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Kinetics of Catalase and Dehydrogenase in Main Soils of Northeast China under Different Soil Moisture Conditions

Lili Zhang, W.U. Zhijie, Lijun Chen, Yong Jiang and L.I. Dongpo
Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

Abstract: Through incubation test, the kinetic characteristics of catalase and dehydrogenase in Black soil (Phaeozem, FAO), albic soil (albic luvisols, FAO), brown soil (Cambisols, FAO) and cinnamon soil (Haplic Greyxems, FAO) were studied under 3 installed soil moisture conditions, i.e., wilting point, 60% of field capacity and saturated water content. The study showed that the kinetics of both catalase and dehydrogenase in test soils exhibited typical Michaelis-Menten kinetic behaviors. The 2 soil intracellular enzymes had larger Km values than corresponding pure enzymes, indicating their weaker enzyme-substrate affinity in soil. For catalase, its Km value was much smaller in black soil and albic soil than in brown soil and cinnamon soil, due to the greater differences of soil organic matter content among the soils and possibly, different origins. The Km and Vmax values of the catalase decreased with increasing soil moisture content and its catalytic ability (Vmax/Km value) was the largest at 60% of field capacity in black soil, albic soil and brown soil and at saturated water content in cinnamon soil. As for dehydrogenase, its Km value had smaller differences under different soil moisture conditions, but its Vmax and Vmax/Km increased with increasing soil moisture content, showing that soil moisture content had less effect on its enzyme-substrate affinity but affected its catalytic ability. There was a significant positive correlation between dehydrogenase Vmax/Km and soil organic matter content, showing that organic soil exhibited a higher dehydrogenase catalytic ability than mineral soil.

Key words: Catalase, dehydrogenase, Km, Vmax, Vmax/Km, soil moisture condition

INTRODUCTION

Soil enzymes are the mediators and catalysts of biochemical processes important in soil functioning such as nutrient mineralization and cycling, decomposition and formation of soil organic matter and decomposition of xenobiotics (i.e., pesticides). Specifically, oxidoreductases can provide information on the status of key reactions that participate in rate limiting steps of oxidation-reduction process of organic and inorganic materials in soils (Trasar-Cepeda *et al*., 2000). Catalase and dehydrogenase are intracellular oxidoreductases associated with aerobic and anaerobic microbial activity separately. They are considered indicators of overall microbial activity of soil (Rodriguez-Kabana and Truelove, 1982; García *et al*., 1994; Masciandaro *et al*., 2000).

The activity of a certain enzyme only provides an indication of its amount and its overall contribution in soil (Farrell *et al*., 1994), while soil enzymatic kinetic study can provide us useful information regarding the origin, existing status (e.g., stability and substrate-affinity) and catalytic properties of a certain enzyme in soil under specific conditions (Perez-Mateos and Gonzales Carcedo, 1985). Catalase and dehydrogenase are intracellular enzymes that are involved in microbial oxidoreductase metabolism and the kinetic parameters (Vmax and Km) are important to establish the efficiency of catalysis by the intracellular enzymes. Masciandaro *et al*. (2000) indicated that dehydrogenase Vmax and Km were useful markers to assess changes in microbial activity of soil, since they represented the "quantity" and the affinity of these enzymes, respectively. It was reported that chernozem and chernozem-like soil had a similar Km value of dehydrogenase, indicating its same origin and in carbonate chernozem, this enzyme had higher Km and Vmax values than in other subgroups of chernozems (Zhou, 1987). Masciandaro *et al*. (2000) found that compared with chemical fertilizer (urea) treatment, dehydrogenase Vmax value markedly increased while Km value did not change in organic fertilizer treatment showing that the use of organic fertilizer caused an increase in dehydrogenase in the active microbial biomass and did not alter the enzyme-substrate affinity.

Soil moisture is an important factor to influence the oxidation-reduction condition. The aerobic and anaerobic microbial activity must be expected to change under

Corresponding Author: W.U. Zhijie, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

different soil moisture contents. Catalase and dehydrogenase are intracellular oxidoreductases associated with aerobic and anaerobic microorganisms. Activities and kinetic characteristics will be influenced by the soil moisture content. In addition, soil is a huge heterogenic system and the diffusion limitations of the substrate may indirectly influence the kinetic parameters of catalase and dehydrogenase (Marx *et al*., 2005). Studies that have evaluated enzyme activities under different soil moisture regimes have been performed largely (Tiquia *et al*., 1996; Subhani *et al*., 2001; Hinojosa *et al*., 2004), but a few were on the kinetic characteristics of catalase and dehydrogenase (Gianfreda and Bollag, 1994; Masciandaro *et al*., 2000).

Black soil, albic soil, brown soil and cinnamon soil are the main cultivated soils in Northeast China. In this area, soil water mainly originates from natural rainfall and there is a fluctuant moisture condition during crop growth period. Studying the effect of soil moisture fluctuation on the kinetic characteristics of catalase and dehydrogenase in these soils is of significance in the agricultural management of this region.

With an incubation test under controlled soil moisture conditions (wilting moisture 60% field moisture capacity and saturation moisture content, respectively), this study studied the kinetic characteristics of catalase and dehydrogenase in the above-mentioned soils of Northeast China, aimed to assess the status of these enzymes in test soils and their response to different soil moisture regimes.

MATERIALS AND METHODS

Soil samples collection and preparation: Soil surface samples 0~20 cm in depth were collected from the black soil and albic soil in Heilongjiang Province and the brown soil and cinnamon soil in Liaoning Province. The soils were all collected from long-term abandoned lands. Ten soil samples were collected from each field, sieved (<8 mm) to remove root and other plant material and combined. They were air-dried and 2 mm-sieved before use and some of their chemical properties were listed in Table 1.

Soil incubation: Three parts of 150 g air-dried soil samples were adjusted to 200 g kg^{-1} moisture content and pre-incubated at 20 for 7 days. Then we adjust their moisture content to 100 g kg^{-1} wilting moisture, 200 g kg^{-1} (60% field moisture capacity) and 300 g kg^{-1} (saturation moisture content), respectively and incubate them at 20 for 14 days. By the end of incubation, the activities of soil dehydrogenase were measured. The incubation process for the catalase was the same but only 10 g soil samples were used to incubate.

Table 1: Some chemical properties of test soils

Soil	pH H_2O (2.5:1)	Organic matter (g kg^{-1})	Total nitrogen (g kg^{-1})	$CaCO_3$ (%)
Black	5.54	46.84	2.22	0.82
Albic	5.81	32.96	1.93	1.11
Brown	5.46	14.47	0.97	1.03
Cinnamon	8.21	10.54	0.93	2.34

Enzyme activities measurement: Catalase activity (mmol $H_2O_2 \cdot g^{-1}soil \cdot h^{-1}$) was measured by the colorimetric determination of remained H_2O_2 after soil samples reacted with H_2O_2 at 20 for 10 min Trasar-Cepeda *et al.* (1999) and dehydrogenase activity (mg $TPF \cdot kg^{-1}soil \cdot 24h^{-1}$) was determined by the reduction of 2, 3, 5-Triphenyl-tetrazolium Chloride (TTC) after the samples reacted with a 3% TTC solution at 37 for 24 h (Tabatabai, 1994). The substrate concentrations for the measurement of catalase and dehydrogenase activities were ranged from 2-16 mM and from 10-90 mM, respectively. For every substrate concentration, triplicate analyses were carried out.

Kinetic parameters determination: The kinetic parameters Vmax and Km were determined by the Lineweaver-Burk transformation of Michaelis-Menten equation

$$\frac{1}{V} = \frac{Km}{Vmax} \cdot \frac{1}{[S]} + \frac{1}{Vmax}$$

Plot 1/V against 1/[S] and solve the Vmax and Km by the slope and intercept.

Statistical analysis: The data were presented as the means of triplicated analyses for each substrate concentration and statistical analysis was performed by using Excel 2000 and SPSS 13.0 program for windows.

RESULTS AND DISCUSSION

Kinetics of soil catalase: The catalase in test soils exhibited typical Michaelis-Menten kinetic behaviors. Several investigations have demonstrated that soil hydrolysis enzymes follow Michaelis-Menten kinetics (McLaren and Packer, 1970; Nannipieri *et al*., 1978; Gianfreda *et al*., 1995), despite soil being considered as a discontinuous, structured and heterogeneous system (Nannipieri *et al*., 2002).

Catalase, typical oxidoreductase also exhibited the same behavior in this research work. Its Km values varied from 0.0422-0.4669 M (Table 2), larger than that of pure catalase extracted from soil (Geng *et al*., 2000). Catalase is an intracellular oxidoreductase and the higher Km value here may be caused by the hindrance in the heterogeneous soil system when the enzyme approached to the substrate.

Table 2: Km and Vmax of soil catalase

Soil	Soil moisture content (g kg^{-1})	Km (M)	Vmax ($mmol_2$ $O_2 \cdot g^{-1}$ $soil \cdot h^{-1}$	Vmax/Km	r^2
Black	100	0.2430	5.6410	23.2144	0.8660*
	200	0.0805	2.6766	33.2496	0.8445*
	300	0.0671	1.6567	24.6900	0.8810**
Albic	100	0.0609	2.6889	44.1527	0.8907**
	200	0.0544	3.1635	58.1525	0.7935*
	300	0.0422	2.4108	57.1279	0.8856**
Brown	100	0.4669	9.5693	20.4954	0.8098*
	200	0.4392	9.9600	22.6776	0.8932**
	300	0.1187	2.5196	21.2274	0.8785**
Cinnamon	100	0.2037	6.6533	32.6612	0.8752**
	200	0.1456	5.5803	38.3262	0.8971**
	300	0.1142	4.0080	35.0963	0.8692**

Other reasons for modification of catalase kinetic characteristics include distortion of the enzyme of the enzyme polymeric configuration or decrease of enzyme accessibility through partial masking of the enzyme`s active site (Quiquampoix, 2000).

Black soil, albic soil, brown soil and cinnamon all exhibited the largest Km value at 100 g kg^{-1} soil moisture content and then decreased with increasing soil moisture content (Table 2). In soil, the typical heterogenic system, substrate diffusion rate affects the Km value largely. The stronger enzyme-substrate affinity (lower Km values) in higher moisture content may be caused by the higher diffusion rate because of more water availability. As for Vmax of catalase, these 4 soils all exhibited the lowest Vmax value at 300 g kg^{-1} soil moisture content. Vmax reflects the amount of enzymes in the medium and the differences in Vmax between the different moisture contents can be interpreted directly in terms of differences in quantity of enzymes. Catalase is an intracellular oxidoreductase associated with aerobic microbial activity (Rodríguez-Kábana and Truelove, 1982). Higher Vmax value in the relatively lower water content may be explained by the improved soil aeration in this condition as a consequence of an increase in soil porosity.

All the 4 soils showed the lowest Vmax/Km values at 100 g kg^{-1} soil moisture content. The extreme dryness in soil limits the solubility and restricts movement of the organic C (the energy source of microbial) (Linn and Doran, 1984), thus limits the microbial respiration and results the lowest Vmax/Km values. According to Dick *et al.* (1996) and Marx *et al.* (2005) enzyme activities are sensitive indicators of climate change than other parameters such as soil organic matter and the conceptual rationale for soil enzyme activity as a soil quality indicator is that enzyme activities are often closely related to important soil quality parameters, soil physical properties and microbial activity or biomass. They can begin to change much sooner than other properties (e.g., soil organic C), thus providing an early indication of the trajectory of soil quality with changes in soil different moisture contents. The determination of enzyme kinetics is a powerful approach to investigate the catalytic properties of enzymes (Perez-Mateos and Gonzales Carcedo, 1985). From our research, we get the catalase catalytic characteristics of main soils of Northeast China under different soil moisture contents and it is meaningful for enzyme research work as indicators of soil quality regarding to natural and artificial soil disturbance.

The Km values of catalase in black soil and albic soil were much smaller than those in brown soil and cinnamon soil. Black soil and albic soil contain much more organic matter (Table 1) and the medium-textured soils exhibit better soil structure that protects the enzyme active site, resulting lower Km values. Another potential reason for lower Km in these soils may be the higher water availability because of higher organic matter content in soils. Farrell *et al.* (1994) pointed out that arylsulfatase had significant positive correlation with Km values, meaning lower enzyme-substrate affinity in higher organic matter content because much of the arylsulfatase in higher organic matter soil occurs in complexes with humic colloids. Arysulfatase is extrocelluar enzyme but catalase is intracellular enzyme, more organic matter content supplied more easily degradable substrates for microorganisms propagation and Km values of catalase were much different with the arylsulfatase in higher organic matter content soils. Indeed, we observed a negative but insignificant correlation between Km values and the organic matter content of the soils (data not shown), which may reflect a change in the location of the enzyme due to changes (both quantitative and qualitative) in the soil organic matter. A lot of previous work had shown that enzyme activities increase with increasing soil organic matter (Dick *et al.*, 1988; Martens *et al.*, 1992; Kandeler and Eder, 1993; Klose *et al.*, 1999; Pascual *et al.*, 1999). Maybe the increased catalase activities were caused by the stronger enzyme-substrate affinity.

The greater difference of the Km values between soil types, especially between brown soil and cinnamon soil, suggests that the bounding status and the origin of the catalase are dissimilar. Different soil groups undergo different pedogenic process and result in much different physical and chemical characteristics and thus, different soils are unlikely to have similar origin catalase.

Kinetics of soil dehydrogenase: The kinetics of dehydrogenase in test soils also exhibited typical Michaelis-Menten kinetic behaviors. Dehydrogenase Km values varied from 0.0253-0.0688 M (Table 3). Dehydrogenase from *Drosophila melanogaster*

Table 3: Km and Vmax (mg TPF·kg^{-1}soil·24 h^{-1}) of soil dehydrogenase

Soil	Soil moisture content (g kg^{-1})	Km (M)	Vmax (mg TPF·kg^{-1} soil·24h^{-1})	Vmax/Km	r^2
Black	100	0.0342	2.4866	72.7102	0.8876**
	200	0.0309	3.3670	108.9644	0.8542**
	300	0.0279	2.7693	99.2580	0.8231**
Albic	100	0.0253	1.7744	70.1343	0.7547*
	200	0.0268	2.3629	88.1716	0.8996**
	300	0.0297	2.9186	98.2693	0.8172*
Brown	100	0.0537	1.7319	32.2513	0.8849**
	200	0.0553	2.1236	38.4031	0.8888**
	300	0.0590	2.9937	50.7411	0.8918**
Cinnamon	100	0.0603	1.7327	28.7356	0.8164*
	200	0.0640	2.1345	33.3522	0.8968**
	300	0.0688	2.3600	34.3031	0.8171*

(Boris and Thomas, 2008) and *Mesorhizobium loti* (Mukherjee *et al*., 2007) were reported to have Km values of 62.7×10^{-6}M and 6.6×10^{-6}M, respectively and increased Km values showed that the enzyme-substrate affinity decreased in soil compared with pure enzyme extracted from plants and microorganisms. Intracellular enzymes exert their catalytic action within the cell, a restricted and compartmentalized system (Burns, 1978; Tabatabai, 1982; Ladd, 1985), Consequently, properties and kinetic behavior of such enzymes very likely will differ from those of the corresponding protein from animal, microbial, or plant origins.

Km values changed slightly with increasing moisture content in the four main soils of Northeast China (Table 3), indicating that the enzyme-substrate affinity was not influenced largely by different soil moisture contents and it was much different from catalase. As for different soils, dehydrogenase also showed relatively lower Km values in higher organic matter soils (black soil and albic soil), but the amplitude of variation was much smaller than catalase. Catalase and dehydrogenase are intracellular enzymes associated with aerobic and anaerobic microorganisms. Maybe slightly and intensely changed Km values of dehydrognease and catalase are attributed to different origins of microorganisms and different existing sites in the microorganisms. Table 3 also shows that the amount of enzymes Vmax in the medium increases with increasing soil moisture content. Dehydrogenase is an intracellular enzyme, functioning as hydrogen carrier. The main source of soil dehydrogenase is anaerobe which propagates rapidly under conditions of higher soil moisture content (Chendrayan *et al*., 1980; Subhani *et al*., 2001). The increased Vmax values may be caused by the propagating anaerobic microorganisms when soil moisture content increases. A lot of researches showed that soil water content was the dominant factor affecting soil dehydrogenase activity and also many researches have indicated the close relationship between its activity and soil moisture content (Dkhar and Misha, 1983; Doran *et al*., 1991; Brzezinska *et al*., 1998). The kinetic research here showed that Vmax values of dehydrogenase changed more intensely than Km values under changed soil moisture content.

The Vmax/Km values of dehydrogenase which represent the catalytic ability in albic soil, brown soil and cinnimma soil increased with the increasing soil moisture content while the black soil exhibited the largest Vmax/Km value at 200 g kg^{-1} soil moisture content (Table 3). Active dehydrogenase is considered to exist in soils and integral parts of intact cells and dehydrogenase activities are thought to reflect the total range of oxidative activities of the soil microflora. The increase of catalytic ability in the case of wet soils showed that moisture played a significant role in the regulation of dehydrogenases enzyme kinetic characteristics in soils. It has been demonstrated that soil moisture significantly alters the microbial population and its activity (Tiwari *et al*., 1987). As most dehydrogenases are of anaerobic origin and are activated by anaerobic conditions, the high catalytic ability with increasing soil moisture may be caused by increased enzyme released into the soil because of faster turnover of the microbial biomass when more water is available (Krämer and Green, 2000). In the case of the low soil moisture content, the decrease may be due to the extreme dryness which becomes unfavorable for most microbial communities and few could survive in the soil (Tiwari *et al*., 1987). As mentioned above, Km changed slightly while Vmax changed largely under different soil moisture contents, this kinetic research demonstrated that the increased dehydrogenase catalytic ability in higher moisture content was due to an increased production of enzymes rather than a production of a more active enzyme system. Table 4 shows that Vmax/Km values exhibit significant positive correlation with soil organic matter (r = 0.955*, 0.993**, 0.939* for 100 g kg^{-1}, 200 g kg^{-1}, 300 g kg^{-1} soil moisture content , respectively) and with total N (r = 0.990*, 0.998*, 0.966* for 100 g kg^{-1}, 200 g kg^{-1}, 300 g kg^{-1} soil moisture content, respectively).

The significant correlation demonstrated that in test soil, organic matter and total N have direct influence to dehydrogenase catalytic ability. Some works have showed that dehydrogenase activity is larger in the soil with higher organic matter (Flieβbach *et al*., 2006). Our work demonstrated the same trend referring to the catalytic ability. Soil organic matter is the basic material for microorganisms propagation and the product of its vital movement. Benitez *et al*. (2005) pointed out that dehydrogenase activity had a strong correlation with pyrophosphate-extractable C (representing easily decomposable organic compounds). In the soil with higher organic matter, there may be more easily decomposable organic compounds that are available as

Table 4: Correlation matrix between soil chemical characteristics and kinetic parameters of soil dehydrogenase under different moisture condition

	Km (g kg^{-1})			Vmax (g kg^{-1})			Vmax/Km (g kg^{-1})		
Parameters	100	200	300	100	200	300	100	200	300
pH	0.613	0.658	0.669	-0.382	-0.419	0.958*	0.565	0.563	0.689
Organic matter	-0.842	-0.903	-0.949	0.845	0.907	0.393	0.955*	0.993**	0.939*
Total N	-0.909	-0.949	-0.975*	0.751	0.832	0.375	0.990*	0.998*	0.966*
CaCO3	0.671	0.726	0.749	0.510	0.549	0.909	0.658	0.670	0.764

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)

substrate for microbial metabolism, maybe resulting stronger dehydrogenase catalytic ability. For soil nitrogen, on the one hand, it is ingredient of soil enzyme; on the other hand, nitrogen accumulated in the soil organic matter decides the amount of enzyme that enters into soil (Zhou, 1987). Here we found the significant correlation between dehydrogenase catalytic ability and soil total N content.

In our study, the kinetic parameters of test enzymes varied between soil types. Guan *et al.* (1986) reported that much different dehydrogenase activity was found in different soil types. Zhou (1987) indicated that dehydrogenase activity changed more intensely in light-textured than in heavy- textured soil and different particle-size soil microaggregates had different enzyme activities. Our test soils differ in soil-forming process and thus, in soil properties, which gives a deep influence on the kinetic characteristics of soil enzymes. Also in this research, information is available that different soil moisture content can result different Km, Vmax and Vmax/Km values.

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