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Urease Enzyme and Its Kinetic and Thermodynamic Parameters in Clay Loam Soil

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In the present work, urease enzyme activity of clay loam soil (pH 7.1, organic matter 2.26%, C/N ratio 16), kinetic (V_{max}, V_m and V_{max}/K_m) values and thermodynamic (E_a, ΔH, ΔS and ΔG) parameters of this enzyme is determined. For this purpose, kinetic and thermodynamic parameters are calculated by determining urease activity in soil in different substrate concentrations (0, 1, 2, 4, 6, 8, 10 and 12%), incubation periods (0, 1, 2, 3, 4, 5 and 6 h) and incubation temp. (0, 10, 20, 30, 40 and 50°C). From the results, it is determined that the speed of reaction starts to become static, while substrate concentration reaches to 8% level and becomes static at 10% level and the highest speed of reaction in substrate concentrations occurs at incubation temperature of 50°C. The highest V_{max}, V_m and V_{max}/K_m values are determined at 40 and 50°C and the highest E_a, ΔH and ΔS values are determined in substrate concentration of 10%. It was also determined that ΔG varies between 1.870–2.145 in all substrate concentrations depending on temperature.

Key Words: Urease, Activation energy, Soil, Michaelis-Menten, Arrhenius, Kinetic, Thermodynamic.

INTRODUCTION

The enzyme urease (urea amidohydrolase EC 3.5.1.5) catalyzes the hydrolysis of urea[1, 2]. The products of the reaction are ammonia and carbonate (Eqn. 1), the latter spontaneously decomposing to ammonia and carbonic acid (Eqn. 2):

$$H_2N—CO—NH_2 + H_2O \xrightarrow{\text{Urease}} NH_3 + H_2N—CO—OH \quad (1)$$

$$H_2N—CO—OH + H_2O \longrightarrow NH_3 + H_2CO_3 \quad (2)$$

The released products, *i.e.*, carbonic acid and ammonia are in equilibrium with their ions (Eqn. 3 and 4), resulting in a net increase in pH of the reaction solution:

$$H_2CO_3 + H_2O \rightleftharpoons H^+ + HCO_3^- \quad (3)$$

$$2NH_3 + 2H_2O \rightleftharpoons 2NH_4^+ + 2OH^- \quad (4)$$

Urease is widely distributed in nature[1-3]. It is found in bacteria, fungi, algae,

some invertebrates, higher plants and in soil as a soil enzyme. By catalyzing the hydrolysis of urea, urease allows organisms to use exogenous and internally generated urea as a nitrogen source. In soils it converts urea to utilizable ammonia. Urease from the plant source *Canavalia ensiformis* (jack bean) was the first enzyme ever crystallized[4] in 1926 and the first nickel containing enzyme identified[5] in 1975. Although ureases from different sources have different protein subunit structures, there is ample evidence provided by amino acids sequence alignments and reaction kinetics that they all are forms of one enzyme having one common structure and catalytic mechanism[6].

Ureases attract attention because of the role they play in human and animal health[7, 8] and in agriculture[9, 10]. Urea, the most extensively used N fertilizer in agriculture[11], undergoes hydrolysis to release nitrogen in the form of ammonia, before being utilized by crop. Hydrolysis of urea, mediated by the enzyme urease is produced by soil micro-organisms and released into the soil for its action. Thus, urease activity in soil is generally regarded as extracellular[12]. Attempts to improve N-efficiency of applied urea fertilizer in farming mostly aim at delaying the rate of urea hydrolysis in soils. Information on the nature of urease activity of a soil and the changes in the urea hydrolysis is beneficial to develop and employ strategies for efficient N management.

The nature and activity of soil urease is affected by many factors, including soil physico-chemical properties and agricultural practices. While urease activity as a function of several soil parameters has been studied in detail[13–15], there is no report on kinetics and thermodynamics of urease in soil. This research studies the kinetics and thermodynamics of urease in a clay soil under laboratory conditions.

EXPERIMENTAL

Soils: Surface soil (0–20 cm) was taken from the experimental station in the campus of Agricultural Faculty, Ondokuz Mayis University. The soil was developed from basalt and contained 31.2% clay, 36.2% silt and 32.6% sand. Soil texture can accordingly be classified as clay loam (CL). The pH of water was 7.1, the oxidizable organic matter content was 2.26% and the soil C : N ratio was 16. The soil samples were air-dried in a laboratory and sieved through 0–2 mm screens. The samples (750 g air-dried soil) were placed in 1 L cylindrical plastic containers. The moisture contents in the soils were adjusted to 60% water holding capacity (WHC) and the containers were incubated at 25 ± 0.5°C for 30 d in the laboratory. The soil moisture was kept at the same level (60% WHC) by adding distilled water at regular intervals throughout the incubation period. At the end of the incubation period, these samples were used to determine urease activity of soils at the moisture condition.

Urease activity in soil: Urease (EC 3.5.1.5) activity (UA) was measured by the method of Hoffmann and Teicher[16]. 0.25 mL toluene, 0.75 mL citrate buffer (pH 6.7) and 1 mL of urea substrate solution were added to the 1 g sample and the samples were incubated. The formation of ammonia was determined spectrophotometrically at 578 nm and the results were expressed as μg N g^{-1} dry sample. All determinations of urease activities were performed in triplicate and all values

reported are averages of the three determinations expressed on an oven-dried soil basis (105°C).

Kinetic and thermodynamic parameters in soil: Kinetic and thermodynamic parameters were determined by using eight different concentrations of the substrate, urea, varying from unsaturated to saturated conditions: 0, 1, 2, 4, 8, 10 and 12 w/v each at different incubation times (0, 1, 2, 3, 4, 5 and 6 h) and incubation temperatures (0, 10, 20, 30, 40 and 50°C). Michaelis-Menten (Eqn. 5) linearized by Lineweaver-Burk (Eqn. 6) is used to determine V_{max}, K_m and V_{max}/K_m kinetic parameters[17–20].

$$V = V_{max}[S]/([S] + K_m) \quad (5)$$

where V = initial velocity, μg N g^{-1} min^{-1}
[S] = substrate urea concentration, %
V_{max} = maximum initial velocity, μg N g^{-1} min^{-1}
K_m = Michaelis constant, μg N g^{-1}

$$[S]/V = [S]/V_{max} + K_m/V_{max} \quad (6)$$

The initial velocity of reaction (V) is calculated in Eqn. (7).

$$V = \Delta C/\Delta t \quad (7)$$

where ΔC = change in amount of output during the beginning of reaction, μg N g^{-1}
Δt = change in time during the beginning of reaction, min

The linearized version (Eqn. 9) of Arrhenius equation (Eqn. 8) is used by assuming k ≈ V in order to determine E_a and A parameters in urease enzymes.

$$k = Ae^{-E_a/RT} \quad (8)$$

$$\ln k = \ln A - \frac{E_a}{R}\frac{1}{T} \quad (9)$$

where k = velocity constant, min^{-1}
T = t°C + 273.15 K
E_a = activation energy, kJ mol^{-1}
A = frequency factor, μg g^{-1} min^{-1}
R = 8.314 J K^{-1} mol^{-1}

The following equations (Eqns. 10–12) are used in order to calculate thermodynamic parameters of enthalphy variance (ΔH), entrophy variance (ΔS) and Gibbs energy (ΔG)[17–20].

$$\Delta H = -aR \quad (10)$$

$$-\Delta S = [b - \ln (2.084 \times 10^{10})]R \quad (11)$$

$$\Delta G(T) = \Delta H - (T \times \Delta S/1000) \quad (12)$$

where a = slope of relationship between ln (k/T) and 1/T
b = ordinate of relationship between ln (k/T) and 1/T on 1/T = 0.

Statistical analysis: The results from urease assays were examined and kinetic and thermodynamic parameters were calculated. Means and standard deviation of triplicates were determined and all the figures presented including standard

errors of the data. Analysis of variance (2 way ANOVA) was carried out using three factors randomized complete plot design. The means were compared using LSD (least significant difference) test with a significance level of $P < 0.01$. All statistical calculations were performed using MSTAT and SPSS 11.0 softwares.

RESULTS AND DISCUSSION

Different substrate concentrations, incubation period and values of urease activity in incubation temperatures of clay loam soils are given in Fig. 1. According to the obtained results, it was determined that there are significant increases in urease activity, while increase in substrate concentrations occurs. Similarly, the significant increases in urease activity arise while incubation temperature increases and incubation period is stretched. Based on results of conducted statistical evaluations, the effect of all factors on urease activity has been found to be significant ($P < 0.01$)

Kinetic parameters: The variance of reaction velocity (V) in different incubation temperatures, which is based on ascending substrate concentration, is given in Fig. 2. The relationships between V and substrate concentration is hyperbolic; velocity of reaction increases as substrate concentration and incubation temperature increase. V starts to become static at [S] > 8% and it becomes static at 10% level. The highest incubation temperature, in which velocity is on the highest level, is 50°C.

Kinetic parameters belonging to urease enzyme activity of clay loam soil and graph of Lineweaver-Burk equation, which is linearized version of Michaelis-Menten equation are given in Table-1 and Fig. 3, consecutively. V_{max} represents velocity of decomposition of enzyme-substrate concentration into enzyme and reaction outputs, high or low value of this parameter implies the occurrence of high or low levels of enzymatic activities in soil[17, 18, 21–23]. V_{max} value of this clay loam soil increases as temperature rises. The increase in V_{max}, in each temperature variation was determined to be 37, 16, 59, 28 and 77% in turn in order. The highest value of V_{max} was observed at 50°C.

TABLE-1
KINETIC PARAMETERS OF CLAY LOAM SOIL IN DIFFERENT INCUBATION TEMPERATURES

Kinetic parameters	Temperature (°C)					
	0	10	20	30	40	50
V_{max}, μg N g^{-1} min^{-1}	0.094	0.129	0.149	0.237	0.304	0.537
K_m, μg N g^{-1}	0.396	0.641	0.744	0.939	1.329	1.177
V_{max}/K_m, min^{-1}	0.236	0.201	0.200	0.253	0.229	0.456

The amount of K_m enzyme has a criterion, which is related with substrate. K_m expresses endurance of enzyme-substrate complex. Moreover, the reverse relationship between endurance of enzyme-substrate complex and K_m values is determined, meaning that the endurance of enzyme-substrate complex is on high level, when the value K_m is low and the endurance of enzyme-substrate complex is on low

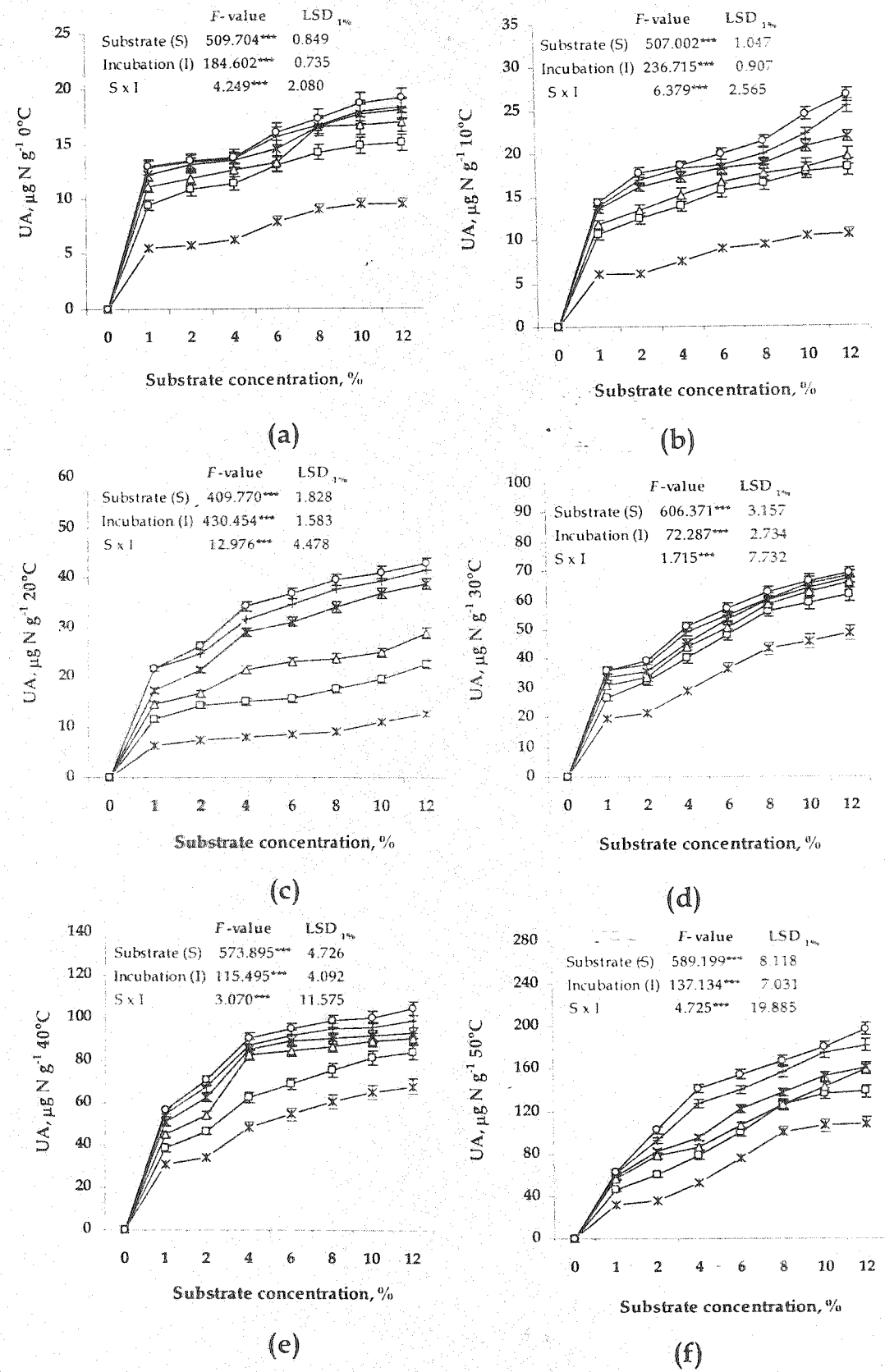

Fig. 1. Variance in urease activity (UA) of clay loam soil determined in different substrate (urea) concentrations and incubation periods (1, 2, 3, 4, 5 and 6 h): (a) 0°C (b) 10°C (c) 20°C (d) 30°C (e) 40°C (f) 50°C. (-*- 1 h; -□- 2 h; -△- 3 h; -×- 4 h; -+- 5 h; -○- 6 h)

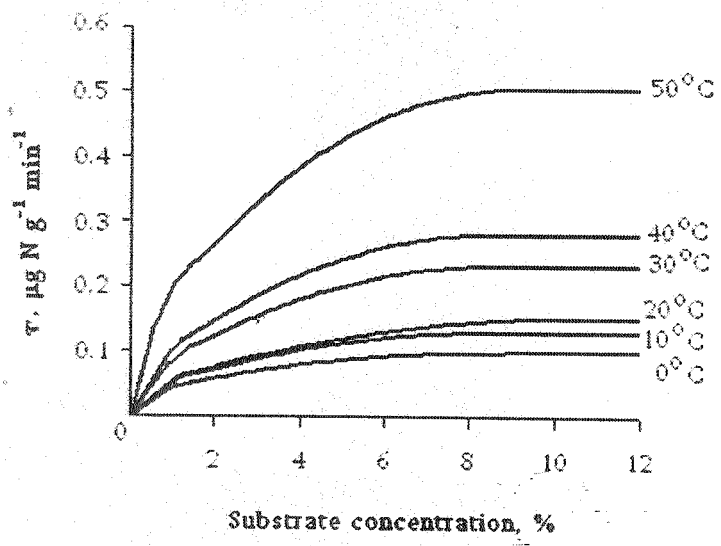

Fig. 2. The variance of reaction velocity (V) based on substrate concentration in different temperatures

level, when the value K_m is high[17, 18, 21–23]. It was determined that K_m value increases as incubation temperature increases in this clay loam soil. However, the highest K_m value was fixed at 40°C.

V_{max}/K_m expresses comparison of formation of enzyme-substrate complex in soil and distribution of that complex. The high value of this ratio implies that the distribution of enzyme-substrate complex is higher than its formation in soil[12, 17, 18, 22, 23]. The highest V_{max}/K_m ratio in clay loam soil was fixed at 50C° temperature.

Thermodynamic parameters: Arrhenius graph between ln k and 1/T used in calculation of E_a and A parameters of urease activity in clay loam soil, the relationship between ln (k/T) and 1/T used in calculation of enthalphy (ΔH) and entrophy (ΔS) in formation of urease activity's enzyme-substrate complex variances and obtained results are given in Figs. 4 and 5 and Table-2, consecutively.

E_a (activation energy) is a minimum kinetic energy of substances entering to reaction essential for constitution of output[20, 24]. The greatest E_a value of clay loam soil was obtained in substrate concentration of 10%. A is measurement unit

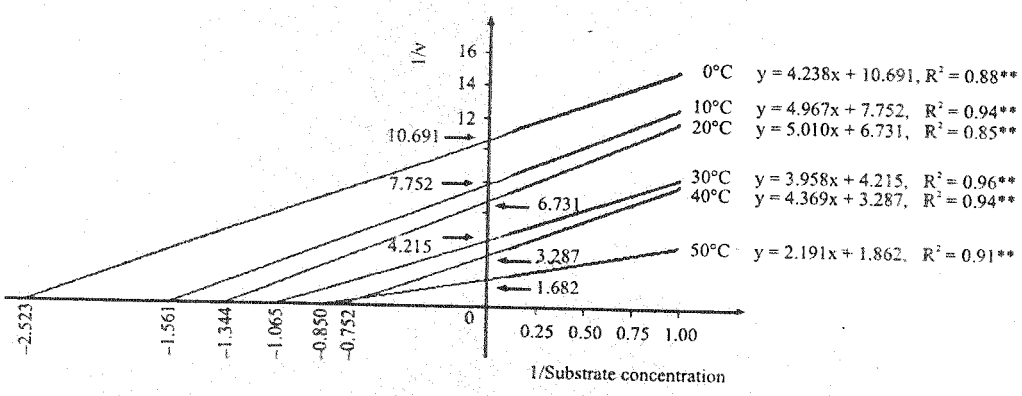

Fig. 3. Curves of Lineweaver Burk Eqn.

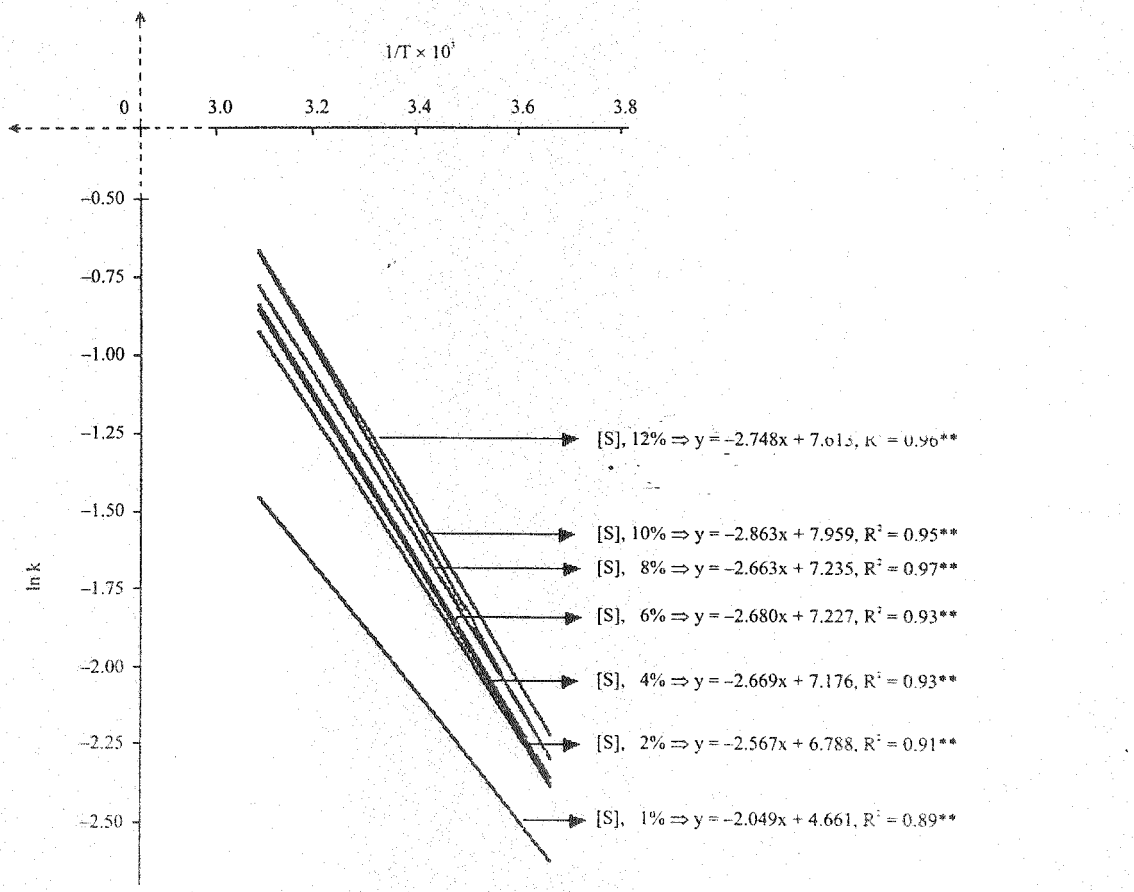

Fig. 4. Arrhenius graph between ln k and 1/T (slope = E_a/R and ordinate at 1/T = 0 equal to ln A)

of velocity of collision occurring between molecules in reaction[19]. The great value of A significantly contributes to velocity of active collisions. The greatest A value in clay loam soil was obtained in substrate concentration of 10% as well, like E_a value. ΔH is the variance of potential energy that the system owns in formation and distribution of substrate complex. In clay loam soil, ΔH increases depending on increase in substrate concentration[19, 20, 24]. In all substrate concentrations the expression of $\Delta H > 0$ is valid and formation of enzyme-substrate complex is an endothermal process. The greatest ΔH values was obtained in substrate concentration of 10%.

ΔS, represents configuration of variance during reaction process[20]. ΔS increases as substrate concentration increases and it becomes static after a certain level. The greatest value of ΔS was again obtained in substrate concentration of 10%.

The differences between Gibbs free energies of different incubation temperatures in each substrate concentration have been determined to be 2.145, 1.968, 1.935, 1.931, 1.930, 1.870 and 1.899 in order and the least difference has fixed in substrate concentration of 10%. This case reveals that substrate concentration of 10% has the most suitable conditions for equilibrium in the formation and distribution of enzyme-substrate complex in substrate concentration of 10%.

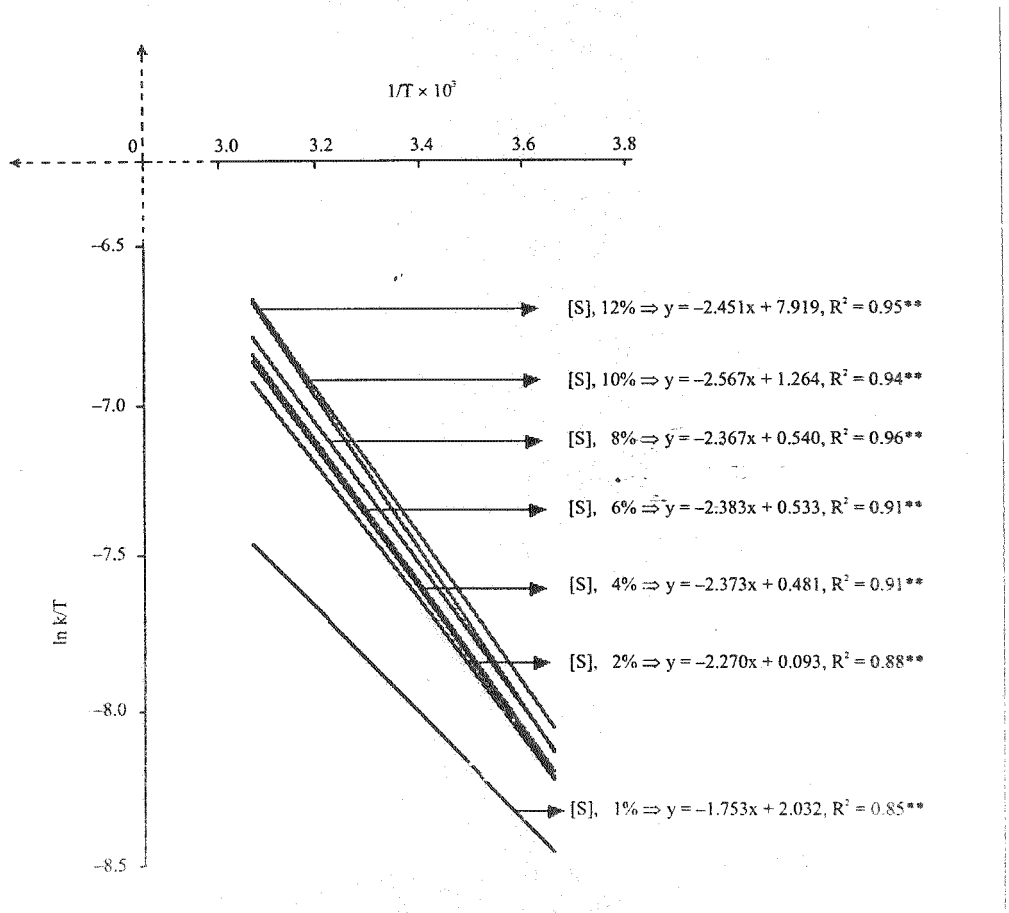

Fig. 5. Relationship between ln (k/T) and 1/T)

TABLE-2
THERMODYNAMIC PARAMETERS OF CLAY LOAM SOILS
IN DIFFERENT INCUBATION TEMPERATURES

Thermodynamic parameters	Substrate concentration (%)						
	1	2	4	6	8	10	12
E_a, kJmol^{-1}	17.035	21.342	22.190	22.282	22.140	23.803	22.847
A, μg g^{-1} min^{-1}	105.742	887.138	1307.667	1376.088	1387.141	2861.210	2024.342
ΔH, kJmol^{-1}	14.574	18.873	19.729	19.812	19.679	21.342	20.378
ΔS, J mol^{-1} K^{-1}	−214.435	−196.759	−193.542	−193.109	−193.051	−187.032	−189.900
ΔG (273.15), kJ mol^{-1}	73.147	72.618	72.595	72.560	72.411	72.430	72.249
ΔG (283.15), kJ mol^{-1}	75.291	74.585	74.530	74.491	74.341	74.300	74.148
ΔG (293.15), kJ mol^{-1}	77.436	76.553	76.466	76.422	76.272	76.170	76.047
ΔG (303.15), kJ mol^{-1}	79.580	78.520	78.401	78.353	78.202	78.041	77.946
ΔG (313.15), kJ mol^{-1}	81.724	80.488	80.337	80.84	80.133	79.911	79.845
ΔG (323.15), kJ mol^{-1}	83.869	82.456	82.272	82.215	82.063	81.81	81.744