Nitrogen Fertilizer – Go or Foe?

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Abstract

Alkaline phosphatase (AP) hydrolyzes esters and anhydrides of phosphoric acid in soil, offering adequate plant nutrition. The enzymatic activity of AP can be changed based on the components of the soil. In Qatar, nitrate fertilizers are commonly used, which improve soil quality, and enhance plant metabolism and growth. It was suggested that nitrate fertilizers can have a decreasing effect on the activity of AP. However, the optimum amount of ammonium nitrate fertilizer that yields the highest AP activity remains unknown. Therefore, our project aims to find the concentration of ammonium nitrate fertilizer at which AP shows the highest activity. This is done using the enzyme kinetic assay, where an enzyme saturation curve, multiple Michaelis-Menten plots, and the rate at varying nitrogen concentrations were plotted. From the Michaelis-Menten plots, important parameters are concluded such as Km and Vmax, which help in the comparison. We hypothesize that AP activity decreases with the increasing addition of liquid nitrate fertilizer, as measured by the Km (affinity) and Vmax (efficiency) constants from enzyme kinetics analyses. This project concludes that nitrogen is indeed an inhibitor for AP, and we conclude that it is a non-competitive inhibitor.

Introduction

Alkaline Phosphatase in Soil

In soil, the enzyme alkaline phosphatase (AP) hydrolyzes esters and anhydrides of phosphoric acid, thereby offering adequate plant nutrition that enhances plant growth. However, AP's activity is extremely sensitive to various agricultural practices and changing environmental conditions.

To increase AP's activity in soil, various activators are added to enhance AP's affinity to its substrate (phosphoric acid) and increase its catalytic efficiency. According to Khadem et al., biochar (a product of biomass pyrolysis at different temperatures at low oxygen conditions) was observed to increase the activity of AP in soil by improving the soil's physical, chemical and biological properties, however, several other studies show no or a negative effect of biochar on AP activity, depending on the soil type, the type of biochar, the experimental conditions and the durations. Khadem et al. found that biochar decreased the affinity of AP to its substrate (high Km value), while maintaining its concentration and efficiency (high Vmax) in clayey soil.

In the state of Qatar, nitrate fertilizers are commonly used instead of biochar. Tomlinson et al. suggested that nitrates lower AP enzyme activity in soil, however, they failed to report the kinetic parameters of enzyme affinity to its substrate (Km value) and enzyme efficiency (Vmax value).

Figure 1: Nitrogen Fertilizer:
Nitrogen fertilizers are widely used around the world, especially in Qatar. Approximately, half the food produced in the world is produced using nitrogen fertilizers. Nitrogen is primarily added to the soil to improve its quality, and to enhance plant metabolism and growth.

Nitrogen Fertilizer. Retrieved from Amazon.

Aim of the Study

This study aims to determine the amount of liquid nitrate fertilizer that maximally activates AP by investigating its effects on AP affinity (Km value) and efficiency (Vmax value) by carrying out an enzyme kinetic assay for three minutes, measured at one second intervals, at each varying concentration.

Hypothesis

We hypothesize that AP activity decreases with the increasing addition of liquid nitrate fertilizer.

Methods: Enzyme Kinetic Assay:

Experimental Conditions for AP Activity Measurement. The enzyme kinetic scans were carried out on the Thermo Fisher UV60 Spectrophotometer, at absorbance 410 nm. The absorbance was measured at one second intervals for 3 minutes. *p*-Nitrophenyl Phosphate (pNPP) was the substrate for this reaction with *E.Coli* AP, and it turns to o-nitrophenol (ONP), which is yellow, and this allows for detection by the spectrophotometer at 410 nm. The buffer used for all the reactions is 1.0M Diethanolamine buffer with 0.50mM Magnesium Chloride, pH 9.8 at 37°C (Vincent, Doonan. 2020). Each scan was performed in triplicates for statistical significance.

Enzyme Saturation Curve. The enzyme saturation curve was determined by varying the concentration of enzyme (0.025 – 0.0015625 Units (U)), and the pNPP concentration was kept at 11.2mM. Information from these scans was used to plot the enzyme saturation curve.

Generating the Michaelis -Menten plot. To find the optimum concentration of pNPP, the enzyme concentration was kept at 0.00625U, and the concentration of pNPP was varied from 0.0012-1.12mM. A Michaelis Menten plot and a Lineweaver-Burk plot were made using that information. The Lineweaver-Burk plot is used to determine Km and Vmax from the equation of the line.

Effect of Nitrogen Fertilizer. To find the effect of the Nitrogen fertilizer on the rate of AP, the concentration of the nitrogen fertilizer stock was varied from 0-20% Nitrogen. The concentration of enzyme used was 0.00625U, and the concentration of pNPP was 0.0616mM. Using this information, a bar chart of nitrogen amount against the rate was plotted. Next, using the two nitrogen percentages that expressed the highest AP rates, the pNPP concentration was varied, and a Michaelis Menten plot and a Lineweaver Burk plot were plotted.

Results and Discussion

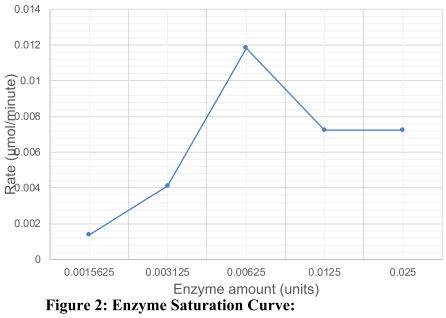

Figure 2: Enzyme Saturation Curve:
The rate of reaction in µmol/minute is plotted against varying enzyme amounts (in units) at a constant substrate (pNPP) concentration. The optimal amount of enzyme (0.00625 U) is used to carry out further experiments, ensuring that the enzyme is not a limiting factor in the experiments.

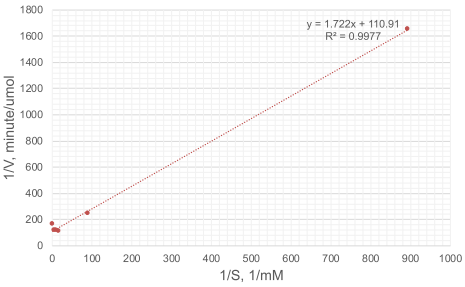

Figure 3: Lineweaver-Burk (LB) plot:
The LB plot is the reciprocal of the Michaelis-Menten plot, keeping the enzyme amount the same (0.00625U) while varying the substrate concentration (pNPP) to find the rate in µmol/minute. The base-line Km and Vmax values are computed (without the addition of nitrogen fertilizer). The Km, corresponding to AP's affinity to pNPP, is 0.0155 mM, while Vmax, or AP's efficiency, is 0.00902 µmol/min.

Table 1: Base-line Km and Vmax values (without the addition of nitrogen) from Figure 3

Vmax	0.00902 µmol/min
Km	0.0155 mM

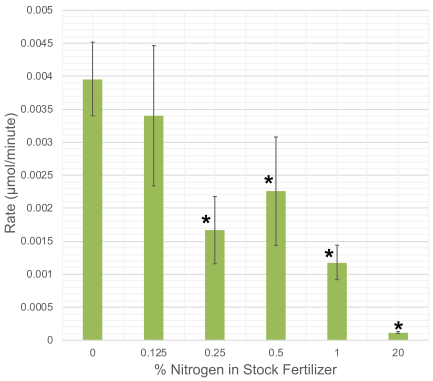

Figure 4: Rate in µmol/min at varying nitrogen concentrations (%).
A bar chart is plotted of the rate of reaction in µmol/min at different concentrations of nitrogen fertilizer (%) added to 0.00625U of enzyme and 0.0616mM of pNPP. The asterisks represent statistically significant data with a p-value below 0.05. The overall observed trend is that increasing the concentration of nitrogen (%) reduces the rate of AP's reaction.

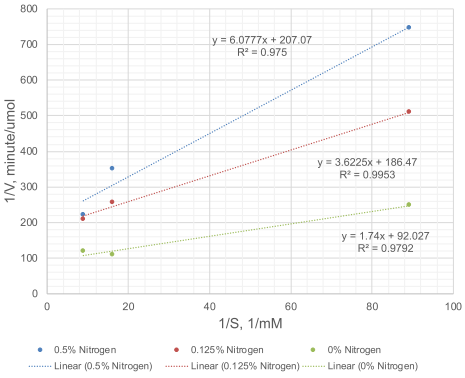

Figure 5: Lineweaver-Burk (LB) plot for varying amounts of Nitrogen fertilizer:
The LB plot is the reciprocal of the Michaelis-Menten plot, keeping the enzyme amount constant at 0.00625U, and varying the substrate concentration. The nitrogen concentrations 0%, 0.125% and 0.5% are assessed and plotted. The Km and Vmax values are reported in table 2 below.

Table 2: Km and Vmax values at different nitrogen concentrations (%).

	Vmax (µmol/min)	Km (mM)
0% Nitrogen	0.0109	0.0189
0.125% Nitrogen	0.00536	0.0194
0.5% Nitrogen	0.00483	0.0294

Non- Competitive Inhibition

Conclusion

According to the plot on figure 5 and table 2's data, we can affirm that increasing the nitrogen concentration in liquid fertilizer decreases the rate of AP. This is because the Vmax decreases as the nitrogen concentration is increased, while the Km stays almost constant, indicating non-competitive inhibition. Non-competitive inhibition is when the inhibitor binds away from the active site of the enzyme independently of the substrate's binding (Keith, P., et al. 2019). This could have implications on the soil quality since nitrogen fertilizer inhibits AP from hydrolyzing esters and anhydrides of phosphoric acid, which prevents the plant from receiving its full nutrition required for growth. Therefore, nitrogen-free fertilizers or fertilizers with little nitrogen could be better for the plant. However, if using a nitrogen fertilizer is essential, the soil could be fortified with nutrients otherwise provided by AP.

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