Peer Review Report

Title: Urease: Kinetic and Thermodynamic Mechanisms and Their Diverse Applications

Reviewer 1

Explain the following aspects

Based on the literature,

Kinetics

1. Which NiK transport is a more specific cofactor for urease?

2. What are the sources for these proteins?

Thermodynamics

3. What is the source of the urease in different applications?

4. What is the relationship between saturation, solubility product constant, and temperature?

Write the only table name and figure name in the respective table and figures.

Kindly write the details of figures and tables in manuscript (in detail).

Reviewer 2

The review can be published with minor revisions to the English, mainly with grammatical errors.

1. Figure 1 requires higher resolution.
2. The rest of the manuscript can be published as is.

Response to the reviewers comments

Thank you for considering the publication of our manuscript for the revision. We are grateful for all queries and suggestions that helped us to improve the original version of the manuscript. We hope that questions, queries, comments and suggestions are appropriately answered.

Reviewer #1:

1. Thank you for your comment and for raising this important point. Nickel (Ni^{2+}) is an essential cofactor for urease, nickel uptake is facilitated by specific transport systems, primarily the NikABCDE system (an ATP-binding cassette (ABC) transporter). In organisms that have urease, the Nik system is considered more specific for supplying nickel to urease compared to other less-specific metal ion transporters.

2. The authors are grateful to the reviewer. The sources for these proteins are as follows. The NikABCDE system, well-characterized in bacteria, is a high-affinity ABC transporter that imports nickel with high specificity. It consists of a periplasmic nickel-binding protein (*NikA*), membrane permeases (*NikB* and *NikC*), ATPases (*NikD* and *NikE*), and sometimes a regulatory protein (*NikR*). This system is highly efficient in nickel-scarce environments and is critical for urease activation in many bacteria, as demonstrated in *Yersinia pseudotuberculosis*, where the homologous YntABCDE system (a Nik-like transporter) mediates nickel uptake at a higher initial rate than the *UreH* system, significantly contributing to urease activity.

3. In response to the reviewer's comment, the sources for urease are given in Table 1. Plant urease like Jack bean (*Canavalia ensiformis*), Soybean (*Glycine max*), Peanut (*Arachis hypogaea*) etc. used in the agriculture sector and medical devices (biosensor, artificial dialysis). Bacterial urease (*Bacillus sphaericus, B. subtilis, Helicobacter pylori, Sporosarcina pasteurii*), is highly demanded for self-healing concrete or bioconcrete production, heavy metals remediation, and soil stabilization. Similarly, fungal urease (*Penicillium chrysogenum, Aspergillus niger, Alternaria tenuissima, Cryptococcus neoformans*), play an important role in nitrogen cycles, soil stabilization.

4. Thank you for your insightful question. A solution at saturation has ion concentrations that satisfy the solubility product constant (K_{sp}) expression. If ion product (Q) = K_{sp} → solution is saturated, $Q < K_{sp}$ → unsaturated, and $Q > K_{sp}$→ supersaturated.

Temperature changes the solubility, and thus the K_{sp}, because dissolution is usually an equilibrium process that absorbs or releases heat.

If dissolution is endothermic, increasing temperature increases solubility → K_{sp} increases.
If dissolution is exothermic, increasing temperature decreases solubility → K_{sp} decreases.
The relationship is governed by the van ’t Hoff equation

$$\ln K_{sp} = -\frac{\Delta H^0}{RT} + constant$$

As per the suggestion of the reviewer, the authors have given the Figure legend name Table name in the revised Figure and revised Table file.

Reviewer #2:

Yes, we do agree with the comment. As recommended by the reviewer, the authors have now corrected the english and grammatical errors.

1. As recommended by the reviewer, authors have added high resolution figure, please check the revised Figure file.

Editorial Decision

The authors have addressed all revisions suggested by the reviewers and the editorial board of Exon has decided to **accept** the manuscript and publish in July 2025 issue.

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