



SDI Review Form 1.6

Journal Name:	British Microbiology Research Journal
Manuscript Number:	2013_BMRJ_8224
Title of the Manuscript:	Improved Cultural Conditions for Methionine Accumulation in Submerged Cultivation of <i>Bacillus cereus</i> S8
Type of the Article	Original Research Article

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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)
Compulsory REVISION comments	<u>Methods</u> • Lines 50-1 The authors state that “uninoculated flasks served as control”—Does this mean that medium alone was used to demonstrate a baseline of methionine production? In figures 4 and 5, it would appear that the medium alone control is able to produce approximately 1.5 mg/mL of methionine. Please clarify. • Line 72 The authors state that one loopful of inoculum contained approximately 3.15×10^6 cells/mL. Was this value generated by direct plate count? How many trials were done to determine this value and what is the standard error? If one loopful contains 3.15×10^6 cells/mL, it is not possible that two loopfuls would be 7.56×10^8 cells/mL as the authors state in line 72. Two loopfuls of 3.15×10^6 per loopful would be 6.3×10^6. Please clarify. <u>Results and Discussion</u> • Line 127 It is stated that 1.40 mg/mL of methionine was	Uninoculated flasks means fermentation medium without <i>Bacillus cereus</i> S8. Control in fig. 4 and 5 means fermentation medium with <i>Bacillus cereus</i> S8 but without growth stimulators and vitamins respectively. The figures were generated experimentally not from extrapolation. One loopful contains 3.15×10^6 cells/ml and two loopfuls contain 7.56×10^8 cells/ml.



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	produced by <i>Bacillus cereus</i> S8 in this study. Does that represent an overall average under all conditions? If so, then the value of the medium only control is responsible for higher levels of methionine at approximately 1.5 mg/mL. Please clarify. - Line 132—Figure 1 No error bars or statistics are presented on Figure 1 or any of the Figures. The reader must be informed of variation. - Lines 155-5, 166-7, 190-5 The study under review reports maximum methionine production using glucose as a carbon source and ammonium sulfate as the nitrogen source. However, the current study makes comparison with a report by Ekwealor and Obeta (2007) who evaluated lysine production by <i>B. megaterium</i> SP 86. While the current authors note that “strain differences” may explain variation, there is actually no appropriate comparison. Ekwealor and Oberta evaluated <i>B. megaterium</i> SP 86 using sucrose as the carbon source and ammonium chloride as a nitrogen source. <i>Bacillus megaterium</i> SP 76 and <i>B. megaterium</i> SP 14 were evaluated using glucose and ammonium sulfate as did the current study. <u>References</u> There needs to be consistency in reference citation style.	Line 127: Corrected. The study generated 3.23mg/ml methionine and not 1.40mg/ml. It was a mistake. The statistical analyses have been included. We disagree with the reviewer’s comment here. Our comparison was not on the carbon and nitrogen sources. We have tried to show that riboflavin does stimulate metabolite production in <i>Bacillus</i> strains. Ekwealor and Obeta (2007) worked on lysine and the influence of riboflavin on the production by <i>B. megaterium</i>. In a similar manner, we worked on methionine and also noted that riboflavin stimulated its production by <i>B. cereus</i> S8
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	There are issues with capitalization, commas, periods, abbreviations, etc. As an example, reference #8 is listed as: Kinoshita S, Udaka S, Shimono M. Amino acid fermentation. 1. Production of L-glutamic acid by various microorganisms. J. Gen. Appl. Microbiol. 1957;3:193-205. The actual citation is: Kinshita S, Udaka S, Shimono M. Studies on the amino acid fermentation. Part 1. Production of L-glutamic acid by various microorganisms. J Gen Appl Microbiol. 2004; 50: 331-43. Reference 17 (Roy <i>et al.</i>, 1989) is surprising since the <i>Journal of Microbiology and Biotechnology</i> did not begin publication until 1991.	Noted and corrected. Noted and corrected. Noted and reference removed.
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<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments	<u>Introduction</u> • Good, complete background provided • Objectives clear <u>General Comments</u> The authors have engaged in an important investigation that will provide useful information to researchers working in this field. By providing more complete methodology, appropriate statistical evaluation, more directed comparisons with other studies and more attention to details of journalistic style, this paper will be improved.	Thank you.