SCIENCEDOMAIN *international*
www.sciencedomain.org

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 Bacillus sonorensis 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)

SDI Review Form 1.6

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	The manuscript entitled "**Purification and characterization of α-amylase from a novel thermoalkalophilic strain of *Bacillus sonorensis* GV2 isolated from mushroom compost**" reports interesting data. 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. In more details, authors should take into account the following comments: 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. 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. 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 x K_m, and [S] > 5 x 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. 4) There are some more, which authors should also repeat in a correct manner. a) Sections, 2.6.1 Effect of pH on the activity and stability, 3.3.1 Effect of pH on a-Amylase enzyme activity and stability, as well as Figure 5: (i) They should be corrected through fitting of the experimental data by means of a suitable equation of the form, $k = \frac{k}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}}$, or its equivalent comprising the p*K*a-values (according to IUBMB recommendations); this is obligatory. (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. 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: (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. (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. 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. d) Sections 2.6.5 Effect of different concentrations of substrate, 2.8 Determination of Kinetic Parameters, Table 7 and Figure 10: (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	

	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. 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".	
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?	*(If yes, Kindly please write down the ethical issues here in details)*	

Reviewer Details:

Name:	***Emmanuel M. Papamichael***
Department, University & Country	***University of Ioannina, Greece***