



**SDI Review Form 1.6**

Journal Name:	<b><u>Biotechnology Journal International</u></b>
Manuscript Number:	<b>Ms_BJI_35030</b>
Title of the Manuscript:	<b>Production of raw starch degrading amylase by <i>Bacillus subtilis</i> TLO3 and its application in bioethanol production using starch-rich flours</b>
Type of the 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, corrects the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<b>Compulsory</b> REVISION comments	<ol style="list-style-type: none"> <li>1) The word "biomass" at line 46 should read: "substrate".</li> <li>2) The phrase of line 38/39 is not understood. The authors should revisit it.</li> <li>3) I totally disagree with the authors on the use of the OVAT (One-Variable-at-Time) method for optimization in an era which much more precise and challenging statistical tools are available for this.</li> <li>4) "Time" and not "duration" at line 117. <b>Time</b> is a parameter with accepted unit as: seconds, hour, day....).</li> <li>5) <b>Temps (h)</b> at the top of figures 3 &amp; 4 is not English. <b>That's French!</b></li> <li>6) All the titles of the Figures do not describe what is going on as process. When the authors write at the X-axis "<b>Reducing sugars released during the saccharification of wheat flour</b>"; they give the impression as if there are identified sugar substances being plotted". "Amounts of reducing sugars released with time of saccharification of wheat flour should be the right title". Similarly, the titles: "<b>Monitoring of ethanol production and reducing sugars.....</b>" of Figures 3 &amp; 4 make no sense. What the authors are reporting should be "Amounts of ethanol produced and reducing sugars fermented with time during....."</li> </ol>	<p>I would like to thank the reviewer for these valuable remarks.</p> <ol style="list-style-type: none"> <li>1. Corrected.</li> <li>2. Indeed, there were a word missing: "Simultaneous saccharification and fermentation with mixed <b>cultures</b> is an effective method for the direct fermentation of starch"</li> <li>3. Authors totally agree with this. This method was used because of lack of time and because we focused on the fermentation process.</li> <li>4. The mistake is corrected, the word duration was replaced with "incubation time".</li> <li>5. Corrected.</li> <li>6. The titles were changed to: "<b>Amounts of reducing sugars released during saccharification of wheat/corn flour.</b>" and "<b>Amounts of ethanol produced and reducing sugars fermented during the fermentation of wheat/corn flour using <i>Saccharomyces cerevisiae</i>.</b>"</li> <li>7. Actually, isolation data was not shown because it is part of a long screening program, which is not the focus of this study. Different soils from different biotopes were collected and isolates were tested for their amylase activity.</li> </ol>



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	7) The paper does not in any way give information as to : “ <b>an amylase hyper-producer strain <i>Bacillus subtilis</i> TLO3 newly isolated from natural soil</b> ” as mentioned by authors in line 10 in the section “abstract” It rather compares amounts of reducing sugars produced or amounts of ethanol produced from wheat and corn flours. No comparison with other strains to show that <b><i>Bacillus subtilis</i> TLO3</b> is truly a hyper-producer of $\alpha$ -amylase.	This strain was the best one among other and was subjected to molecular identification.
<b>Minor</b> REVISION comments	<ol style="list-style-type: none"> <li>1) Line 48. What is actually fermented is an <i>aqueous extract of sugars</i> and not just sugars</li> <li>2) The sub-heading at line 115: “<b>Reducing sugars fermentation and ethanol production</b>” should read thus: <b>Fermentation of reducing sugars and ethanol production.</b></li> </ol>	<ol style="list-style-type: none"> <li>1. Corrected to: “the released sugars in solution were fermented using the yeast.”</li> <li>2. Corrected.</li> </ol>
<b>Optional/General</b> comments	At the end of Introduction, the authors affirm that comparison of the substrates used and alcohol production from the said substrates were compared. This can lead readers to think this was the objective of the work; whereas it’s very clear the authors’ objective here is reporting strain <i>B. subtilis</i> TLO3 as potentially a powerful producer of high amounts of $\alpha$ -amylase for hydrolysing starch for the production of ethanol during yeast fermentation. I will propose they rewrite the sentence at lines 49 and 50.	The sentence was changed to: “. The results obtained for the two flours were compared to determine the effect of amylase pre-treatment on each substrates concerning starch hydrolysis and thus ethanol production.”