



**SDI Review Form 1.6**

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| Journal Name:            | <a href="#">British Microbiology Research Journal</a>                                                                                                                 |
| Manuscript Number:       | Ms_BMRJ_20766                                                                                                                                                         |
| Title of the Manuscript: | CHARACTERISATION OF A PURIFIED LOW MOLECULAR WEIGHT AND THERMO-ALKALINE TOLERANT XYLANASE PRODUCED BY <i>Aspergillus brasiliensis</i> IN SUBMERGED FERMENTATION (SmF) |
| 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.

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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)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
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| <b>Compulsory</b><br>REVISION comments | <ul style="list-style-type: none"> <li>The manuscript represents a very valuable study on characterisation of enzymatic activities and other characteristics of endo-1,4-<math>\beta</math>-xylanase isolated from <i>Aspergillus brasiliensis</i> ATCC 16404 strain. The enzyme activity is precisely characterised with respect to several factors such as temperature, pH, metal ions, chelating agents and organic solvents.</li> <li>The presented experimental data represent a valuable and a complex set; however, I have one important notice on the presented data that lies in missing statistical evaluation. As I understand, all the presented data are just mean values without no data variability shown and evaluated. However, the experimental data cannot be presented in such form.</li> <li>Adequate statistical evaluation, i.e., analysis of variance (ANOVA) and a post-hoc test (e.g., LSD 0.05, Tukey, Fisher, Duncan's multiple range test, etc.) has to be carried out to evaluate the data variability and to determine significant differences between the individual samples practically to all presented data, especially the column graphs in Figures 1, 2, 3, 10, 13, 14, 15).</li> <li>Moreover, regarding the data presented in Figs. 8 and 9 on the effect of a broad temperature or pH range on xylanase enzymatic activity, I miss any data on the time period of the presented temperature or pH treatments after which the enzymatic activity was determined. The data on the incubation period of the enzyme under the given temperature or pH conditions have to be added to the figure legends as well as to the corresponding text.</li> <li>Due to an absence of statistical evaluation of the presented data, I have to recommend a major revision of the manuscript.</li> <li>Otherwise, I have only a few formal comments on the manuscript.</li> <li>A minor linguistic revision of the manuscript should be performed.</li> <li>Remove "the" before the general terms such as "xylanase activity"</li> <li>Abstract, line 9: Use the verb form "catalyzes" instead of "catalyse" in the sentence "Xylanase, also known as endo-1,4-<math>\beta</math>-xylanase, is a glycosidase that catalyzes the conversion of xylan to xylose..."</li> <li>Abstract, line 35: Use the verb "was found" instead of "was detected" in the sentence "...while <math>Al^{3+}</math> was found to be the toughest inhibitor.</li> </ul> | <p>Most of the characterization study on the xylanase activity and stability was analysed without statistical analysis (ANOVA and post-hoc tests). In fact, some data did not even performed as the mean value and SD or SE. Here are the examples of journals:</p> <ul style="list-style-type: none"> <li>According to Rajashri and Anandrao (2012), the presented data on the effects of the different temperatures and pH on the activity and stability of xylanase from <i>Bacillus arsenicelenatis</i> DSM 15340 was not analyzed by statistical analysis (Reference: Rajashri D. K. and Anandrao R. J. Isolation, purification and characterization of xylanase produced by a new species of <i>Bacillus</i> in solid state fermentation. International Journal of Microbiology. 2012. <a href="http://dx.doi.org/10.1155/6683155">http://dx.doi.org/10.1155/6683155</a>).</li> <li>Additionally, Shin et al. (2010), also did not performed the statistical analysis on the substrate specificity, effects of pH, temperature, metals and reagents on the xylanase activity of <i>Fomitopsis pinicola</i> in their characterization study (Reference: Shin K., Marimuthu J., Jung-Kul L. and Yeong-Suk K. Purification and characterization of a thermostable xylanase from <i>Fomitopsis pinicola</i>. Journal of Microbiology and Biotechnology. 2010; 20(10): 1415–1423. doi: <a href="https://doi.org/10.4014/jmb.1003.03031">10.4014/jmb.1003.03031</a>).</li> </ul> <p>Besides that, here are more examples without statistical analysis in the characterization study on xylanase. No statistical analysis was conducted on:</p> <ul style="list-style-type: none"> <li>Substrate specificity and activity of xylanase at alkaline pH condition (Reference: Sonia K. G., Bhupinder S. C, Badhan A. K., Saini H. S. and Bhat M. K. Identification and characterization of diverse xylanases from thermophilic and thermotolerant fungi. Bioresources. 2006; 1(1): 18-33).</li> </ul> |



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|                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <p>concentrations; enzyme concentrations; metal ions on xylanase activity and thermostability of xylanase (Reference: Pawan K. S and Duni C. Purification and characterization of thermostable cellulase free xylanase from <i>Pseudomonas</i> sp. XPB-6. Advances in Microbiology. 2012; 2: 17-25.<a href="http://dx.doi.org/10.4236/aim.2012.21003">http://dx.doi.org/10.4236/aim.2012.21003</a>).</p> <p>The incubation time of xylanase activity for 30 min at different temperatures and pH was discussed in the section of Methodology. The incubation time of xylanase activity is performed according to Bailey et al. (1992) as listed below:</p> <ul style="list-style-type: none"> <li>• Xylanase activity assay was performed using 3,5-dinitrosalicylic (DNS) by measuring the reducing sugar of xylose according to Bailey et al. (1992). 0.1 mL of supernatant was added into 0.9 mL of 1% xylan and incubated in water bath at 50°C for 30 min. Subsequently, 1.5 mL of DNS reagent was added into the mixture and incubated at 90°C for 5 min. 0.5 mL of 40% potassium sodium tartrate was added and cooled to room temperature before measuring the absorbance at 575 nm using spectrophotometer. The amount of xylose obtained was calculated using xylose standard curve. (Reference: Bailey MJ, Biely P, Poutanen K. Laboratory testing of methods for assay of xylanase activity. Journal of Biotechnology. 1992;23: 257-270).</li> </ul> <p>The corrections and revision were performed accordingly.</p> |
| <b>Minor</b> REVISION comments   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Optional/General</b> comments | <p>The manuscript provides a complex study on the investigation of factors affecting enzymatic activity of xylanase isolated from <i>Aspergillus brasiliensis</i>; however, I miss adequate statistical evaluation of the presented data, i.e., not only mean values and SD or SE, but also ANOVA and post-hoc tests of statistical significance to determine significant differences between the different data sets compared. Otherwise, I have only a few formal comments on the manuscript. Relevant statistical evaluation of the presented data (ANOVA analysis leading to a determination of significant differences).</p> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |