Letter to the Editor

Comment on the review by German et al. (2011) “Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies” [Soil Biology & Biochemistry 43: 1387–1397]

German et al. (2011) have written a valuable, practical guide to reproducibly measuring extracellular enzyme activities, which we believe will be useful to researchers working in both terrestrial and aquatic environments. Adoption of these recommendations will enable the research community more effectively to compare enzyme activities among environments.

The authors state that “with the exception of phenol oxidase and peroxidase...the majority of DEs measured in ecosystem studies are hydrolytic enzymes, which generally follow Michaelis–Menten kinetics.” This is true, but exceptions to Michaelis–Menten kinetics are also common, and can have important implications both for the interpretation of reaction velocity data and for understanding ecosystem function. Here we discuss three important ways in which enzyme kinetics can deviate from Michaelis–Menten kinetics – substrate inhibition, cooperative binding, and the coexistence of isofunctional enzymes (Leskovac, 2004) – and we describe the implications of those deviations for ecosystem studies.

Substrate inhibition, in which reaction velocity decreases at high substrate concentrations, is widespread. It has been observed in more than 79 classes of enzymes (Kaiser, 1980; Reed et al., 2010), including environmentally relevant enzymes such as polyphenol oxidase (Golbeck and Cammarata, 1981), xanthine oxidase (Rubbo et al., 1991), cellobiose oxidase (Morpeth, 1985), xylanase (Sreenath and Joseph, 1982), α-glucosidase (de Burlet and Sudaka, 1977), trypsin (a protease; Nakata et al., 1972), and carboxypeptidase (Lumry et al., 1951). We have observed dramatic substrate inhibition in natural alkaline phosphatase measured using 4-methylumbelliferyl phosphate in surface seawater from an offshore site in the Gulf of Mexico, coastal waters in Bogue Sound, NC, and in commercially-obtained alkaline phosphatase (Fig. 1).

The possibility of substrate inhibition complicates comparisons among samples. For these samples we define [S]_{opt}, the substrate concentration at which reaction velocity is maximized, and V_{opt}, the corresponding velocity. [S]_{opt} varies by roughly an order of magnitude among the three samples shown in Fig. 1, from ~10 μM in the Gulf of Mexico to 100–500 μM in Bogue Sound. Furthermore, in the Gulf of Mexico and the commercial phosphatase, reaction velocity was quite sensitive to [S] at substrate concentrations close to [S]_{opt}. For this reason, in substrate-inhibited systems it is particularly important to publish a detailed saturation curve so that readers can evaluate how well [S]_{opt} has been constrained. To avoid confusion in such situations, we recommend reporting measured maximum velocities as V_{opt} rather than V_{max}.

In the context of measuring oxidase activity using L-DOPA, German and colleagues state that “It is likely...that swamping the sample with an overabundance of substrate (e.g. 25 mM substrate concentration) will allow the enzymatic reaction to proceed at some maximal rate”. The example of Fig. 1 shows that, when substrate inhibition is important, the use of too-large substrate concentrations will lead to underestimates of maximum reaction velocity and increased likelihood of Type II error (false negatives), just as use of too-low substrate concentrations will.

Although substrate inhibition is sometimes viewed as an artifact of unrealistic substrate concentrations, it can have adaptive value. In the human brain, for instance, substrate inhibition in tyrosine hydroxylase buffers the concentration of the reaction product (L-DOPA) when concentrations of substrate (L-tyrosine) change rapidly (Reed et al., 2010). Observation of substrate inhibition in environmental samples could similarly yield clues about the biochemistry and ecology of enzyme-producing microbes.

Cooperative binding, in which reaction velocity deviates from the Michaelis–Menten model at low substrate concentrations, resulting in a sigmoidal kinetic function, does not affect estimates of V_{max}. Reaction velocity at low [S] may be more environmentally relevant than V_{max}, however, because typical substrate concentrations in situ may also be low. In such systems microbial communities may respond to environmental change by altering the cooperativity of binding, rather than V_{max}. Cooperative binding has been observed among polyphenol oxidases (Srivastava and van Huystee, 1977), proteases (Reiskind et al., 2011), polysaccharide hydrolases (Hrnova and Fincher, 1998), and phosphatases (St. Martin and Wittenberger, 1979), among others. Cooperative binding often (but not always) indicates that enzymes consist of multiple subunits (Leskovac, 2004). Analysis of enzyme kinetics at low [S] therefore has the potential to offer clues about the physical nature of the dominant enzymes in an environmental sample. Since isolation of enzymes from environmental samples is often a major challenge, those clues can be valuable.

Finally, non-Michaelis–Menten kinetics can be due to the simultaneous presence in the environment of multiple, isofunctional enzymes, each of which individually obeys Michaelis–Menten kinetics with substantially different K_{m} values. Such isoenzymes indicate an enzyme-producing community adapted to a wide range of substrate concentrations (Hollibaugh and Azam, 1983; Nannipieri et al., 1982; Tarpgaard et al., 2011), possibly due to rapid

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fluctuations in substrate availability, microenvironments, or enzymes operating outside of their intended medium (e.g. excretion of metazoan gut enzymes, Bochdansky et al., 1995).

We fully support German and colleagues’ recommendations concerning the selection of appropriate reaction conditions and procedures to maximize the reproducibility and comparability of enzyme activity data. With this comment, we hope to convince researchers to consider the possibility of non-Michaelis-Menten kinetics in environmental samples, since the observation of such kinetics can affect the correct interpretation of reaction velocity data, and can lead the way to new discoveries about the function of enzymes in the environment.

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References


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