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Parameter estimation for models of ligninolytic and cellulolytic enzyme kinetics

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ABSTRACT

While soil enzymes have been explicitly included in the soil organic carbon (SOC) decomposition models, there is a serious lack of suitable data for model parameterization. This study provides well-documented enzymatic parameters for application in enzyme-driven SOC decomposition models from a compilation and analysis of published measurements. In particular, we developed appropriate kinetic parameters for five typical ligninolytic and cellulolytic enzymes (β-glucosidase, cellobiohydrolase, endo-glucanase, peroxidase, and phenol oxidase). The kinetic parameters included the maximum specific enzyme activity (V_{max}) and half-saturation constant (K_m) in the Michaelis–Menten equation. The activation energy (Ea) and the pH optimum and sensitivity (pH_{opt} and pH_{sen}) were also analyzed. pH_{sen} was estimated by fitting an exponential-quadratic function. The V_{max} values, often presented in different units under various measurement conditions, were converted into the same units at a reference temperature (20 °C) and pH_{opt}. Major conclusions are: (i) Both V_{max} and K_m were log-normal distributed, with no significant difference in V_{max} exhibited between enzymes originating from bacteria or fungi. (ii) No significant difference in V_{max} was found between cellulases and ligninases; however, there was significant difference in K_m between them. (iii) Ligninases had higher Ea values and lower pH_{opt} than cellulases; average ratio of pH_{sen} to pH_{opt} ranged 0.3–0.4 for the five enzymes, which means that an increase or decrease of 1.1–1.7 pH units from pH_{opt} would reduce V_{max} by 50%. (iv) Our analysis indicated that the V_{max} values from lab measurements with purified enzymes were 1–2 orders of magnitude higher than those for use in SOC decomposition models under field conditions.

1. Introduction

Recent developments in modeling of soil organic carbon (SOC) and plant litter decomposition have explicitly taken into account the role of enzymes (Allison et al., 2010; Lawrence et al., 2009; Moorhead and Sinsabaugh, 2006; Schimel and Weintraub, 2003). Enzyme-driven decomposition is usually characterized by the Michaelis–Menten (M–M) kinetics (Allison et al., 2010; Gershenson et al., 2009; Grant et al., 1993; Manzoni and Porporato, 2009) or the reverse M–M kinetics (Lawrence et al., 2009; Moorhead and Sinsabaugh, 2006; Schimel and Weintraub, 2003). Both model mechanisms are parameterized by two kinetic parameters, maximum specific reaction rate (V_{max}), and half-saturation constant (K_m).

V_{max} is modified by temperature and pH (Tabatabai, 2003), in addition K_m is also positively dependent on temperature (Gershenson et al., 2009). Not all enzymes have the same temperature and pH optimum. Activation energy (Ea) is a key parameter in the Arrhenius equation describing the reaction rate response to temperature under optimal conditions (Calsavara et al., 2001; McClaugherty and Linkins, 1990). Obtaining and comparing values for the three parameters (V_{max}, K_m, and Ea) can be difficult because they require numerous enzyme assays at multiple temperature and pH values, and current assay protocols are quite varied (Han and Srinivasan, 1969). In addition, reported measurements of enzyme parameters are often provided in different units. For example, V_{max} was reported as U mg^{-1} Enz (Vila-Real et al., 2010; Yague and Estevez, 1988), nkat mg^{-1} Enz (Sue et al., 2000), U μmol^{-1} Enz (Chauve et al., 2010), mmol $mmol^{-1}$ Enz s^{-1} (Wallecha and Mishra, 2003), $\mu g\ mg^{-1}$ Enz min^{-1} (Takahashi et al., 2010), or $\mu g\ g^{-1}$ soil h^{-1} (Eivazi and Tabatabai, 1988).

Useful and documented parameter estimates are necessary to ensure appropriate parameters for use in SOC models. In particular, proper understanding of lignocellulose-degrading enzyme kinetics is essential for modeling the decomposition of plant litter and soil organic matter (SOM) because of the carbohydrate- and phenolic-based structures of SOM and plant materials (Caldwell, 2005; McGuire and Treseder, 2010; Sinsabaugh et al., 2002). Cellulolytic enzymes (cellulases) are hydrolases that catalyze the breakdown of glycosidic bonds in cellulose. The degradation of cellulose requires at least three enzymes: β-glucosidase (BG), cellobiohydrolase

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(CBH), and endo-glucanase (EG) (Baldrian, 2009; Baldrian and Stursova, 2011; Shi, 2011). Ligninolytic enzymes (ligninases) are oxidoreductases that modify phenolic-containing organics including polyphenols and lignin (Dashtban et al., 2010; Levasseur et al., 2008; Shi, 2011). Typical ligninase includes peroxidase (PER) and phenol oxidase (POX) (Caldwell, 2005; Sinsabaugh et al., 2008; Tabatabai, 2003).

The objective of this study was to provide well-documented enzymatic parameters for application in enzyme-driven SOC decomposition models by compiling a database of kinetic parameters for typical ligninases and cellulases through a literature research and data synthesis.

2. Materials and methods

2.1. Literature review

We compiled a database of kinetic properties of 5 ligninases and cellulases from the literature (Table 1). Kinetic parameters (i.e., maximum specific enzyme activity (V_{max}) and half-saturation constant (K_m)) and corresponding experimental conditions (origin, substrate, temperature, and pH) were collected from 202 observations of 73 publications (Table 2). V_{max} and K_m are important kinetic parameters to characterize the enzyme-driven reactions in the M–M equation (Allison et al., 2010; Molz et al., 1986):

$$v = V_{max}\frac{E\cdot S}{K_m + S} \quad (1)$$

where v is the reaction rate; V_{max} and K_m are the maximum specific enzyme activity and the half-saturation constant, respectively; and E and S are the concentration of enzyme and substrate, respectively.

Various assumptions for K_m relative to the substrate concentration (S) led to different simplifications of Eq. (1) (Manzoni and Porporato, 2009). If $K_m >> S$, Eq. (1) can be reduced to the simple first-order kinetics:

$$v = \frac{V_{max}}{K_m}\cdot E\cdot S \quad (2)$$

Otherwise, if $K_m << S$, then

$$v = V_{max}\cdot E \quad (3)$$

Most of the studies adopted the Lineweaver–Burk (LB) transformation to estimate V_{max} and K_m in Eq. (1) (Han and Srinivasan, 1969), although other transformations (i.e., Hanes-Wolf (HW) and Eadie-Hofstee (EH)) or methods (e.g., Eisenthal & Cornish-Bowden and Wilkinson) have been shown to be superior to LB (Atkins and Nimmo, 1975; Dowd and Riggs, 1965). One publication used HW or EH transformation (Patchett et al., 1987), another one employed an optimization approach called the Nelder-Mead simplex (Gusakov et al., 2005), and four papers mentioned nonlinear regressions (Bratkovskaja et al., 2004; Jeya et al., 2010; Kempton and Withers, 1992; Nikolova et al., 1997). Since LB was most often used to derive the kinetic parameters, we did not distinguish among the data using different regression methods.

The optimum temperature and pH values for enzyme activity, the *Ea* and molecular weight (*MW*), if mentioned in these publications, were also recorded. 18 and 17 additional publications were reviewed to collect more data of *Ea* and *MW*, respectively. The *MW* (in units of kDa) (Plant et al., 1988) was used to convert the units of V_{max} in the case that the enzyme protein was quantified in units of mole. The final dataset included 78 observations from 39 papers for *Ea* and 107 observations from 72 papers for *MW*.

2.2. Response functions of temperature and pH

The kinetic properties of enzymes are usually measured under specific temperature and pH conditions. The influences of temperature and pH on the kinetic properties, especially on maximum specific enzyme activity, were examined in nearly all of the studies. The modification of maximum specific enzyme activity by soil temperature and pH can be described as follows:

$$V_{max} = V_{max0}\cdot f(T)\cdot f(pH) \quad (4)$$

where V_{max} is the maximum specific enzyme activity at temperature (T) and pH; V_{max0} is the maximum specific enzyme activity at a reference temperature (T_{ref}) and optimum pH (pH_{opt}).

The influence of soil temperature is calculated by the Arrhenius equation (Gonçalves et al., 2007; Wallenstein et al., 2011):

$$f(T) = \exp\left[-\frac{Ea}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right] \quad (5)$$

where T and T_{ref} are the temperature and reference temperature (K), respectively; Ea is the energy of activation (kJ mol^{-1}); and $R = 8.314$ J mol^{-1} K^{-1}, is the universal gas constant. In addition, Q_{10} is widely used to express the temperature sensitivity (Davidson et al., 2006; Ise and Moorcroft, 2006):

$$f(T) = Q_{10}^{\frac{T-T_{ref}}{10}} \quad (6)$$

where Q_{10} is the factor by which enzyme activity is multiplied when temperature increases by 10 °C.

Combining Eqs. (5) and (6), we can find the relationship between Q_{10} and Ea:

$$Q_{10} = \exp\left[\frac{Ea}{R\cdot T_{ref}}\cdot\frac{10}{T}\right] \quad (7)$$

The response function of pH can be expressed by an exponential-quadratic function (Reth et al., 2005):

$$f(pH) = \exp\left[-\left(\frac{pH - pH_{opt}}{pH_{sen}}\right)^2\right] \quad (8)$$

where pH_{opt} is the optimum pH that gives the maximum reaction rate; and pH_{sen} refers to the sensitivity of the reaction rate to deviation from pH_{opt}.

Table 1
Enzymes studied for lignocellulose degradation.

Substrate	Enzyme[a]	Abbr.	EC#[b]
Cellulose	β-glucosidase; Cellobiase	BG	3.2.1.21
	Cellulose 1,4-β-cellobiosidase; Cellobiohydrolase; Exoglucanase	CBH	3.2.1.91
	Cellulase; Endo-glucanase; Carboxymethylcellulase	EG	3.2.1.4
Lignin	Peroxidase	PER	1.11.1.7
	Laccase; Phenol oxidase	POX	1.10.3.2

[a] For each enzyme, the first is the recommended name, and the others are synonyms.
[b] EC number: Enzyme Commission number authorized by the International Union of Biochemistry.

Table 2
Summary of data used for the analysis of ligninolytic and cellulolytic enzyme kinetics (the associated full data series are presented in the Supplementary Table 1).

Enzyme[a]	Origin[b]	V_{max}[c]	K_m[d]	Reference
BG	Bacteria	8.9–837.4	0.02–120	(Ait et al., 1982; Han and Srinivasan, 1969; Kempton and Withers, 1992; Namchuk and Withers, 1995; Patchett et al., 1987; Plant et al., 1988)
	Fungi	9.1–1480.4	0.2–24.0	(Amouri and Gargouri, 2006; Chauve et al., 2010; Issam et al., 2003; Jeya et al., 2010; Joo et al., 2009; Kaur et al., 2007; Le Traon-Masson and Pellerin, 1998; Rajoka et al., 2006; Riou et al., 1998; Saha and Bothast, 1996; Saha et al., 1994; Wallecha and Mishra, 2003; Wei et al., 1996)
	Plant	186.3	1.2	(Sue et al., 2000)
	Soil	165–3973	1.6–56.9	(Deng and Tabatabai, 1994; Eivazi and Tabatabai, 1988)
CBH	Bacteria	17.2	0.7	(Nikolova et al., 1997)
	Fungi	0.04–39.41	0.1–24.3	(Gusakov et al., 2005; Lahjouji et al., 2007; Lee et al., 2011; Schmidhalter and Canevascini, 1993; Shin et al., 2010; Takahashi et al., 2010; Voutilainen et al., 2008)
EG	Bacteria	61.7–1084.1	0.1–88.6	(Cho et al., 2000; Lin and Stutzenberger, 1995; Mejia-Castillo et al., 2008; Ng and Zeikus, 1981; Pérez-Avalos et al., 2008; Pétré et al., 1986; Warner et al., 2010)
	Fungi	0.3–7901.0	0.01–71	(Bhat et al., 1989; Christakopoulos et al., 1995; Elshafei et al., 2009; Elvan et al., 2010; Jabbar et al., 2008; Javed et al., 2009; Karnchanatat et al., 2008; Kaur et al., 2007; Lee et al., 2010; Li et al., 2003; Lim et al., 1991; Onyike et al., 2008; Saqib et al., 2010; Siddiqui et al., 2000; Tong et al., 1980; Yoon et al., 2008)
PER	Fungi	1.3–3144.6	0.03–0.79	(Bourbonnais and Paice, 1988; Bratkovskaja et al., 2004; Farrell et al., 1989; Kersten et al., 1990; Ryu et al., 2008; Tien et al., 1986)
	Plant	490.9	5.3	(Guida et al., 2011)
	Fungi[e]	67.3	0.1	(Kersten et al., 1990)
	Plant[e]	1625.5	NA	(Lavery et al., 2010)
	Animal[e]	1527–1773	12.1–21.1	(Targovnik et al., 2010)
POX	Fungi	1.0–2119.4	0.01–8	(Aktas et al., 2001; Chakroun et al., 2010; Farnet et al., 2010b; Kersten et al., 1990; Liers et al., 2007; Lu et al., 2007; Munoz et al., 1997; Palmeiri et al., 1993; Shleev et al., 2004)
	Plant	92.6	NA	(Niemetz and Gross, 2003)
	Soil	826.1	NA	(Floch et al., 2007)
	Animal	3.4–12.4	0.5–1.9	(Dittmer et al., 2009)

[a] Enzymes: BG: β-1,4-glucosidase; CBH: Cellobiohydrolase; EG: Endo-glucanase; PER: Peroxidase; POX: Phenol oxidase.
[b] Origin of enzyme.
[c] Range of V_{max}: maximum specific enzyme activity (mg C mg^{-1} Enz h^{-1}) at 20 °C and optimum pH.
[d] Range of K_m: Half-saturation constant (mM), "NA" indicates no data available.
[e] data for Horseradish-PER.

2.3. Enzymatic data analysis

The majority of kinetic parameters were measured by laboratory experiments using purified enzymes originating from fungi or bacteria (Table 2). There was not enough data from enzymes derived from non-fungal or non-bacterial sources to distinguish their potential differences. Thus V_{max} and K_m were analyzed based on those data with enzymes from bacteria and fungi. The data from other sources (animal, plant, and soil) were excluded from the statistical analysis. The significance of difference in V_{max} or K_m between bacteria and fungi was tested for BG and EG since the data from both sources were available. The BGs purified from yeast (Saha and Bothast, 1996; Wallecha and Mishra, 2003) were included in the category of fungi. In addition, only the data from lignin-PER were included in the analysis for PER. Statistical analyses of parameters were conducted in R software (R Development Core Team, 2011). Descriptive statistics of parameters include mean, median, standard deviation, minimum, and maximum. If the standard deviations depended on means of the parameter, data transformations (e.g., logarithmic, square-root, or reciprocal) were implemented to stabilize the variances (Bland and Altman, 1996; Keene, 1995; Lin et al., 2008). The significance of difference in kinetic parameters between two enzyme origins (fungi and bacteria) was tested by ANOVA (Devore, 2008). The difference in kinetic parameters of various enzymes was tested by the Fisher's least significant difference (LSD) (de Mendiburu, 2010) or non-parametric Kruskal–Wallis (KW) test (Giraudoux, 2011; Ott and Longnecker, 2010) at a significance level of $\alpha = 0.05$.

To compare V_{max} from various experiments under different temperature and pH conditions, we converted V_{max} to the same units, mg C mg^{-1} Enz h^{-1}, at a T_{ref} of 20 °C (Doiron, 2007) and pH_{opt}. "Enz" in the units denotes a particular enzyme protein. The parameters V_{max} at T_{ref} and pH_{opt} can be easily used in lignocellulose-degrading models provided that the response functions of temperature and pH (Eqs. (5) and (8)) are known. The conversion of V_{max} included two steps: (i) convert V_{max} to the same units, i.e., mg C mg^{-1} Enz h^{-1}; and (ii) modify V_{max} to a reference temperature (we set it to 20 °C) and pH_{opt} using Eqs. (4), (5) and (8) with corresponding temperature and pH at which the V_{max} was measured. While applying Eqs. (5) and (8) in Step (ii), we must notice that: (a) the inverses of Eqs. (5) and (8) should be used since we want to obtain V_{max0} at T_{ref} and pH_{opt} from V_{max} at the experimental temperature and pH; and (b) the average Ea and pH_{opt} from our database would be used if they were not presented in the literature.

The effect of pH on V_{max} was usually presented by data plots in the publications we studied. We estimated pH_{sen} using Eq. (8) through curve fitting of the pH response data. We redrew the data points via normalizing the V_{max} by the largest observed V_{max}. Thus the normalized V_{max} values fell into the range between 0 and 1. The values of pH_{sen} from the curve fitting (50 observations) and the pH_{opt} from the larger samples (162 observations) were combined to characterize the pH response function for the enzymes.

Most of the K_m values were expressed in mM (mmol L^{-1}) in the lab experiments. When K_m was in mg mL^{-1}, the units were converted to mM by using the appropriate substrate molecular weight. We use a molecular weight of 282 g mol^{-1} for the substrate carboxymethyl cellulose (CMC) (Ichikawa et al., 2005).

2.4. Estimation of maximum specific enzyme activity in soil

The maximum specific enzyme activity under soil conditions could be estimated based on a steady-state analysis of a simplified SOC dynamics:

$$\frac{dSOC}{dt} = I_S - \frac{V_{max,S} \cdot E_S \cdot SOC}{K_{m,S} + SOC} \tag{9}$$

where SOC (mg C g^{-1} soil) and E_S (mg Enz g^{-1} soil) denote the contents of SOC and associated enzymes in soil; $V_{max,S}$ and $K_{m,S}$ are the maximum specific enzyme activity (mg C mg^{-1} Enz h^{-1}) and the half-saturation constant (mg C g^{-1} soil) under soil conditions, respectively; and I_S (mg C g^{-1} soil h^{-1}) is the average carbon input rate to soil. The second term in the right hand of Eq. (9) refers to the decomposition of SOC.

The steady-state solution of Eq. (9) gives

$$V_{max,S} = \frac{K_{m,S} + SOC_{eq}}{SOC_{eq}} \cdot \frac{I_S}{E_S} \quad (10)$$

where SOC_{eq} is the steady-state SOC content.

The values of $K_{m,S}$ are thought to be comparable to (more commonly greater than) the average substrate concentrations in soil (German et al., 2011). This implies that the M–M equation, i.e., Eq. (1), is fully functional. Thus the magnitude of $V_{max,S}$ can be determined by I_S/E_S. We collected some data on I_S and E_S to quantify the ratio of these two items.

3. Results

3.1. Activation energy and optimum temperature

The average *Ea* values were 42, 32, 34, 53, and 54 kJ mol^{-1} for BG, CBH, EG, PER, and POX, respectively. Based on Eq. (7), the above *Ea* values corresponded to Q_{10} of 1.8, 1.6, 1.6, 2.1, and 2.1 with an increase from 20 °C to 30 °C. According to the LSD test, no significant difference was found among POX, PER, and BG, nor among BG, EG, and CBH (Fig. 1a). There was significant difference in *Ea* between cellulases and ligninases, i.e., 37 ± 15 and 53 ± 17 kJ mol^{-1}, respectively.

The average T_{opt} ranged 48–60 °C. The T_{opt} for PER was significantly lower than that for the others (Fig. 1b). A significant difference (p-value = 0.0002) was found between the mean T_{opt} values of cellulases and of ligninases, i.e., 59 ± 9 and 54 ± 8 °C, respectively.

3.2. Optimum pH and response function

The average pH_{opt} values ranged 4.1–5.6 (Fig. 1c). No significant difference was found within each group (cellulases and ligninases), but the pH_{opt} for cellulases was higher than that for ligninases (5.3 ± 0.9 vs. 4.2 ± 0.9).

The curve fitting results of pH response functions are shown in Fig. 2. The good performance of curve-fittings indicates that Eq. (8) can be used to describe the effect of pH on the V_{max}. The average pH_{sen} ranged from 1.4 to 2.1, which indicated an average ratio of pH_{sen} to pH_{opt} of 0.3–0.4 across all enzymes (Table 3).

3.3. Maximum specific enzyme activity (V_{max})

V_{max} at T_{ref} (20 °C) and pH_{opt} showed great variability spanning several orders of magnitude from 10^{-2}–10^{4} mg C mg^{-1} Enz h^{-1}. The coefficient of variation (CV) of V_{max} was greater than 1.0 (between 1.1 and 2.1). The Kolmogorov–Smirnov goodness-of-fit (KS) test (p-value < 10^{-4}) rejected the null-hypothesis of a normal distribution of V_{max} (Giraudoux, 2011). Fig. 3a indicates that the standard deviation was proportional to mean of V_{max}. Therefore, a logarithmic-transformation was used to eliminate the dependence, which was evidenced by the plot of standard deviation versus mean of $\log(V_{max})$($\log_{10}$ of V_{max}) in Fig. 3b (Bland and Altman, 1996). In addition, the KS test of $\log(V_{max})$ failed to reject the null-hypothesis (p-value = 0.34 > 0.05) implying a normal distribution of $\log(V_{max})$. Therefore, the significance of difference was tested in terms of $\log(V_{max})$ instead of V_{max}.

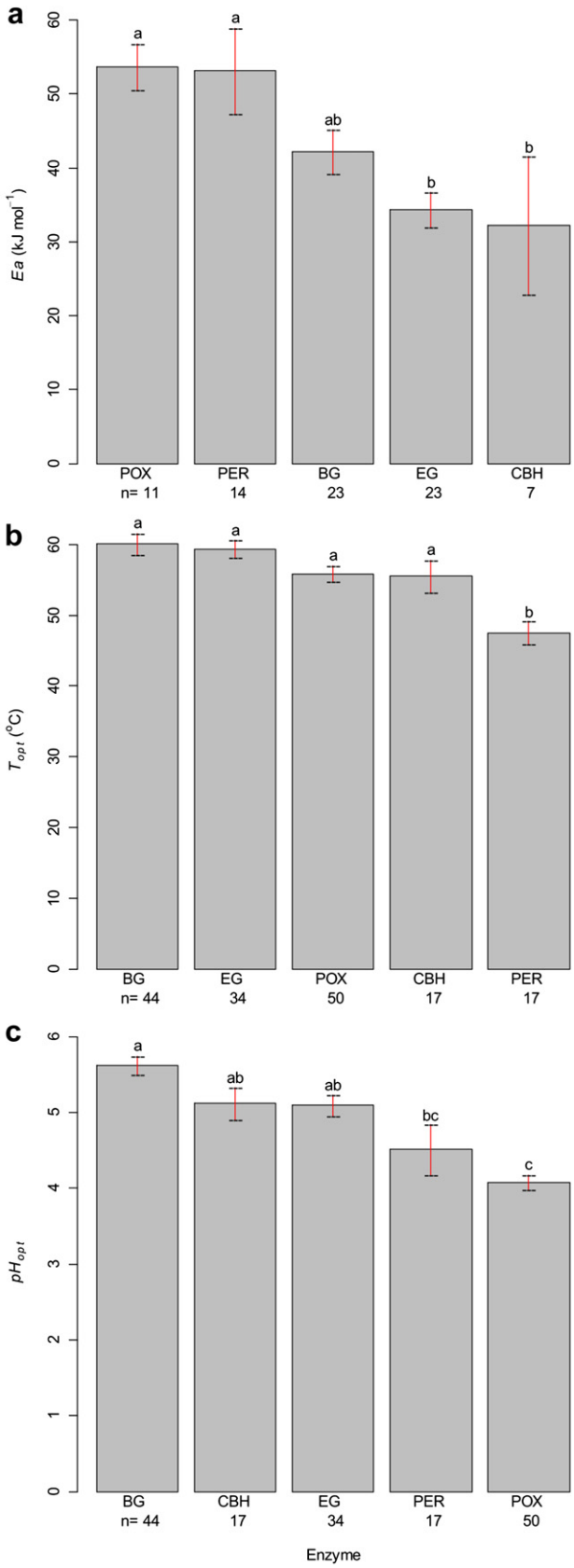

Fig. 1. (a) Activation energy (*Ea*, kJ mol^{-1}), (b) Optimum temperature (T_{opt}, °C), and (c) Optimum pH (pH_{opt}) for enzymes BG: β-glucosidase, CBH: cellobiohydrolase, EG: endo-glucanase, PER: peroxidase, and POX: phenol oxidase; the numbers below enzyme names denote the sample sizes (n); the error bars refer to standard errors; different letters on error bars indicate significantly different means at $P < 0.05$ according to the LSD test.

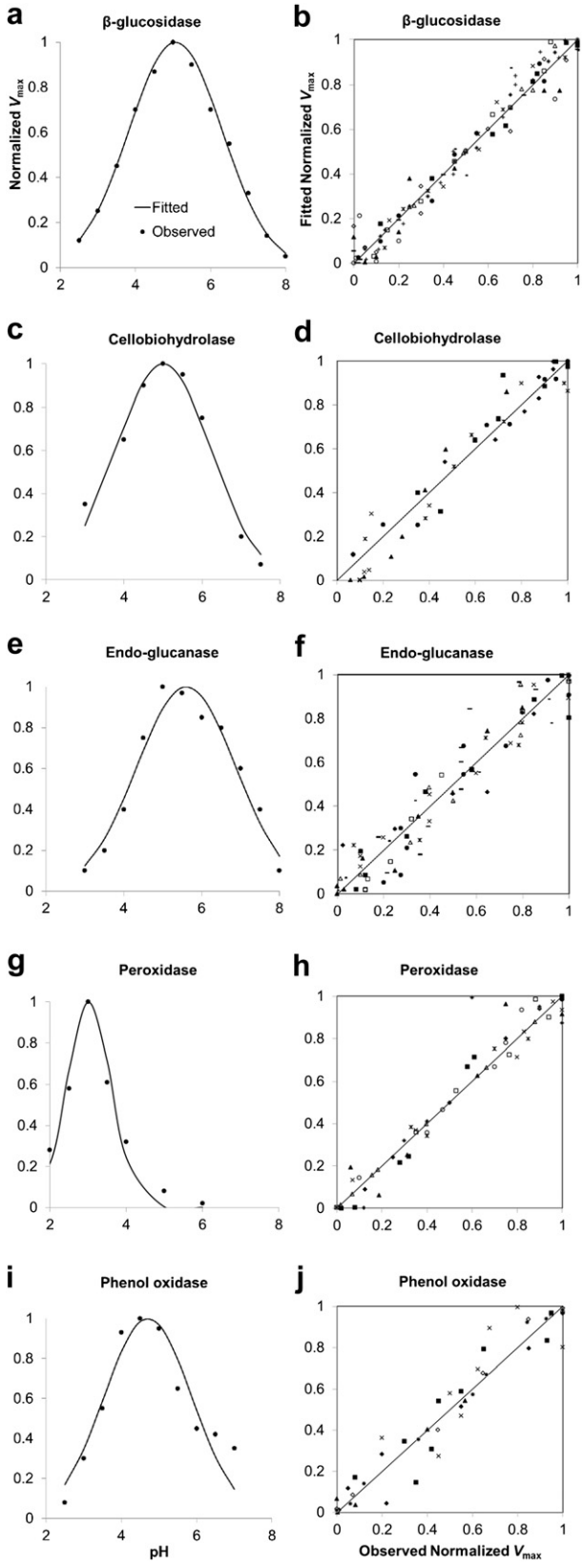

Fig. 2. Effect of pH on maximum specific enzyme activity; (a), (c), (e), (g), and (i): example of curve fitting of response function; (b), (d), (f), (h), and (j): comparison between fitted and observed normalized V_{max} with data from some publications in Table 2.

Table 3
Optimum pH (pH_{opt}) and sensitivity of pH (pH_{sen}) in the pH response function (Eq. (8)) for different enzymes.

Enzyme	pH_{opt}	pH_{sen}
BG	5.6 ± 0.8 (44)[a]	1.7 ± 0.7 (13)
CBH	5.1 ± 0.9 (17)	2.1 ± 1.2 (6)
EG	5.1 ± 0.8 (34)	1.6 ± 0.4 (10)
PER	4.5 ± 1.4 (17)	1.5 ± 0.7 (9)
POX	4.1 ± 0.7 (50)	1.4 ± 0.4 (12)

[a] mean ± standard deviation (sample size).

There were 31 and 27 observations of kinetic parameters for BG and 8 and 26 observations for EG from bacteria and fungi, respectively. The ANOVA tests showed that there were no significant differences in $\log(V_{max})$ between enzymes from bacteria and fungi, where the p-values were 0.70 and 0.73 (>0.05) for BG and EG, respectively. The LSD test on $\log(V_{max})$ indicated that the mean of CBH was significantly lower than the mean values of others (Fig. 4). No significant difference was found between ligninases and cellulases (p-value = 0.27). The values of $\log(V_{max})$ were 1.91 ± 1.03 and 1.75 ± 0.90, corresponding to the mean V_{max} of 81 and 56 and the one-std-intervals of (8, 871) and (7, 447) mg C mg^{-1} Enz h^{-1} for cellulases and ligninases, respectively. The one-std-interval for V_{max} is the back-transformed interval from the one-standard deviation interval of $\log(V_{max})$. The two-std-intervals of V_{max} were 0.7–9333 and 0.9–3548 mg C mg^{-1} Enz h^{-1} for cellulases and ligninases, respectively. The KW tests on V_{max} and $\log(V_{max})$ produced the same comparison results as the LSD test on $\log(V_{max})$, which demonstrated no requirement of equal-variances or normal distribution for the KW test (Ott and Longnecker, 2010).

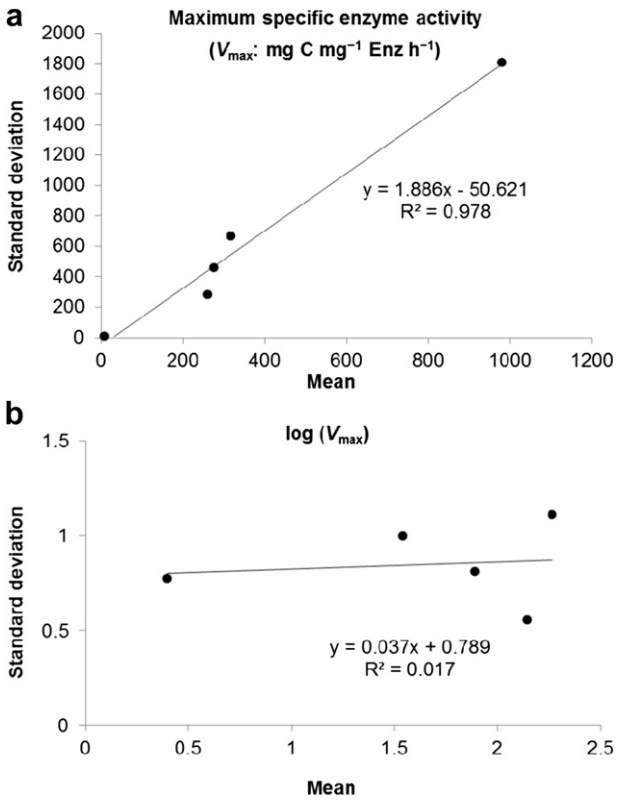

Fig. 3. (a) The relationship between standard deviation and mean of maximum specific enzyme activity (V_{max}, mg C mg^{-1} Enz h^{-1}); (b) Standard deviation versus mean of logarithmic-transformed V_{max}, $\log(V_{max})$.

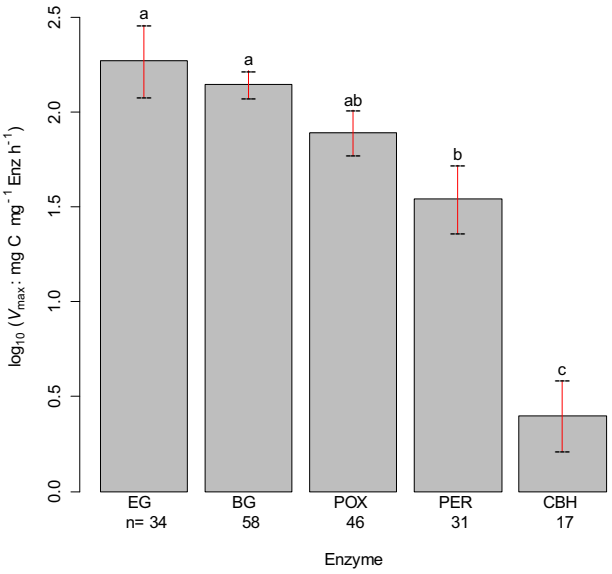

Fig. 4. Comparison of logarithmic-transformed maximum specific enzyme activity (V_{max}, mg C mg^{-1} Enz h^{-1}) at 20 °C and optimum pH; the numbers below enzyme names denote the sample sizes (n); the error bars refer to standard errors; different letters on error bars indicate significantly different means at $P < 0.05$ according to the LSD test.

3.4. Half-saturation constant (K_m)

K_m spanned several orders of magnitude from 10^{-3}–10^{2} mM. The KS test of K_m (p-value $< 10^{-4}$) rejected the null-hypothesis of a normal distribution of K_m. A logarithmic-transformation (p-value $= 0.67 > 0.05$ for normality test) was also implemented for further statistical analysis (Fig. 5).

The ANOVA tests showed that there were no significant differences in log(K_m) between enzymes from bacteria and fungi, where the p-values were 0.76 and 0.06 (>0.05) for BG and EG, respectively. The ANOVA test also showed the mean log(K_m) of ligninases was significantly lower than that of cellulases (p-value$<10^{-4}$). The values of log(K_m) were 0.26 ± 0.91 and -0.67 ± 0.74, corresponding to the mean K_m of 1.82 and 0.21 and the one-std-intervals of (0.22, 14.79) and (0.04, 1.17) mM for cellulases and ligninases, respectively. Additionally, the two-std-intervals of K_m were 0.03–120.23 and 0.01–6.46 mM for cellulases and ligninases, respectively. There was no significant difference in log(K_m) between PER and POX, but the mean log(K_m) of EG was significantly higher than that of BG and CBH (Fig. 6).

3.5. Maximum specific enzyme activity in soil ($V_{max,S}$)

The concentration of lignocellulolytic enzymes in soils (E_S) is 10^{-5}–5×10^{-3} mg Enz g^{-1} soil (Tabatabai, 2003). Based on the literature data in Table 4, the carbon input rate followed a log-normal distribution (p-value $= 0.69$) and log(I_S) $= -3.85 \pm 0.28$. The back-transformed two-std-interval of I_S ranged 4×10^{-5}–5×10^{-4} mg C g^{-1} soil h^{-1}. Thus I_S/E_S yielded a range of 0.008–50 mg C mg^{-1} Enz h^{-1}. With the previous assumption that $K_{m,S}$ is likely close to SOC_{eq}, the range of $V_{max,S}$ was estimated as 10^{-2}–10^{2} mg C mg^{-1} Enz h^{-1}.

4. Discussion

4.1. Sensitivities of enzyme kinetics to temperature and pH

We have studied the statistical features of Ea and T_{opt} that describe the impact of temperature on enzyme activity. The

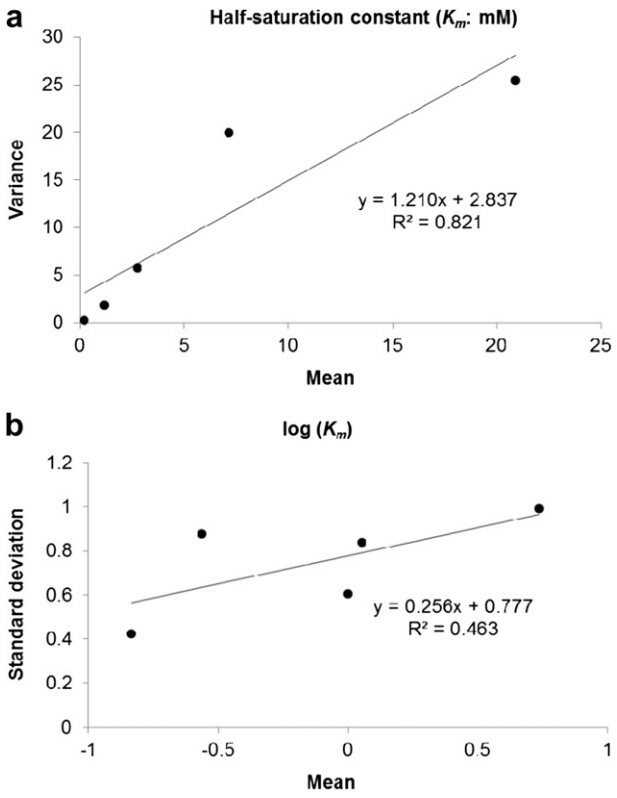

Fig. 5. (a) The relationship between standard deviation and mean of half-saturation constant (K_m, mM); (b) Standard deviation versus mean of logarithmic-transformed K_m, log(K_m).

equivalent Q_{10} values from purified enzymes fell into the range of 1.64–2.27 derived from experiments using soil as enzyme sources (Stone et al., 2011). The Ea values were significantly higher for ligninases than for cellulases. This was consistent with the carbon-

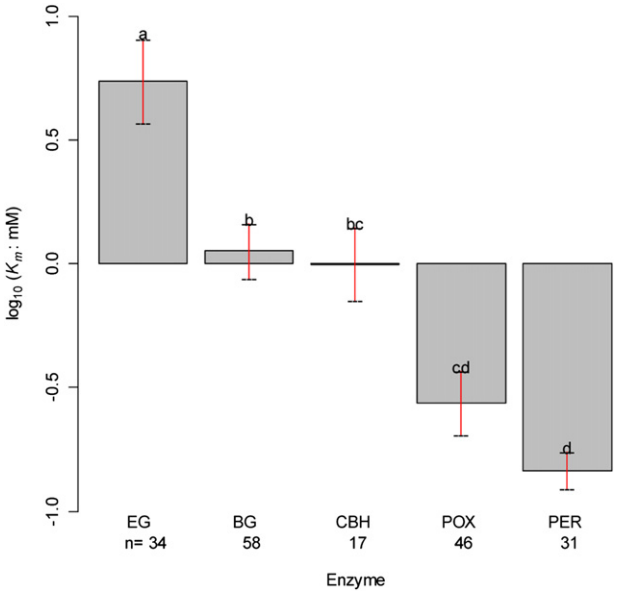

Fig. 6. Comparison of logarithmic-transformed half-saturation constant (K_m, mM); the numbers below enzyme names denote the sample sizes (n); the error bars refer to standard errors; different letters on error bars indicate significantly different means at $P < 0.05$ according to the LSD test.

Table 4
Summary of external carbon input rate to the soil (the associated full data series are presented in the Supplementary Table 2).

Carbon input rate[a] (10^{-4} mg C g^{-1} soil h^{-1})	Land use	Reference
1.32	cultivated	(Jastrow, 1996)
0.46–2.81	wheat, barley	(Jenkinson et al., 1992; Keith et al., 1986; Kuzyakov and Domanski, 2000)
0.22–4.22	pasture, grass	(Jastrow, 1996; Jenkinson et al., 1992; Kuzyakov and Domanski, 2000)
0.95–5.23	forest, woodland	(Godbold et al., 2006; Jenkinson et al., 1992; Lugo and Brown, 1986)
5.00	–	(Allison et al., 2010)

[a] Assuming a 10-cm soil depth (Jastrow, 1996).

quality-temperature hypothesis, which assumes that the enzymatic reactions on low-quality C substrates require higher net activation energies (Kleber, 2010). It also implied that the temperature-driven changes in enzyme activity were greater for ligninases than for cellulases. T_{opt} values (48–60 °C) were much higher than *in situ* soil temperatures and might be regarded as a measure of the relative stability of soil enzymes (Wallenstein et al., 2011), although their ecological relevance is disputable (Wallenstein and Weintraub, 2008). While being used to model the dependence of decomposition rate on temperature, *Ea* is usually regarded invariant, although both *Ea* and T_{opt} values may be modified by the environmental interactions between enzymes and organic and inorganic particles (Wallenstein et al., 2011). For example, *Ea* for enzyme-driven decomposition also changes with pH (Kleber, 2010), which implies the effects of temperature and pH may confound each other. pH changes might affect the catalytic reactions by means of changing the binding of substrate to enzyme and altering the conformation of the enzyme protein (Jakubowski, 2010).

The mean pH_{opt} of POX was below 5, which was in accordance with the study by Bollag and Leonowicz (1984) and the conclusion that fungal POX typically has an acidic pH_{opt} (Baldrian, 2006). When the exponential-quadratic function (Eq. (8)) was used to describe the pH impact on enzyme activity, the average ratio of pH_{sen} to pH_{opt} was 0.34 (ranged 0.3–0.4). This means that a pH increase or decrease of 1.1–1.7 pH units ($\approx 0.3 \times pH_{opt}$) from pH_{opt} would reduce the specific enzyme activity by 50%, and an increase or decrease of 2.0–3.2 ($\approx 0.5 \times pH_{opt}$) would result in a decrease of 90% in specific activity. The pH response function used herein was a symmetric one. However, asymmetric responses of V_{max} to pH have been observed for BG and POX in our database (e.g., Joo et al., 2009; Patchett et al., 1987; Rogalski et al., 1999; Wei et al., 1996). This asymmetry might be caused by measurement errors or changes in buffers to obtain the desired pH values, where a buffer could stimulate or inhibit the enzyme activity (Wei et al., 1996).

Very few experiments examined the sensitivities of K_m to temperature or pH in the literature. Consistent trends were not observed in terms of the changes in K_m with temperature (Nedwell, 1999). Stone et al. (2011) concluded that K_m (Q_{10} = 1.04–1.93) exhibited lower temperature sensitivities than V_{max} in terms of hydrolytic enzymes in soils of hardwood forests. Another study was conducted on a chitin-degrading N-acetyl-β-D-glucosaminidase (NAG, EC number: 3.2.1.14) by Buchholz and Vetter (1993), in which the response curves of K_m to temperature had U-shapes or piecewise-linear-increase-shapes, and the variations were only between a narrow range of substrate concentrations (0.1–0.6 mM). Some studies discussed the temperature response of K_m using the Q_{10} (Atkin and Tjoelker, 2003; Davidson and Janssens, 2006; Davidson et al., 2006). Specifically, Davidson et al. (2006) used a $Q_{10} = 2$ for V_{max} and $Q_{10} = 2$, 1.5, or 1.0 for K_m. In Allison et al. (2010), a linear-increase function was used to express the temperature dependence of K_m. From these limited studies, we might conclude that K_m is positively dependent on temperature but is perhaps less sensitive to temperature compared to V_{max}.

4.2. Comparison of kinetic parameters between lab and field-soil conditions

From Fig. 6, average K_m for EG was significantly higher than the others. This is, to a great extent, because CMC was almost always used as substrate for EG in the experiments, and it is a good substrate for EG (Ichikawa et al., 2005). At this point it is difficult to link K_m measured in the laboratory with K_m in field soils, where enzymes and SOC are not fully mixed and SOC is protected against decomposition by enzymes under certain conditions like sorptive interactions with minerals and physical separations (Kleber, 2010; Kleber et al., 2007). In addition, the lab-derived K_m is measured under solution conditions and expressed in units of mM or mg L^{-1} solution, which is difficult to convert to the units of mg C g^{-1} soil or mg C cm^{-3} soil required in the SOC decomposition models because of their incomparability.

Generally, the few observations of V_{max} measured in lab with enzymes from other sources (plant, animal, or soil, see Table 2) fell into the two-std-intervals derived from bacterial and fungal enzymes. The V_{max} values for BG originating from rye (Sue et al., 2000) and soil (Deng and Tabatabai, 1994) were included in the one-std-interval. The V_{max} values of Horseradish-PER from the horseradish (*Armoracia rusticana*) (Lavery et al., 2010) and animal (*Spodoptera frugiperda*) (Targovnik et al., 2010) were greater than the one-std-interval but still within the two-std-interval of lignin-PER. The V_{max} values of POX from the animal (Manduca sexta) (Dittmer et al., 2009) were close to the lower bound of the one-std-interval of bacterial and fungal POX. The V_{max} is also affected by the substrate used (Wallenstein and Weintraub, 2008). The measurements for POX with ABTS as substrate generally had higher V_{max} due to its role as oxidation mediator and its higher extinction coefficient (German et al., 2011).

Enzyme activities from purified enzymes and abundant substrates are likely much greater than field enzyme activities (Wallenstein et al., 2011; Wallenstein and Weintraub, 2008). Our data analysis showed that V_{max} from lab experiments ranged 10^0–10^4 (mean: 10^2) mg C mg^{-1} Enz h^{-1}, which was higher than the V_{max} (10^{-2}–10^2) for the SOC decomposition. Considering the effects of environmental factors such as soil temperature, pH and water, we might conclude that the lab-measured V_{max} are 1–2 orders of magnitude higher than the *in situ* values. The above calculations were based on wide ranges of I_S and E_S, which provided quantitative information on the magnitude of the scaling factors that should be used for model simulations. However, appropriate scaling factors should be used for specific ecosystems and different SOC pools. The reasons for the discrepancy of enzyme kinetics in the soil from those in idealized lab experiments might include: (i) the adsorption of enzymes to organic or mineral surfaces (Wallenstein and Weintraub, 2008; Zimmerman and Ahn, 2011); (ii) the complexing of reactive polyphenolics (e.g., tannins) with both enzymes and substrates (Niemetz and Gross, 2003; Zimmerman and Ahn, 2011); (iii) substrate solubility (Farnet et al., 2010b); (iv) the more complex soil environment than the enzyme assays (Zimmerman and Ahn, 2011); and (v) the more

complex lignocellulolytic material than the simple substrate used in lab resulting in the competition among substrates (Baldrian, 2006).

The inhibition of substrate or product is not considered in the aforementioned M–M kinetics. For example, glucose, as a product of cellulose-degradation, is an inhibitor for BG-catalytic reactions (Chauve et al., 2010). This kind of product inhibition is called competitive inhibition (Jakubowski, 2010; Maguire, 1977) and Eq. (1) can be modified to:

$$v = V_{\max}\frac{E\cdot S}{K_m(1+P/K_P)+S} \tag{11}$$

where P is the initial product concentration, K_p is the inhibition constant, $K_m(1+P/K_p)$ is called apparent half-saturation constant. For BG purified from *Aspergillus niger*, $K_m/K_p = 0.26$ and 0.21 for substrate cellobiose and pNPG, respectively (Chauve et al., 2010). $K_m/K_p = 0.32 \pm 0.19$ ($n = 10$) for BG with various substrates according to the data from Table 1 of Chauve et al. (2010). Consideration of inhibition adds additional complexity that we do not consider here.

4.3. Effect of soil water

Water is not a limiting factor in the lab enzyme assays. Thus it is not studied in these lab experiments. The cellulases are hydrolytic enzymes thus the importance of water as a solvent of reaction in lab experiments is obviously huge. Actually, it is currently not possible to directly detect the effect of soil water since we cannot directly measure the enzyme activities in field soils (Wallenstein and Weintraub, 2008). However, the effect of soil water on SOC decomposition rates cannot be neglected because the incorporation of soil water response function significantly improves the model performance (Baldrian and Stursova, 2011; Ise and Moorcroft, 2006). A soil water content (SWC) reduction of 10% in Mediterranean evergreen forests could lead to a 10–80% decrease in BG activity (Sardans and Penuelas, 2005). In the correlation analysis of BG and EG activities against temperature, pH, and moisture content of the litter, only the coefficients for moisture were statistically significant (p-value < 0.05) and the enzyme activities were positively correlated to the moisture contents (Criquet et al., 2002). However, saturated water-filled pore space (WFPS) would reduce the enzyme activity of POX due to oxygen deficiency (Freeman et al., 2001).

New techniques are needed to design and conduct the experiments regarding the soil water effects on enzyme activities; specifically, on the kinetic parameters, $V_{\max}$ and K_m. Under certain laboratory conditions, for instance with enzymes such as lipases, enzyme activities can be detected using organic solvents (Farnet et al., 2010a; Goujard et al., 2009). Rogalski et al. (1999) observed that the POX activities decreased with the increasing of concentrations of organic solvents. This may be a solution to overcome the issues of using aqueous phase for enzyme assays and thus to test the effect of water availability. The organic solvents affect an enzyme by interacting with the enzyme-bound water instead of with the enzyme itself (Zaks and Klibanov, 1988). The same water content in different organic solvents could result in completely different amounts of water bounded on the enzymes due to the solvent's hydrophobicity (Zaks and Klibanov, 1988). Thus water activity, instead of water content, has been recommended to characterize the effect of water on enzyme activities in multi-phase nonconventional media where water does not participate in the reaction (Goujard et al., 2009; Halling, 1994). Water activity reflects the distribution of water between various phases that compete in binding water and the amount of water available for microorganisms (Halling, 1994). This is an important parameter in soils under arid climate conditions where water is a limiting factor.

5. Conclusions

We compiled a kinetic parameter database for five typical lignocellulolytic enzymes through an analysis of literature data. The variance analysis and normality test indicated that both $V_{\max}$ and K_m were log-normal distributed. No significant differences in $V_{\max}$ of BG and EG were found between enzymes from bacteria and fungi. There was significant difference in lab-measured K_m between groups (cellulases > ligninases). No significant difference in $V_{\max}$ was found between them. The one-std-interval of $V_{\max}$ (back-transformation from logarithmic values) fell within the range of 10^1–10^3 (mean $\approx 10^2$) mg C mg^{-1} Enz h^{-1}. Significant difference in activation energy existed between cellulases and ligninases (37 ± 15 and 53 ± 17 kJ mol^{-1}, respectively). Both cellulase and ligninase activities were optimal under acidic conditions. The exponential-quadratic function can be used to describe the effect of pH on the specific enzyme activity. The average ratio of pH_{sen} to pH_{opt} ranged 0.3–0.4 for all enzymes and the pH_{opt} for ligninases was significantly lower than pH_{opt} for cellulases. Our analysis indicated that the $V_{\max}$ values for SOC decomposition under field conditions could be 1–2 orders of magnitude lower than the laboratory measurements using purified enzymes. Finally, there is still a lack of knowledge about the conversion of K_m and its response to temperature and pH, as well as the effects of soil water on the kinetic parameters.

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Appendix. Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2012.01.011.

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