

Dear Editors,

Thank you very much for reviewing the manuscript. We changed the manuscript as per the queries raised by you. Following are our replies to the queries raised.

Kindly note that all the changes have been made and are in the “TRACK CHANGES” mode in the word file

Query 1: Please note that the section numbers and step numbers have been formatted to fit the Journal standard. Please review.

Reply: As suggested by the editors, the manuscript was reviewed for the formatting changes.

Query 2: How is this done? Please add more details regarding retrieving and maintenance of cultures.

Reply: As suggested by the editors, relevant details have been added for the retrieval and maintenance of the cultures.

Query 3: How is subculturing done. Please add the steps for subculturing. A citation would suffice.

Reply: As suggested by the editors, the methodology for the subculturing has been added .

Query 4: Artificial seawater? Is this prepared? Any specific composition?

Reply: The artificial seawater broth was purchased from Himedia , Bangalore, India. The specifications have been added in the text.

Query 5: If the details for each parameter mentioned in this step is detailed below, please reference the section numbers of the specific methods in this step?

Reply: The details of the section has been added in the text.

Query 6: The sentence s incomplete. Please revise.

Reply: The suggested modifications have been incorporated.

Query 7: Revised. Please check.

Reply: The corrections were made in the revised sentence.

Query 8: How?

Reply: The reference used for melanin estimation has been incorporated in the test.

Query 9: How is the bioprocess harvested?

Reply: The sentence has been modified to mention the harvesting step.

Query 10: How is the absorbent washed. Is any centrifugation or vortexing involved? Any specific volume of water and ethanol used? Any specific time duration for each washing step?

Reply: The centrifugation details required for washing have been incorporated in the text.

Query 11: What is added to reduce the pH? Please specify the added constituent and the volume added to achieve the mentioned pH.

Reply: The details of the HCL used for the acidification have been added. The volume of the HCl will depend on the melanin yield, hence the endpoint pH has been mentioned.

Query 12: Any centrifugation or vortexing involved? What is the time duration of each wash?

Reply: The details of the centrifugation have been incorporated in the manuscript.

Query 13: Is this stimulate or simulate?

Reply: The conditions were simulated for computer modeling.

Query 14: Please cite the reference number

Reply: The citation has been added in the text.

Query 15: How is the culture collected? How is the sterility ensured? What is the volume collected each time to measure the OD?

Reply: The details of the query raised has been addressed and incorporated in the manuscript.

Query 16: After how long of culturing is the culture centrifuged?

Reply: The samples were collected at an interval of 4h. This is mentioned in the preceding step.

Query 17: Sample? Please specify.

Reply: It is a commercial DNA sample and has been mentioned in the manuscript.

Query 18: What is the dilution?

Reply: The dilution factor has been included in the text.

Query 19: Is this commercial or prepared in the lab? If its prepared, please include a citation. In case it is purchased, ensure that it is included in the Table of Materials.

Reply: The estimation was done using the commercial kit from Himedia and has been mentioned in the manuscript.

Query 20: Volume?

Reply: The volume utilized has been added in the text.

Query 21: What is the volume of water used? How much of melanin is dispersed?

Reply: The volume has been included in the text.

Query 22: Volume?

Reply: The volume has been included in the text.

Query 23: Is this performed according to the manufacturer's protocol? Please cite.

Reply: The changes have been incorporated as suggested.

Query 24: How? Using an oven or dry at RT? For how long is the melanin dried?

Reply: The melanin is kept in the oven for 8h for drying. The conditions have been included.

Query 25: How is the sample loaded to the TEM sample matrix

Reply: The protocol as specified by the manufacturer was followed. This has been mentioned.

Query 26: Please check if the section number is correct.

Reply: The section no. as suggested has been corrected.

Query 27: Is this correct?

Reply: The notation of the units have been changed from 1/h to h⁻¹

Query 27: Please check if the section numbers are correct.

Reply: The section number mentioned are correct.

Query 28: Please include a title for each figure.

Reply: The title has been included as suggested.

Query 29: Please include a brief description for the Table

Reply: The changes as suggested have been incorporated in the main text.

Query 30: As we are a methods journal, please ensure that the Discussion explicitly covers the following in detail in 3-6 paragraphs with citations: a) Critical steps within the protocol, b) Any

modifications and troubleshooting of the technique, c) Any limitations of the technique, d) The significance with respect to existing methods, e) Any future applications of the technique.

Reply: The text explicitly speaks about the suggested points.