



SDI Review Form 1.6

Journal Name:	<u>Biotechnology Journal International</u>
Manuscript Number:	Ms_BJI_35030
Title of the Manuscript:	Production of raw starch degrading amylase by <i>Bacillus subtilis</i> TLO3 and its application in bioethanol production using starch-rich flours
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.

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	<p>The work is promising in many ways. However, some corrections are necessary that should fit the authors. I will cite some points raised during my review. I would like to remind you that these points may or may not be accepted by the authors.</p> <p>Line 35: Punctuation needs to be corrected;</p> <p>Line 41: This reference was not found;</p> <p>Line 44-45: Both references used are very old: Reference 7 is from an article from 1997 and reference 8 is from 2004. There are many current works that prove the same concept: "To increase the production of ethanol, it is necessary to breed a microorganism By a genetic manipulation ... "</p> <p>Line 49 and 57: The authors used the yeast <i>S. cereviceae</i>, however "What is the strain?" - What was the company that provided this yeast?</p> <p>Line 59: The culture medium needs to be better described. It is necessary to discriminate in detail the concentration of all the components, using the correct concept of concentration.</p> <p>Ex.: "v/v, w/v, w/w. % volume per volume (v/v), % weight per volume (w/v) and % weight per weight (w/w)."</p>	<p>I would like to thank the reviewer for these valuable remarks.</p> <ul style="list-style-type: none"> • Line 35: corrected • Line 41: the reference was added • Line 44: the references were replaced with Sakuragi <i>et al.</i> 2011. • Line 49 and 57: The strain is <i>S. cerevisiae</i> S288C • Line 59: This paragraph was just to introduce this section and medium detailed composition is described below • Line 63-71: Corrected 5g starch and 2g yeast extract per 1000 ml distilled water (w/v);Inoculum size (0,5, 1, 2, 3, 4, 5% (v/v)) • Line 63-71: Corrected • Line 74: Corrected • Line 77 and 79: The value of amylase activity is mentioned in the



SDI Review Form 1.6

	<p>Line 63-71: Is this percentage based on the final volume of the culture medium? Thus, Would be v/v (v.v-1) or w/v (w.v-1)?</p> <p>Ex.: "v/v, w/v, w/w. % volume per volume (v/v), % weight per volume (w/v) and % weight per weight (w/w)."</p> <p>Line 63-71: Punctuation needs to be corrected. For example: " ... (2,5 , 5, 10, 15, 20%, 25%)";</p> <p>Line 74: Is this percentage based on the final volume of the culture medium? Thus, Would be v/v (v.v-1) or w/v (w.v-1)?</p> <p>Ex.: "v/v, w/v, w/w. % volume per volume (v/v), % weight per volume (w/v) and % weight per weight (w/w)."</p> <p>Line 77 and 79: - It is necessary to discriminate the value of amylase activity (U/mL or U/g) achieved in the production;</p> <p>- The authors did not explain how the enzymatic activity evaluation was done. "What were the reagents and techniques (reference of the original article) used to determine the activities of amylases?"</p> <p>- It is necessary to discriminate the volume of the crude supernatant used;</p> <p>- Suggestion: Partial purification could have been done, such as simple filtration or evaporation.</p> <p>Line 80: Writing revision is required: 150rpm or 150 rpm.</p> <p>Line 82: Writing revision is required: 10min or 10 min.</p>	<p>results section ; activity = 367 U/ml.</p> <ul style="list-style-type: none"> • The assay used is that of Miller (1959) and it is detailed in material and method section IN: amylase production optimization. • Partial purification was done and is a part of another work which is almost ready for publication. • Line 80-82 : corrected • Line 86: PYG medium composition was detailed • Line 87: v/v • Line 94: revised • Line 95-96: revised • Line 100 : activity = 367 U/ml • The assay used is that of Miller (1959) and it is detailed in material and method section IN: amylase production optimization. • Results are gathered in Table 1 • Line 100: Percentages were expressed in v/v or w/v
--	--	--



SDI Review Form 1.6

	<p>Line 86: It is necessary to discriminate in detail (concentration of each component - v.v-1 or w.v-1) the culture medium (Peptone-yeast-glucose medium) or to refer to the original article.</p> <p>Line 87: Concentration: v.v-1 or w.v-1.</p> <p>Line 94: Writing revision is required: 5ml or 5 mL.</p> <p>Line 95: Writing revision is required: 1min or 1 min.</p> <p>Line 96: Writing revision is required: 120min or 120 min.</p> <p>Line 100: "...The highest amylase production was obtained ..."</p> <p>- It is necessary to discriminate the value of amylase activity (U/mL or U/g) achieved in the production;</p> <p>- The authors did not explain how the enzymatic activity evaluation was done. "What were the reagents and techniques (reference of the original article) used to determine the activities of amylases?";</p> <p>- The authors did not present the values of amylases produced (minimum and maximum), and a discussion about the results;</p> <p>- Or the authors need to present some work or previous article by discriminating those values and study.</p> <p>Line 100: Is this percentage based on the final volume of the culture medium? Thus, Would be v/v (v.v-1) or w/v (w.v-1)?</p> <p>Ex.:</p>	<ul style="list-style-type: none"> • Line 104: results are in Table 1 • No microbial control agent was added; we just used an autoclaved solution and worked in aseptic conditions. • Line 114: some newer references were added • Line 126: has been referenced • Line 127-128: only total reducing sugars were amount was analysed, thus the sentence "glucose in particular" was removed. • Line 134: References were added to the discussion • Line 148-149: That is right , but is it hard to exactly predict the optimal conditions to achieve the best ethanol production. An investigation work must be done.
--	---	--



SDI Review Form 1.6

	<p>"v/v, w/v, w/w. % volume per volume (v/v), % weight per volume (w/v) and % weight per weight (w/w)."</p> <p>Line 104: "... are promising results for application at large scale allowing high amylase production and consequently elevated concentrations of fermentable sugars for bioethanol production..."</p> <p>- What results? No figures, data, or information about these results were submitted.</p> <p>- Suggestion: In future studies, the authors could do a purification of crude extracts</p> <p>Line 107: Topic: 2. Wheat and corn flours amylase pre-treatment</p> <p>- Has any microbial control agent been added to the pre-treatment (eg, antibiotic or any chemical antimicrobial agent)? Otherwise, how can the authors prove that there was no microbial growth based on the released sugars?</p> <p>- Has any kind of microbial growth control been done during wheat and corn flours saccharification?</p> <p>Line 114: Old references. It is necessary to have a better bibliographic survey and discussion.</p> <p>Line 126: "... be explained by a secretion of amylase by the yeast <i>S. cerevisiae</i>."</p> <p>- Authors need to reference this information.</p> <p>Line 127-128: "This decrease indicates clearly that the yeast transformed the reducing sugars, glucose in</p>	
--	--	--



SDI Review Form 1.6

	<p>particular...”</p> <ul style="list-style-type: none">- The authors have done any analysis of total glucose? If yes, it is necessary to describe the technique used (refer to the original article) and the data obtained. Otherwise, some scientific work should be cited to confirm this statement;- A better discussion about point of work is needed. <p>Line 134: “This decrease could be due to a contamination by an acetic acid bacteria...”</p> <p>Have the authors done any analysis of possible acids produced by microbial contaminants? If yes, it is necessary to present the techniques used (refer to original articles) and data obtained. Otherwise, a better discussion is needed, based on current scientific work. In this case, the authors used only one article from 2015, this type of discussion is weak and easily discredited.</p> <p>Line 148 -149: “The bioethanol production conditions could be optimized to achieve a successful scale-up to industrial level production.”</p> <p>This statement is correct and possible, given the data provided by the authors. However, the authors could offer possible optimization conditions for scale up.</p>	
--	---	--



SDI Review Form 1.6

<u>Minor</u> REVISION comments	<p>The work is very promising, because it seeks an alternative to the energetic problems of society. However, a more up-to-date bibliography is needed, as well as a more detailed discussion.</p> <p>Some data may have been taken from the paper. If so, these data need to be presented.</p>	
<u>Optional/General</u> comments	<p>Improve bibliographic references;</p> <p>Check writing and grammatical scores;</p> <p>Improve discussion;</p> <p>Provide missing data.</p>	