A study of the effect of temperature on the activity of the glutaminase enzyme and the kinetic values in calcareous soil

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Abstract

The study was carried out in Department of Soil Sciences and Water Resources Lab, College of Agriculture and Forestry, to determine the kinetic parameters (maximum speed Vmax and Substrate matter concentration Km) and to show how temperature influences the activity and performance of the Glutaminase enzyme. therefore, clay loam soil from the Rashidieh district was selected, and it was treated with various Substrate enzyme concentrations (0.15, 0.25, 0.5, 0.75, and 1) molar under various temperature influences (10, 20, 30, 40, 50, 60, and 70) C^{o}.

The results demonstrated: a definite influence of the substrate on the enzyme's activity, as the enzyme's activity increased as the amount of added substrate got in concentration. The greatest value for the Glutaminase enzyme activity [S]/ V was (0.019) microgram NH4-N g^{-1}. 2 hr^{-1}, which came as a result of the substrate's concentration of (1) mmol. The enzyme Glutaminase [S] /V had the lowest activity, measured in micrograms (0.004) NH4-N g^{-1}. $2h^{-1}$, as a result of the lowest substrate concentration (0.1) mmol. According to the research results, the Vmax value was reached (0.815) micrograms of NH4-N g^{-1} $2hr^{-1}$. Km had a value of (0.033) mmole. The Glutaminase enzyme's activity increased as the incubation temperature was raised when the substrate concentration was (0.5) molar. The highest value for Glutaminase enzyme activity was (213.70) micrograms of (NH4-N g^{-1} $2hr^{-1}$), which was obtained from incubating the soil at the highest temperature (60) C^{o}, as the Glutamines enzyme activity values increased.

Keywords: Micrograms, Glutaminase enzyme, Substrate, Vmax, Temperature.

Introduction

Soil enzymes are the main key to the processes of analyzing organic compounds, nutrient availability, and other biochemical processes in the soil [3]. They are also cofactors composed of highly specialized proteins with catalytic properties that increase the rate of the reaction without changing the enzyme properties after the reaction is completed [21].

Enzymes are highly sensitive to environmental changes and often react to soil management changes faster than other soil variables, which makes enzymes an attractive option for determining the state of soil fertility. They also show an integrated biological assessment of the activities of soil, particularly those that catalyze a variety of activities, including Asparaginase, Urease, Phosphatase, and Dehydrogenase [16]. Glutaminase is a widespread enzyme in nature, secreted primarily by microorganisms [19], also by many animals [27], and plants [10]. It is important in breaking the C-N bonds of straight non-peptide amides [29]. Galstyan and Saakyan [15] investigated the efficiency and function of this enzyme and discovered that it hydrolyzes the Amino acid L-glutamine, producing Glutamic acid and Ammonia. According to Almeida et al. [4], microbial species release enzymes into the environment to decompose complex organic molecules into simple absorbable molecules. Soil enzymes, as

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a result, stimulate and increase biochemical reactions [17], which result in the decomposition of organic wastes and their conversion into mineral forms available for plants, as well as soil aggregates forming [8]. Microorganisms also play an important role in soil by controlling an extensive number of important soil processes [13], [12]. According to Malherbe and Marais [23], controlling the constituents of agricultural soil is a crucial aspect of the soil's biological composition. According to Kayler et al. [20], ventilation plays a significant influence in the growth of microorganisms. In order to prepare nutrients and preserve soil fertility, living things and microbial diversity are crucial [26]. According to Srilatha et al. [28], because of their quick reactions and connection to the soil environment, enzymes play a significant role in recycling nutrients and stimulating biochemical reactions in the soil. Regarding changes to soil management. Considering enzymes are vital in the cycling of elements and the ability to operate in the soil, measurements of soil enzyme activity are significant for identifying the condition of the soil's surroundings. Soil enzymes have an essential function as a marker of good or negative changes in the handling of soils due to their extreme reactivity to these alterations and simplicity of quantification [9], [18].

Because enzymes are critical to many soil transformations that directly influence soil fertility, such as the breakdown of biological nutrients and their transfer of minerals into their biological state to other forms, [25] and [22] have suggested that enzymatic activity is a measure of soil fertility. Enzymes produced by pathogens, particularly Microbes as well as fungi, are the only means by which the plant can obtain the inorganic form. The enzyme protease breaks down proteins to release nitrogen in a form of acid, whereas the enzyme phosphatase breaks down organic phosphorus like phytin, DNA, and RNA to produce phosphoric acid that is ready for plant, and so on.

The Urease enzyme's function lies in the breakdown urea added to the soil as a fertilizer or to decompose animal and plant manure producing carbon dioxide and ammonia with a slight increase in soil pH [5]. The phosphatase enzyme is important as well as efficient in the soil ecosystem [14], as it mineralizes organic phosphorus and converts it to available form for the plants .

Materials and Methods

Soil sampling and preparing for physical and chemical analyses:

Soil has been taken out from the Rashidiya area at a depth of (0-15) cm for the study. The soil was transported in plastic bags to the laboratory, air-dried, ground with a wooden hammer, and sieved with a 2 mm diameter sieve. The soil was refrigerated until used in the laboratory experiment, and its chemical, physical, and biological properties were estimated, as shown in Table (1).

Table (1): Physical and chemical characteristics of the study soil.

Properties	Value	Unit
pH	7.3	/
EC	0.43	dS m^{-1}
OM	11.4	g kg^{-1}
CEC	22.4	$Cmol_c$ kg^{-1} soil
$CaCO_3$	239	$g.kg^{-1}$

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Available N	24.3	mg kg^{-1}
Available P	6.5	mg kg^{-1}
Available K	282	mg kg^{-1}
Field Capacity	240	g kg^{-1}
Bulk Density	1.29	*M*g m^{-3}
Sand	153.3	g kg^{-1}
Silt	520.5	g kg^{-1}
Clay	326.2	g kg^{-1}
Texture	SiCL	/
No. of Bacteria	54 x 10^5	Cfu g^{-1} dry soil
No. of fungi	25.4 x 10^3	Cfu g^{-1} dry soil

L-Glutaminase enzyme activity:

Enzyme activity was determined according to Tabatabai and Bremner [30] by incubating 5 g of soil with 0.2 ml of toluene, 9 cm^3 of buffer solution (Tris Hydroxy methyl Amino THAM Methane) with pH 10, and 1 ml of Substrate 0.5 N Glutamine solution at different temperatures (10, 20, 30, 40, 50, 60, and 70)°C for two hours, after which a 2.5 molar KCl solution containing 100 $mg.L^{-1}$ of Ag_2SO_4 to inhibit enzyme activity. Then ammonium ion arising from enzyme production was calculated using distillation with steam, as stated by Bremner and Edwards (1965) using heavy magnesium oxide (MgO), , by receiving the ammonia with boric acid as the indicator, then extracting with hydrochloric acid 0.005 N.

- Study of kinetic parameters.

The kinetic parameters of the glutaminase enzyme, including the maximum speed of the enzyme (Vmax) and the Michaelis constant (Km), were investigated for the studied soil at (0-15) cm depth using various substrate amounts (0.1, 0.25, 0.5, 0.75, 1) molar. After estimating enzyme activity, the values of Vmax and Km were calculated using the Hanes-Woolf transformation formula, which was modified from the Michaelis-Menten, [24] equation:

$$\frac{s}{v} = \frac{km}{v\,max} + \frac{1}{v\,max}[S] \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Were:

V: reaction velocity (hr)

Vmax: maximum speed of the enzyme

Km: Michaelis constant

S: Substrate concentration (mol. L^{-1})

The kinematic parameters Km and Vmax were extracted from the slope of the straight line equation representing 1/(v max) and the intercept representing km/(v max).

-Statistical analysis :

Complete Randomized Design (CRD) was used to analyze the characteristics studied in the laboratory experiment for three replications using analysis of variance using the SAS program (2010).

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The averages were compared using the least significant difference (L.S.D) at the significant level (0.05) [5].

Results and discussion

Influence of temperature on Glutaminase enzyme activity

Figure (1) showed an increase in Glutaminase enzyme activity as the incubation temperature for the study soil increased from 10 to 60 °C. Increases in incubation temperature above 60 °C resulted in a significant decrease in enzyme activity. The results show that the glutaminase enzyme's activity increased significantly until incubation. The soil at (60) °C achieved the lowest value for glutaminase enzyme activity (52.80) micrograms of NH4-N g^{-1} soil 2 hr^{-1}, which was obtained by incubating the soil at the lowest temperature of (10) °C. While the highest value for glutaminase enzyme activity was (213.70) micrograms of NH4-N g^{-1} soil 2 hr^{-1}, which was the result of soil incubating at (60) °C, glutaminase activity values continued to increase even at (60) °C. Then, at 70°C, there was a decrease. At the 0.05 comparison level, the least significant difference (LSD) was (0.000281), indicating the presence of large significant differences between the effects of the coefficients.

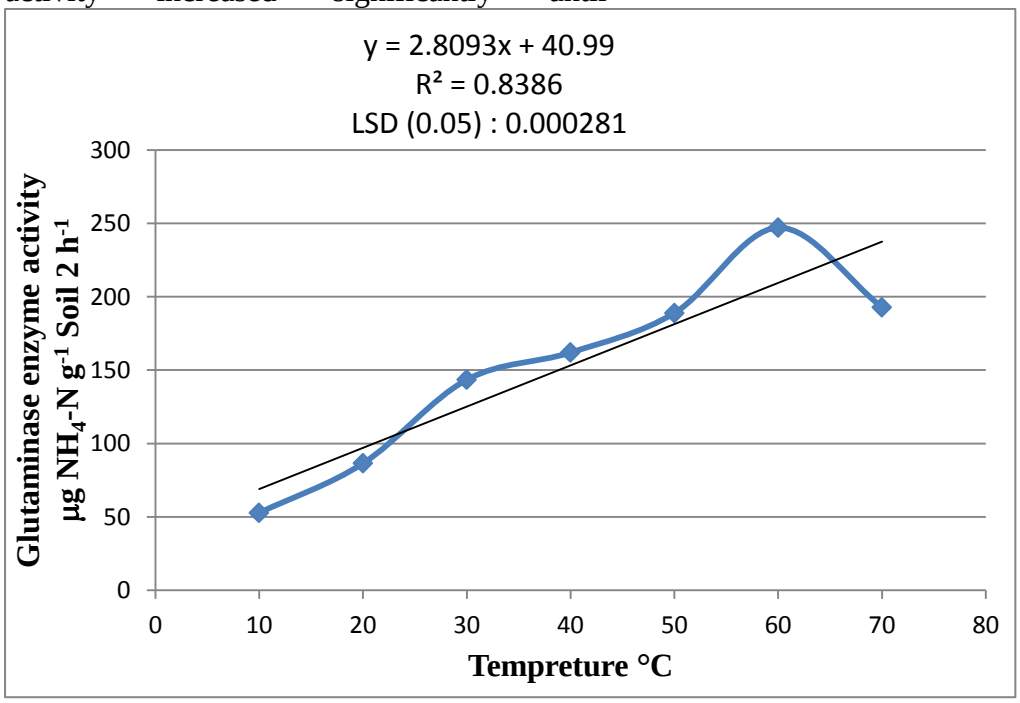

Figure (1) Relationship between temperature and Glutaminase enzyme activity.

Most chemical reactions speeds up as temperature increases, because rising temperatures give reacting molecules more kinetic energy, resulting in more collisions per unit time. Enzymatic reactions are affected similarly until certain temperature limits are reached, at which point the glutaminase enzyme is negatively affected due to a change in enzyme composition [2]. These findings suggest that glutaminase enzyme activity can increase to a point and then begin to decrease as the temperature rises. [2] realized an increase in the activity of the basic and acid phosphatase enzymes with an increase in the incubation temperature from (10-60) °C for all soils studied for both depths from (0-15) cm to (15-30) cm, and that an incubation temperature increased more than 60 °C led to a sharp significant decrease in enzyme activity. These findings indicate that the alkaline phosphatase enzyme will be very active when the temperature rises above

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(30)°C, and these result are consistent with the findings of [7], [3].

Influence of substrate concentration on kinetic parameters of the glutaminase enzyme:

Figure (2) depicts the linear relationship between substrate glutamine [S] concentration and enzyme glutaminase activity [S]/V in the study soil at (37)°C using the Hanes-Woolf formula. The results demonstrate that the enzyme's effectiveness increases with increasing substrate concentrations. The highest value for glutaminase [S]/V enzyme activity was (0.019) microgram NH4-N g^{-1} 2 hr^{-1}, which resulted from the effect of (1) mill molar substrate concentration.

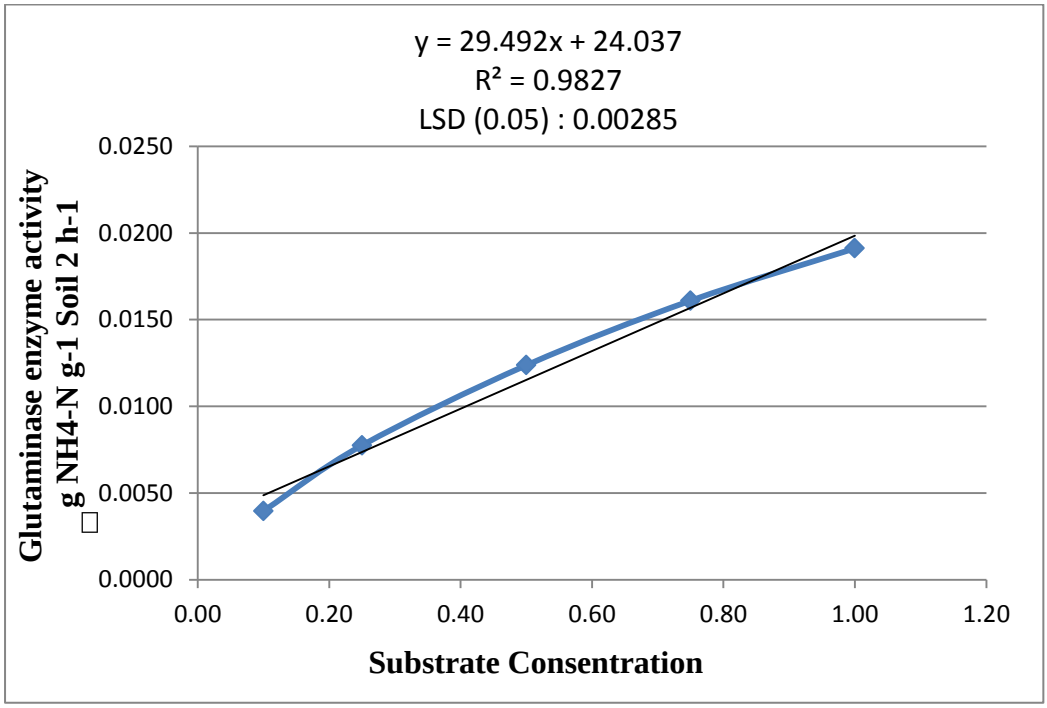

Figure (2) Relationship between the substrate glutamine concentration and glutaminase enzyme activity

The effect of the lowest substrate concentration (0.1) mill molar produced the lowest value for glutaminase enzyme activity [S]/V, which was (0.004) micrograms g^{-1} $2h^{-1}$. It additionally indicates that the glutaminase enzyme effectiveness did not appear to decrease even at the higher concentration used, suggesting that the glutaminase enzyme effectiveness may increase in the event that higher substrate concentrations are being used.

The straight-line equation for the relationship between substrate concentrations and glutaminase enzyme activity was used to calculate the value of Vmax, which is equal to 1/slope, and the value of Km, which is the outcome of multiplying the value of Vmax by the secant. (0.815) micrograms of N-NH4 g^{-1} 2 hr^{-1} was the Vmax value. While the value of Km reached (0.033) mill molar (Figure 3), this result was less than what [7] and [6] obtained. In general, the value of Km indicates the affinity between the substrate and the enzyme. Low Km values indicate a high affinity between the glutaminase enzyme and the substrate, as the enzyme requires less substrate to reach its maximum speed. The Km values did not follow a consistent trend across all soils and studied area, which could be attributed to the different sources of enzyme preparation on the one hand, and the fact that these values do not depend on enzyme

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concentration in the soil on the other hand. The low values between field and orchard soils in different Diwaniyah Governorate locations weren't in line with a specific trend, indicating a clear difference in enzyme preparation sources, the basis of which are the prevailing organisms and root secretions [3]. [6] discovered the enzyme alkaline phosphatase in some soils in southern Iraq, and pointing out that the Michaelis constant values ranged from (0.5-1.4) mill molar and the maximum velocity values ranged from (400-500) micrograms of PNP g^{-1} soil 1 hr^{-1}.

[7] proved a difference in Vmax and Km values for the Amidase enzyme among ten soils studied, stating that the Km values fluctuated between (2.56-44.19) mill molar to (12.88-446.68) microgram-N NH4 g-1 soil.2 hr-1 The Vmax and Km values for the Amidase enzyme in the Rhizosphere soil of wheat and bean plants were also different, with Vmax values of (205.88 and 216.59) micrograms of N - NH4 g^{-1} soil 2 h^{-1} and Km values of (10.78 and 9.86) mill molar, respectively. The difference is attributed to variability in the nature of plant root secretions, which is followed by variation in the number and type of microorganisms that secrete the enzyme. Large significant differences exist between the effects of the coefficients, as indicated by the value of the least significant difference (LSD) at the 0.05 comparison level, which was (0.00285). The high Vmax value denotes both the high efficacy of the enzymes in the soil and the variation in their composition and sources, which can be attributed to the substantial amounts of organic matter in the study soil, the variety and quantity of microorganisms in the forest soil, the amount of root secretions, and the types of clay minerals [7].

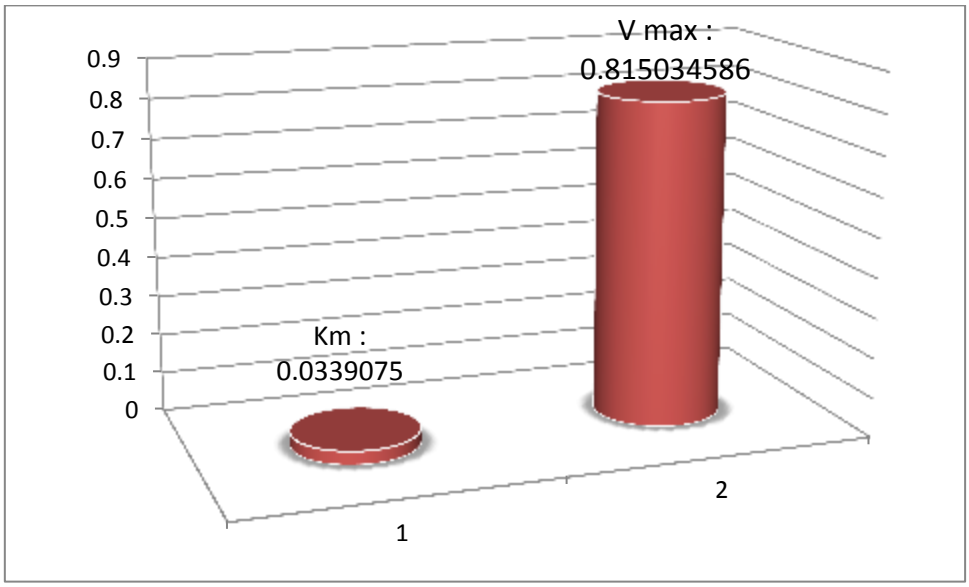

Figure (3) Vmax and Km values for glutaminase enzyme activity in the study soil.

Conclusion

Investigate the activity of the Glutaminase enzyme in various soils and plant covers, as well as the effect of different salinity levels on the activity and effectiveness of the Glutaminase enzyme in soil, and the effect of incubating the Glutaminase enzyme for a longer period of time. Conducting agricultural investigations on the function of macronutrient chemical fertilization and its influence on the efficacy of enzymes in the soil, as well as the role of enzymes in soil fertility.

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