

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 [39]. 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, USA).

Results

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

	Pristine biochar	Bacteria immobilised biochar
Proximate analysis (wt% d.b)		
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 soil 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.

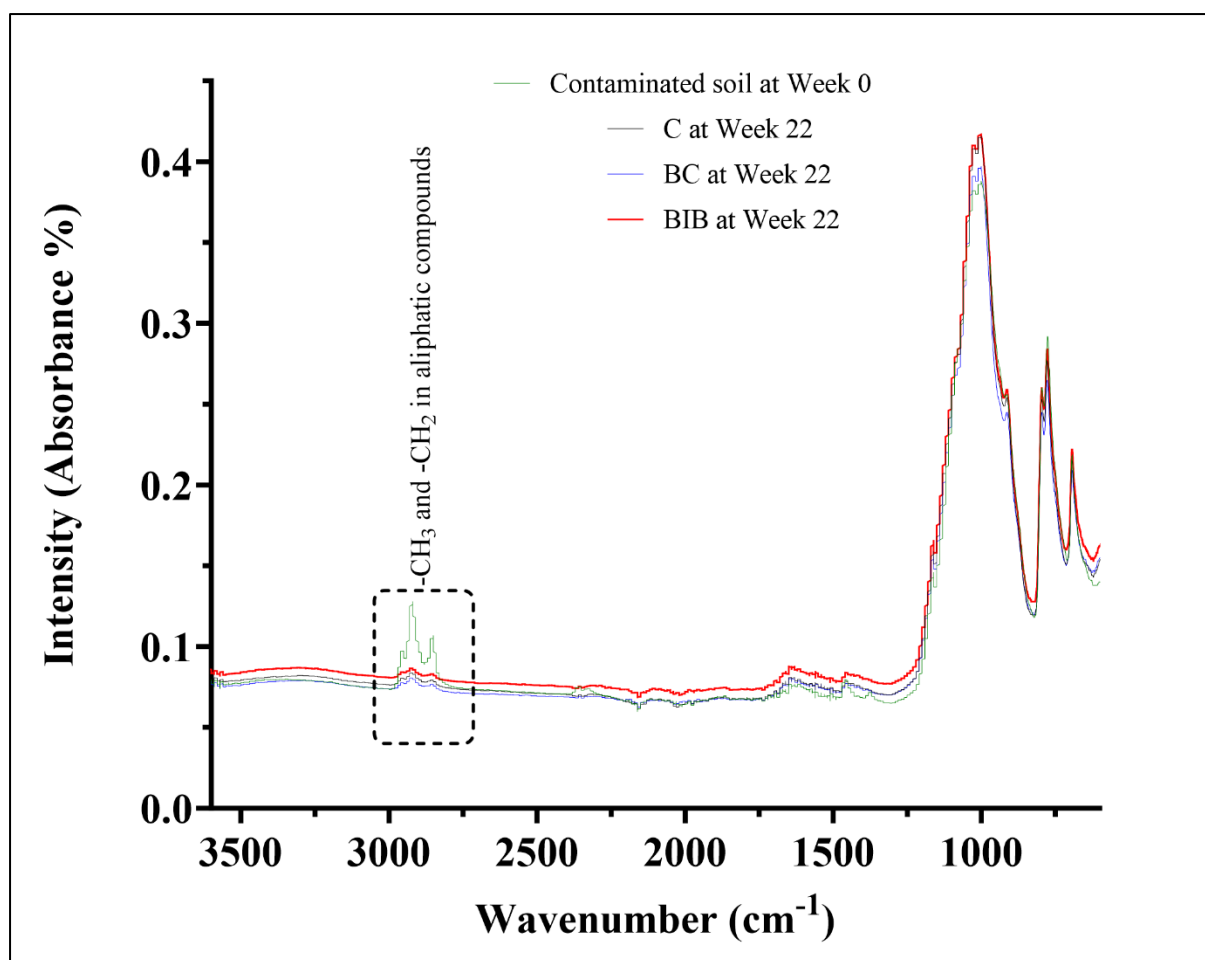


Figure S1: FTIR spectra of the C, BC, and BIB treatment at week 22 versus the contaminated soil at week 0.

C: Control; BC: 5% w/w Biochar; and BIB: Bacteria immobilised biochar

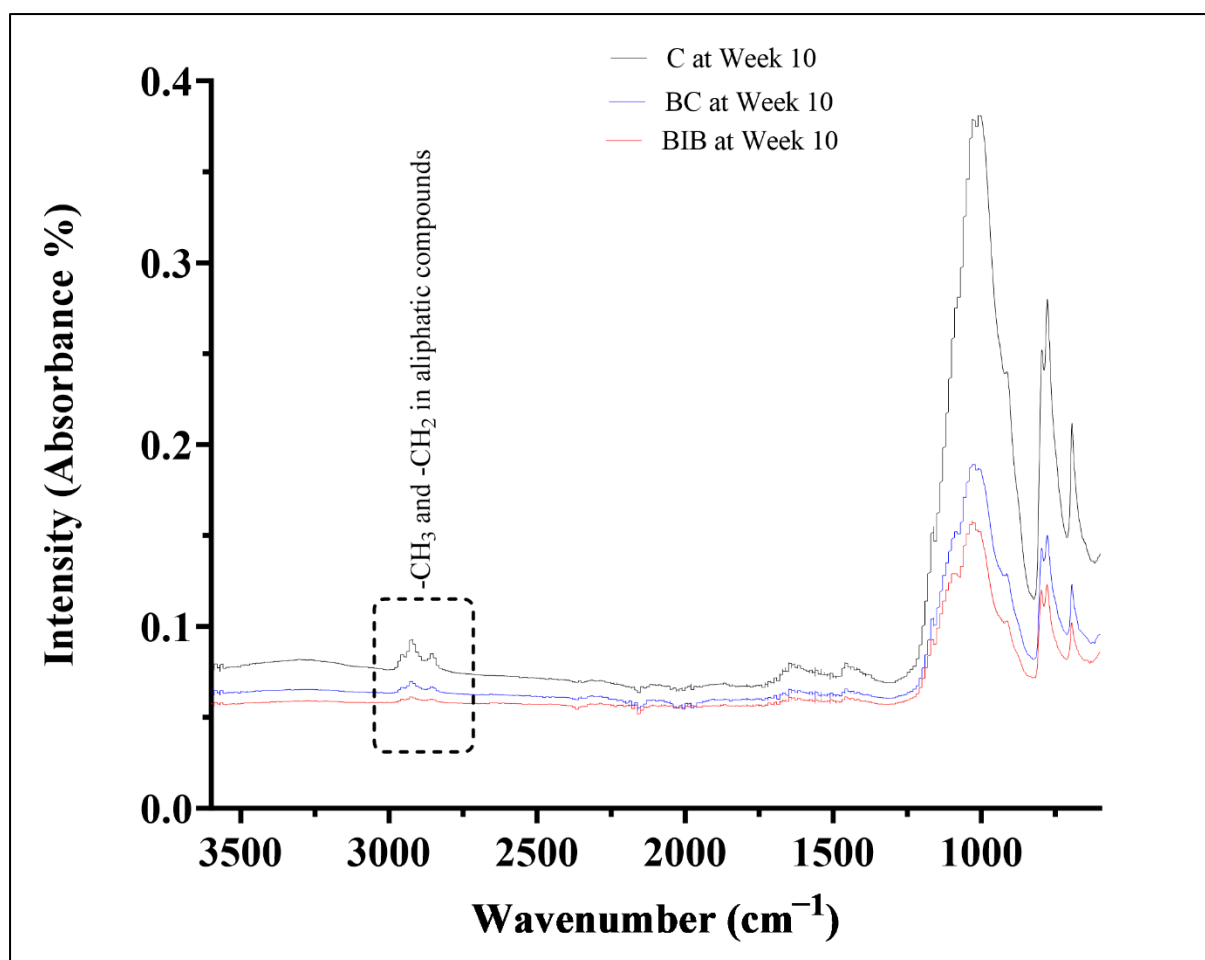


Figure S2: FTIR spectra of the treatment C, BC, and BIB at week 10.

C: Control; BC: 5% w/w Biochar; and BIB: Bacteria immobilised biochar