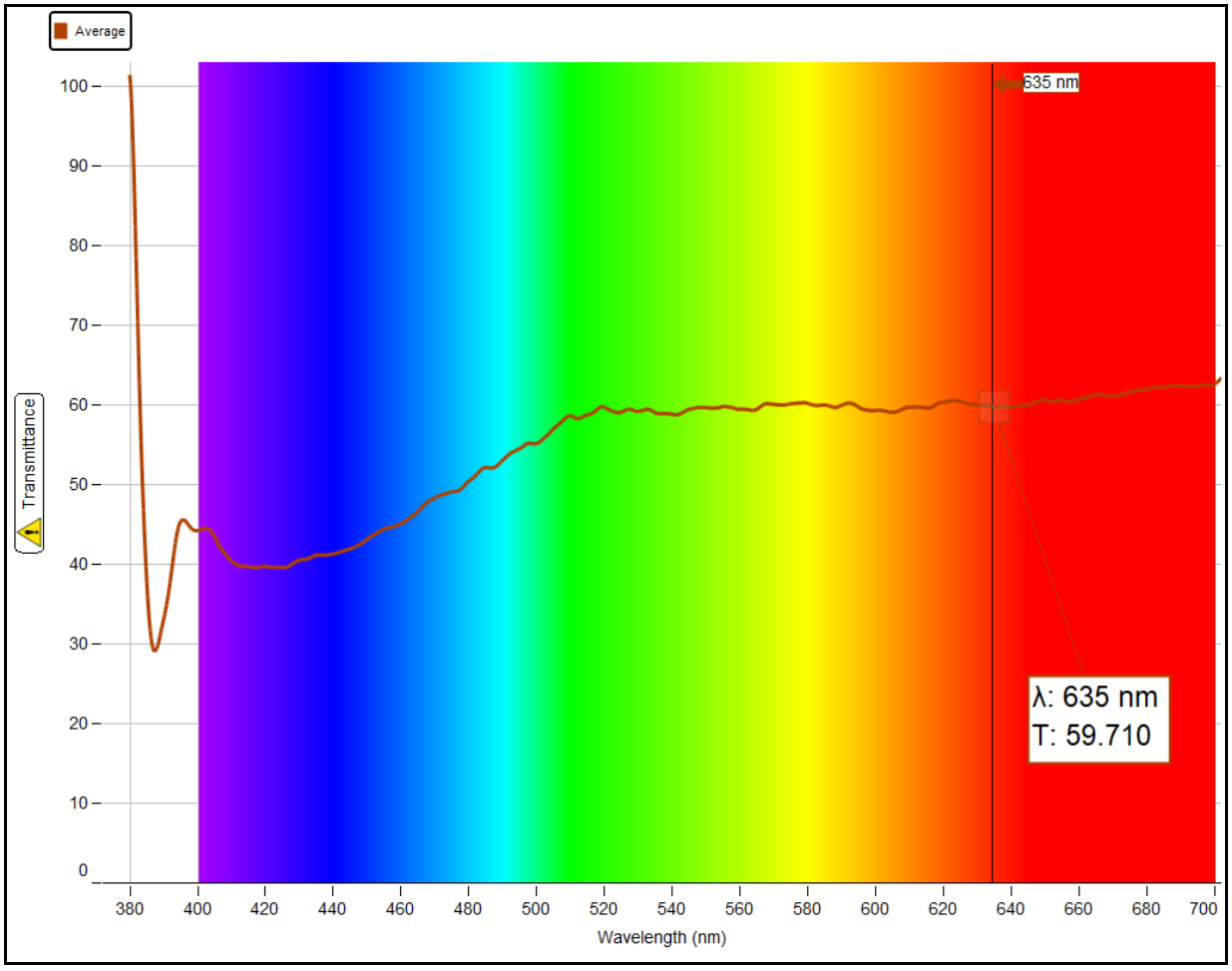

Figure 4 - Average Spectrometer Graph for all 7 solutions

Table 1: Trial 1 - Transmittance (635 nm) vs Selenite Concentration							
	Concentration						
Time (hours)	**0 mM**	**10 mM**	**20 mM**	**40 mM**	**60 mM**	**80 mM**	**100 mM**
0	100	100	100	100	100	100	100
24	100	99.93	99.76	99.63	99.53	99.32	98.74
48	100	99.85	99.52	99.32	99.16	98.56	98.22
72	100	99.7	99.4	98.81	98.00	98.01	97.95

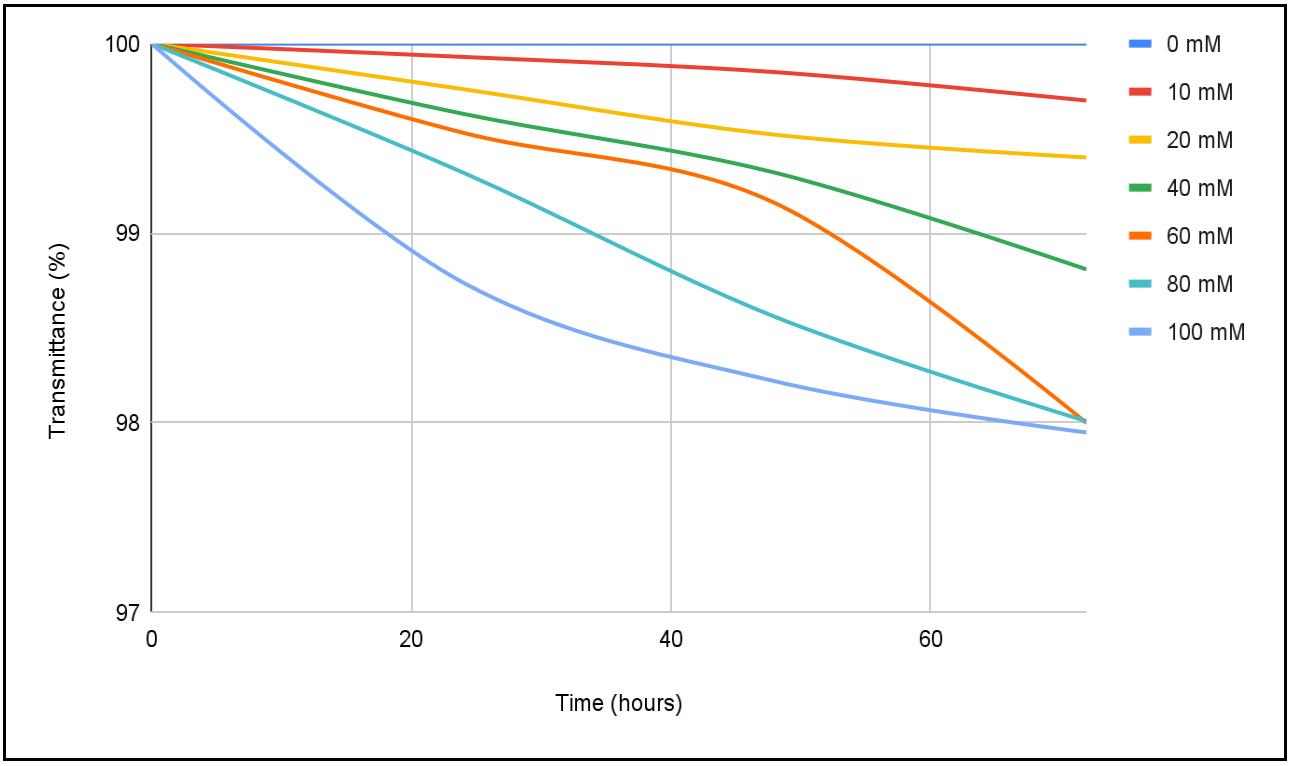

Figure 5 - Trial 1: Transmittance (635 nm) vs. Selenite Concentration

Table 2: Trial 2 - Transmittance (635 nm) vs Selenite Concentration							
	Concentration						
Time (hours)	**0 mM**	**10 mM**	**20 mM**	**40 mM**	**60 mM**	**80 mM**	**100 mM**
0	100	100	100	100	100	100	100
24	100	99.89	99.79	99.63	99.51	99.45	98.72
48	100	99.83	99.63	99.45	98.75	98.53	98.34
72	100	99.68	99.49	98.76	98.01	97.95	97.89

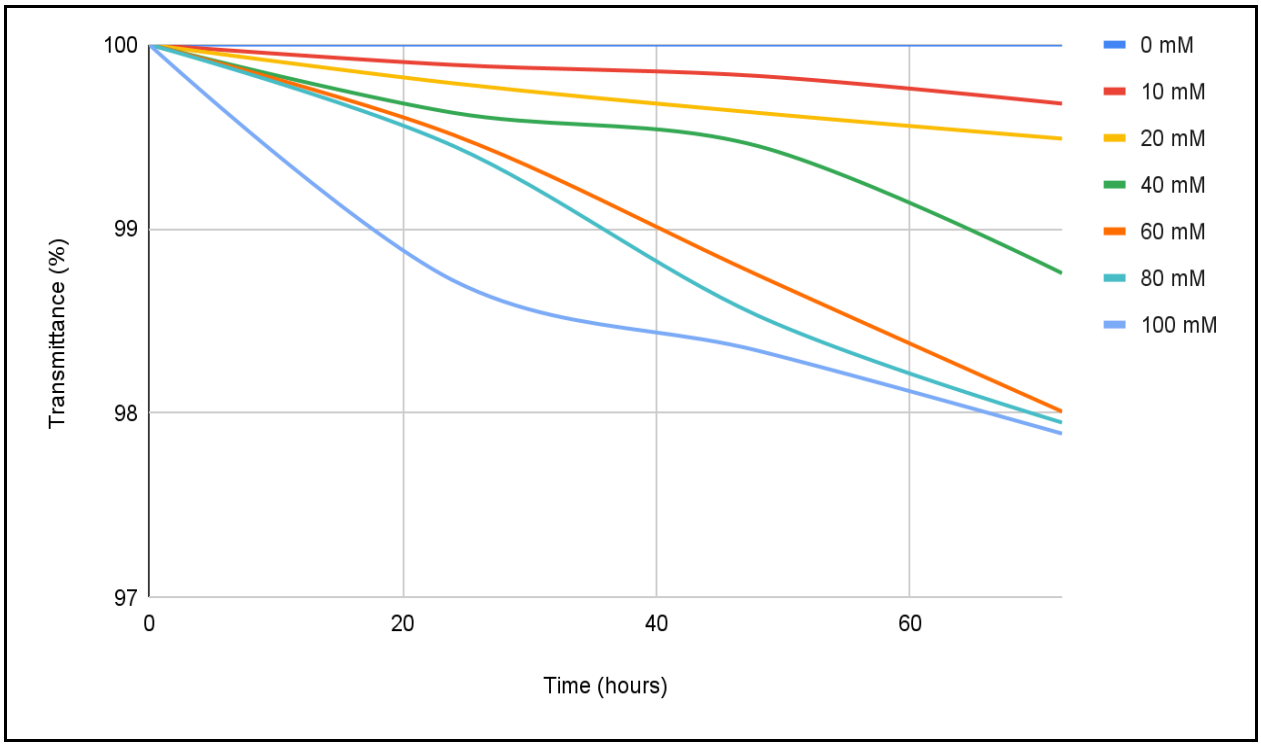

Figure 6 - Trial 2: Transmittance (635 nm) vs. Selenite Concentration

Table 3: Trial 1 - Beer–Lambert Law and Efficiency

Selenite Concentration (mM)	Absorbance	Selenium Concentration (mM)	Efficiency
0	0	0	0.000000%
10	0.0013048	0.0000932	0.000932%
20	0.0026136	0.0001867	0.000933%
40	0.0051991	0.0003714	0.000928%
60	0.0087739	0.0006267	0.001045%
80	0.0087296	0.0006235	0.000779%
100	0.0089955	0.0006425	0.000643%

Table 4: Trial 2 - Beer–Lambert Law and Efficiency

Selenite Concentration (mM)	Absorbance	Selenium Concentration (mM)	Efficiency
0	0	0	0.000000%
10	0.0013919	0.0000994	0.000994%
20	0.0022205	0.0001586	0.000793%
40	0.005418	0.0003871	0.000968%
60	0.0087296	0.0006235	0.001039%
80	0.0089955	0.0006425	0.000803%
100	0.0092616	0.0006615	0.000662%

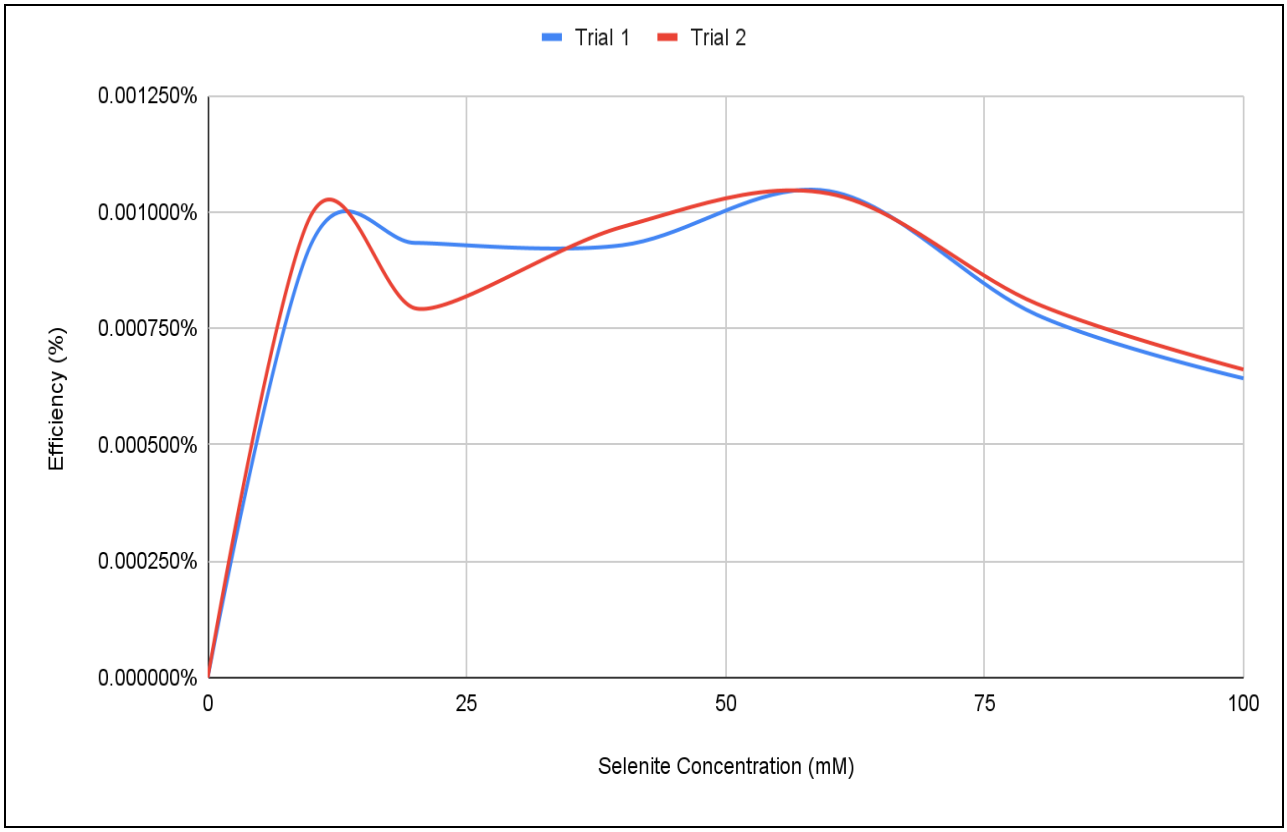

Figure 7 - Selenite Concentration vs. Efficiency

Overall, the results of the spectrometer analysis demonstrated that the bacterial culture solutions containing selenium had a distinct color, and the optimal wavelength for measuring the intensity of the color by a colorimeter was determined to be 635 nm.
The efficiency of the conversion process increased with increasing selenite concentration up to 60 mM, where it reached a peak of 0.001045% in Trial 1 (Figure 8) and 0.001039% in Trial 2 (Figure 9). However, the efficiency then decreased at higher selenite concentrations.

There was some variation in the efficiency between the two trials, but the general trend of increasing efficiency up to 60 mM and then decreasing efficiency at higher concentrations is consistent between the trials.

Overall, these results suggest that there is an optimal selenite concentration for the conversion process, and that concentrations above this optimum may actually hinder the efficiency of the reaction.

DISCUSSION

The results of this study indicate that *Vibrio natriegens* can effectively synthesize selenium in the presence of sodium selenite. The concentration of selenium in the bacterial culture solutions increased with increasing concentration of sodium selenite up to a maximum concentration of 60 mM. Beyond this concentration, the concentration of selenium in the cultures did not increase significantly. This could be caused by the increased concentration being toxic to the bacteria, which would decrease their population count and thus, decrease their ability to produce selenium.

These findings suggest that 60 mM is the optimal concentration of sodium selenite for the biosynthesis of selenium by *Vibrio natriegens* under the conditions studied. This information could be useful for optimizing the production of selenium using microbial biosynthesis as a potential source of this important element.

Overall, this study provides valuable insights into the biosynthesis of selenium by *Vibrio natriegens* and highlights the potential of using microbial biosynthesis

as a sustainable and cost-effective method for producing selenium. Further research is needed to explore the potential applications of selenium biosynthesis and to optimize the production process.

FUTURE PERSPECTIVES

Further research in this area could focus on several aspects related to the biosynthesis of selenium by *Vibrio natriegens*. For example, one possible direction for future studies could be to investigate the mechanism by which *Vibrio natriegens* is able to synthesize selenium, and to identify the enzymes and pathways involved in this process. This could provide insights into the genetic basis of selenium biosynthesis in bacteria, and could potentially lead to the development of novel biotechnological applications.

In addition, further research could also focus on optimizing the conditions for selenium biosynthesis by *Vibrio natriegens*, such as the pH, temperature, and nutrient composition of the growth medium. By identifying the optimal conditions for selenium biosynthesis, it may be possible to increase the yield and efficiency of this process, which could have important implications for large-scale production.

Overall, there are many opportunities for further research in the area of selenium biosynthesis by *Vibrio natriegens*, and this research has the potential to make important contributions to our understanding of microbial metabolism, biotechnology, and human health.

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