

REVIEWER'S REPORT

Manuscript No.: IJAR-58802

Title: Isolation and Molecular Characterisation of Biosurfactant-Producing Bacteria from Petroleum-Contaminated Sites and Their Application in Mesocarbon-Amalgamated Oil Absorption Systems.

Recommendation:

Accept as it is

Accept after **minor revision**.....

Accept after major revision

Do not accept (*Reasons below*)

Rating	Excel.	Good	Fair	Poor
Originality	Excellent			
Techn. Quality	Excellent			
Clarity	Excellent			
Significance	Excellent			

Reviewer's ID: JPR- 115

Detailed Reviewer's Report

Manuscript Title: Isolation, Molecular Characterisation of Biosurfactant-Producing Bacteria from Petroleum-Contaminated Sites and Their Application in Mesocarbon-Amalgamated Oil Absorption Systems

The manuscript addresses an important environmental problem and presents an interesting approach combining microbial biosurfactant production with mesocarbon-based materials for oil removal. The study has potential practical significance; however, several methodological, scientific, and presentation-related issues should be addressed before publication.

Major Comments

- Title:** The title is lengthy and contains the term “biosurfactant2,” which appears to be a typographical error. The title should be carefully revised for clarity and grammar.
- Abstract:** The abstract provides a useful overview, but quantitative results are largely absent. Important numerical data such as oil absorption capacity, emulsification index (E24), oil-spreading zone, and comparative efficiency of sheets and beads should be included.
- Bacterial identification:** The manuscript states that the isolate was identified as *Stenotrophomonas maltophilia*, but the molecular identification methodology appears inconsistent. The primers ITS1 and ITS4 are generally associated with fungal/internal transcribed spacer amplification, whereas bacterial

REVIEWER'S REPORT

identification normally requires appropriate bacterial 16S rRNA gene primers. The authors should clarify and correct this issue.

4. **GenBank accession:** The GenBank accession number PX624000 should be verified and supported with the corresponding sequence information and database record. The percentage similarity, query coverage, and closest related strain should also be reported.
5. **Phylogenetic analysis:** The manuscript mentions MUSCLE, Gblocks, PhyML, and TreeDyn, but the phylogenetic tree and its statistical support values should be clearly presented. Bootstrap or aLRT values should be indicated on the tree.
6. **MSM composition:** The reported composition of MSM requires clarification. In particular, the inclusion of 10% glucose conflicts with the statement that different hydrocarbons were used as the sole carbon sources. If glucose was present, the authors should explain its purpose and revise the experimental design accordingly.
7. **Hydrocarbon concentration:** The use of 3% hydrocarbon source should be justified. The authors should clearly state whether this concentration was used for all oils and diesel and whether the experiments were performed in biological replicates.
8. **Biosurfactant extraction:** The extraction procedure needs more detail, including the actual culture volume, centrifugation conditions, acid volume/concentration, precipitation yield, washing procedure, drying method, and final biosurfactant yield.
9. **Screening assays:** The oil-spreading, E24, and foam-forming assays should be described quantitatively. Appropriate equations for calculating E24 and oil absorption capacity should be provided.
10. **Experimental controls:** Suitable controls are required. The study should include mesocarbon without biosurfactant, polymer matrix without mesocarbon, and preferably a biosurfactant-only control to demonstrate the specific contribution of each component.
11. **Oil absorption experiment:** The experimental procedure is difficult to reproduce because terms such as "Turf A" and "Turf B" are introduced without adequate explanation. The authors should replace these terms with clearly defined containers or experimental chambers.
12. **Oil absorption capacity:** The manuscript should report oil absorption capacity quantitatively, preferably as g oil/g material, along with removal efficiency (%). The number of replicates, mean $\pm$ standard deviation, and statistical significance should be provided.
13. **Statistical analysis:** No clear statistical analysis is reported. The authors should include the number of biological/technical replicates and appropriate statistical tests to determine whether differences between treatments are significant.

REVIEWER'S REPORT

14. **Characterization of materials:** The claim that the sheets and beads possess porous structures should be supported by appropriate characterization, preferably SEM analysis. FTIR alone is insufficient to establish morphology and porosity.
15. **FTIR interpretation:** The reported C–H, C=C, C–O, and C–O–C groups should be accompanied by specific wavenumbers and proper interpretation. FTIR spectra of relevant controls should also be provided for comparison.
16. **Reusability claim:** The manuscript states that the materials may be suitable for repeated use and reusability, but actual reuse experiments are not presented. This claim should either be experimentally demonstrated through adsorption–desorption cycles or appropriately moderated.
17. **Biodegradability:** The manuscript describes the materials as “biodegradable,” but no biodegradation experiment is reported. The authors should provide evidence or avoid making a definitive biodegradability claim.
18. **Mechanism of oil removal:** The manuscript would benefit from a clearer explanation of the mechanism by which biosurfactant and mesocarbon interact to enhance oil adsorption. The proposed role of surface porosity, hydrophobicity, and biosurfactant-mediated oil interaction should be discussed.
19. **Comparison with existing materials:** The oil absorption performance should be compared with previously reported carbon-based, polymer-based, or biosurfactant-functionalized oil sorbents to establish the novelty and effectiveness of the developed material.
20. **Environmental safety:** Since *Stenotrophomonas maltophilia* can be an opportunistic human pathogen, the authors should discuss the biosafety implications of using this organism or its crude biosurfactant in environmental applications. The application should be carefully distinguished from direct release of viable bacteria.

Minor Comments

1. The manuscript requires thorough English-language editing for grammar, punctuation, spacing, and sentence construction.
2. Scientific names should consistently be italicised, including *Stenotrophomonas maltophilia*.
3. Units and symbols should be standardised throughout the manuscript (e.g., °C, rpm, mL, g/L, % w/v).
4. Several sections contain inconsistent spacing and formatting, such as “Mineral Salt Medium(MSM)” and “biosurfactantwere.” These should be corrected.
5. The numbering of sections and figures should be checked carefully because several numbers appear to be missing from the provided text.

International Journal of Advanced Research

Publisher's Name: Jana Publication and Research LLP

www.journalijar.com

REVIEWER'S REPORT

6. References should be checked for consistency and completeness according to the journal's reference style.
7. Figures showing the bacterial screening assays, molecular identification, phylogenetic tree, mesocarbon sheets/beads, FTIR spectra, and oil absorption experiments should contain clear legends and scale information where appropriate.
8. Tables summarising bacterial isolates, screening results, hydrocarbon utilization, biosurfactant yield, and oil absorption efficiency would substantially improve the presentation.