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# Kinetic parameters of phosphatase: A quantitative synthesis

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ABSTRACT

Phosphatases play an important role in mineralization of organic phosphorus, soil phosphorus availability and global phosphorus cycling. Release of phosphorus in different ecosystems is important for plant growth and microbial function, and may be simulated by modeling organic phosphate mineralization. The half-saturation constant ($K_m$) and the maximum enzyme activity ($V_{max}$) in the Michaelis–Menten equation are the two important kinetic parameters in these models, but their values have not been systematically investigated. In this study, we compiled a database of kinetic parameters of phosphatase from 139 publications, estimated the means, variations and distributions of the kinetic parameters, and tested the differences in kinetic parameters of phosphatases of different types, origins and under different incubation conditions. We also analyzed the activation energy ($E_a$), temperature sensitivity ($Q_{10}$), optimum pH ($pH_{opt}$) and sensitivity of pH ($pH_{sen}$) of phosphatase activity. Our results indicated that: 1) Both $V_{max}$ and $K_m$ were log-normal distributed with large variations; 2) There was no significant difference in $K_m$ between the acid or alkaline phosphatases, but a significantly higher $V_{max}$ for acid phosphatases was found compared with alkaline phosphatases; 3) $K_m$ and $V_{max}$ varied with the origins of enzymes and under different incubation conditions. Plant originated enzymes had the highest $V_{max}$ while soil originated enzymes had the lowest $V_{max}$. Larger variation in $V_{max}$ was found among the incubation times than among the incubation temperatures; 4) The mean values of $E_a$ for acid and alkaline phosphatases were 36.30 and 23.61 kJ mol$^{-1}$, respectively, with an overall mean of 34.40 kJ mol$^{-1}$. The mean value of estimated $pH_{opt}$ for acid phosphatase was 5.2 while that for alkaline phosphatase was 9.5. The information generated in this study will be useful for phosphorus mineralization modeling and uncertainty analysis.



## 1. Introduction

Phosphorus (P) is an essential macronutrient utilized by all organisms for energy transport and growth (Krämer and Green, 2000). It is involved in many critical biological processes, such as energy metabolism, synthesis of nucleic acids and membranes, and photosynthesis (Raghothama, 1999; Vance et al., 2003). Globally, P is still a major nutrient that limits crop production and plant productivity in many different ecosystems, especially in highly weathered, acidic or calcareous soils (Holford, 1997; Sánchez and Salinas, 1981; Chapin and Kedrowski, 1983; Krämer and Green, 2000). P also interacts with other essential elements such as carbon (C) and nitrogen (N) in regulating biological processes, and the ratio of C:N:P is considered as an important indicator in estimating C and nutrient fluxes in global circulation models.

Availability of P may be regulated by many factors. In natural ecosystems, P may originate from the mineralization of plant litter, algae, soil organic matter and sediments (Eivazi and Tabatabai, 1977; Bae and Barton, 1989; Yadav and Yadav, 1996; Joner et al., 2000; Vance et al., 2003). P exists in either inorganic (*Pi*) or organic (*Po*) forms in the soil, where *Po* and *Pi* are operationally defined based on the Hedley fractionation (Cross and Schlesinger, 1995). *Po* is believed to be biologically assimilable while *Pi* is believed to be geochemically bound. Plants can only utilize *Po* after hydrolysis (Adams and Pate, 1992; Tarafdar and Claassen, 1988). Phosphatases include a group of enzymes that can hydrolyze the ester-phosphate bonds in soil organic P, which releases the phosphate into soil solution for uptake by nearby roots or microbes (Tarafdar and Claassen, 1988; Pant and Warman, 2000; Duff et al., 2006). There are two major extracellular enzymes of phosphatase: acid and alkaline phosphatases, classified according to their most effective pH. Acid phosphatase activity, in particular, may provide a large portion of *Pi* for plants (Harrison and Pearce, 1979; Kroehler and Linkins, 1988; Moorhead and Reynolds, 1993). Both of

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them significantly contribute to the *Pi* release in the soils and nutrient cycling on the earth.

Phosphatase activity can be detected using a quantitative measurement of hydrolysis of a substrate, either measured as disappearance of the substrate or as formation of one of the two resulting products. Since its introduction decades ago (Tabatabai and Bremner, 1969), the use of p-nitrophenyl phosphate (*p*NPP) as a substrate in quantitative measurements of endogenous soil phosphatase and extracellular phosphatase of plants and microorganisms has dominated due to its convenience (Joner et al., 2000). This activity assay method measures activities of extracellular enzymes, which are released by microorganisms to initially cleave organic matter into smaller molecules (Tabatabai and Bremner, 1969; Williams et al., 1973; Juma and Tabatabai, 1988; Coolen and Overmann, 2000; Waldrop et al., 2004; Wallenstein and Weintraub, 2008; Henry, 2012; Burns et al., 2013). In the case of substrate *p*NPP, p-nitrophenol (*p*NP) formed after hydrolysis will be subsequently extracted with chemical such as sodium hydroxide (NaOH) and then measured spectrophotometrically. From such measurements, the affinity and potential rate of extracellular hydrolysis of biopolymers can be inferred, using the Michaelis–Menten (M–M) kinetics (Michaelis and Menten, 1913; Tabatabai and Bremner, 1971; Williams et al., 1973; Juma and Tabatabai, 1988; Grant et al., 1993; Nannipieri and Gianfreda, 1998; Manzoni and Porporato, 2009; Allison et al., 2010; Wang and Post, 2013; Wang et al., 2012, 2013):

$$V = V_{\max}\frac{S}{K_m + S} \quad (1)$$

where $V$ is the enzyme reaction rate; $V_{\max}$ and $K_m$ are the maximum enzyme activity and the half-saturation constant, respectively; and $S$ is the concentration of substrate. Both $K_m$ and $V_{\max}$ can be derived using a series of substrate concentrations at certain pH and temperature. Enzymatic activity usually varies with temperature and pH and enzymes often have different optimum temperature and pH. The response of enzyme activity to temperature change can be described using the Arrhenius equation in which the activation energy ($E_a$) is a key parameter (Johnston, 1975; Feng et al., 1990; McClaugherty and Linkins, 1990; Scrutton et al., 2001; Calsavara et al., 2001). These model parameters and the influences of temperature and pH can be built into mineralization models to simulate P release and cycling.

Many studies have been conducted on phosphatase, including kinetics, regulation of enzymatic activity by temperature and pH, influence by substrate concentrations, inhibitors, and effects of many different treatments (Verchot and Borelli, 2005). But there is a lack of syntheses of the kinetic parameters which is necessary to enable modeling. Although current phosphorus models mostly estimate mineralized phosphorus by either converting from mineralized carbon or nitrogen using C:P or N:P ratio, or through a rate coefficient for mineralization (Jones et al., 1984; Treseder and Vitousek, 2001; Wang et al., 2010; Henry, 2012; Runyan and D'Odorico, 2012), some newly developed ecosystem models tend to consider the kinetics of enzyme activity and include enzyme pools in the simulation (Davidson et al., 2012; Moorhead et al., 2012; Sinsabaugh and Shah, 2012; Wang et al., 2013). For example, Sinsabaugh and Shah (2012) proposed a biogeochemical equilibrium model that combines the kinetics of enzyme activity and community growth under different resource limitations, based on metabolic and ecological stoichiometry theory. They suggest that phosphorus, rather than nitrogen, might be a constraint on microbial metabolism and call for better estimates of enzyme related model parameters and their variations.

The objective of this study was to document enzymatic parameters of phosphatase through a literature research and data synthesis. We have compiled a database of kinetic parameters for phosphatases and analyzed the means, variations and distributions. The influences of enzymatic activities of phosphatase by temperature and pH were also investigated. The database and information obtained in this study will be useful for enzyme-driven soil organic/sediment decomposition models to simulate nutrient release and nutrient cycling, and has important implications for plant growth and nutrient dynamics and cycling.

## 2. Materials and methods

### 2.1. Literature review and data collection

We compiled a database of kinetic properties of phosphatases including acid phosphatases and alkaline phosphatases from the literature up to July 2012. We searched the online databases (i.e. Web of Science and ScienceDirect) using the keywords kinetic, phosphatase, $K_m$ and $V_{\max}$. We included papers that reported both the half-saturation constant ($K_m$) and maximum enzyme activity ($V_{\max}$). For a small number of papers that the $K_m$ and $V_{\max}$ were reported in figures, we manually digitalized them. If multiple values were reported in one paper under different treatments, we recorded all values with the treatment conditions. In total, we recorded 930 pairs of $K_m$ and $V_{\max}$ and the corresponding experimental conditions (i.e. origin of enzyme, type of enzyme, substrate, maximum substrate concentration ($S_{\max}$), buffer, incubation temperature, pH and parameter estimation method) from 139 publications (Table 1).

### 2.2. Enzymatic activity assay and kinetic analysis

Several different methods were used in the literature to measure enzymatic activity (Joner et al., 2000), including a histochemical method of precipitating a Fast Blue RR salt with P from alpha-naphthyl acid phosphate to indicate metabolically active cells or cell components (Tisserant et al., 1993), a qualitative visualization employing phenolphthalein phosphate in agar plates to demonstrate extracellular phosphatase activity (Trolldenier, 1992), and a quantitative measurement of hydrolysis of a substrate, either measured as disappearance of the substrate or as formation of one of the two resulting products. But since Tabatabai and Bremner (1969) proposed to use *p*NPP as a substrate instead of the PP (phenyl phosphate), most of the studies adopted this method.

Conditions of the assay must be controlled with respect to temperature, duration, pH, and ionic strength of the solution (Tabatabai, 1994; Verchot and Borelli, 2005). Some investigators used different incubation conditions such as incubation temperatures, times, buffers, or used a slightly different wavelength from 410 nm for color absorption measurements. For kinetic analysis, enzymatic activity was measured under a series of different substrate concentrations. The starting substrate concentration was close to 0 mM, and the high level of substrate concentration varied from close to 1 mM to more than 500 mM, with majority between 5 mM and 50 mM.

Depending on the pH of the incubation medium, one can distinguish acid phosphatase activity from alkaline phosphatase activity, measured respectively at pH around 5 and 8 (Bae and Barton, 1989; van Aarle and Plassard, 2010).

### 2.3. Estimation of kinetic parameters using Michaelis–Menten (M–M) model

As important kinetic parameters, $V_{\max}$ and $K_m$ have been extensively investigated to characterize the enzyme-driven