WHY USE ENZYMES IN MOIST-SOLID REACTION MIXTURES?

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Enzymes are typically manipulated as dilute aqueous solutions. This is however remote from their natural environment. Whereas cellular enzymes operate in a highly concentrated milieu, with little bulk water available, other enzymes (*e.g.* cellulases, chitinases, and cutinases) are naturally secreted into the environment by microorganisms and have evolved to operate on a surface exposed to air moisture. From this perspective, we hypothesized that such conditions could be emulated using moist-solid reaction mixtures and accelerated with mechanical mixing (mechanoenzymology). Our main technique, coined RAging, consists of brief, intermittent periods of mixing of these moist-solids between longer periods of static incubation (*i.e.* aging). This led to an unconventional and more sustainable biocatalytic approach that is especially promising for poorly soluble, chemically recalcitrant substrates such as biomass and plastics. Remarkably, under such conditions enzymes not only proceed with higher yields than in bulk water, but also generate a cleaner product and can catalyze the direct depolymerization of highly crystalline substrates, which would otherwise require a pre-treatment step. Additional advantages of this unorthodox approach range from circumventing solubility issues and minimizing wastewater production, to avoiding the need for pH control. We have applied this method to an increasing number of reactions. This presentation will describe both the biocatalytic applications and mechanistic studies.