

Lanthanides as Product Growth Modulators in Enzyme-Catalyzed Reactions

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Abstract

Lanthanides, known for their unique coordination chemistry, can modulate enzymatic activity by interacting with active or allosteric sites. This study investigated the effects of lanthanides on alkaline phosphatase activity, a model enzyme essential for phosphate metabolism. Reactions were conducted in 96-well microplates using a 0.1 M tris hydrochloride buffer, with sequential additions of enzyme, lanthanide, and phosphatase substrate. Product formation was monitored at 415 nm, and reaction rates were calculated using linear regression. Most lanthanides, including Cerium, Praseodymium, and Neodymium, exhibited inhibitory effects, reducing reaction rates by 12.1%, 14.8%, and 19.8%, respectively. In contrast, Europium and sodium nitrate (NaNO₃) demonstrated modest enhancements of 3.7% and 5.2%, respectively. These findings highlight the dual role of lanthanides as inhibitors and enhancers, suggesting potential applications in enzymatic engineering and biocatalysis. Further studies are needed to elucidate the molecular mechanisms underlying these interactions.

Keywords

Lanthanides, Enzyme Modulation, Alkaline Phosphatase, Enzymatic Inhibition, Enzymatic Enhancement, Biocatalysis, Coordination Chemistry, Enzymatic Engineering, Product Formation

1. Introduction

Lanthanides, a group of rare earth elements known for their unique electronic configurations and reactivity, have recently emerged as key players in biochemical research [1]. Historically, lanthanides were primarily valued for technological applications, such as in electronics and optics. However, their ability to engage in

coordination chemistry with biological molecules has revealed potential roles in enzymatic catalysis and molecular stabilization [2] [3]. Despite this growing interest, the specific mechanisms by which lanthanides influence enzymatic activity remain unexplored, leaving critical gaps in the understanding of their biochemical applications.

Enzymes rely on interactions at their active and allosteric sites to achieve catalytic efficiency. Lanthanides, with high charge densities and flexible coordination geometries, may stabilize reaction intermediates, alter active site conformations, or interfere with substrate binding [4]. These interactions can either enhance or inhibit enzymatic activity, depending on the specific properties of the lanthanide and its mode of interaction with the enzyme. Such dual roles position lanthanides as both promising tools for modulating enzymatic reactions and potential disruptors of biochemical pathways.

Alkaline phosphatase serves as the model enzyme for investigating these interactions. This enzyme plays a critical role in bone mineralization by hydrolyzing pyrophosphate to promote calcium and phosphate deposition [5]. It also facilitates phosphate metabolism by liberating inorganic phosphate from organic molecules, underscoring its essential role in cellular processes [6]. Clinically, alkaline phosphatase functions as a biomarker for liver function and bone disorders, further highlighting its physiological importance [7]. These attributes make it an ideal candidate for examining the effects of lanthanides on enzymatic activity.

This research addresses the knowledge gap by systematically evaluating the effects of all lanthanides on the growth of reaction products in an enzyme-catalyzed system. The experimental approach involves the sequential addition of a 0.1 M tris hydrochloride buffer, alkaline phosphatase, a lanthanide, and a phosphatase substrate, with the reaction ultimately terminated by adding sodium hydroxide (NaOH). Controls of no lanthanide and sodium nitrate (NaNO_3) are utilized to isolate the specific effects of lanthanides. The hypothesis posits that lanthanides exhibit varying effects on alkaline phosphatase activity, ranging from inhibition to enhancement, depending on their coordination chemistry and interactions with the enzyme. Elucidating these effects aims to provide new insights into the biochemical roles of lanthanides and explore their potential applications in industrial biocatalysis and enzymatic engineering.

2. Materials and Methods

2.1. Materials

- 0.1 M hydrochloride pH 8.5 + 1 mM
- Alkaline phosphatase (lyophilized powder)
- Phosphatase substrate (5 mg tablets)
- Sodium hydroxide (NaOH)

2.2. Preparation of Enzyme and Substrate Solutions

The enzyme solution was prepared by dissolving 1.0 mg of alkaline phosphatase

in 1000 mL of 0.1 M tris HCl buffer to create the stock (1:1) solution. A 1:10 intermediate solution was then prepared by combining 100 μL of the stock with 900 μL of buffer, followed by a final 1:100 working solution prepared by mixing 100 μL of the 1:10 dilution with 900 μL of buffer. Based on supplier specifications, the alkaline phosphatase used has an approximate activity of ≥ 10 units/mg, resulting in an estimated final activity of ~ 0.01 units/mL in the working solution. Although exact enzymatic activity was not experimentally quantified, the same stock solution was used for all experimental batches and stored at 4°C to maintain consistency across trials.

2.3. Preparation of Lanthanide Solutions

Lanthanide solutions were prepared by dissolving 3 mg of the desired lanthanide nitrate salt ($\text{Ln}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$) in the appropriate volume of buffer to achieve a final concentration of 0.01 M. The required buffer volume was calculated using the formula $\text{g/L} = \text{molar mass} \times \text{M}$, where M is the desired molarity. Solutions were mixed thoroughly to ensure homogeneity.

2.4. Preparation of NaNO_3 Solution

The sodium nitrate (NaNO_3) solution, used exclusively in the second batch of experiments, was incorporated after initial results raised the possibility that nitrate ions may influence enzymatic activity independently of the lanthanide cation. It was prepared by dissolving 4.25 mg of NaNO_3 in 5 mL of 0.1 M tris hydrochloride buffer, achieving a final molarity of 0.01 M. The solution was gently mixed by tilting the container to ensure complete dissolution. Stored at room temperature, the NaNO_3 solution was incorporated into the experimental protocols alongside lanthanide solutions to evaluate whether NO_3^- from $\text{Ln}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ acted as a confounding variable.

2.5. Experimental Setup

The experiments were conducted using dual-chamber reagent reservoirs containing enzyme solution, substrate solution, buffer, NaOH, and lanthanide solution. Reactions were performed in 96-well microplates using a multichannel micropipette, with each well containing a total reaction volume of 220 μL . For the no-lanthanide control group, wells designated for the 0-minute time point contained 180 μL of buffer, 20 μL of NaOH, and 20 μL of substrate solution. For the 2-minute, 3-minute, 5-minute, and 10-minute time points, wells received 160 μL of buffer, 20 μL of enzyme solution, and 20 μL of substrate solution, with the reaction stopped by the addition of 20 μL of NaOH at the designated time.

For experiments involving lanthanides and NaNO_3 , the setup was modified as follows: wells designated for the 0-minute time point contained 160 μL of buffer, 20 μL of NaOH, 20 μL of lanthanide solution, and 20 μL of substrate solution. For the other time points, wells received 140 μL of buffer, 20 μL of enzyme solution, 20 μL of lanthanide solution, and 20 μL of substrate solution. The enzyme and

lanthanide solutions were incubated together for 3 minutes prior to the addition of substrate to allow for potential interactions. After the designated reaction time, the reaction was terminated by adding 20 μL of NaOH.

2.6. Controls and Experimental Design

The no-lanthanide control group was performed each day to establish baseline enzyme activity. Temperature and other confounding variables were kept constant. The experiments were conducted at a room temperature of 20°C. Lanthanide-treated samples from the same day were compared to their respective daily controls to account for potential variability in experimental conditions. Each experiment included five time points (0 minutes, 2 minutes, 3 minutes, 5 minutes, and 10 minutes) conducted in quadruplicate to ensure statistical reliability.

2.7. Data Collection and Analysis

Absorbance was measured at 415 nm using a microplate reader immediately after the addition of NaOH. For each well, the mean absorbance of replicates was calculated, and the blank (0-minute) absorbance was subtracted to obtain corrected values. Corrected absorbance values were plotted against time, and linear regression was performed to calculate the reaction rate (slope). Percent differences in reaction rates were calculated relative to the no-lanthanide control to quantify the impact of each lanthanide on enzymatic activity.

3. Results

3.1. Overview

The effects of lanthanides on alkaline phosphatase activity were evaluated across two experimental batches, with no-lanthanide controls serving as baseline conditions. Product formation rates were monitored over time, revealing the extent to which different lanthanides influenced enzymatic activity.

In the first batch, the no-lanthanide control established a baseline growth rate of 0.0519 absorbance units per minute, which served as a reference for assessing the lanthanides' impact. The majority of tested lanthanides, including Cerium (Ce), Praseodymium (Pr), and Neodymium (Nd), demonstrated inhibitory effects on product formation, with growth rates slower than the control. In contrast, Europium (Eu) exhibited a modest enhancement of product formation.

In the second batch, the no-lanthanide control produced a baseline growth rate of 0.0308 absorbance units per minute. The majority of lanthanides tested in this batch, including Dysprosium (Dy), Holmium (Ho), and Thulium (Tm), demonstrated slight inhibitory effects compared to the control. Notably, Sodium Nitrate (NaNO_3) and Erbium (Er) displayed slightly faster growth rates, indicating minor enhancements likely influenced by ionic effects.

Across both batches, standard deviations were minimal, reflecting the consistency of measurements within replicates.

3.2. Control: No Lanthanide (First Batch)

In the first batch of experiments, product formation increased over time, as shown in **Figure 1**. The average corrected absorbance values at 2, 3, 5, and 10 minutes were 0.1175, 0.205, 0.3125, and 0.5225, respectively. This control condition established a baseline growth rate with a slope of 0.0519 absorbance units per minute ($R^2 = 0.9837$). Standard deviations across replicates were minimal, indicating consistent measurements.

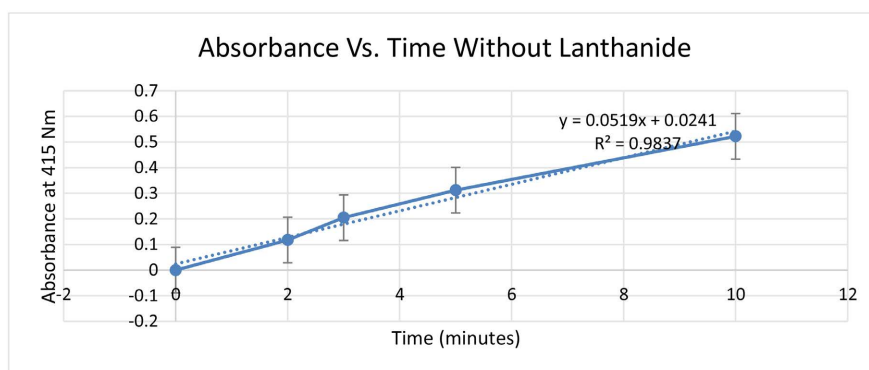


Figure 1. Absorbance vs. Time Without Lanthanide (First Control). Product formation was measured at 415 nm, serving as the baseline for the first set of lanthanide experiments conducted in the first batch. Data represent the average of three replicates, with error bars indicating standard deviation.

3.3. Cerium (Ce)

Cerium-treated reactions exhibited a growth rate of 0.0456 absorbance units per minute ($R^2 = 0.9871$), which is approximately 12.1% slower than the first batch control conditions (no lanthanide, 0.0519 absorbance units per minute), as shown in **Figure 2**. Corrected absorbance values at 2, 3, 5, and 10 minutes were 0.135, 0.1425, 0.265, and 0.465, respectively. Standard deviations across replicates were minimal, indicating consistent measurements. These results suggest that Cerium may inhibit product formation compared to baseline conditions.

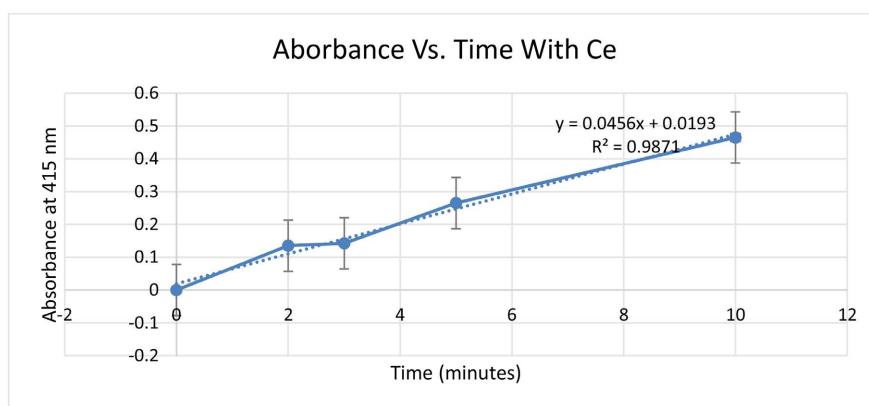


Figure 2. Absorbance vs. Time With Cerium. Product formation was measured at 415 nm, showing a reduced growth rate compared to the first batch control conditions. Data represent the average of three replicates, with error bars indicating standard deviation.

3.4. Praseodymium (Pr)

Praseodymium-treated reactions exhibited a growth rate of 0.0442 absorbance units per minute ($R^2 = 0.985$), which is approximately 14.8% slower than the first batch control conditions (no lanthanide, 0.0519 absorbance units per minute), as shown in **Figure 3**. Corrected absorbance values at 2, 3, 5, and 10 minutes were 0.0725, 0.1675, 0.205, and 0.445, respectively. Standard deviations across replicates were low, indicating reliable and consistent measurements. These results suggest that Praseodymium may inhibit product formation compared to baseline conditions.

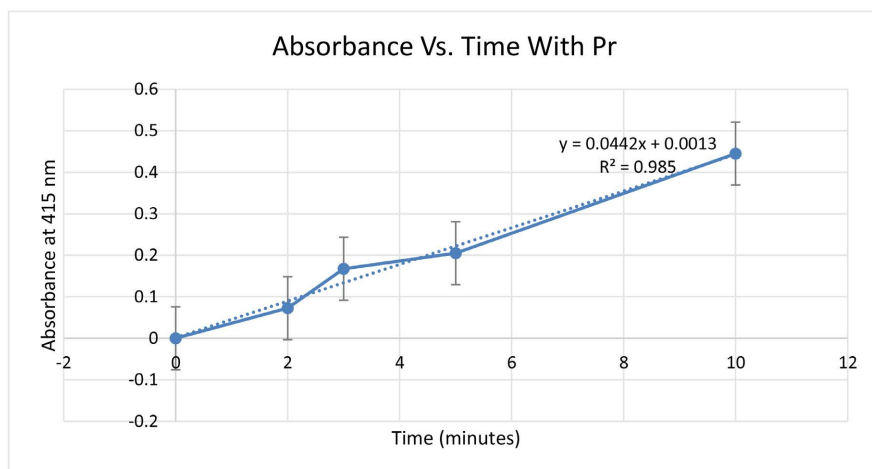


Figure 3. Absorbance vs. Time With Praseodymium. Product formation was measured at 415 nm, showing a reduced growth rate compared to the first batch control conditions. Data represent the average of three replicates, with error bars indicating standard deviation.

3.5. Neodymium (Nd)

Neodymium-treated reactions exhibited a growth rate of 0.0416 absorbance units per minute ($R^2 = 0.9748$), which is approximately 19.8% slower than the first batch control conditions (no lanthanide, 0.0519 absorbance units per minute), as shown in **Figure 4**. Corrected absorbance values at 2, 3, 5, and 10 minutes were 0.075, 0.1425, 0.26, and 0.4075, respectively. Standard deviations across replicates were minimal, indicating consistency in the results. These results suggest that Neodymium may inhibit product formation compared to baseline conditions.

3.6. Samarium (Sm)

Samarium-treated reactions exhibited a growth rate of 0.0486 absorbance units per minute ($R^2 = 0.9861$), which is approximately 6.4% slower than the first batch control conditions (no lanthanide, 0.0519 absorbance units per minute), as shown in **Figure 5**. Corrected absorbance values at 2, 3, 5, and 10 minutes were 0.135, 0.1625, 0.2225, and 0.505, respectively. Standard deviations across replicates were minimal, indicating reliable and consistent measurements. These results suggest that Samarium may slightly inhibit product formation compared to baseline conditions.

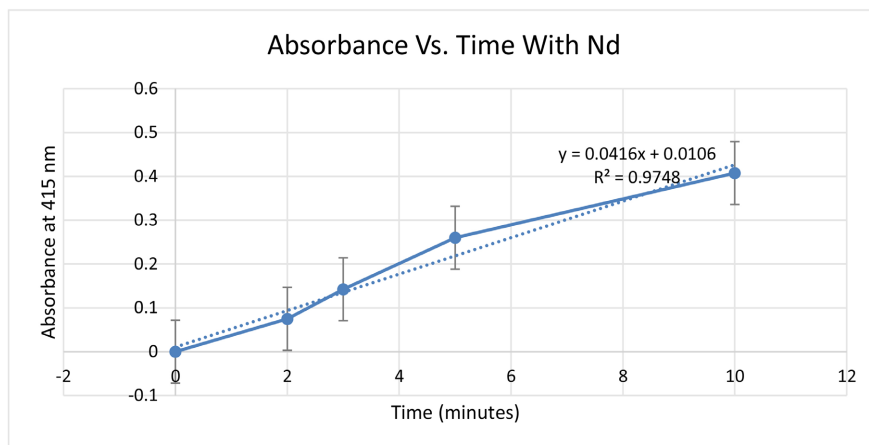


Figure 4. Absorbance vs. Time With Neodymium. Product formation was measured at 415 nm, showing a reduced growth rate compared to the first batch control conditions. Data represent the average of three replicates, with error bars indicating standard deviation.

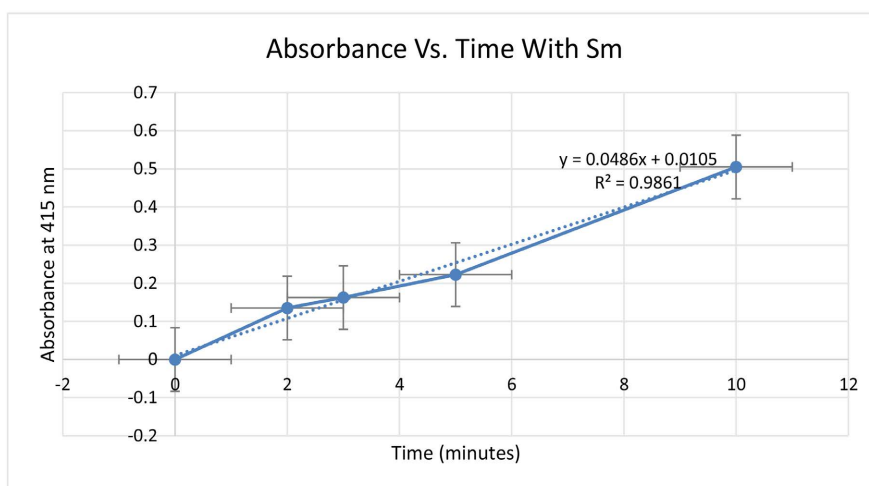


Figure 5. Absorbance vs. Time With Samarium. Product formation was measured at 415 nm, showing a slightly reduced growth rate compared to the first batch control conditions. Data represent the average of three replicates, with error bars indicating standard deviation.

3.7. Europium (Eu)

Europium-treated reactions exhibited a growth rate of 0.0538 absorbance units per minute ($R^2 = 0.9878$), which is approximately 3.7% faster than the first batch control conditions (no lanthanide, 0.0519 absorbance units per minute), as shown in **Figure 6**. Corrected absorbance values at 2, 3, 5, and 10 minutes were 0.16, 0.205, 0.3175, and 0.555, respectively. Standard deviations across replicates were minimal, indicating reliable and consistent measurements. These results suggest that Europium may slightly enhance product formation compared to baseline conditions.

3.8. Gadolinium (Gd)

Gadolinium-treated reactions exhibited a growth rate of 0.0444 absorbance units

per minute ($R^2 = 0.989$), which is approximately 14.5% slower than the first batch control conditions (no lanthanide, 0.0519 absorbance units per minute), as shown in **Figure 7**. Corrected absorbance values at 2, 3, 5, and 10 minutes were 0.125, 0.1675, 0.2625, and 0.455, respectively. Standard deviations across replicates were minimal, indicating consistency in measurements. These results suggest that Gadolinium may inhibit product formation compared to baseline conditions.

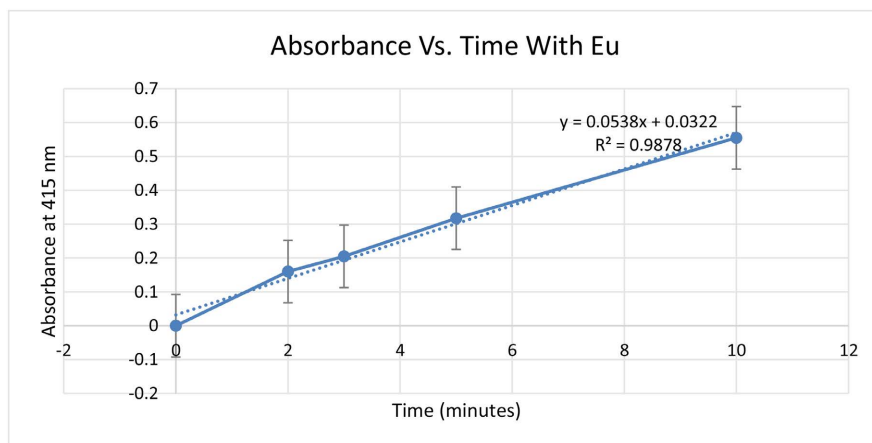


Figure 6. Absorbance vs. Time With Europium. Product formation was measured at 415 nm, showing a considerably enhanced growth rate compared to the first batch control conditions. Data represent the average of three replicates, with error bars indicating standard deviation.

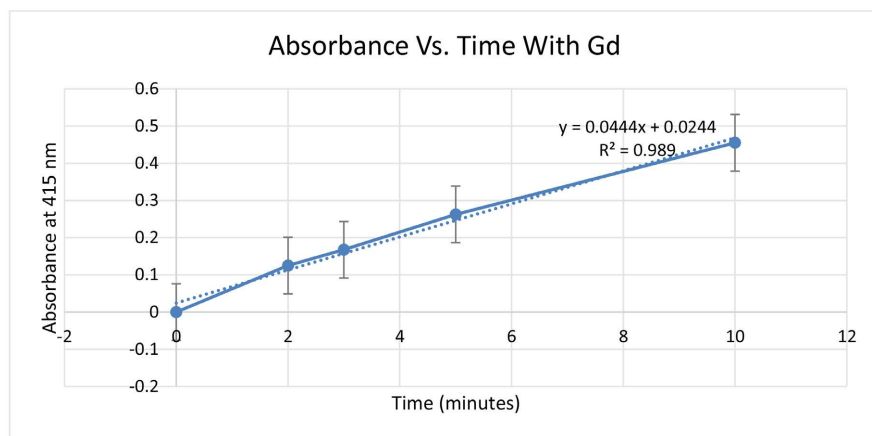


Figure 7. Absorbance vs. Time With Gadolinium. Product formation was measured at 415 nm, showing a reduced growth rate compared to the first batch control conditions. Data represent the average of three replicates, with error bars indicating standard deviation.

3.9. Control: No Lanthanide (Second Batch)

In the absence of lanthanides, product formation exhibited a linear increase over time, as shown in **Figure 8**. The average corrected absorbance values at 2, 3, 5, and 10 minutes were 0.08, 0.1025, 0.16, and 0.315, respectively. This control condition established a baseline growth rate with a slope of 0.0308 absorbance units per minute ($R^2 = 0.9967$). Standard deviations across replicates were minimal, indicating

consistent measurements.

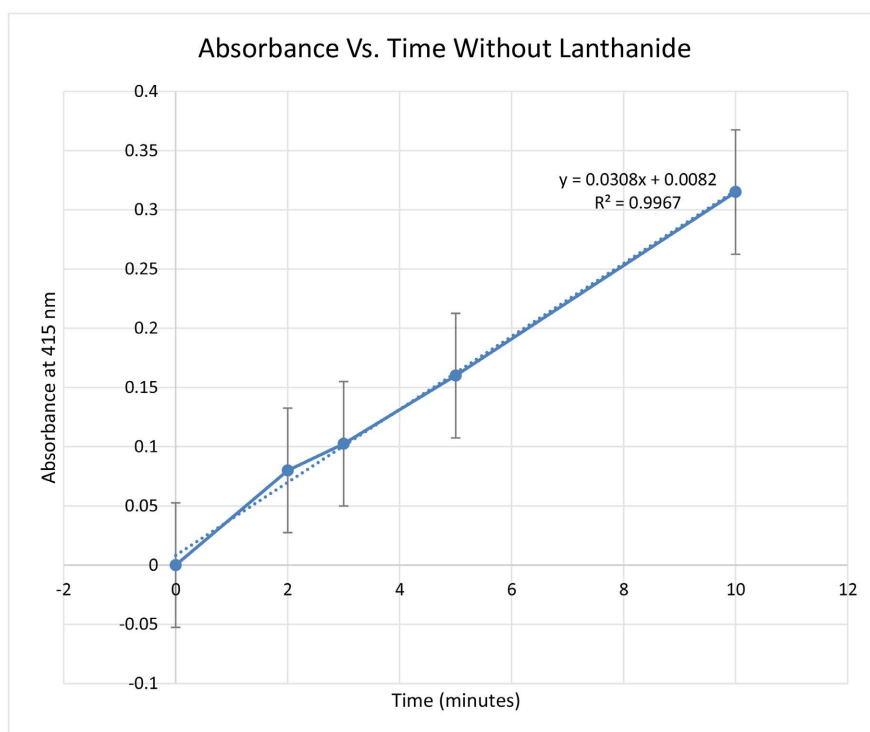


Figure 8. Absorbance vs. Time Without Lanthanide (Second Control). Product formation was measured at 415 nm, serving as the baseline for the second set of lanthanide experiments conducted in the second batch. Data represent the average of three replicates, with error bars indicating standard deviation.

3.10. Control: Sodium Nitrate (NaNO_3)

In the presence of NaNO_3 , product formation exhibited a growth rate of 0.0324 absorbance units per minute ($R^2 = 0.9965$), which is approximately 5.2% faster than the second batch control conditions (no lanthanide, 0.0308 absorbance units per minute), as shown in **Figure 9**. Corrected absorbance values at 2, 3, 5, and 10 minutes were 0.0775, 0.1, 0.155, and 0.33, respectively. Standard deviations across replicates were minimal, indicating consistent measurements. These results suggest a minor enhancement of product formation compared to baseline conditions, likely due to the ionic effects of NaNO_3 .

3.11. Dysprosium (Dy)

Dysprosium-treated reactions exhibited a growth rate of 0.0279 absorbance units per minute ($R^2 = 0.9789$), which is approximately 9.4% slower than the second control conditions (no lanthanide, 0.0308 absorbance units per minute), as shown in **Figure 10**. Corrected absorbance values at 2, 3, 5, and 10 minutes were 0.095, 0.115, 0.1675, and 0.2925, respectively. Despite the lower growth rate, the results were consistent across replicates, with standard deviations remaining within acceptable limits. This suggests a mild inhibitory effect of Dysprosium on alkaline

phosphatase-mediated product formation.

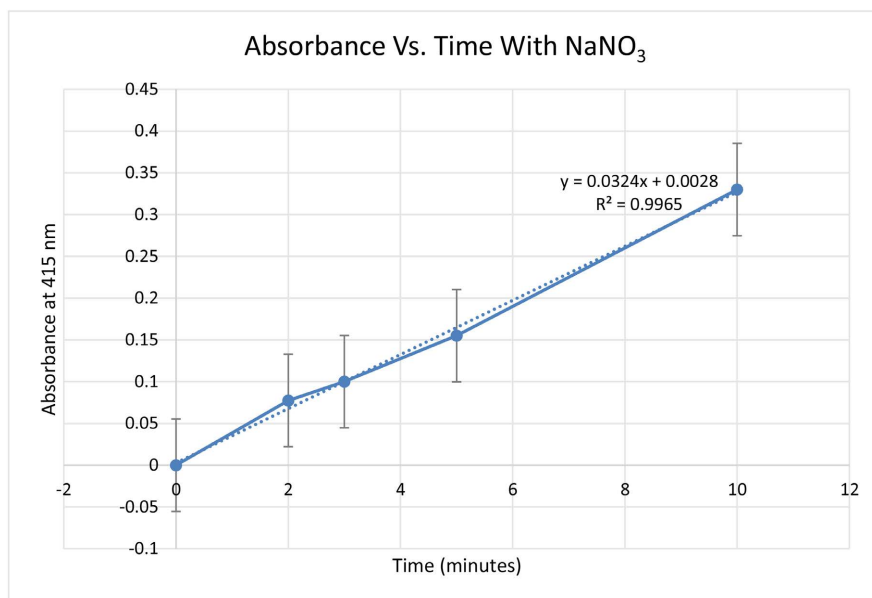


Figure 9. Absorbance vs. Time With Sodium Nitrate. Product formation was measured at 415 nm, showing a slightly higher growth rate compared to the second batch control without lanthanides. Data represent the average of three replicates, with error bars indicating standard deviation.

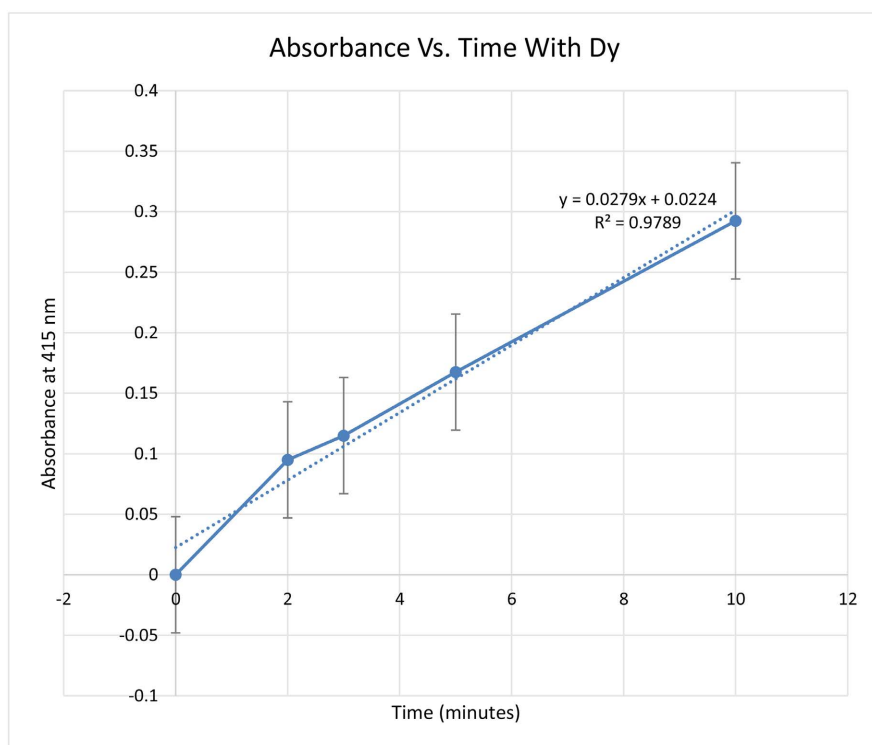


Figure 10. Absorbance vs. Time With Dysprosium. Product formation was measured at 415 nm, showing a reduced growth rate compared to the second batch control conditions. Data represent the average of three replicates, with error bars indicating standard deviation.

3.12. Holmium (Ho)

Holmium-treated reactions exhibited a growth rate of 0.0275 absorbance units per minute ($R^2 = 0.9894$), which is approximately 10.7% slower than the second batch control conditions (no lanthanide, 0.0308 absorbance units per minute), as shown in **Figure 11**. Corrected absorbance values at 2, 3, 5, and 10 minutes were 0.04, 0.06, 0.1125, and 0.27, respectively. Standard deviations across replicates were relatively small, indicating consistent measurements. These results suggest that Holmium may inhibit product formation compared to baseline conditions in the second batch.

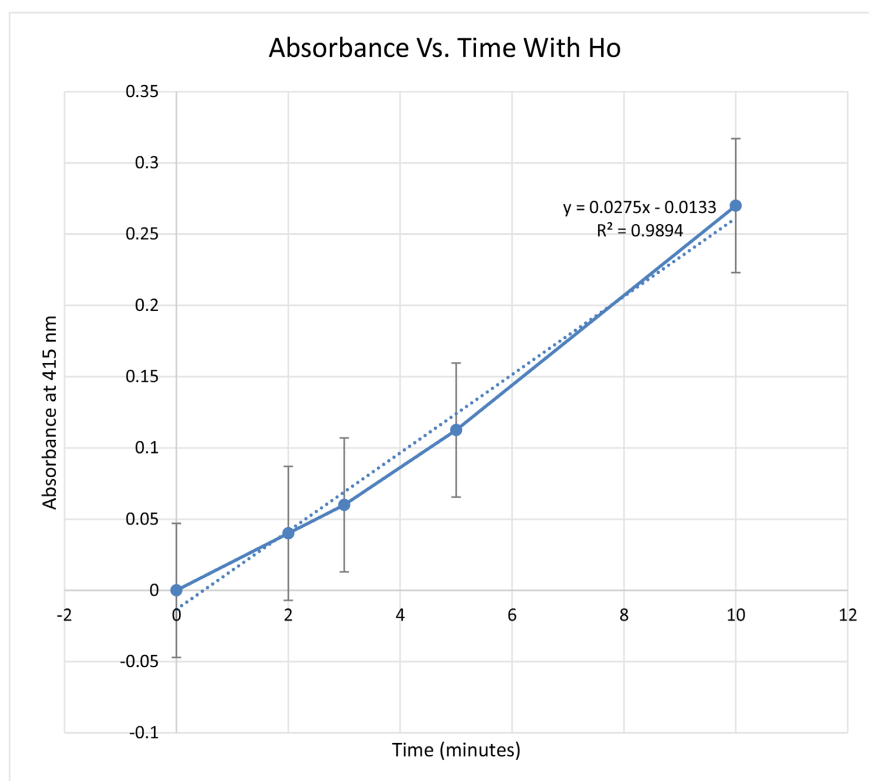


Figure 11. Absorbance vs. Time With Holmium. Product formation was measured at 415 nm, showing a reduced growth rate compared to the second batch control conditions. Data represent the average of three replicates, with error bars indicating standard deviation.

3.13. Erbium (Er)

Erbium-treated reactions demonstrated a growth rate of 0.0324 absorbance units per minute ($R^2 = 0.9983$), which is approximately 5.2% faster than the second batch control conditions (no lanthanide, 0.0308 absorbance units per minute), as shown in **Figure 12**. Corrected absorbance values at 2, 3, 5, and 10 minutes were 0.0675, 0.0875, 0.165, and 0.3225, respectively. Standard deviations across replicates were small, indicating consistent measurements. These results suggest a minor enhancement of product formation compared to baseline conditions in the second batch.

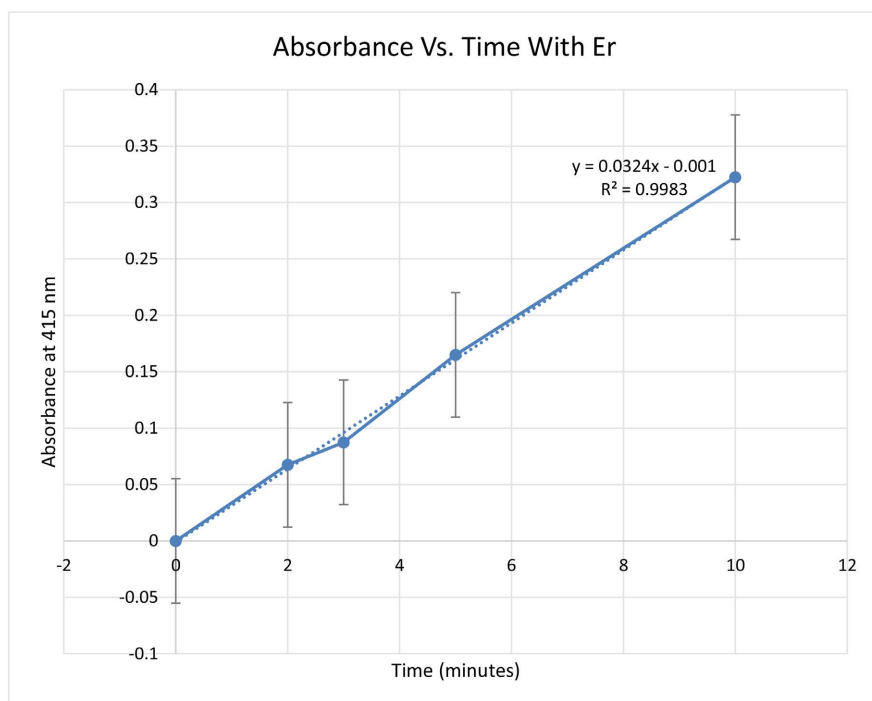


Figure 12. Absorbance vs. Time With Erbium. Product formation was measured at 415 nm, showing a growth rate comparable to the second batch control conditions. Data represent the average of three replicates, with error bars indicating standard deviation.

3.14. Thulium (Tm)

Thulium-treated reactions exhibited a growth rate of 0.0317 absorbance units per minute ($R^2 = 0.9897$), which is approximately 3.6% slower than the second batch control conditions (no lanthanide, 0.0308 absorbance units per minute), as shown in **Figure 13**. Corrected absorbance values at 2, 3, 5, and 10 minutes were 0.085, 0.1275, 0.17, and 0.3275, respectively. Standard deviations across replicates were consistent, indicating reliable measurements. These results suggest that Thulium may slightly inhibit product formation compared to baseline conditions in the second batch.

3.15. Ytterbium (Yb)

Ytterbium-treated reactions exhibited a growth rate of 0.0299 absorbance units per minute ($R^2 = 0.9813$), which is approximately 2.9% slower than the second batch control conditions (no lanthanide, 0.0308 absorbance units per minute), as shown in **Figure 14**. Corrected absorbance values at 2, 3, 5, and 10 minutes were 0.0775, 0.0727, 0.13, and 0.305, respectively. Standard deviations across replicates were relatively small, indicating reliable measurements. These results suggest that Ytterbium may slightly inhibit product formation compared to baseline conditions in the second batch.

3.16. Lutetium (Lu)

Lutetium-treated reactions exhibited a growth rate of 0.0298 absorbance units per minute ($R^2 = 0.9981$), which is approximately 3.2% slower than the second batch

control conditions (no lanthanide, 0.0308 absorbance units per minute), as shown in **Figure 15**. Corrected absorbance values at 2, 3, 5, and 10 minutes were 0.0625, 0.085, 0.14, and 0.3, respectively. Standard deviations across replicates were consistent, indicating reliable measurements. These results suggest that Lutetium may slightly inhibit product formation compared to baseline conditions in the second batch.

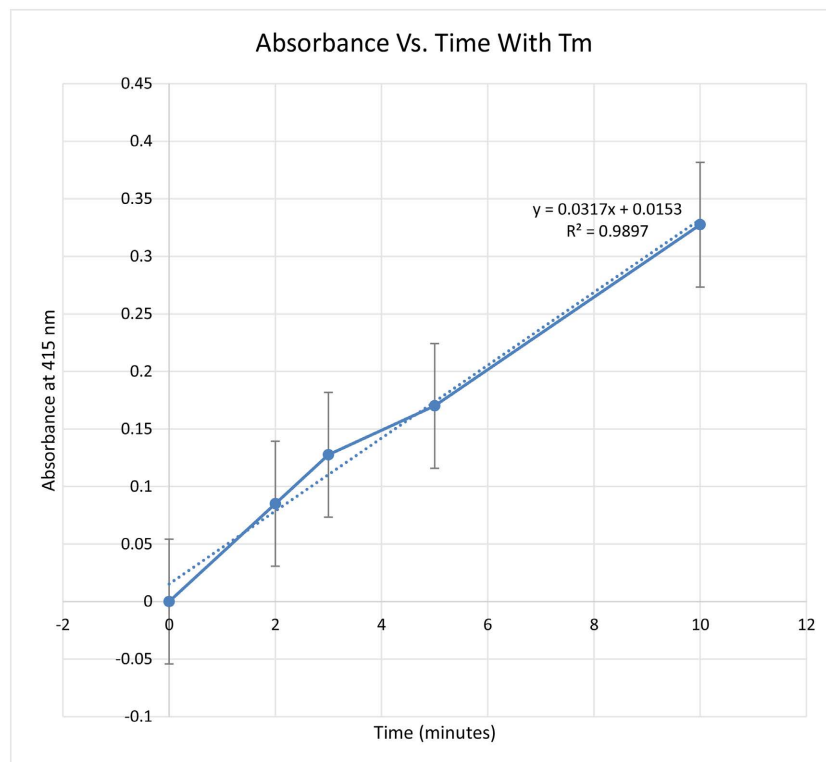


Figure 13. Absorbance vs. Time With Thulium. Product formation was measured at 415 nm, showing a growth rate comparable to the second batch control conditions. Data represent the average of three replicates, with error bars indicating standard deviation.

4. Discussion

The results demonstrate varying impacts of individual lanthanides, ranging from inhibition to slight enhancement of enzymatic activity. These findings contribute to our understanding of how lanthanides interact with enzymes and suggest potential biochemical and industrial applications [2] [3].

4.1. Interpretation of Results

The inhibitory effects observed for most lanthanides align with the hypothesis that lanthanides can interfere with enzymatic function by binding to active or allosteric sites, thereby altering the enzyme's conformation [4]. For example, Cerium (Ce), Praseodymium (Pr), and Neodymium (Nd) reduced growth rates by 12.1%, 14.8%, and 19.8%, respectively, relative to the first batch control (no lanthanide). Such inhibition could result from lanthanides displacing essential cofactors or directly competing with the substrate for access to the enzyme's active site, disrupting catalytic turnover and slowing product formation [2] [6].

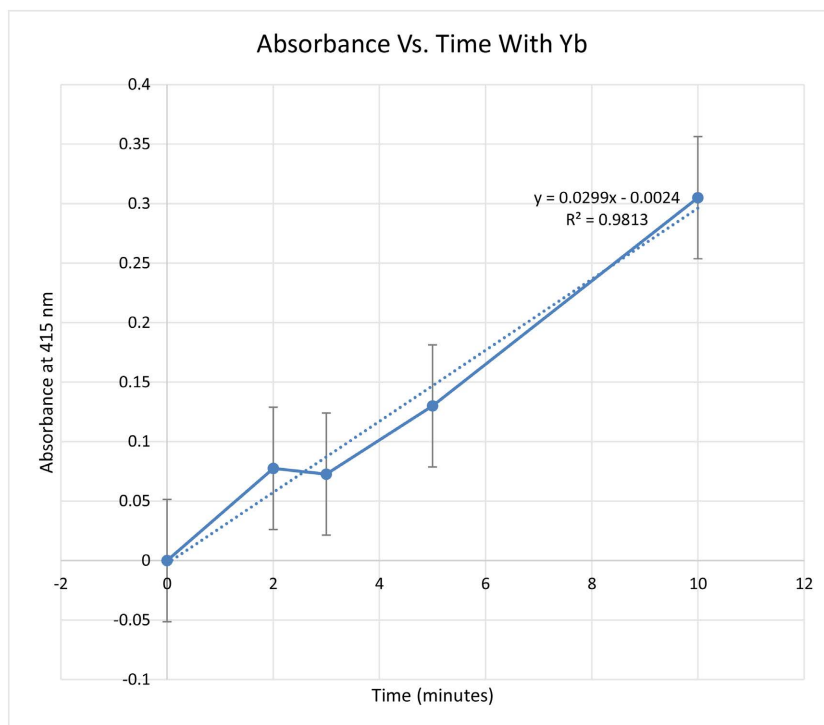


Figure 14. Absorbance vs. Time With Ytterbium. Product formation was measured at 415 nm, showing a reduced growth rate compared to the second batch control conditions. Data represent the average of three replicates, with error bars indicating standard deviation.

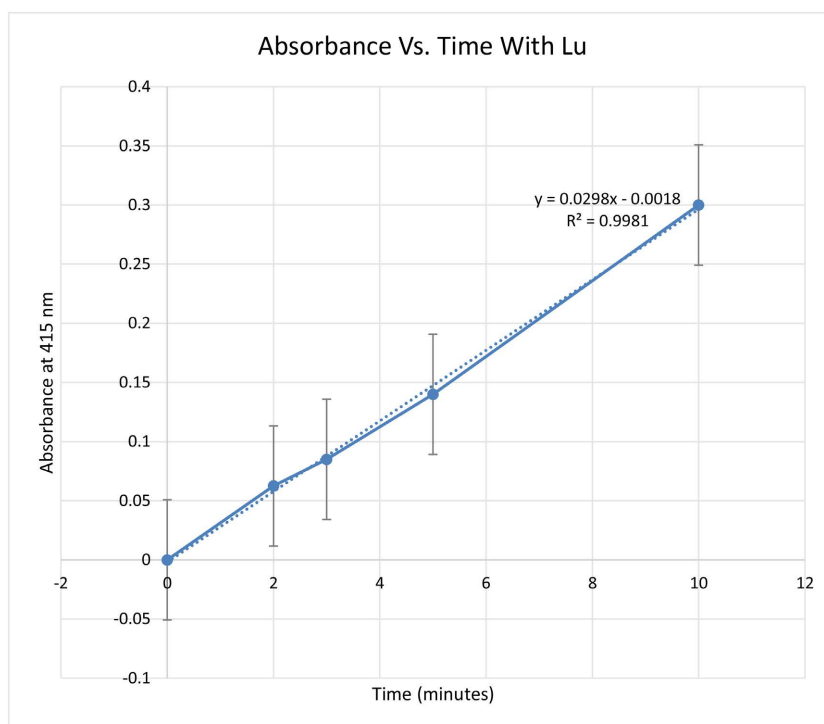


Figure 15. Absorbance vs. Time With Lutetium. Product formation was measured at 415 nm, showing a slightly reduced growth rate compared to the second batch control conditions. Data represent the average of three replicates, with error bars indicating standard deviation.

Similarly, Dysprosium (Dy) and Holmium (Ho), tested in the second batch, exhibited slower growth rates compared to the second batch control, with reductions of 9.4% and 10.7%, respectively. These findings suggest a consistent trend of inhibition across the lanthanides tested, underscoring their potential to negatively affect enzymatic efficiency. However, the extent of inhibition varied, likely reflecting differences in the chemical properties of individual lanthanides, such as ionic radii and coordination chemistry [3] [4].

Interestingly, Europium (Eu) deviated from the general trend, showing a slight enhancement of product formation with a growth rate 3.7% higher than the first batch control. This suggests that under specific conditions, certain lanthanides may stabilize the enzyme-substrate complex or facilitate more efficient catalysis. Sodium nitrate (NaNO_3), included as a control for ionic effects, exhibited a 5.2% increase in growth rate compared to the second batch control, indicating that ionic interactions also modulate enzymatic activity, though to a lesser extent than the broader effects of lanthanides [1].

Erbium (Er), which exhibited a growth rate identical to the second batch control, demonstrates that not all lanthanides significantly impact enzymatic activity. Similarly, Thulium (Tm) and Ytterbium (Yb) showed only slight reductions in growth rates (3.6% and 2.9%, respectively), indicating that their interactions with the enzyme may not disrupt catalytic efficiency to the same extent as other lanthanides.

4.2. Biochemical Implications

The variability in lanthanide effects highlights the complex nature of their interactions with enzymes. The inhibitory effects observed for most lanthanides suggest they may interfere with steps in the catalytic cycle, such as substrate binding, intermediate stabilization, or product release [4] [5]. These findings are consistent with prior studies showing that lanthanides can bind tightly to oxygen donor ligands, which may mimic functional groups in enzyme active sites [2]. Such interactions could disrupt the balance of forces required for optimal enzymatic function.

In contrast, the modest enhancement observed with Europium and NaNO_3 suggests potential applications in optimizing enzymatic activity under specific conditions. Europium's ability to enhance growth rates suggests that it could stabilize transition states or create a more favorable electrostatic environment for catalysis [3]. These properties could be leveraged in industrial biocatalysis, where even small improvements in reaction efficiency can have significant practical benefits.

4.3. Limitations and Future Directions

The dual roles of lanthanides as both inhibitors and enhancers of enzymatic activity present significant opportunities and challenges for their application in industrial biocatalysis. While the inhibitory effects observed for most lanthanides highlight potential limitations, their selective ability to enhance enzymatic activity

under certain conditions underscores their promise as tools for fine-tuning enzymatic reactions. Europium, for instance, demonstrated a modest enhancement of product formation, suggesting potential applications in optimizing enzyme performance [2].

One limitation of this study is the variability observed between the first and second batch controls. Although standard deviations were minimal within replicates, differences between the batches suggest potential inconsistencies in experimental conditions, which could influence the reproducibility of the results. Minor differences in environmental conditions, such as temperature fluctuations, enzyme activity degradation over time, or pipetting precision, may have contributed to this discrepancy. To account for this, all lanthanide-treated samples were analyzed relative to their respective same-day controls, ensuring valid intra-batch comparisons. Addressing these issues will require standardizing experimental protocols and ensuring consistent conditions across batches in future studies. This is particularly important for industrial applications, where reproducibility and scalability are critical [6].

Another limitation of this study is that the NaNO₃ control was only included in the second batch. This control was introduced after preliminary data from the first batch suggested that nitrate ions, present in all lanthanide nitrate salts, could act as a confounding variable. Although comparisons within the second batch helped isolate the potential effects of NO₃⁻, including the NaNO₃ control in all batches would have allowed for more consistent cross-batch comparisons. Future experiments should incorporate appropriate ionic controls across all conditions to fully distinguish lanthanide-specific effects from those attributable to accompanying counterions.

The inhibitory effects observed for most lanthanides emphasize the need for a deeper understanding of how these elements interact with enzymes. Mechanistic studies, including structural techniques like X-ray crystallography or nuclear magnetic resonance (NMR), could elucidate how lanthanides alter enzyme conformation and function. Computational modeling could complement these approaches, providing predictive tools for designing lanthanides that enhance, rather than inhibit, enzymatic activity [3].

Expanding the scope of lanthanide research beyond alkaline phosphatase to include other enzymes and reaction types could uncover additional opportunities for their application. Certain enzymes may exhibit unique sensitivities to lanthanides, making them suitable for targeted applications in synthetic biology, metabolic engineering, or biomanufacturing [5]. Investigating the effects of lanthanides in whole-cell systems could also reveal their broader impact on cellular metabolism and open new avenues for biotechnological innovation.

5. Conclusions

This study investigated the role of lanthanides in modulating product growth during enzyme-catalyzed reactions, specifically using alkaline phosphatase as a model

system. The results demonstrate that most lanthanides exert inhibitory effects on enzymatic activity, likely through disruption of substrate binding or active site conformation. However, certain lanthanides, particularly Europium, were found to enhance product formation under specific conditions.

These findings highlight the dual nature of lanthanides as both potential inhibitors and enhancers of enzymatic function. This duality underscores the importance of evaluating individual lanthanides for tailored applications, especially in industrial biocatalysis where fine-tuning enzyme performance can lead to significant efficiency gains. Additionally, the observed effects of sodium nitrate suggest that ionic context plays a non-negligible role in enzymatic modulation and should be carefully controlled in future studies.

To fully harness the potential of lanthanides in biotechnology, future research should aim to standardize experimental conditions, explore mechanistic interactions at the molecular level, and extend these investigations to a broader range of enzymes and reaction systems. If these challenges are addressed, lanthanides could serve as powerful tools for enhancing and regulating enzymatic processes in both research and industry.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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