



SDI FINAL EVALUATION FORM 1.1

PART 1:

Journal Name:	British Microbiology Research Journal
Manuscript Number:	Ms_BMRJ_22959
Title of the Manuscript:	OVERPRODUCTION OF XYLANASE FROM MUTANTS OF <i>Bacillus subtilis</i> USING BARLEY HUSK AS THE PRIME CARBON SOURCE IN SUBMERGED FERMENTATION AFTER RANDOM MUTAGENESIS OF ETHYL METHANE SULFONATE (EMS) AND ACRIDINE ORANGE (AO)
New Title:	OVERPRODUCTION OF XYLANASE FROM MUTANTS OF <i>Bacillus subtilis</i> WITH BARLEY HUSK AS THE PRIME CARBON SOURCE UNDER SUBMERGED FERMENTATION AFTER RANDOM MUTAGENESIS USING ETHYL METHANE SULFONATE (EMS) AND ACRIDINE ORANGE (AO)
Type of Article:	Original research Articles

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	Authors' response to final evaluator's comments
The paper addresses a matter of relevance, namely within the scope of industrial biotechnology, particularly when the development of sustainable bioprocesses is considered Most of the comments raised by the reviewer were adequately addressed. Still the issue of oxygen limitation is not fully clear. In order for production to be optimized, evidence should be provided that for the cell concentration achieved, and under the growth condition, oxygen does not become a limiting factor. Moreover since the authors state that "oxygen saturation and agitation of the culture medium which possess significant effects on yield of xylanases are optimized in submerged fermentation". Or otherwise the authors should indicate that detailed studies on this matter should be carried out prior to scale up. Clearly highlight the relevance of the present work given the context of the literature. As the authors refer, "bacterial strain of <i>B. subtilis</i> BS05. As a result, the maximum xylanolytic activity of 439.5 ± 2.84 U/mL" from ref [45], in the present work the activity is roughly 100 fold lower There are still some glitches in writing, that make the manuscript difficult to read e.g. "Enzymes are generally exhibited"; "The biodegradation characteristic of xylanase on plant cell wall described this enzyme possesses one essential character along with many significant applications", the sentence is not clear "Xylanase has also used in clarification of fruit juices and wines" should be "Xylanase has also been used in clarification of fruit juices and wines " "Xylan hydrolyzing enzymes are one of the crucial industrial enzymes" singular mixed with plural "Notably, many studies have also been evaluated using barley husk for production of ethanol" performed or carried out rather than evaluated "On the other hand, total of" should be "On the other hand, a total of" "The xylan hydrolysis might be varied depends on their different tones of discoloration" please clarify, it is considered that the authors mean to say that the extent of hydrolysis can be qualitatively identified by the tone of discoloration Also on this, the authors refer to other works where the zone of clearance was measured, did they proceed in the same way? "in which xylanases are notably peculiar significance and industrial importance" please tone down and check verbs, maybe "xylanases are relevant for industrial applications" "When <i>Bacillus</i> spp that isolated by Annamalai et al" remove "that" "we could suggested that all mutant strains" should be "we could suggest that all mutant strains" "Apparently, it is diversely known for fermentation of aerobic microorganisms especially in the production of xylanase" please clarify "such as protease into the fermentation medium could be the reasons to inhibit and terminate xylanase activity" terminate is not the suitable verb	According to Corral and Villaseñor-Ortega (2006), significant fermentation conditions such as temperature, medium pH, oxygen saturation and agitation of the culture medium that possess significant effects on yield of xylanases are commonly optimised in submerged fermentation. The advantages of using submerged fermentation are the ease of handling various parameters including agitation whereby they can be monitored and controlled. According to Kavya and Padmavathi (2009), low xylanase activity was obtained in non-agitated flasks, most probably due to oxygen or mass transfer limitations, while on agitation, high xylanase activity was produced due to good nutrients and oxygen supply and transfer. (References: 1. Corral OL, Villaseñor-Ortega F. Xylanases. <i>Advances in Agricultural and Food Biotechnology</i>. 2006;305-322. 2. Kavya V, Padmavathi T. Optimisation of growth conditions for xylanase production by <i>Aspergillus niger</i> in solid state fermentation. <i>Polish Journal of Microbiology</i>. 2009;58:125-130.) Irfan et al, 2012 investigated vast agricultural-based substrates for xylanase production from bacterial strain of <i>B. subtilis</i> BS05. As a result, the maximum xylanolytic activity of 439.5 ± 2.84 U/mL was attained using sugarcane bagasse in the production medium constituents containing sucrose and yeast extracts. Different species of <i>Bacillus</i> produce different amounts of xylanase using different types of substrates. (Reference: Irfan M, Nadeem M, Syed Q, Baig S. Effect of medium composition on xylanase production by <i>Bacillus subtilis</i> using various agricultural wastes. <i>American-Eurasian Journal of Agricultural and Environmental Sciences</i>. 2012;12(5):561-565.) The xylan hydrolysis might be varied depends on their different tones of discoloration. Some of bacteria species might produce darker zone color compared to those from <i>Bacillus</i> spp. Other works also performed the plate hydrolysis assay to confirm the xylanase activity on the xylan agar. Apparently, submerged fermentation is known for fermentation of aerobic microorganisms especially in the production of xylanase. Enzyme is not primary metabolite. The husk which also known as the hull represents 10% to 30% of the barley kernel is composed of hemicelluloses. Xylan degradation of barley requires xylanase to liberate xylose. The mutant strain accessed the hemicelluloses of xylan in barley husk after the substrate was pre-treated in an oven at 65°C. Conclusion has been reduced. Rewrite: In the current study, EMS and AO induced visible and lethal mutations in the xylanase genes, causing alkylation of guanine residues which resulted the permanent lesions within the DNA molecules. All corrections have been carried out accordingly. Please refer to the latest revised manuscript.



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“An increase in cell count resulted in a simultaneous increase in xylanase secretion into the culture medium which suggested that xylose was actively utilized by the wild type and mutants of B. subtilis during the growth phase” so xylanases can be considered a primary metabolite, correct?

“Sugumaran et al. [4] exhibited 7.5 U/mL of xylanase activity” should be “Sugumaran et al. [4] reported 7.5 U/mL of xylanase activity”

“Copious studies divulged” Several rather than copious

“enhancing its xylanase productivity several fold in comparison to the wild type [8].” Please be more specific, 2-fold, 10 -fold, as to more effectively compare with the present work

“xylanase activity could also owing to the ability of the mutant strain in gaining easier access of the hemicelluloses of xylan in barley husk [51]” should be “xylanase activity could also be ascribed to the ability of the mutant strain in gaining easier access of the hemicelluloses of xylan in barley husk [51]”. Still, the authors should clarify how the access of the mutant strains is enhanced

Conclusions section is still too long, occasionally includes matters that should rather be in the discussion or introduction, please summarize highlighting the key findings of the work

“The result findings might attribute to the fact that EMS and AO induced” please improve writing

“five mutants of B. subtilis ATCC 6633 were successfully showed the” please replace by “five mutants of B. subtilis ATCC 6633 displayed the”

“highest xylanase producing mutant of MXB 4 attained after subjected the wild type” should be “highest xylanase producing mutant of MXB 4 was obtained after subjecting the wild type”

“substrate are potentially be cost effective” please remove “be”