

Supplementary File

Effect of the Application of *Ochrobactrum* sp.- Immobilised Biochar on the Remediation of Diesel- Contaminated Soil

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Materials and Methodology

Text S1: Polymerase chain reaction (PCR) and gel electrophoresis for primer

Polymerase Chain Reaction (PCR) was carried out in triplicate using the initial denaturation of 94 °C (4 min), followed by 35 cycles of 94 °C (30 s), 56 °C (30 s), and 72 °C (1 min) with a final extension of 15 min at 72 °C [1]. An aliquot (25 µL) was used for PCR, comprising of 2 x MIFI (12.5), 4 µL primer mix (10 µM each primer), 2 µL of template DNA of the bacteria and 8.5 µL PCR grade water. Following completion of the PCR run, a 5 µL PCR mixture was used to check for amplification on a 2% agarose gel in 1 x Tris-acetate-EDTA (TAE) buffer stained with SYBR safe DNA gel stain (Invitrogen, Massachusetts, US). Processing of the gel was carried out in the ChemiDoc (Bio-Rad, California, US) [2]

Results

Table S1: Proximate analysis of pristine biochar and bacteria immobilised biochar

| Proximate analysis (wt% d.b) | Pristine biochar | Bacteria immobilised biochar |
|------------------------------|------------------|------------------------------|
| Moisture content (%) | 0.42 ± 0.26 | 0.49 ± 0.30 |
| Volatile matter (%) | 3.15 ± 0.21 | 3.95 ± 0.21 |
| Fixed carbon (%) | 20.18 ± 5.26 | 25.26 ± 0.34 |
| Ash content (%) | 76.26 ± 4.79 | 70.30 ± 0.85 |

Values are mean of duplicate and the standard deviation of the mean.

Table S2: First-order kinetics equation, rate constant (k), half-life ($t_{1/2}$) and R^2 and of the different treatments

| Treatments | First order kinetic equation | k (day ⁻¹) | $t_{1/2}$ (days) | R^2 |
|------------|------------------------------|------------------------|------------------|-------|
| C | $y=-0.0044x+11.096$ | 0.0044 | 157 | 0.97 |
| B | $y=-0.0047x+11.108$ | 0.0047 | 147 | 0.95 |
| F | $y=-0.0038x+11.176$ | 0.0038 | 182 | 0.84 |
| BC | $y=-0.0049x+11.006$ | 0.0049 | 141 | 0.96 |
| BCF | $y=-0.0046x+11.114$ | 0.0046 | 151 | 0.95 |
| BIB | $y=-0.0053x+10.991$ | 0.0053 | 131 | 0.99 |
| BIBF | $y=-0.0043x+11.092$ | 0.0043 | 161 | 0.98 |

C: Control; B: Bacteria; F: 2% Fertiliser; BC: 5% w/w Biochar; BCF: 5% w/w Biochar + 2% Fertiliser; BIB: Bacteria immobilised biochar; BIBF: Bacteria immobilised biochar + 2% Fertiliser.

Table S3: Estimated time to achieve a concentration of 995 – 997 mg/kg, which is lower than the EPA Victoria fill material threshold (1,000 mg/kg) in the different treatments

| | Time (weeks) | TPH conc at that time (mg/kg) |
|------|---------------------|--------------------------------------|
| C | 134 | 996 |
| B | 126 | 996 |
| F | 155 | 997 |
| BC | 120 | 996 |
| BCF | 128 | 997 |
| BIB | 111 | 996 |
| BIBF | 137 | 995 |

C: Control; B: Bacteria; F: 2% Fertiliser; BC: 5% w/w Biochar; BCF: 5% w/w Biochar + 2% Fertiliser; BIB: Bacteria immobilised biochar; BIBF: Bacteria immobilised biochar + 2% Fertiliser.

Table S4: The intensity of peaks associated with -CH₃ and -CH₂ in aliphatic compounds (peaks 2923 and 2853 cm⁻¹) in the different treatments

| Wave number (cm⁻¹) | Treatments | Intensity (Absorbance %) |
|--------------------------------------|--------------------------|---------------------------------|
| 2923 | Week 0 contaminated soil | 0.034 ± 0.0019 |
| | C – Week 10 | -0.014 ± 0.0066 |
| | BC – Week 10 | -0.0039 ± 0.01 |
| | BIB – Week 10 | -0.016 ± 0.0079 |
| | C – Week 22 | -0.018 ± 0.0035 |
| | BC – Week 22 | -0.017 ± 0.0052 |
| | BIB – Week 22 | -0.018 ± 0.0031 |
| 2853 | Week 0 contaminated soil | 0.020 ± 0.0021 |
| | C – Week 10 | -0.017 ± 0.0072 |
| | BC – Week 10 | -0.0078 ± 0.0089 |
| | BIB – Week 10 | -0.018 ± 0.0065 |
| | C – Week 22 | -0.0194 ± 0.0040 |
| | BC – Week 22 | -0.018 ± 0.0051 |
| | BIB – Week 22 | -0.019 ± 0.0030 |

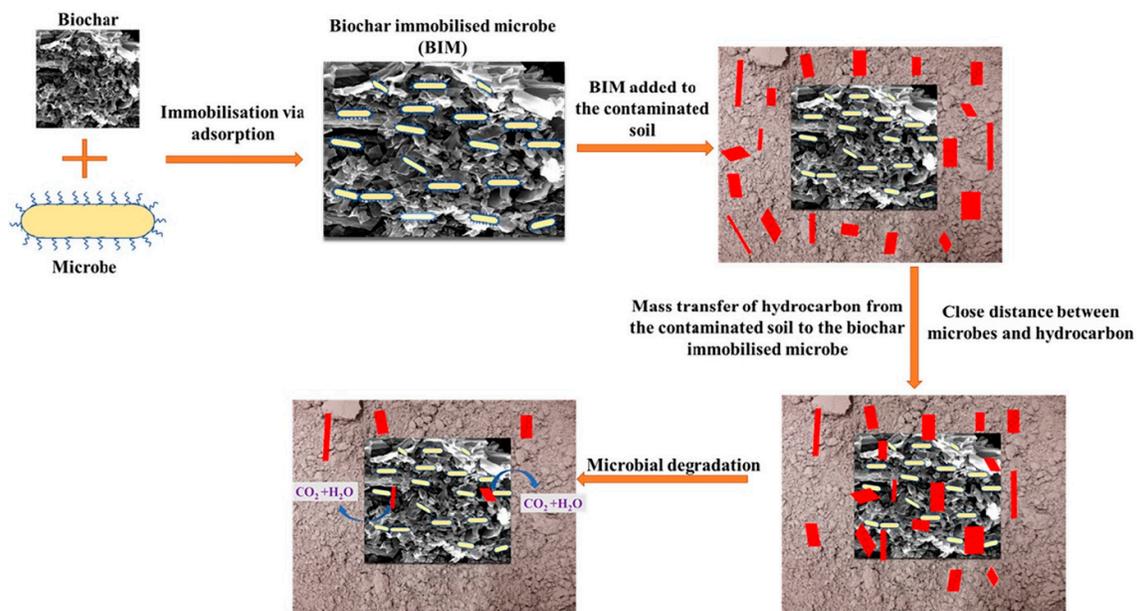


Figure S1: Mechanism for hydrocarbon removal in soils amended with biochar immobilised microbe [3] (Copyright permission obtained)

References

- [1] Z. Liang, G. Li, B. Mai, H. Ma, T. An, Application of a novel gene encoding bromophenol dehalogenase from *Ochrobactrum* sp. T in TBBPA degradation, *Chemosphere*, 217 (2019) 507-515.
- [2] G.K. Satyapal, S.K. Mishra, A. Srivastava, R.K. Ranjan, K. Prakash, R. Haque, N. Kumar, Possible bioremediation of arsenic toxicity by isolating indigenous bacteria from the middle Gangetic plain of Bihar, India, *Biotechnology reports*, 17 (2018) 117-125.
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