

## Supplementary file

### Effect of the application of *Ochrobactrum* sp.-immobilised biochar on the remediation of diesel-contaminated soil

Charles Chinyere Dike<sup>12\*</sup>, Alka Rani<sup>123</sup>, Leadin Khudur<sup>12</sup>, Kamrun Nahar<sup>4</sup>, Andy Ball<sup>12</sup>

<sup>1</sup>ARC Training Centre for Transformation of Australia's Biosolids Centre, RMIT University, Bundoora, Melbourne, Vic 3083, Australia

<sup>2</sup>School of Science, RMIT University, Bundoora, Melbourne, Vic 3083, Australia

<sup>3</sup>Environment Protection Authority Victoria, Centre for Applied Sciences, Ernest Jones Drive, Macleod Victoria, 3085, Australia

<sup>4</sup>School of Engineering, RMIT University, Melbourne, Vic 3000, Australia

\*Corresponding author: [charles.dike@student.rmit.edu.au](mailto:charles.dike@student.rmit.edu.au)

## **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
Moisture content (%)	$0.42 \pm 0.26$	$0.49 \pm 0.30$
Volatile matter (%)	$3.15 \pm 0.21$	$3.95 \pm 0.21$
Fixed carbon (%)	$20.18 \pm 5.26$	$25.26 \pm 0.34$
Ash content (%)	$76.26 \pm 4.79$	$70.30 \pm 0.85$

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 <sup>-1</sup> )	t <sub>1/2</sub> (days)	R <sup>2</sup>
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**

	<b>Time (weeks)</b>	<b>TPH conc at that time (mg/kg)</b>
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.

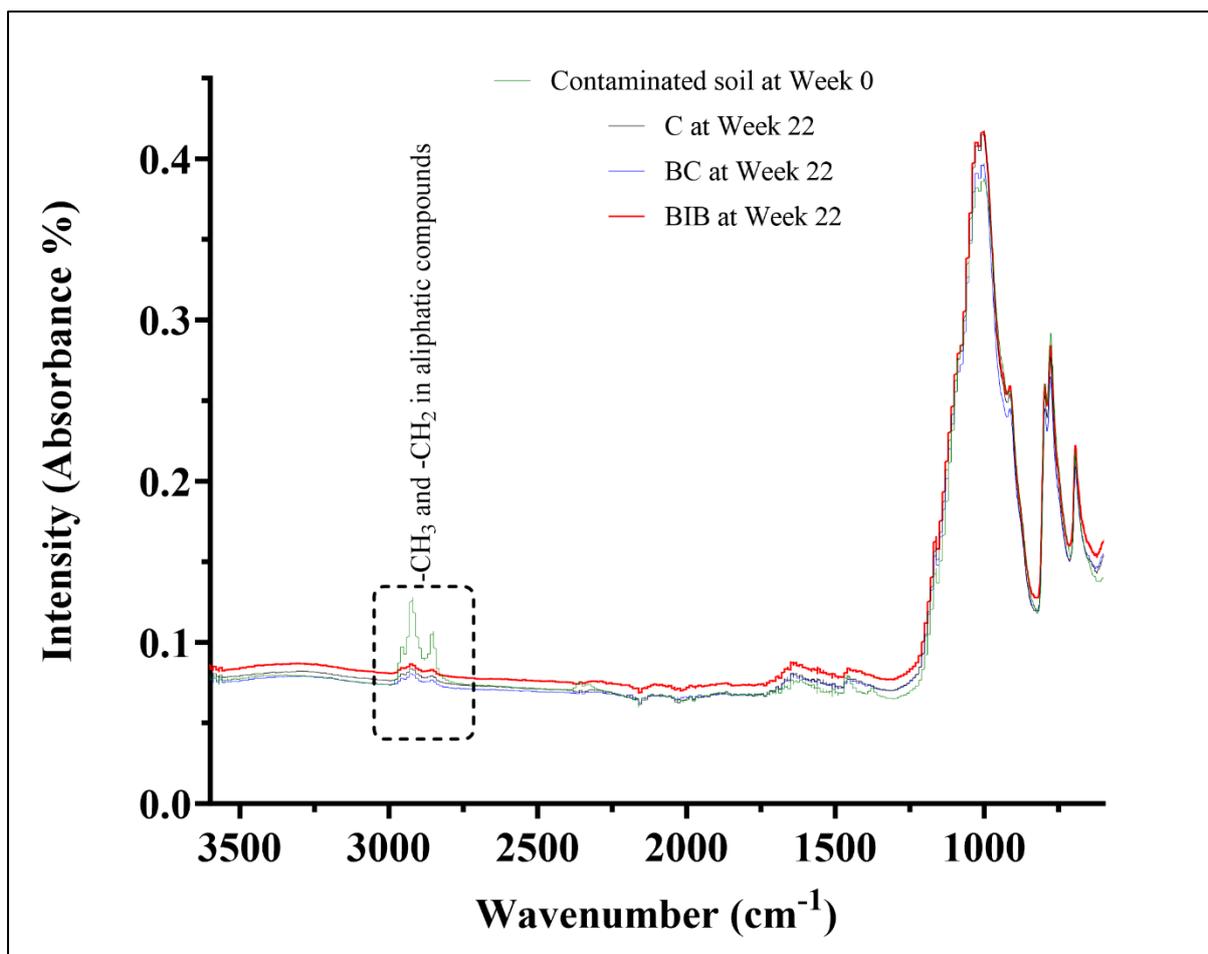


Figure S1: FTIR spectra of the C, BC, and BIB treatment at week 22 versus the contaminated soil at week 0.

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

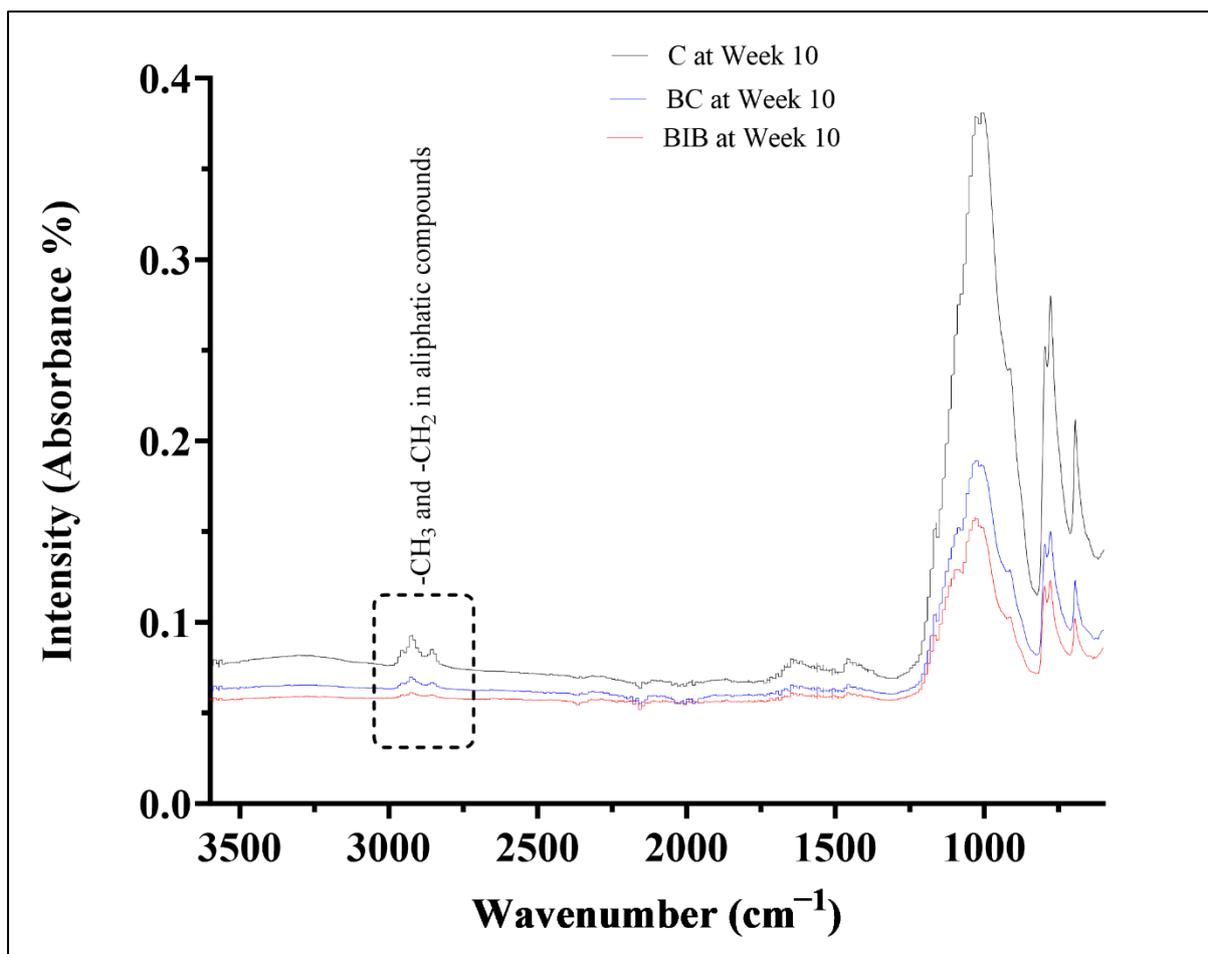


Figure S2: FTIR spectra of the treatment C, BC, and BIB at week 10.

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