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Thermodynamic parameters of inorganic Pyrophosphatase in marshland south of Iraq

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Abstract

Marsh land (south of Iraq) is of great international interest at present time. Studying some microbial properties including enzyme activities , can be useful as indicator of soil quality of this area. General conclusion of enzyme reaction in soil can be reached from the thermodynamic parameters of the enzyme – soil system. Hence , this experiment was conducted to study thermodynamic parameters (Q10 , Ea , ΔHa , ΔSa and ΔGa) for inorganic pyrophosphatase enzyme in this soil as compared with their counter parts of other soils of south of Iraq (Al-Zubair and Abul-Khasib soils). Results indicated that Q10 and ΔGa values of marsh land did not significantly differs from other soils under study with average values of about 1.11 and 14.55 KJmol $^{-1}$, respectively. Ea values of marsh land , Al-Zubair and Abul-Khasib soil were 8.72 , 7.47 and 9.53 KJmol $^{-1}$, respectively. ΔSa values for marsh land soil (–10.49 J deg $^{-1}$ mol $^{-1}$) were lower than those of Abul-Khasib (–7.85 J deg $^{-1}$ mol $^{-1}$) but higher than those of Al-Zubair (–14.55J deg $^{-1}$ mol $^{-1}$).

ΔHa values were in order of Abul-Khasib (12.08 KJmol $^{-1}$) > marsh land (11.27 KJmol $^{-1}$) > Al-Zubair soil (10.02 KJmol $^{-1}$).

1- Introduction

It is well known that biochemical reaction involve in nutrient cycling in soils are mediated by soil enzymes. McLatchey and Reddy (1998) reported that soil enzymes are known to be involved in the cycling of nutrients can be used as potential indicators of nutrients cycling processes. Complex structure of organic

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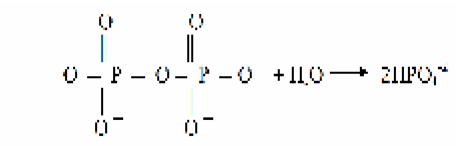
compounds in soils must be first hydrolyzed through the activity of enzymes into low molecular weight compounds can be directly transfered to cells oxidized and use as an energy source (Wright and Reddy, 2001).

Several phosphatase enzymes occur in soil, among them is pyrophosphatase (pyrophosphate phosphohydrolase, EC 3.6.1.1.) which catalyze the hydrolysis of pyrophosphate synthesized by microorganisms (Pepper *et al*., 1976) or added to soil as fertilizers (Dick and Tabatabai, 1978) to orthophosphate. The overall reaction is:



Pyrophosphatase is widely distributed in nature and its activity in bacteria , insect , mammalian and plants has been reported by several workers as reported by Tabatabai (199). Pyrophosphatase activity in soils has been reported by Gilliam and Sample (1968) and Hossner and Phillips (1971). The activity of this enzyme in soil needs special attention , because its substrate , pyrophosphate is used as fertilizer. Pyrophosphatase activity in soils , like other soil enzymes affected by soil properties such as soil type , ion strength and pH.

General conclusion of enzyme reaction in soil can be reached from the thermodynamic parameters of the enzyme – soil system. Such parameters include activation of energy (Ea), change in enthalpy (ΔHa) and in entropy (ΔSa) , and free energy of activation (ΔGa) (Perucci and Scarponi , 1984).

Marsh land (south of Iraq) is of great international interest at present time. Studying some of microbiological properties , including enzyme activities can be useful as indicator of soil quality in this area. A search of literatures revealed no previous studies on enzyme activities in this area. Therefore , series of experiments were conducted to study enzyme activities in this area as compared with their counter parts of other soils namely Al-Zubair and Abul-Khasib in south of Iraq. The purpose of this paper is to present the thermodynamic parameters of inorganic pyrophosphatase in marsh land and other soils under study. Characteristics of this enzyme in all soils under study was reported previously (Al-Ansari *et al*., 2007).

2-Materials and Methods

Surface soil samples were collected from marsh land (south of Iraq) to study thermodynamic parameters: activation of energy (Ea) , change in enthalpy (ΔHa) and in entropy (ΔSa) as well as free energy of activation (ΔGa) in such a soil as compared with their counter parts of soils collected from other sites namely Al-Zubair and Abul-Khasib both located at south of Iraq. Soils were collected to obtain a wide range in organic

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matter , total nitrogen, $CaCO_3$, electrical conductivity (E.C.) and soil texture. Soil E.C. range from 13 dSm^{-1} at marsh land to 5.8dSm^{-1} at Abul-Khasib area. Organic materials and total nitrogen (gm kg^{-1}) range from 35.0 and 5.0 to 22.0 and 1.5 at marsh land and Abul-Khasib area , respectively. While texture were clay at marsh land and silty clay at Abul-Khasib area. Soil properties of Al-Zubair area were electrical conductivity 9.01 dSm^{-1} , organic materials 2.05 gm kg^{-1} , total nitrogen 0.30 gm kg^{-1} and with a texture of loamy sand. pH of soils under study was about 7.8 . Above reported soil properties were determined as described by Page *at el*.,(1982)

To study the effect of temperature on inorganic pyrophosphatase activity, soil samples treated with 50mM pyrophosphate solution as substrate and incubated at different temperatures (10, 20, 30, 40, 50, 60, 70 and 80ºC) for five hours , then enzyme activity was assayed following procedure of Dick and Tabatabai (1978) described by Tabatabai (1994).

The activation energy (Ea) of inorganic pyrophosphatase in soils under study was calculated from enzyme activity obtained at 50 m pyrophosphate but temperature of incubation varied from 10 to 50 ºC (Tabatabai , 1994). The activation energy were calculated using the Arrhenius equation :

$$\mathbf{K = A . \exp (- Ea / RT)}$$

Where A is the preexpontial factor , Ea is the energy of activation , R is the gas constant and T is the temperature in degree Kelvin. The temperature coefficient (Q10) was calculated by the following equation as described by Frankenberger (1983) :

$$Q10 = \exp \frac{10000\ Ea}{8.314\ T\ (T + 10)}$$

Changes in enthalpy (ΔHa) and entropy (ΔSa) of activation values have been calculated from the following equations as described by Perucci and Scarponi (1984) :

$$\mathbf{\Delta Ha = Ea + RT}$$

$$\mathbf{\log K/T = 10.319 + \Delta Sa/4.574 - \Delta Ha/ 4.574T}$$

The free energy of activation (ΔGa) values calculated from equation:

$$\mathbf{\Delta Ga = \Delta Ha - T\ \Delta Sa}$$

All results reported are average of three replicates expressed on air – dry basis.

3-Results and Discussion

Fig. (1) shows that at various temperatures of incubation inorganic pyrophosphatase activity of marsh land was higher than those of Al-Zubair soil but lower than Abul-Khasib soil. Enzyme activity in all soils under study increased by increasing temperature from 10 to 60 ºC. However , increasing temperature of incubation beyond 60 ºC decreased inorganic pyrophosphatase activity. Studies on the effect

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of temperature on enzyme activities in soils have shown that most soil enzymes are inactivated (denaturated) at temperature between 60 and 70 ºC (Tabatabai , 1994). The stability of enzymes increased as temperature decreased below the optimum temperature for activity. Khaziev (1975) reported that at low temperature , free and weakly bound soil enzymes are most active , but with increasing temperature the immobilized enzymes became more active. He also reported that the changes in temperature in thermodynamic characteristics indicate heterogeneity in the composition and state of soil enzymes. O'Toole and Morgan (1984) suggested existence of a number of types of organic materials – urease complexes in soils , each possessing distinct characteristics including thermal stability.

The temperature dependence of the rate constant of an enzyme reaction can be described by Arrhenius equation which could expressed as :

$$\textbf{Log K} = -(\textbf{Ea} / 2.303\ \textbf{RT}) + \textbf{Log A}$$

the constant A is a factor related to the frequency of collisions of the reactants (eg. substrate) with the enzyme. The collisions frequency is influenced by the concentration of the reactant molecules and their kinetic energy (Gray, 1977). The activation energy was obtained from an Arrhenius equation plot of Log K against 1/T at lower temperature generally between 10–50 ºC (Tabatabai , 1994) where Ea is determined from the negative slop. The Arrhenius plots for inorganic pyrophosphatase activity values (μg $PO4^{\equiv}$–P/gm soil /5 hrs.) of the three soils under study were linear between 10 and 60 ºC (Fig. 2).

The activation energy values of the reaction catalyzed by inorganic pyrophosphatase expressed in KJ mol^{-1} were 7.47 , 9.53 and 8.75 for Al-Zubair , Abul-Khasib and marsh land soils , respectively. The values obtained in this study were lower than those of other phosphatase enzyme activities (eg. Alkaline phosphatase) in the soils under study (Al-Ansari *et al*., 1999). Perucci and Scarponi (1984) reported that Ea values reflect the affinity of enzyme to substrate and low Ea values may be correlated with easier enzyme – substrate complex formation. The results of their study showed that the Ea values of Arylsulphatase enzyme in soil amended with plant residue were lower than that for unamended soil , but the differences are not significant.

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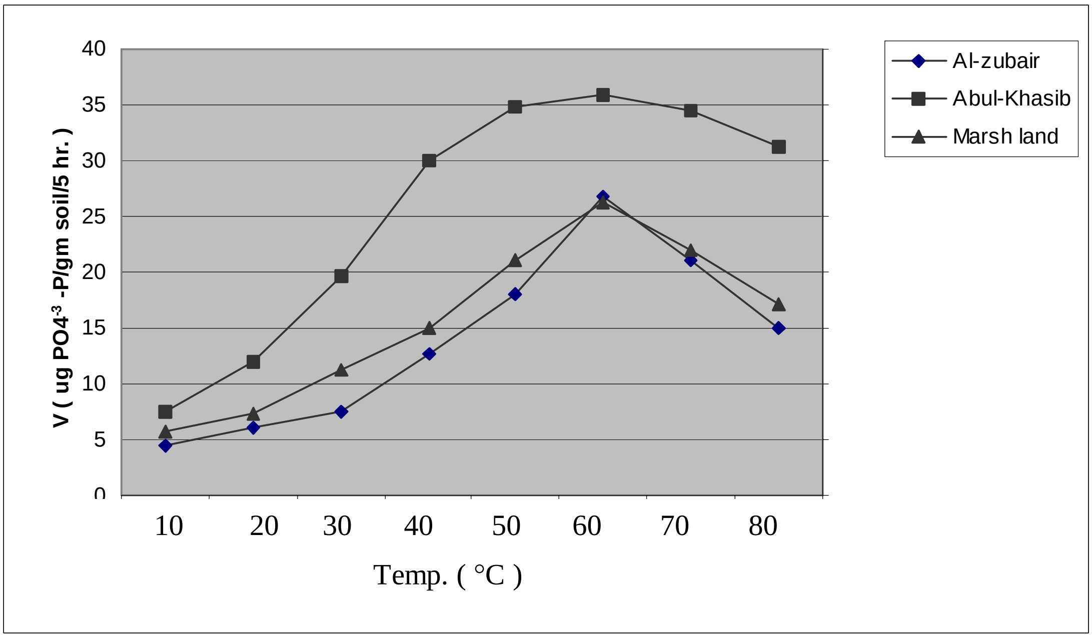

Fig.(1): Effect of temperature of incubation on inorganic pyrophosphatase activity in different soils.

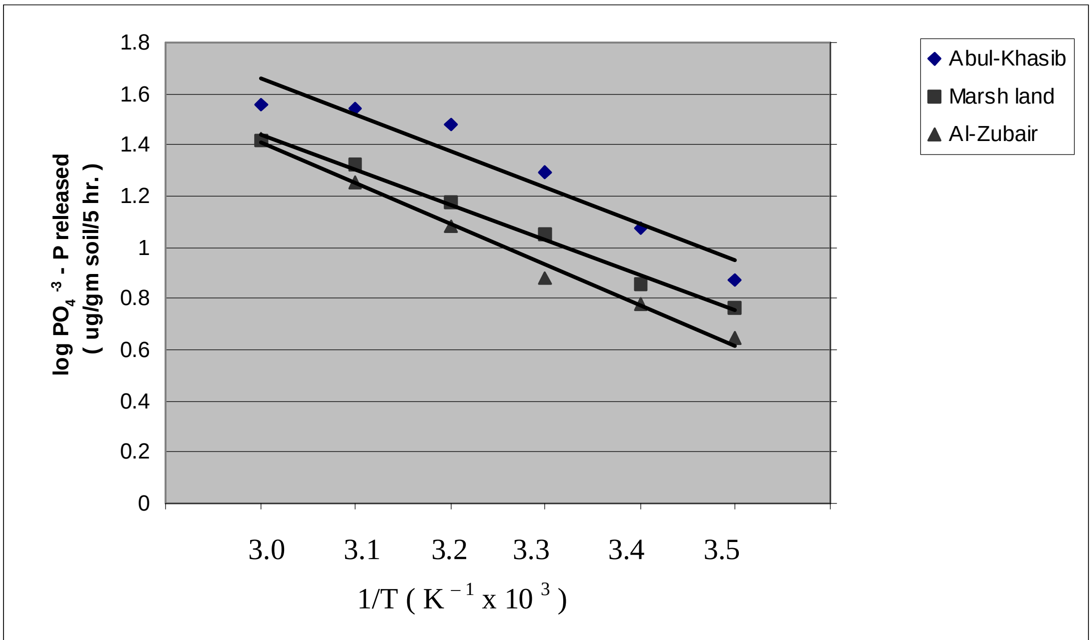

Fig.(2): Arrhenius equation plot of inorganic pyrophosphatase activity of different soil.

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Temperature coefficient (Q10) most often , is used to study the effect of temperature on enzyme – catalyzed reaction . Enzymes catalyzed reactions , however , are less sensitive to temperature changes than their uncatalyzed counter partes , whereas the uncatalyzed rate may double with every 10 ºC elevation of temperature , the enzyme – catalyzed reaction rate will increase by a factor of < 2 (Zafren and Hall , 1973). Temperature coefficient (Q10) is related to the activation energy (Ea) and can be expressed as suggested by Segel (1976) :

$$\text{Log Q10} = \frac{10\ Ea}{2.303\ T\ (T + 10)}$$

Table (1) : Temperature coefficient (Q10) of inorganic pyrophosphatase in different soils.

ºC	AL-ZUBAIR	ABUL-KHASIB	MARSH LANDS
10	1.13	1.15	1.13
20	1.11	1.14	1.13
30	1.00	1.13	1.12
40	1.09	1.12	1.11
50	1.09	1.11	1.10
60	1.08	1.10	1.10

Temperature coefficient of inorganic pyrophosphatase for soils under study were calculated according to equation described by Frankenberger (1983) and presented in table (1). The average of Q10 for inorganic pyrophosphatase activity for temperature ranging from 10 to 60 ºC were , 1.11 , 1.13 and 1.08 for marsh land , Abul-Khasib and Al-Zubair , respectively. The Q10 values of soil enzymes is an indication of kinetics energy required for the reaction catalyzed by enzyme in soils. The lower the Q10 value , the lower the kinetics energy required for a reaction. Segel (1975) reported that a Q10 of 2 is equivalent to an Ea of about 12600 Cal mol^{-1} (53 $KJmol^{-1}$) at 25 ºC. the Q10 values of the energy under study were lower than those of other phosphatase (eg. Alkaline phosphatase) in all soils under study (Al-Ansari *et al*., 1999).

Thermodynamic parameters (ΔHa , ΔSa and ΔGa) were calculated and presented in table (2).The ΔHa values for inorganic pyrophosphatase activity for temperature ranging from 10 to 60 ºC were 11.27 $KJmol^{-1}$ for marsh land , 12.08 and 10.02 $KJmol^{-1}$ for Abul-Khasib and Al-Zubair soils , respectively.

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All ΔSa values for soils under study were negative and values of Abul-Khasib soil were higher than of marsh land and Al-Zubair soil. The average ΔSa were – 7.85 , – 10.49 and – 14.55 Jdeg $^{-1}$ mol $^{-1}$ for Abul-Khasib , marsh land and Al-Zubair soils , respectively. Perucci and Scarponi (1984) reported that amendment soil with crop residue lower the steric effect and stabilized the formation of enzyme – substrate complex.

Table (2) : Thermodynamic parameters ; ΔHa , ΔSa and ΔGa of studied soils under different temperature of incubation.

	AL-ZUBAIR			ABUL-KHASIB			MARSH LANDS		
ºC	ΔHa*	ΔSa*	ΔGa*	ΔHa	ΔSa	ΔGa	ΔHa	ΔSa	ΔGa
10	9.82	−12.50	13.35	11.88	−5.21	13.35	11.07	−8.07	13.35
20	9.90	−13.39	13.82	11.96	−6.37	13.82	11.15	−9.13	13.82
30	10.00	−14.14	14.28	12.04	−7.45	14.30	11.23	−10.12	14.29
40	10.07	−15.04	14.72	12.13	−8.44	14.77	11.32	−11.02	14.76
50	10.15	−15.79	15.25	12.21	−9.39	15.24	11.40	−11.90	15.24
60	10.23	−16.49	15.72	12.29	−10.29	15.71	11.48	−12.72	15.71

*ΔHa and ΔGa are expressed as KJmol $^{-1}$ and ΔSa is expressed as Jdeg $^{-1}$ mol $^{-1}$.

The ΔGa values reported in table (2) showed that the free energy of the inorganic pyrophosphatase in each soil samples are similar in spite of differences in ΔHa and ΔSa values obtained for different soils under study. Similar trends for arylsulphatase activity in soils amended with different crop residues were reported by Perucci and Scarponi (1984).

4- References

Al-Ansari , A. M. S. ; M. A. Abdulkareem and L. A. Omar (1999).Characteristics of enzymes in recently reclaimed land. III. Kinetics parameters of phosphatase. Iraqi J. Agric. Sci. 30 : 573 – 582.

Al-Ansari , A. M. S., M. A. Abdlkareem and L. A. Omar (1999). Characteristics of enzymes in recently reclaimed land : II. Alkaline and acid phosphatase activity. Iraqi J. Agric. Sci. 30 : 563 – 572.

Al-Ansari , A. M. S.; M. A. Abdulkareem and S.J. Khadem (2007). Study of characteristics of inorganic pyrophosphatase activity in marshland , south of Iraq. Marsh Bull. 2 : 1 – 10.

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Dick , W. A. and M. A. Tabatabai (1978) . inorganic pyrophosphatase activity of soils. Soil Biol. Biochem. 10 : 59 - 65.

Frankenberger , W. T. JR. (1983). Kinetic properties of L-Histidine ammonia-Lyase activity in soils. Soil Sic. Soc. Amer. J. 47 : 71 – 74.

Gilliam , J. W. and E. C. Sample (1968). Hydrolysis of pyrophosphatase in soils : pH and biological effects. Soil Sci. 106 : 352 – 357.

Gray C. L. (1971). Enzyme-catalyzed reactions. Van Nostrand Rein-hold Co. London.

Hossner , L. R. and D. P. Phillips (1971). Pyrophosphate hydrolysis in flooded soil. Soil Sci. Soc. Amer. Proc. 354 : 379 – 383.

Khaziev, F. (1975). Thermodynamic characteristics of enzymic reactions in soil. Boil. Nauki 10 : 121 – 127.

McLatchey , G. P. and K. R. Reddy(1998). Regulation of organic matter decomposition and nutrient release in a wetland soil. J. Environ. Qual. 27 : 1268 – 1274.

O'Toole , P.and M. A. Morgan (1984). Thermal stabilities of urease enzymes in some Irish soils. Soil Biol. Biochem. 16: 471 – 474.

Page , A.L. ; R. H. Miller and D. R. Keeny (1982). Methods of soil analysis. Part 2. 2nd ed. , Madison , Wisconson , U. S. A.

Pepper , I. L. ; R. H. Miller and C. P. Ghonsikar (1976). Microbial inorganic polyphosphatase : factors influencing their accumulation in soil. Soil Sci. Soc. Amer. J. 40 : 872 – 875.

Perucci, P. and L. Scarponi (1984). Arylsulphatase activity in soils amended with crop residues kinetic and thermodynamic parameters. Soil Biol. Biochem. 16 : 605 – 608.

Segel , I. H. (1975). Enzyme kinetics behavior and analysis of rapid equilibrium and steady-state enzyme systems. John Wiley & sons. Inc. New York.

Segel , I. H. (1976). Biochemical calculations. P. 278. John Wiley & Sons. Inc. New York.

Tabatabai, M. A. (1994). Soil enzymes. In : Weaver, R. W.; J. S. Angle and P. S. Bottomley (eds.) Methods of soil analysis. Part 2. SSSA.Book series No. 5 , Madison , Wis. PP 775 – 833.

Wright , A. L. and K. R. Reddy (2001). Phosphorus loading effects on extracellular enzyme activity in Everglades wetland soils. Soil Sci. Soc. Amer. J. 65 : 588 – 595.

Zaffren , E. and P. L. Hall (1973). The study of enzyme mechanisms. John Wiley & Sons. Inc. New York.

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المقاييس الثرموديناميكية لأنزيم البايروفوسفتيز المعدني في تربة الاهوار جنوبي العراق

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الملخص

لقد نالت ترب مناطق أهوار جنوب العراق أهتماماً عالمياً متزايداً في الاونة الاخيره وأن دراسة صفاتها المختلفه ومن ضمنها الصفات المايكروبايولوجية ومنها النشاط الانزيمي يعد ضرورياً كمؤشر للدلالة على خواص ترب هذه المناطق . ونظراً لأن دراسة الصفات الثرموديناميكية لنظام التربه ـــ الانزيم يمكن أن تعطي تصوراً عاماً عن تفاعل الانزيم في التربة فقد نفذت هذه التجربة لدراسة الصفات الثرموديناميكية (Q10 و Ea و ΔHa و ΔSa و ΔGa) لأنزيم البايروفوسفتيز المعدني (inorganic pyrophosphatase) في تربة الاهوار ومقارنتها بتربتين أخرتين من جنوب العراق (تربة الزبير وتربة أبي الخصيب) . أوضحت النتائج أن قيم Q10 و ΔGa لتربة الاهوار لم تختلف معنوياً عما هي للتربتين الاخريتين قيد الدراسه فقد بلغ معدلهما 1.11 و 14.55 كيلوجول مول $^{-1}$ على التوالي. أما بالنسبه لقيم Ea فقد بلغ معدلهما 8.72 و 7.47 و 9.53 كيلوجول مول $^{-1}$ لترب الاهوار والزبير وأبو الخصيب على التوالي. معدل قيم ΔSa لتربة الاهوار (– 10.49 جول درجه $^{-1}$ مول $^{-1}$) كان أقل مما هو لتربة أبي الخصيب (– 7.85 جول درجه $^{-1}$ مول $^{-1}$) لكنه أعلى مما هو لتربة الزبير (– 14.55 جول درجه $^{-1}$ مول $^{-1}$) . أما قيم ΔHa للترب المدروسة فكانت ضمن التسلسل : أبو الخصيب (12.08 كيلوجول مول $^{-1}$) > الاهوار (11.27 كيلوجول مول $^{-1}$) > الزبير (10.02 كيلوجول مول $^{-1}$).

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