

1 Running Title: PETROLEUM REMOVAL FROM SOIL

2
3 **Combining Solvent Extraction and Bioremediation for Removal of**
4 **Weathered Petroleum in Soil** ^{*1}

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6 WU Guozhong ^{1,2,a}, F. COULON ², YANG Yuewei ¹, LI Hong ^{1,3}, SUI Hong ^{1,3,*2}

7
8 ¹ School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072 (China)

9 ² Department of Environmental Science and Technology, School of Applied Sciences, Cranfield
10 University, Cranfield MK43 0AL (UK)

11 ³ National Engineering Research Centre for Distillation Technology, Tianjin 300072 (China)

12 ^a Present address: Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055 (China)

13
14 **ABSTRACT**

15
16 This study aimed to remediate a highly contaminated soil with weathered petroleum using an
17 integrated technology combining solvent extraction and biodegradation. Soils were washed using a
18 composite organic solvent consisted of hexane and pentane (4:1 v/v) and then bioremediated in
19 microcosms which were bioaugmented with *Bacillus subtilis* FQ06 strains and/or rhamnolipid. The
20 optimal solvent extraction conditions were determined as extraction for 20 min at 25 °C with
21 solvent-soil ratio of 6:1 (v/w). On this basis, total petroleum hydrocarbon was decreased from 140,000
22 to 14,000 mg kg⁻¹, which was further reduced to < 4000 mg kg⁻¹ by subsequent bioremediation for
23 132 days. Sustainability assessment of this integrated technology showed its good performance for
24 both short- and long-term effectiveness. Overall results encouraged its application for remediating
25 contaminated sites especially with high concentration weathered hydrocarbons.

26
27 **Key Words:** solvent extraction, TU-A solvent, bioremediation, *Bacillus subtilis*, rhamnolipid

28
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33
34 **INTRODUCTION**

35
36 Most of the existing oil fields in China have been exploited for more than ten years and
37 residual petroleum hydrocarbons concentrations in some soils can exceed 10 000 mg kg⁻¹ (Zhou *et*
38 *al.*, 2005). Remediating these contaminated sites could provide more land available for housing
39 development, which is a continued concern in China due to the sustained growth of population and
40 the lack of residential land. Therefore, there is an increasing demand for development of soil

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*² Corresponding author: suihong@tju.edu.cn

1 remediation technologies that offer both pragmatic cost effective solution and sustainable
2 regeneration of contaminated land.

3 Among the various remediation technologies currently available, solvent extraction is one of
4 the most attractive ones due to its high efficiency. A great deal of investigations on solvent
5 extraction showed that oil removal efficiencies ranged between 75 and 99% (Heemken *et al.*, 1997;
6 Khodadoust *et al.*, 2000; Saifuddin and Chua, 2003; Silva *et al.*, 2005; Risdon *et al.*, 2008).
7 Typically, either an individual solvent or a mixture of solvents is used to extract or wash petroleum
8 from contaminated soils (Risdon *et al.*, 2008; Gan *et al.*, 2009). Advancements within this field
9 include using non-toxic and biodegradable extraction agents such as cyclodextrins and vegetable oil
10 as well as using supercritical and subcritical fluids (Wu *et al.*, 2011). The two steps involved in the
11 extraction of the chemicals of concern (CoC) from a solid matrix are desorption from the binding
12 site in (or on) the solid matrix followed by elution from the solid into the extraction fluid, which
13 implies that the CoC are only transferred from the solid phase to the liquid phase. Therefore, the
14 extracting fluid and the residual soil need further treatment to remove or destroy the remaining
15 CoC.

16 In order to address this drawback, solvent extraction complemented with bioremediation is an
17 attractive approach because bioremediation has the ability to inexpensively treat wide range of
18 organics in all environmental media, generating little or no residues with a low carbon footprint,
19 and causing minimal, if any, ecological effects (Atlas and Cerniglia, 1995; Allard and Neilson, 1997;
20 Lohner *et al.*, 2009). But it is generally perceived that soil contaminated with high TPH
21 concentrations will not be amenable to most biotreatment approaches if pretreatment(s) are not
22 employed to lower concentration and toxicity (Khodadoust *et al.*, 2000). Therefore, this study
23 investigated a combined approach where solvent extraction was coupled to surfactant addition and
24 bioaugmentation in order to improve the performance of conventional soil remediation process for
25 heavily oil contaminated sites.

26 Benefits of such combined approach have been previously demonstrated with coal
27 tar-contaminated soils that were pre-extracted with acetone and ethanol in a batch mode bioreactor
28 (Lee *et al.*, 2001). However, it remains unclear whether this approach would be efficient with high
29 level contamination, as the hydrocarbons concentrations were relatively low ($< 5\ 000\ \text{mg kg}^{-1}$ soil)
30 compared to those reported in our previous study which were $\geq 140\ 000\ \text{mg kg}^{-1}$ (Wu *et al.*, 2011).
31 To the best of our knowledge, there are not yet any combined approaches that have been
32 successfully used to remediate highly contaminated sites with weathered oil. This is partly due to
33 two challenges: (i) ageing processes result in the incorporation of organic compounds to soil
34 organic matter, slow dissolution into nano- and micro-pores, and formation of semi-rigid films
35 around the non-aqueous phase liquid (NAPL) that causes a high resistance to mass transfer
36 (Trindade *et al.*, 2005); and (ii) the explicit NAPL would become one of the most significant
37 compartments for distribution of hydrocarbons, which results in the infinite degradation half-lives
38 because of the toxicity to microorganisms, lack of water, nutrients and oxygen to support
39 degradation (Coulon *et al.*, 2010).

40 Therefore, the objectives of this study were: (i) to evaluate the efficacy of a combined remedial
41 approach based on solvent extraction and biological transformation of soils contaminated with high
42 levels of weathered hydrocarbons, and (ii) to assess the practicality and sustainability of such
43 approach.

1 MATERIALS AND METHODS

2

3 *Soil*

4 The soils used in this study were obtained from a typical oilfield in China that had a long
5 history of contamination with high concentration of petroleum hydrocarbons. Soil samples were
6 collected at depth of 20 cm approximately using spade, transported and stored in tarpaulin bags at
7 4 °C. Soil samples were air-dried, protected from sunlight to minimize any potential reaction, and
8 homogenized by screening through 40 mesh sieves to remove extra vegetable roots before
9 physicochemical characterization.

10 Soil pH was measured by 1 M NaCl on pH meter. Water content was determined
11 gravimetrically after drying soil samples at 105 °C for 8 h. Soil organic matter (SOM) content was
12 measured by placing samples (2 g) in the crucible, weighed and heated in a furnace at 500 °C for 2
13 h to allow the organic matter to burn away. After removal from the oven, the crucibles were allowed
14 to cool before being weighed again. The weight after baking was subtracted from the initial weight
15 to give the weight of organic matter in each sample. Total organic carbons (TOC) was tested by
16 weighing 5 g of sample into a small silver-foil capsule and adding 4 M hydrochloric acid drop by
17 drop until any visible reaction stopped. After dried at 90 °C for 4 h, the capsule was packed into a
18 larger aluminium-foil capsule and was heated at 900°C on a flow of oxygen gas. TOC was
19 determined by carbon dioxide released that was measured by thermal conductivity detector. Bulk
20 density, tap density and soil grain size were tested using Laser Particle Sizer (LG-Malvern).

21

22 *Solvent extraction process*

23 Composite TU-A solvent used in this study consisted of hexane and pentane (4:1) as described by
24 Lian *et al.* (2008). The influence of operating parameter settings including temperature, extraction
25 time and solvent volume on solvent extraction efficiency were evaluated as described by previous
26 studies (Urum *et al.*, 2004; Han *et al.*, 2009).

27 The solvent extraction process is shown in Fig. 1. Briefly, soil samples (10 g) were weighed into
28 a cylinder (50 mm × 50 mm internal diameter) filled with a certain amount of organic solvent as
29 shown in Table I. After extraction at a constant temperature for 5, 10, 15, 20, 25 and 30 min on a
30 side-to-side shaker, the concentration of TPH remaining in the soil was measured. Based on these
31 experimental results, the optimal temperature, solvent volume and extraction time were determined.
32 Samples for subsequent bioremediation were prepared by an extra set of extractions under the
33 optimized conditions.

34

35 

36 Fig. 1 Schematic of the solvent extraction and biodegradation experiments

37

38 TABLE I

39

40 Operation parameters in solvent extraction

Parameters	Values
------------	--------

	1	2	3	4	5	6
Temperature (°C)	20	30	40	50	-	-
Solvent volume (mL)	20	40	60	80	-	-
Extraction time (min)	5	10	15	20	25	30

1

2 *Analysis of TPH*

3 TPH concentration in the soil was determined using infrared spectrophotometric method which
4 was a rapid and cheap technique for measuring TPH in soil and water (US EPA Method 418.1).
5 Briefly, soil samples (0.25 g) were chemically dried using 0.25 g of anhydrous Na₂SO₄ and
6 ultrasonically extracted with 10 mL of CCl₄ at 20 °C for 20 min followed by vacuum filtration in
7 sand core funnel fitted with glass receiver tube. This step was repeated twice. After rinsing the
8 conical flask with 10 mL of CCl₄ in triplicate, the extract was adjusted to 50 mL with CCl₄. The
9 silica gel cleanup was then performed by passing the extract through a silica gel column (200 mm ×
10 10 mm internal diameter) before analysis on F2000-I Infrared Spectrophotometer (China Jiling Oer,
11 Ltd.). Calibrations were carried out using oil standards dissolved in CCl₄ solution ranging from 80
12 to 400 M.

13 Fractionation of TPH into aromatic, aliphatic, asphaltene and NSO (nitrogen, sulfur and
14 oxygen-containing compounds) was done by silica gel column chromatography followed by
15 gravimetric analysis (Das and Mukherjee, 2007). TPH extracts were dissolved in *n*-pentane and
16 separated into soluble and insoluble fractions (asphaltene). The soluble fraction was loaded on the
17 top of a silica gel column. The alkane fraction was eluted with 100 mL of hexane followed by the
18 aromatic fraction with 100 mL of toluene. The NSO fraction was eluted with 100 mL of methanol
19 and chloroform.

20

21 *Batch biodegradation experiments*

22 *Bacillus subtilis* (*B. subtilis*) FQ06 strains obtained from Liao Oilfield, China, was used in this
23 study due to its ability of degrading crude oil (> 2 g mL⁻¹) under laboratory conditions (Yang, 2010).
24 The processes of bacteria screening and acclimating, and the preparation and maintenance of
25 bacteria suspension were carried out as described by Yang (2010).

26 As shown in Fig. 2, triplicate microcosms were established in sterile Bunsen beakers (100 mL)
27 containing 60 g of solvent washed soil and 15 mL of sterile water (natural attenuation: Scenario 1); 60
28 g of solvent washed soil and 15 mL of *B. subtilis* FQ06 strains suspension with a cell density of 10⁸
29 cell mL⁻¹ (bioaugmentation: Scenario 2); and 60 g of solvent washed soil, 13 mL of *B. subtilis* FQ06
30 strains suspension and 2 mL of rhamnolipid solution (bioaugmentation/biostimulation: Scenario 3).
31 The rhamnolipid (3 g L⁻¹) was bought from Wotaisi Chemicals Co., Ltd., China.

32 The beakers were well sealed by multi-layer gauze to prevent cross contamination with other
33 microorganism and incubated at 25 °C. The pH value ranged from 7.2 to 7.6 during all the
34 experiments. Sterile water was supplemented periodically to saturate the soils which drained to the
35 field capacity without leaching. The soil slurry system was thoroughly agitated manually every 10
36 days. Residual TPH concentration was determined before the onset of the incubation and after 7, 12,
37 20, 25, 35, 45, 54, 64, 74, 84, 94, 105, 118 and 132 days.

1

2 *Modelling biodegradation kinetics*

3 The first-order degradation reaction equation was used to predict the biodegradation kinetics
4 with absence of surfactant (Scenarios 1 and 2) as follows:

5

$$6 \quad dC/dt = -k \cdot C \quad (1)$$

7

8 where K represents the first-order proportionality constant. C is TPH concentration.

9 Integrating this equation over time yields:

10

$$11 \quad \ln C = -k \cdot t + A \quad (2)$$

12

13 where A is Napierian logarithm of TPH concentration at t_0 .

14 In the case of Scenario 3, it was assumed that a residual amount of contaminants (C_m)
15 partitions into the surfactant micellar phase and thereby it is not bioavailable to the bacteria (Guha
16 and Jaffé, 1996). In other words, degradation occurs only in water phase. On this basis, the term C
17 of the Eq. (2) was substituted by an effective concentration defined as the concentration (C)
18 subtracting the concentration of contaminants bound to micellar phase (C_m). The mass balance
19 equation can then be written as follows:

20

$$21 \quad \ln(C - C_m) = -k \cdot t + A \quad (3)$$

22

23 The goodness of fit of the models was assessed by calculating t-statistic, correlation coefficient
24 squared R^2 and F-ratio via analysis of variance (ANOVA; Field, 2009).

25

26 *Statistical analysis*

27 ANOVA was carried out using Tukey test to evaluate the effects of factors on the efficiency of
28 solvent extraction and the changes of TPH during biodegradation. The difference between outcomes
29 was recognised as significant where $P < 0.05$. All tests were performed using SPSS 13.0 for
30 Windows.

31

32 RESULTS

33 *Soil characterization*

34 The physicochemical properties of the sandy soil are presented in Table II. The high TOC
35 implies that the bioavailability of the TPH could possibly be enhanced by the addition of an organic
36 solvent and a biosurfactant, as there is a strong positive correlation between the solubilisation
37 effectiveness of solvent on petroleum hydrocarbons and the logarithm of the organic carbon
38 partition coefficients ($\log K_{oc}$) (Lee *et al.*, 2001). The relatively low moisture content and SOM
39 suggests that such type of soil would not be considered as promising candidates for bioremediation
40 without pre-treatments due to the unfavourable conditions for microbial degradation. Therefore,
41 potential strategies would be the addition of nutrients that limit the growth or activity of the
42 microorganisms and the amendment of microorganisms with desired bioremediation capabilities.

43 The level of TPH contamination was estimated to be $140,000 \pm 20,000 \text{ mg kg}^{-1}$ of soil. The

1 alkanes (50%) represented the largest fraction of the solvent extracted TPH, followed by the
2 aromatic (21%), the NSO (18%) and the asphaltene fractions (11%).

3
4 TABLE II

5
6 Physicochemical characteristics of the soil

pH	SOM ^{a)} (%)	CEC ^{b)} (mmol·kg ⁻¹)	Moisture content (%)	TOC ^{c)} (%)	Soil class (%)			BD ^{d)} (g·cm ⁻³)	TD ^{e)} (g·cm ⁻³)
					Clay	Sand	Silt		
8.0	0.7	164.0	1.7	7.0	25.3	40.4	34.3	1.4	2.8

7 ^{a)} Soil organic matter, ^{b)} Cation exchange capacity, ^{c)} Total organic matters, ^{d)} Bulk density, and ^{e)} Tap density

8
9 *Effects of solvent extraction parameters*

10 Removal efficiencies at different extraction time indicated oil removal was a very fast process
11 and over 80% of TPH was removed within 5 min (Fig. 2a). Maximum removal rate (up to 96%)
12 was reached after 15 min and barely increased ($P > 0.05$) after 20 min, suggesting that the
13 equilibrium time was reached.

14 The oil removal rate was enhanced from 88% to 96% as the temperature increased from 20 °C
15 to 50 °C (Fig. 2b). The increase of temperature resulted in a decrease of oil viscosity, which could
16 improve oil mobility (Urum *et al.*, 2004). In the meantime, the addition of heat was observed to
17 alter the component molecules of natural SOM resulting in phase transitions of SOM from the
18 condensed glassy to the expanded rubber state (Pignatello, 1998). Consequently, the hydrocarbons
19 previously bound to SOM are more easily desorbed and released to the solvent phase. However,
20 temperature higher than 40 °C is unfavourable in this study as hexane and pentane would be lost by
21 evaporation. Therefore, operating design at room temperature was recommended in practical terms.

22 The oil removal was significantly enhanced ($P < 0.05$) as solvent volume increased from 20 to
23 80 mL (Fig. 2c). The raise of liquid-solid ratio facilitated the diffusion of the desorbed contaminants
24 into the aqueous phase, thus improved the oil removal. On the other hand, using excessive solvents
25 would increase the cost of subsequent solvent recycling. Accordingly, a liquid-solid ratio of 6:1 (v:
26 w) is suggested in terms of practicality.

27 Based on these findings, the optimal solvent extraction conditions were determined at ambient
28 temperature (25 ± 2 °C) for 20 min with an initial solvent concentration of 6 mL g⁻¹ soil. Thereby an
29 extra set of triplicate solvent extraction was carried out in which TPH concentration was reduced to
30 $14\ 000 \pm 3\ 000$ mg kg⁻¹.

31
32 **Fig. 2**

33 Fig. 2 Influence of extraction time, temperature and solvent volume on the removal efficiencies of TPH.

34
35 *Bioaugmentation effectiveness on the degradation of the residual TPH*

36 As expected, the natural attenuation (Scenario 1) was very slow and the greatest degradation
37 rate was only 18% by day 118 (Fig. 3). In contrast bioaugmentation of the microcosms with *B.*
38 *subtilis* (Scenario 2) significantly enhanced the TPH removal ($P < 0.01$) suggesting that the

1 proportion of the hydrocarbon-degrading bacteria within the indigenous community was
 2 insufficient to effectively sustain the degradation of a complex mixture of hydrocarbons. It also
 3 suggests that *B. subtilis* can significantly accelerate the degradation of heavy hydrocarbons in
 4 contaminated soils. This finding is in good agreement with Das and Mukherjee (2007).

5 Addition of rhamnolipids (Scenario 3) further enhanced the removal of TPH by one order of
 6 magnitude compared to the natural attenuation (Scenario 1). This suggested that the bioavailability
 7 of the substrate has been enhanced by reducing the surface tension of phase boundaries. The
 8 biosurfactant contributed to wreck the stable structure of asphaltene coagulation formed by
 9 presence of TU-A solvent as discussed previously, which facilitated the transport of residual oil
 10 from soil and non-aqueous phases to aqueous phase where microorganisms are present.

11  Fig. 3

12 Fig. 3 Percentage degradation of TPH over 132 days
 13
 14

15 Biodegradation kinetics

16 The kinetic equations and associated parameters are shown in Table III. Confidences in the
 17 hypothesis that the predictor variable contributes significantly to the ability of the model to estimate
 18 concentration of contaminants during biodegradation process were obtained by t-statistic test ($P <$
 19 0.001). The first order biodegradation proportionality constant k increased from 0.008 to 0.051 d^{-1} in
 20 presence of biosurfactant. In addition the calculated half-life of the bioaugmented microcosms was
 21 decreased from 347 to 87 days and further decreased to 20 days by addition of biosurfactant (Table
 22 III).

23
 24 TABLE III

25
 26 Calculated parameters of the regression equations

Scenario	Equation	Parameter	Estimated	Std. Error	t-statistic	Sig.	Half-life (d)	R ²	Adjusted R ²
1	$\ln C = -kt + A$	K (d ⁻¹)	0.002	0.000	16.070	0.000	346.6	0.952	0.948
		A (mg g ⁻¹)	2.638	0.007	372.639	0.000			
2	$\ln C = -kt + A$	K (d ⁻¹)	0.008	0.001	34.156	0.000	86.6	0.848	0.836
		A (mg g ⁻¹)	2.281	0.067	0.850	0.000			
3	$\ln(C-C_m) = -kt + A$	K (d ⁻¹)	0.051	0.005	-	-	20.2	0.995	0.979
		A (mg g ⁻¹)	2.336	0.044	-	-			
		C _m (mg g ⁻¹)	2.965	0.211	-	-			

27
 28 Concentrations predicted were similar to those measured as presented in Fig. 4. A smaller
 29 correlation coefficient squared (0.848) was observed in Scenario 2. The F-ratios of the three models
 30 (Table IV) were much greater than 1 indicating that the improvement due to fitting the regression
 31 models is much greater than the inaccuracy within the model and there was less than 0.1% chance
 32 that such large F-ratios would happen if the null hypothesis were true ($P < 0.001$). Therefore, it is
 33 concluded that the regression models overall predict the biodegradation kinetics significantly well.

Fig. 4

Fig. 4 Comparison of the calculated TPH concentration with the observed data

TABLE IV

Analysis of variance of the kinetic models

	Model	Sum of squares	Degree of freedom	Mean squares	F-ratio	P-value
1	Regression	0.064	1	0.064	258.230	0.000
	Residual	0.003	13	0.000		
	Total	0.067	14			
2	Regression	1.599	1	1.599	72.248	0.000
	Residual	0.288	13	0.022		
	Total	1.887	14			
3	Regression	553.284	3	184.428	723.247	0.000
	Residual	3.056	12	0.255		
	Total	556.340	15			

DISCUSSION

This study clearly indicated that TU-A solvent is a promising mixture providing significant decrease of extraction time compared to other studies (Khodadoust *et al.*, 2005; Ahn *et al.*, 2008; Han *et al.*, 2009) in which the solvent washing of weathered oil required considerable time (1--48 h) to reach equilibrium. It can be inferred that all of the aliphatic and aromatic fractions have been removed after solvent extraction, although not being quantitatively confirmed, and the remaining insoluble fractions (14 000 mg kg⁻¹) were most likely asphaltenes as 15 000 mg kg⁻¹ (11% of TPH) were quantified before extraction. This is evidenced by the chemical structure and properties of asphaltenes, which is believed to be polycyclic aromatic ring compounds bearing alkyl side chains composed of small amounts of heteroatoms (S, N, and O) and traces of vanadium and nickel (Ali and Alqam, 2000; Ancheyta *et al.*, 2002). They are soluble in liquids having a surface tension above 25 dynes cm⁻¹ (such as benzene, pyridine, carbon disulfide and CCl₄), but insoluble in nonpolar solvents with a surface tension lower than 25 dynes cm⁻¹ (such as petroleum ether, pentane, isopentane and hexane) (Speight and Moschopedis, 1994). Therefore, TU-A solvent was inefficient to remove asphaltenes by solubilisation. Moreover, coagulation of asphaltenes tend to be stimulated by low molecular weight hydrocarbons (i.e. hexane and pentane) that would take up the 'stable space' previously formed when colloid molecules in the crude oil were adsorbed on the surface of asphaltenes (Leontaritis and Mansoori, 1989; Chang and Fogler, 1994). This led to the formation of a thermodynamic stable structure, in which asphaltenes were sequestered and became hardly transferable from the inner parts to the outer parts of the soil particles (Hu *et al.*, 2000). Future works are needed to better understand the solvent-asphaltenes interaction mechanisms and develop strategies for regenerating the solvent sequestered in the formulated coagulation.

Results revealed that bioaugmentation with *B. subtilis* FQ06 and rhamnolipid was beneficial for TPH removal. However, use of biosurfactant for the purpose of improving biodegradation

1 should be further investigated as various studies reported contradicting results. For instance, the
2 addition of rhamnolipids to hexadecane stimulated the biodegradative capabilities of *P. aeruginosa*
3 strains UG2, ATCC15528 and PG201 as well as *R. erythropolis* ATCC 19558 while the degradative
4 activity of *R. erythropolis* DSM 43066 was inhibited and no effect on the strain BCG112 was
5 observed (Bruheim *et al.*, 1997; Noordman and Janssen, 2002). This demonstrated that stimulation,
6 inhibition or no effects on biodegradation by exogenous addition of biosurfactants depend on the
7 property of surfactant added and the type of biosurfactant producing organisms in the soil.

8 Biodegradation dynamics suggested that oil degradation was co-limited by the number of oil
9 degraders and hydrocarbon bioavailability at the early stage. After 54 days, neither the addition of
10 bacteria nor the emulsification of hydrocarbons via addition of biosurfactant would increase the
11 degradation (Fig. 3), suggesting that hydrocarbon bioavailability was no longer a co-limiting factor
12 at the final stage. It is likely that nutrients became a limiting factor since the carbon source
13 available to microorganisms was gradually reduced and harmful substances resulted by metabolism
14 were continuously accumulated. This was supported by McKew *et al.* (2007) that increasing
15 bioavailability of the oil by emulsification will have a minimal effect if nutrients were limiting.

16 The first order biodegradation rate constants (Table III) were one order of magnitude lower than
17 that of Lee *et al.* (2001). Therefore, the residual TU-A solvent was inferred to inhibit biodegradation,
18 although biodegradation experiments without pre-extraction were not carried out in this study because
19 such a high initial TPH concentration was toxic to microorganisms and would cause loss of
20 degradation efficiency. This finding sounds opposite to several studies (Brusseau *et al.*, 1991;
21 Pignatello and Xing, 1996; Lee *et al.*, 2001) in which organic solvents resulted in the enhancement of
22 PAH bioavailability by solubilisation and consequently increased the first order biodegradation rate
23 constants. This may be attributed to two concomitant effects resulted from TU-A solvent addition: (i)
24 enhancement of bioavailability by solubilisation that causes faster biodegradation, and (ii) coagulation
25 phenomena that makes hydrocarbons less or unavailable to microorganisms. The latter is likely the
26 dominant kinetic factor limiting the transfer of highly recalcitrant hydrocarbons from solvent to water
27 phase. Therefore, removal of the residual TU-A solvent in the extracted soil before bioremediation
28 could be an important strategy since bioavailability is a co-limiting factor at the early stage of
29 biodegradation. In order to gain more insights into the selectivity on different hydrocarbons during
30 biodegradation, future works are needed to focus on biodegradation and bioavailability of various
31 hydrocarbon fractions and indicator compounds instead of TPH when implementing this technology.

32 Overall results demonstrated both short-term and long-term effectiveness of the integrated
33 technology. The short-term performances include: (i) more than 90% of TPH was removed within 15
34 min at room temperature, which was much more effective than previously reported solvent extraction
35 methods with an extraction time raging from 1 to 48 h and temperature ranging from 70 to 100 °C
36 (Khodadoust *et al.*, 2000; Khodadoust *et al.*, 2005; Ahn *et al.*, 2008; Han *et al.*, 2009); (ii) instruments
37 used in traditional methods such as ultrasonic probes, freeze-dryer and Soxhlet equipments were not
38 required, which kept capital outlay low and minimized the consumption of energy and other natural
39 resources; (iii) significant amount of oil could be recovered by solvent extraction if the field-scale
40 application of this method is successful to a site previously contaminated with extremely high
41 concentration of oil; and (iv) negative impacts on environments associated with the utilization of
42 solvent extraction are overcome as the subsequent biodegradation allows more complete removal of
43 the unsolved waste, which is consistent with the recognition by the US and UK Sustainable
44 Remediation Forums (SURF) encouraging the use of remedial technologies that permanently destroy

1 contaminants (Ellis and Hadley, 2009; Bardos *et al.*, 2011).

2 The long-term performances are as follows: (i) the ultimate residues remaining in the soil are
3 water and the untreated asphaltenes ($< 4000 \text{ mg kg}^{-1}$), because up to 99% of the solvent used in the
4 solvent extraction could be recycled and reused by water wash technology (Wu *et al.*, 2011); (ii) water
5 is recognized as 'green solvent' and its disposal is regarded as benign with little affect on the
6 environment; (iii) although toxicity and carcinogenicity of asphaltenes have been recognized (Wess *et*
7 *al.*, 2004), the potential toxicity of highly recalcitrant asphaltenes would diminish as they become less
8 available to ecological receptors in soil (Alexander, 2000). Further bioaccumulation of asphaltenes
9 would be highly unlikely because of their low water solubility and high relative molecular weight.
10 The residual contaminants may potentially be left in place without creating additional environmental
11 risk.

12 13 CONCLUSIONS

14
15 The performance of an integrated remedial approach coupling solvent extraction to
16 bioremediation to treat soils contaminated with high concentrations of weathered petroleum
17 hydrocarbons was investigated using TU-A solvent and *B. subtilis* FQ06 strains. The solvent
18 extraction was proved to be fast and effective which removed over 80% of TPH in 5 min at room
19 temperature. All of extraction time, temperature and solvent volume significantly influenced the
20 extraction efficacy. The optimal extraction conditions were determined at 25 °C for 20 min with a
21 solvent-soil ratio of 6:1 (v/w). On this basis, TPH were decreased from 140,000 to 14,000 $\text{mg}\cdot\text{kg}^{-1}$
22 d.w. soil. However, an inherent limitation of the solvent extraction lies in the fact that some extremely
23 recalcitrant hydrocarbons such as asphaltenes are insoluble in the TU-A solvent. Therefore the
24 residual oil was further removed by biodegradation. Bioaugmentation and surfactant addition
25 enhanced significantly the degradation of the residual TPH ($< 4000 \text{ mg}\cdot\text{kg}^{-1}$) after 132-day of
26 experiment. Biodegradation kinetics demonstrated that removal of residual solvent in the extracted
27 soil at the early stage of degradation could be an important strategy. The overall results of the
28 integrated remedial technology are in good agreement with the US and UK SURF-frameworks that
29 incorporate sustainable concepts throughout the remedial action process. Importantly, it is easy and
30 simple to reproduce. It should be encouraged at contaminated sites for reducing remediation time,
31 enhancing efficacy, improving sustainability and restoring previous contaminated sites especially with
32 high concentration weathered hydrocarbons.

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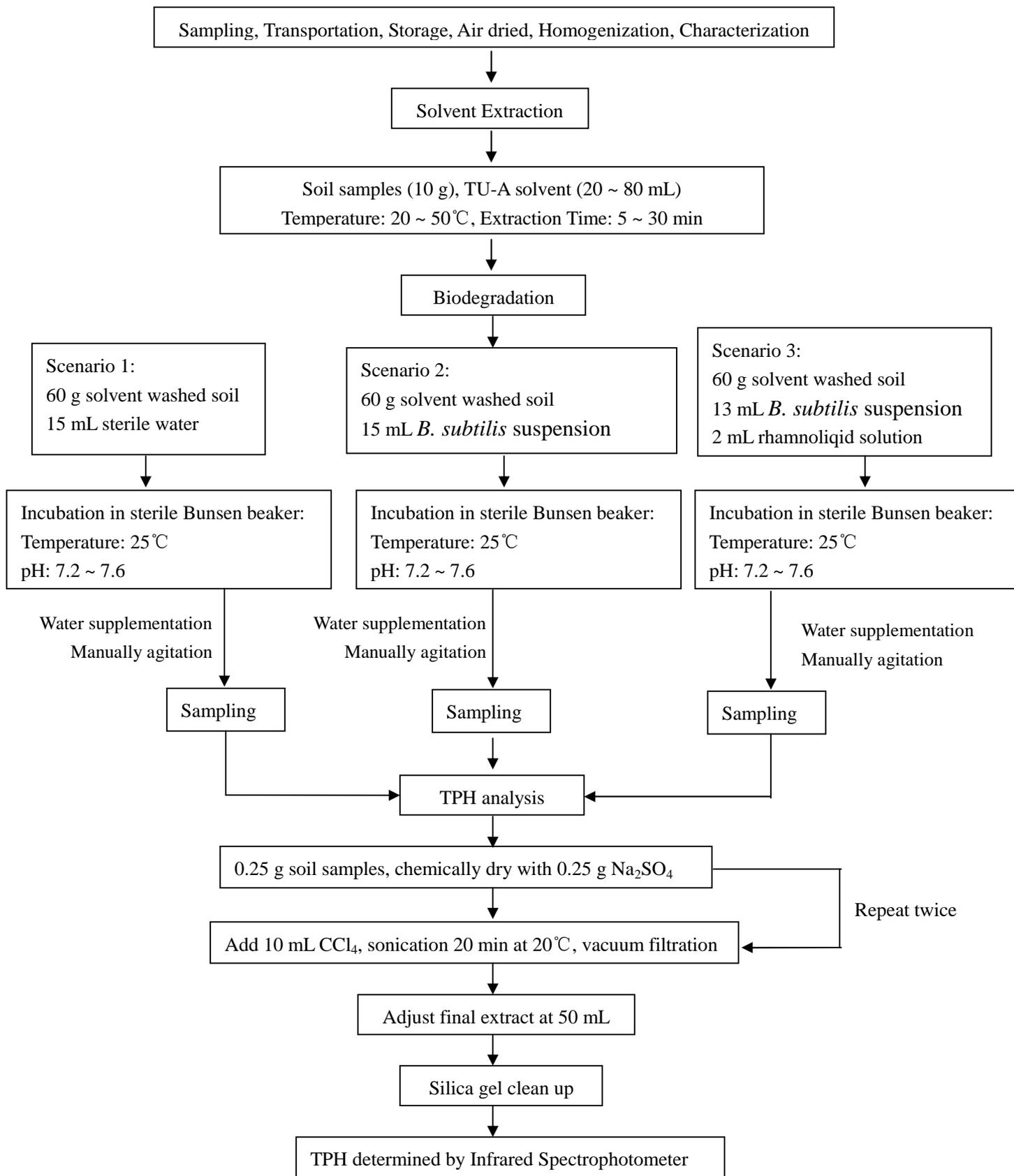
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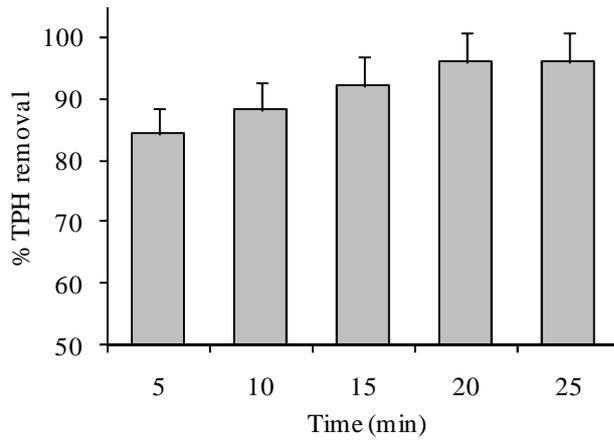
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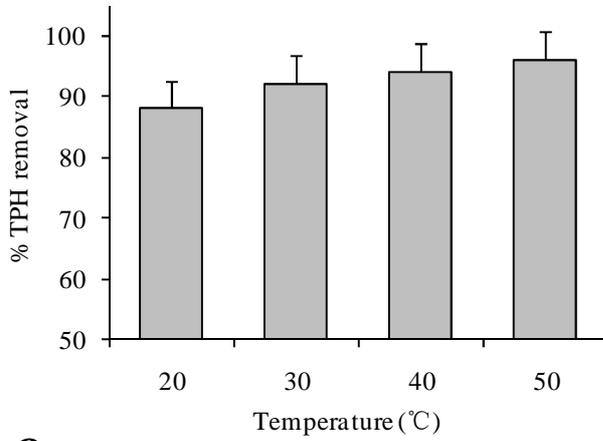
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Fig. 1 Schematic of the solvent extraction and biodegradation experiments

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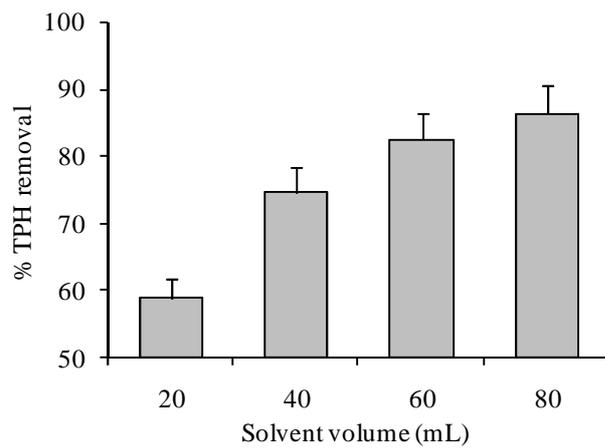
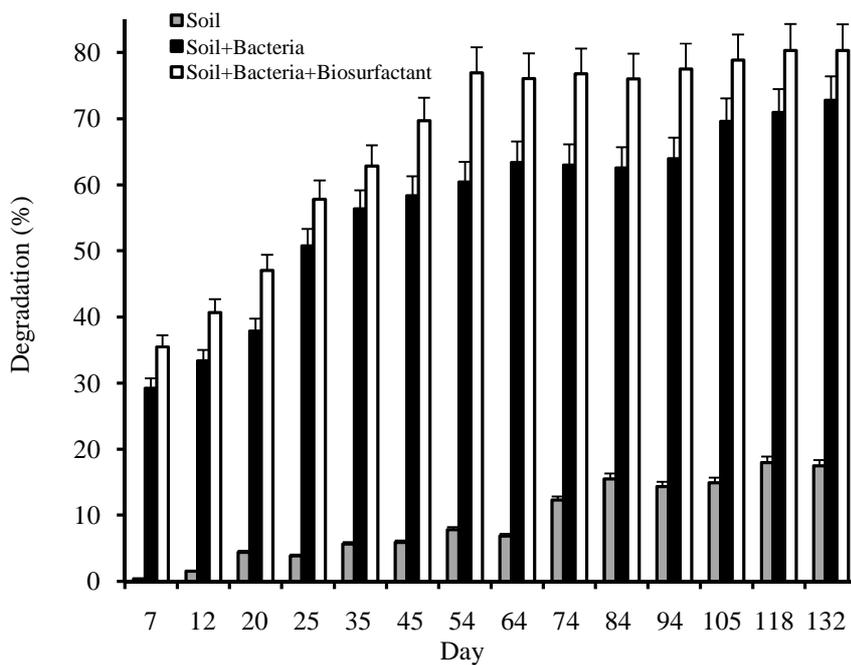


Fig. 2 Influence of extraction time, temperature and solvent volume on the removal efficiencies of TPH (Each value represents the mean removal percentage of TPH from duplicate samples \pm standard deviation)

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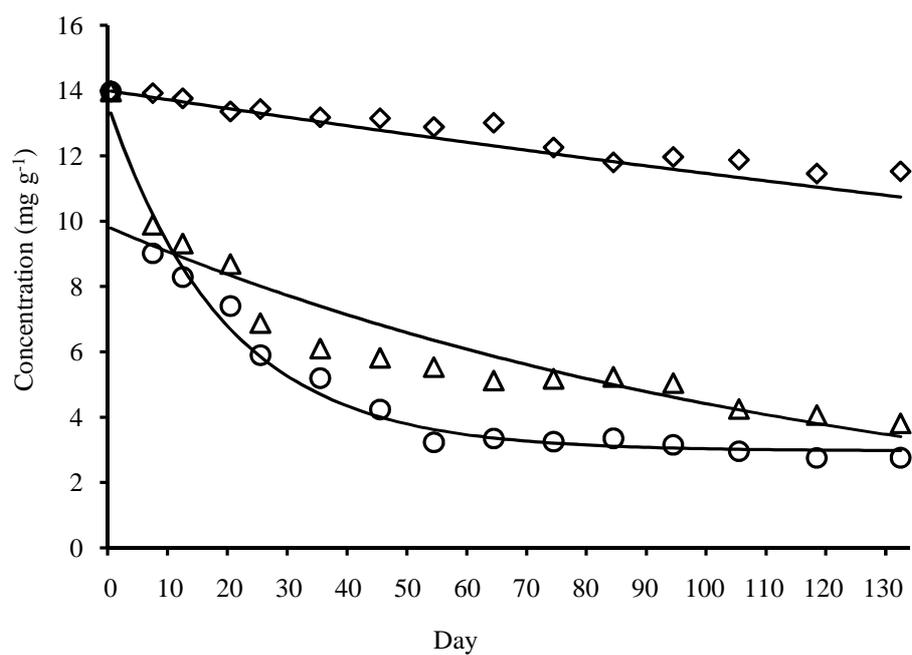
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3 Fig. 3 Percentage degradation of TPH over 132 days (Each value represents the mean degradation percentage of
4 TPH from duplicate samples \pm standard deviation).

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1
 2 Fig. 4 Comparison of the calculated TPH concentration with the observed data. The symbols (\diamond : Soil, Δ : Soil +
 3 Bacteria; \circ : Soil + Bacteria + Biosurfactant) represent the experimental data and the continuous lines are the
 4 model results of TPH concentration.