



SDI Review Form 1.6

Journal Name:	International Research Journal of Pure and Applied Chemistry
Manuscript Number:	Ms_IRJPAC_50060
Title of the Manuscript:	Purification and characterization of α -amylase from a novel thermoalkalophilic strain of <i>Bacillus sonorensis</i> GV2 isolated from mushroom compost
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	<p>The manuscript entitled “Purification and characterization of α-amylase from a novel thermoalkalophilic strain of <i>Bacillus sonorensis</i> GV2 isolated from mushroom compost” reports interesting data.</p> <p>Nevertheless, it seems more likely to me that authors ignore basic issues concerning the IUBMB recommendations on enzyme nomenclature and kinetics [Perspectives in Science (2014) 1, 74–87; https://www.qmul.ac.uk/sbcs/iubmb/, etc.]. Therefore, authors should conform and revise accordingly their manuscript before its publication. I would like also to point out that the ignorance and the consequent not applying the IUBMB recommendations leads to incorrect results and conclusions.</p> <p>In more details, authors should take into account the following comments:</p> <p>1) To re-write the manuscript and correct it according to English grammar and syntax; in the present version, the text is not followed well.</p> <p>2) The novelties of the manuscript are “buried” by the authors who make use of a useless and meaningless (among enzymologists) quantity (i.e. “Relative activity %”); this comment is referred to Figures 5-(pH-profile), 6-(temperature profile), 7-(thermostability profile), 8-(Shelf stability of purified α-amylase at 4°C), and 9-(Shelf stability of purified α-amylase at room temperature), as well as to Tables 2, 3, 4, and 5.</p> <p>3) The abovementioned “Relative activity %” is meaningless, as within the text nowhere is referred the size of the [S], which was used in the corresponding measurements vs. the magnitude of the parameter K_m. The only acceptable conditions for reliable measurements are for $[S] < 20 \times K_m$, and $[S] > 5 \times K_m$; in the former case the studied Michaelis-Menten parameter is the k_{cat}/K_m (or V_{max}/K_m if it is not known the $[E]_{total}$), while in the latter case is the k_{cat} (or V_{max}) parameter. Therefore, authors should repeat their experiments under both the aforementioned conditions and redraw all the related figures, as well as correct the Tables 2, 3, 4, and 5, accordingly.</p> <p>4) There are some more, which authors should also repeat in a correct manner.</p> <p>a) Sections, 2.6.1 Effect of pH on the activity and stability, 3.3.1 Effect of pH on α-Amylase enzyme activity and stability, as well as Figure 5:</p> <p>(i) They should be corrected through fitting of the experimental data by means of a suitable equation of the form, $k = \frac{V_{max}}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}}$, or its equivalent comprising the pKa-values (according to IUBMB recommendations); this is obligatory.</p> <p>(ii) Obviously, the ordinates of the new pH-profiles should be replaced by either k_{cat}/K_m (or V_{max}), or k_{cat} (or V_{max}), i.e. two figures are necessary, whereas the “Relative activity %”, should be rejected.</p> <p>b) Sections 2.6.2 Temperature optimum and thermal stability, 3.3.2 Effect of temperature on activity of purified α-amylase, as well as Figure 6:</p> <p>(i) They should be corrected through fitting of the experimental data by means of a suitable Eyring equation, considering the use of absolute temperatures (according to IUBMB recommendations); this is obligatory.</p> <p>(ii) Obviously, the ordinates of the new temperature-profiles should be replaced by either k_{cat}/K_m (or V_{max}), or k_{cat} (or V_{max}), i.e. two figures are necessary, whereas the “Relative activity %”, should be rejected.</p> <p>c) The “Relative activity %”, should be replaced accordingly in all figures, as well as in all of its occurrences in the text or in figures' captions or Tables etc.</p> <p>d) Sections 2.6.5 Effect of different concentrations of substrate, 2.8 Determination of Kinetic Parameters, Table 7 and Figure 10:</p> <p>(i) The experimental data, which are related to Michaelis-Menten kinetics, were fitted by the old-fashion and statistically the most erroneous and incorrect Lineweaver - Burk double-reciprocal equation (it has been published long ago, e.g. “Statistical</p>	<p>Grammatical corrections have been made</p> <p>Corrected as suggested by you</p> <p>Revised according to the comments</p> <p>Revision made</p> <p>Done</p>



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	<p>Estimations in Enzyme Kinetics”, by G. N. WILKINSON, 1960); authors used an approach which has been scientifically rejected before 59 years! Therefore, authors should use the non-linear fitting of their experimental data by means of the Michaelis-Menten equation, or to use the reasonable linearization (non parametric) approach introduced by A. Cornish-Bowden, and redraw accordingly figure 10, as well as to correct the corresponding text in their manuscript.</p> <p>Overall: I recommend a major revision, according to the above comments, before the publication of this manuscript in the journal “International Research Journal of Pure and Applied Chemistry”.</p>	Done
Minor REVISION comments		
Optional/General comments		

PART 2:

	Reviewer’s comment	Author’s comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Are there ethical issues in this manuscript?	<u>(If yes, Kindly please write down the ethical issues here in details)</u>	