RESEARCH ON LEARNING

How the Cereal Crumbles: A Hands-On Activity for Enzyme Kinetics and Thermodynamics in Introductory Biology

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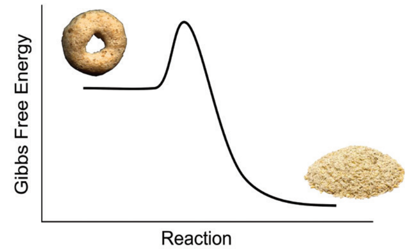

Abstract

Concepts relating to enzymes and energy are central to understanding chemical and biological processes at the molecular level. Student learning of these crosscutting concepts can be challenging, so it is important to identify misconceptions and remediate them early, especially in introductory classes. Here we describe an activity in which undergraduate introductory biology students timed themselves crushing pieces of cereal to simulate and quantify the progress of an enzymatic reaction in the presence of competitive and noncompetitive inhibitors, and we asked students to connect the cereal analogy to concepts of thermodynamics. We developed an assessment and short surveys to evaluate the impact of the activity and to identify persistent misconceptions. Measurable improvements of assessment scores and qualitative student survey responses demonstrate the value of including a hands-on activity along with other modes of instruction.

Keywords: *enzymes, enzyme kinetics, energy, active learning.*

○ Introduction

Several evidence-informed practices exist to promote student learning and success, including active learning (Freeman et al., 2014; Theobald et al., 2020), spaced retrieval practice (Hopkins et al., 2016; Karpicke & Roediger, 2008), and interleaving topics (Rohrer, 2012; Carvahlo & Goldstone, 2019). This study investigates the use of these practices on student learning and confidence of enzyme kinetics and thermodynamics in an introductory biology course. A wide variety of active learning methods exist for teaching enzyme kinetics; they include use of toothpicks, beans (Hinckley, 2012), marbles (Runge et al., 2006), nuts and bolts (Junker, 2010; Lechner, 2011; Silverstein, 2011), pennies (House et al., 2016), modeling clay (Friedman & Terry, 2020), or candy (Berndsen et al., 2020). Assessment of an activity using plastic building bricks in a cell biology course demonstrated student improvements in interpreting and discussing Michaelis-Menten and Lineweaver-Burk graphs and the effects of substrate concentration and addition of competitive and noncompetitive inhibitors (Darling et al., 2021), as well as improvements in students visualizing V_{max} and K_m. The visualization observations are consistent with an association between a similar activity and visualizing V_{max} and turnover number (Runge et al., 2006). An additional recent report describes survey data showing improved student confidence in analyzing enzyme kinetics data, calculating kinetic constants, and making appropriate figures of numerical data (Berndsen et al., 2020).

This cereal-crushing activity can serve as a useful analogy for enzymes that bridges concepts of enzymes and thermodynamics.

While these active learning methods have been shown effective in various courses, kinetics activities in introductory classes were underrepresented in the literature despite the importance of these classes for early identification and remediation of misconceptions (Halim et al., 2018). Furthermore, introductory biology courses commonly teach enzymes together with energy concepts and thermodynamics; the aforementioned enzyme kinetics activities do not emphasize these concepts or their connections. We therefore developed an activity and assessment plan that explicitly seeks to teach and evaluate connections between enzymes and energy at the introductory level.

Interviews, concept inventories, and writing exercises conducted by several research groups have identified common challenges and misconceptions relating to energy, enzyme kinetics, and enzymatic interactions (Bretz & Linenberger, 2012; Halim et al., 2018) that we have taken into consideration in our instructional plan. Challenges include visualizing V_{max} and K_m (Runge et al., 2006; Darling et al., 2021; Berndsen et al., 2020), interpreting graphs with rates or unfamiliar representations of time (Rodriguez et al., 2019; Rodriguez & Towns, 2020), and identifying if energy is absorbed or released when bonds form or are broken (Bain & Towns, 2018). Fostering student reasoning of core concepts that relate to diverse disciplinary contexts may help to reinforce learning of Gibbs

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energy and enzyme kinetics that are often presented as separate topics in texts (Bain & Towns, 2018; Bearne, 2012). In introductory biochemistry courses, instructors are counseled to focus enzyme kinetics on particulate-level descriptions of competitive and noncompetitive inhibition, with less attention placed on uncompetitive and mixed models (Rodriguez & Towns, 2019). Other recommendations promote using multiple instructional approaches, including analogies and laboratory exercises (Srinivasan, 2021).

Here we describe an activity in which introductory biology students timed themselves crushing pieces of cereal to simulate and quantify the progress of an enzymatic reaction in the presence of competitive and noncompetitive inhibitors, and we asked students to connect the cereal analogy to concepts of thermodynamics. We introduced the activity before a separate laboratory exercise in which students monitored and quantified the progress of a turnip peroxidase–catalyzed reaction. We developed a concept inventory based on misconceptions and suggested assessment items, and we administered the concept inventory and short surveys to assess student perceptions of the cereal-crumbling activity and to identify persistent misconceptions. We expected that the cereal-crushing activity would improve students' understanding of enzymes and energy. Because enzymes and energy are difficult for many students, we expected to see student learning continue to improve with additional instruction after the cereal-crushing activity.

○ Methods

Students & Course Description

This activity was used in the second semester of a two-course Introduction to Biology sequence typically taken in the sophomore year at a medium-sized regional comprehensive university. Two semesters of general chemistry, one semester of introductory biology, and precalculus were prerequisites for the course. This five-credit course included an additional one-credit recitation. Students with different instructors or accommodations may have had different experiences accessing and participating in the course. Seventy-nine students completed the concept inventory and survey in fall 2020. Because of COVID accommodations, for 51 students the course was hybrid, with remote instruction and in-person labs with a teaching assistant, and 1 student had accommodations for the course to be fully remote. The other 27 students were in the classroom and laboratory with the instructor for lecture, lab, and recitation.

Cereal-Crumbling Activity

The cereal crumbling activity was implemented near the start of the unit on energy and enzymes. Students worked alone or in groups of two to three. Before the activity, students were assigned a prelab assignment. For the activity, two bowls, representing substrate and product, were placed before the student(s). During the first iteration, a single piece of noncolored cereal was placed in the substrate bowl. With each successive iteration, the number of cereal pieces was increased to model increasing substrate concentrations. Students selected one piece of cereal at a time from the substrate bowl, used one hand to crumble the cereal into the product bowl, repeating for up to 15 seconds or until all of the cereal pieces had been crumbled, and recorded the number of substrates converted. To represent an increase in enzyme concentration, the experiment was repeated with instructions to use two hands to transfer/crush cereal pieces. To represent the presence of a reversible, competitive inhibitor, five colored cereal pieces were included, with instructions to randomly select a cereal piece and if a colored cereal piece was selected, to not crush it but return it to the substrate bowl and randomly select again. To represent the presence of a noncompetitive inhibitor causing a conformational change, students were instructed to impede their manipulation by not rotating their wrist and/or elbow. Students graphed their data to generate a Michaelis-Menten plot to determine K_m and V_{max}. Activity instructions and instructor notes are provided in Appendix 1, available as Supplemental Material with the online version of this article.

Post-Activity Instruction

Instruction on energy, enzymes, enzyme kinetics, thermodynamics, cellular respiration, and photosynthesis included a wet lab experiment and report; lecture, discussion, and other classroom activities; and reading and other homework assignments. The wet lab experiment has been a common lab activity in the course, similar to those discussed in Latourelle et al., 2019, and Pitkin, 1992, in which turnip peroxidase catalysis of hydrogen peroxide is detected using a colorimetric reaction. Groups of three to four students worked together to graph data and write a lab report. Additional instruction associated with the lecture and recitation portions of the course took many forms, including lectures, clicker questions, case studies (e.g., Baines et al., 2004), and discussions with teaching assistants, peers, and professors. Outside-of-class activities included Mastering Biology (Campbell Biology, 11th ed.) and Learning Management System assigned readings, videos, quizzes, and interactive assignments (e.g., Bergan-Roller et al., 2017), as well as student use of additional resources, such as reviewing learning objectives and taking advantage of visiting office hours.

Assessment Strategy

A survey of student confidence in chemical thermodynamics and enzymes was administered before any instruction, and again after all instruction and exams were complete. Short reflective prompts about the cereal-crushing and other course activities were added to the final survey. A concept inventory, consisting of 24 multiple choice and two multiple select questions, was administered three times: before any instruction, immediately after the cereal-crushing activity, and after all instruction on enzymes, energy, and metabolism (three to four weeks after the cereal activity). The questions were influenced by recent studies of student reasoning and misconceptions (Rodriguez et al., 2019; Rodriguez & Towns, 2020; Shi et al., 2017; Halim et al., 2018; Bain & Towns, 2018) and included questions using cereal as analogy for thermodynamic concepts. Some assessment items were from the Enzyme-Substrate Interactions Concept Inventory (ESICI) (Bretz & Linenberger, 2012). Individual questions are described in more detail in the Analysis section.

Data Analysis

Personally identifiable information was removed prior to coding and analysis, pursuant to Mercer University Institutional Review Board H2007190. Qualitative responses were coded independently by authors JPS and MDP and then iteratively compared with reach consensus codes.

We examined student performance on the concept inventory by performing a logistic regression with a generalized linear mixed effects model (binomial errors) with test (pre-activity, post-activity, and post-instruction) as a fixed effect and student identifier as a random effect. All data were analyzed using R (R Core Team, 2019). The generalized linear mixed effect models were conducted using the *lme4* package (Bates et al., 2015). Tests of fixed effects were obtained using the *car* package (Fox & Weisberg, 2011).

We also asked whether students' self-efficacy related to chemistry and thermodynamics changed, using survey questions completed before and after the activity. These data were examined with Fisher's exact test or G-test of independence using the *RVAideMemoire* package (Hervé, 2022).

○ Analysis

This cereal-crushing activity can serve as a useful analogy for enzymes that bridges concepts of enzymes and thermodynamics. Student scores on the concept inventory showed significant improvement immediately after completing the activity ($\chi^2 = 74.14$, df = 1, $p < 0.001$) and continued improvement after further instruction ($\chi^2 = 123.54$, df = 1, $p < 0.001$) compared with before the activity. Students were 1.73 times more likely to answer a question correctly on the post-activity than on the pre-activity concept inventory, and they were 2.06 times more likely to answer a question correctly on the post-instruction than on the pre-activity concept inventory. Analysis of concept inventory questions by topics and the qualitative survey responses demonstrate recurring themes related to enzymes, energy, visual representations of data, and using hands-on activities to visualize concepts.

Student Perceptions

Students were asked, "Which course activities do you feel most helped your understanding of enzymes?" They were asked the same question about enzyme kinetics and then about thermodynamics, and then they were asked what helped them least. The two most common responses to what was helpful identified the cereal activity and classroom activities, broadly described as any activity taking place in a classroom setting, including recitations, online lectures, and discussions (Figure 1). Other responses identified the turnip peroxidase lab and lab activities and the study resources, including reading and homework assignments, as helpful. Fewer students identified the cereal activity as being helpful for thermodynamics than they did for enzyme kinetics. The largest fraction of responses did not identify any unhelpful activities, and the most commonly mentioned activity was the lab. Although we did not ask students directly about the course format, 4 of the 52 students who interacted with the course in a remote or hybrid format described the format as a barrier to learning.

When asked, "If you feel the cereal activity helped your understanding of enzymes (and enzyme kinetics or thermodynamics), please describe how," students commonly described the activity as a simple, hands-on activity that helped with visualization (Table 1). Three misconceptions were identified among the responses.

Students reported increased confidence in applying chemical thermodynamics to biological reactions and in understanding how enzymes function (Figure 2). Two questions showed

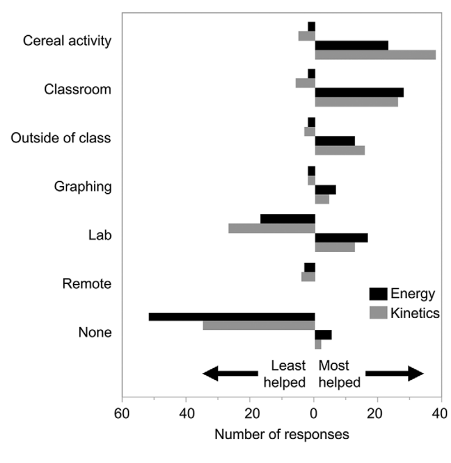

Figure 1. Responses to the prompts, Which course activities do you feel most (or least) helped your understanding of enzymes (and enzyme kinetics or thermodynamics)?

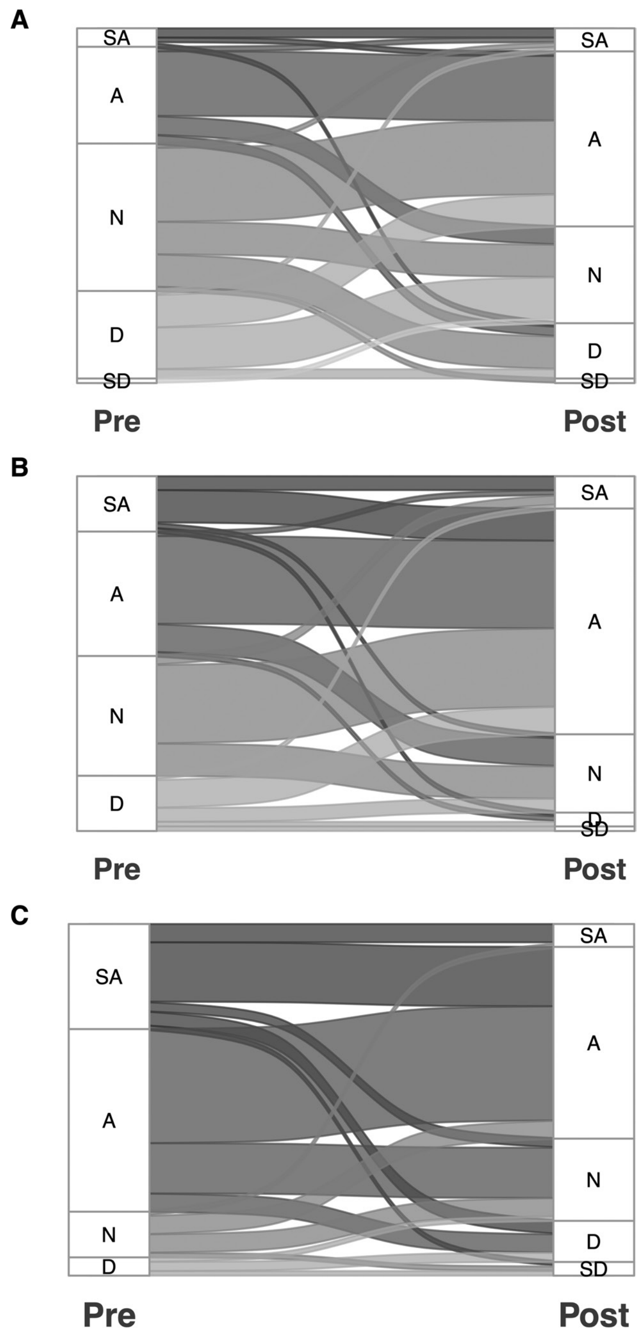

Figure 2. Student confidence, shown by survey responses on a five-point scale, ranging form strongly agree (SA) to strongly disagree (SD): (A) thermodynamics; (B) enzyme function; (C) interpreting graphs.

Table 1. Summary of responses to the prompts, If you feel the cereal activity helped your understanding of enzymes (and enzyme kinetics or thermodynamics), please describe how.

Coding Descriptions	Representative Response for each Coding Description (some responses may match multiple coding descriptions)
Visualizing, picturing, or imagining	"Understanding the thermodynamics aspect was slightly more conceptual rather than the more hands-on understanding of enzyme kinetics simply due to the fact that it is more of a visual understanding (can't see the release of energy, stability, etc)"
Hands-on and other tactile descriptions	"Being able to actually see how the enzyme functioned and worked by using my own hand helped me bring the idea out of the textbook."
Simple representations of complex ideas	"I didn't quite understand the kinetics and free energy connection without considering the actual crushing of cereal. It helped me to associate the energy change with an action."
References to making or interpreting graphs	"By using something so simple it made it very simple to relate that to rather complex concepts like Km and graphical representations."
Specific terms and concepts related to enzymes and kinetics (see Table 2)	"I did not expect the regular enzyme and the enzyme faced with a competitive inhibitor to have the same Vmax. Of course it makes perfect sense so maybe if I would have thought through the entire process i could have figured that out but it is something to really think about"
Specific terms and concepts related to energy and thermodynamics (see Table 2)	"I would say that the cereal lab helped mostly with inhibitors, but thermodynamic stability also played some part in the cereal lab. The more crushed the cereal was the more stable it was which helped in my understanding of thermodynamic stability."
Descriptions of usefulness for studying, reviewing, or connecting to other concepts and courses	"At first I did not think it was very helpful. Then we had class and talked about enzymes and it made sense when I related what I learned from the lab to the lecture."
Didn't help, uncertain, or N/A	"I feel as though the cereal activity helped my understanding of enzymes more than it did thermodynamics. I think it's hard for me to make the connection between the enzymes and their relationship to thermodynamics with the cereal activity."
Misconception: Defining kinetics as change of energy	"It was helpful for me to visualize the enzyme, the inhibitors and the 'crushing' of the enzyme in kinetics (change of energy)."
Misconception: Thinking enzymes do not have a V_{max}	"It showed us how enzymes have a rate at which they function and no matter how much substrate is added, the enzyme can work faster."
Misconception: Conflating entropy in a system with reaction progress	"I think this also helped with my understanding of thermodynamics because the cereal lab upheld the two laws of thermodynamics: 1. Energy cannot be created or destroyed within a reaction and 2. The entropy within a system always increases as a reaction progresses."

statistically significant gains when comparing the before-activity survey and the after-instruction survey: (A) "I can confidently apply chemical thermodynamics to biological reactions" ($p = 0.0417$); (B) "I am confident in my understanding of how enzymes function" ($p = 0.0017$).

In contrast, students shifted to significantly less positive responses to a third question: (C) "I am confident in my ability to interpret graphical representations of data" ($p = 0.0004$). Additionally, a small number of students described the act of graphing as helpful, and a similar number described the act of graphing as unhelpful (Figure 1). Understanding the reasons for student attitudes about graphing is beyond the scope of this study, though challenges could include interpreting graphs with less familiar axis values (i.e., rates) and object-oriented thinking about Michaelis-Menten plots (Rodriguez & Towns, 2021).

Enzyme Concepts

When asked, "If you feel the cereal activity helped your understanding of enzymes (and enzyme kinetics), please describe how," several of the responses identified specific aspects of enzymes. Of these responses, the majority described inhibitors (Table 2). On two questions from the ESICI, there was an increase in the number of correct responses identifying the effect of inhibitor binding, but not for identifying the inhibitor binding partner (Bretz & Linenberger, 2012). Inhibitors were also represented in the concept inventory by a set of questions in which students were asked to match each of three lines in a Michaelis-Menten plot to an uninhibited reaction, a competitively inhibited reaction, or a noncompetitively inhibited reaction. The percentage of students who correctly identified all lines increased from 12% to 36% after the activity, and to 47% after instruction. Using the same plot, students were asked to decide if V_{max} and K_m were higher, equal, or lower, when comparing the uninhibited reaction with the effects of both inhibitors. The group of students who correctly identified both lines for V_{max} increased from 39% to 61% to 70%. Correlating K_m was more challenging, as respectively 4%, 6%, and 23% of respondents correctly matched both lines. This pattern is consistent with reports that students found a similar hands-on activity using marbles (Runge et al., 2006) or plastic building bricks (Darling et al., 2021) to be more helpful for visualizing and understanding V_{max} than for K_m.

Identifying the units for K_m was also challenging for students, as after the activity, 49% of responses described K_m as a concentration; by the end of instruction only 35% did. The smaller improvement may underscore the need for reinforcement of challenging concepts using spaced retrieval, because K_m was addressed primarily in the cereal activity and the lab, but less emphasized in lecture and the textbook. K_m as a rate was the most commonly selected of the distractors. A corresponding question asked students to correlate their response to the cereal analogy (i.e., cereal pieces per bowl, pieces per time, number of whole or crushed pieces). Approximately half of the responses consistently matched the units to their response. Of the students who identified K_m as a concentration, a majority did not correlate their response to the cereal analogy (i.e., did not select cereal pieces per bowl). This outcome is consistent with other reports of concentration as a challenging concept to first-year undergraduate chemistry students (de Berg, 2012) and a report identifying that approximately half of first-year undergraduate students have not mastered a conceptual understanding of molarity (Raviolo et al., 2021).

On a select-all-true question, a large majority of students correctly identified the role of enzymes in affecting the rate of a reaction in all three iterations of the concept inventory. However, distractors were often selected, even after all instruction: over a third of respondents chose "enzymes provide energy for chemical reactions," approximately a third of respondents chose "enzymes change the chemical equilibrium of reactions," and a smaller number of respondents chose "enzymes are required to make chemical reactions happen." Conflating enzymatic effects of rate with equilibrium agrees with previous findings (Shi et al., 2017).

Thermodynamics Concepts

When asked, "If you feel the cereal activity helped your understanding of enzymes and thermodynamics, please describe how," students identified several different aspects of thermodynamics, with potential energy and anabolic and catabolic reactions most frequently cited (Table 2). These topics also represented the largest gains from among the five thermodynamics concept inventory questions that use cereal as an analogy for glucose catabolism (Figure 3). Entropy also showed improvement after the activity, with no further change after all instruction. Gibbs free energy was consistently answered correctly, and thermodynamic stability did not show much improvement until the end of all instruction.

After each concept inventory, the share of students correctly identifying the effect of enzymes on the free energy of the transition state increased (Figure 4). However, a select-all-true question format for this question identified misconceptions among these students, as approximately one-third of students selected the correct answer but also responded that enzymes change the free energy of the reactants and/or products. Our assessment does not attempt to evaluate student reasoning for selecting these incorrect responses, but their reasoning is consistent with student misconceptions of thermodynamic forces (Bain & Towns, 2018).

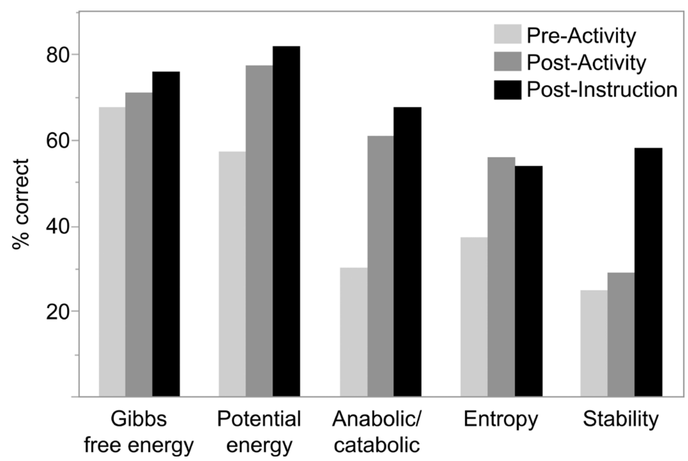

Figure 3. Summary of thermodynamics concept questions in which students identified the correct response on both parts (glucose catabolism and its corresponding cereal analogy).

Table 2. Enzyme kinetics concepts and thermodynamics concepts described in response to the prompt, If you feel the cereal activity helped your understanding of enzymes (and enzyme kinetics or thermodynamics), please describe how.

Enzyme kinetics concept or term	Number of responses	Energy concept or term	Number of responses
Inhibitors	24	Anabolic/catabolic	4
Rates and V_{max}	9	Potential energy	4
Enzyme-substrate interactions	4	Entropy	3
K_m	2	Laws of thermodynamics	2
		Transition state, activation energy, exergonic, stability	1 each

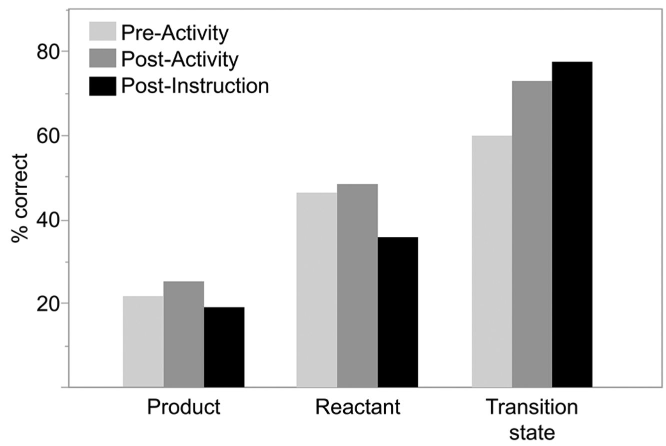

Figure 4. Summary responses for, Which accurately describes the thermodynamic effect of enzymes in chemical reactions (choose all that are correct)? Enzymes change the free energy of the: products/transition state/reactants.

○ Conclusions

Students performed better on the concept inventory immediately following the cereal-crushing activity, indicating that it is beneficial in promoting student learning about enzyme kinetics and thermodynamics. Additional instruction in class, lab, and homework activities further improves student learning, showing that it is helpful for students to encounter these difficult concepts multiple times in different formats. Students responded positively to the cereal activity, identifying it most frequently as the course activity that helped their understanding of enzyme kinetics. Its hands-on nature helped students to visualize the role of enzymes and inhibitors in catalyzing reactions. Students also reported benefits for their understanding of thermodynamics concepts, although these concepts are harder to visualize. After the activity, students described themselves as more confident in their understanding of how enzymes function in biological contexts. However, there appeared to be persistent challenges in understanding concentrations, rates and their graphical representations, and K_m, despite prior instruction on some of these concepts in general chemistry. Although it is beyond the scope of our study to evaluate how introductory biology and chemistry instruction on these topics impacted student understanding in subsequent courses, research into spaced retrieval practice (Hopkins et al., 2016) suggests that a simple analogy may benefit students when referred to repeatedly within a course, when connecting lecture and lab activities, and across multiple courses. We encourage instructors to seek opportunities for forging these connections with students. For example, in the laboratory activity that followed the cereal activity, we are considering introducing inhibitors to match the inhibitors used in the cereal analogy. Additionally, we positioned the cereal activity at the start of instruction to evaluate its impact separate from other instructional activities. We suggest that the analogy could be more useful if it is first established by discussion and other active instruction, prior to students conducting the data collection and analysis. When teaching K_m, it may benefit students to emphasize K_m as a descriptor of affinity and a property of the enzyme itself, and to relate this concept to turnover number (Runge et al., 2006). The cereal analogy provides a visual representation of submicroscopic matter, which is important for comprehending concentration (de Berg, 2012), and we recommend using a tactile analogy, whether cereal or any of the other various analogies, to bolster instruction on this fundamental concept. Finally, because assessments and their interpretations may be biased (Rodriguez & Towns, 2021), we recommend using student assessments that allow for an evaluation of student reasoning, both in the context of the activity at hand, and in assessing underlying concepts.

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