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Science of the Total Environment
Editor

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Dear Editor:

Attached you will find the submitted manuscript “*Kinetic modelling of a diesel polluted clayey soil bioremediation process*” by Engracia Lacasa Fernández, Elena Moliterni Merlo, Lourdes Rodríguez Mayor and José Villaseñor Camacho (corresponding author: jose.villasenor@uclm.es) in order to be reviewed for a possible publication as original research article in *S.T.E.* (suggested classification: bioremediation).

The work described has not been published previously, in whole or in part, and it is not under consideration for publication elsewhere. All authors are aware of, and accept responsibility for, the manuscript, and they mutually agree that it should be submitted to *S.T.E.* The manuscript was not previously submitted to *S.T.E.* and it was revised by a professional English Language editing service.

This is an original work reached out by the authors in the Chemical Engineering Department of the University of Castilla La Mancha (Address for corresponding author: Research Institute for Chemical and Environmental Technology (ITQUIMA). University of Castilla La Mancha, 13071, Ciudad Real, Spain; phone: 34-926295300).

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Rationale for relevance to *S.T.E.*:

The present work offers valuable and novel information about the mathematical modelling of a complex process such as the bioremediation of hydrocarbon polluted soils. The proposed model tries to couple both transport phenomena, and hydrocarbons distribution among four different phases, with biodegradation phenomena. It has been made an effort to obtain background information about modelling of bioremediation processes, in order to compare with the proposal by the authors. Only few mathematical models that couple mass transfer with Monod or first-order kinetics for hydrocarbon biodegradation are found in the literature. The model predictions have been validated through the comparison with experimental data that had already been reported in the current literature by the same authors.

We consider that the work is directly related to the *S.T.E.* objectives and it could be classified in the “bioremediation” section. The work has been developed in a laboratory scale following correct scientific methods.

Yours sincerely

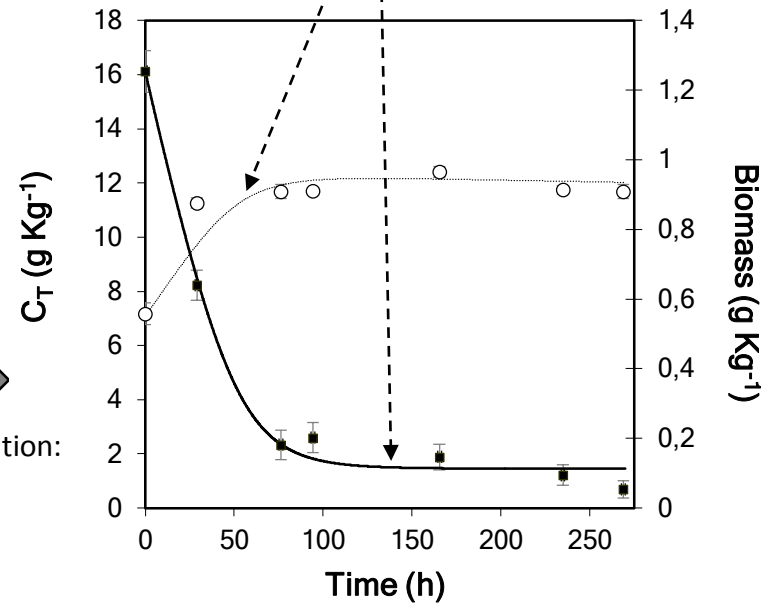
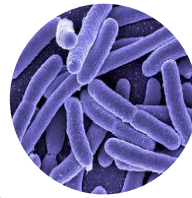
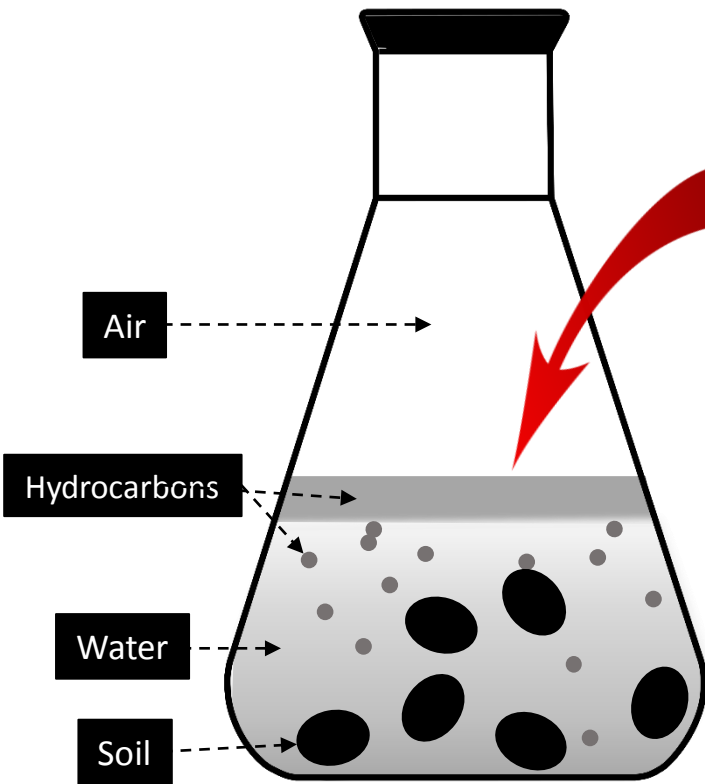
Dr. José Villaseñor Camacho

1. Hydrocarbons (HC) polluted soil suspension in water.

2. Addition of Diesel degrading microorganisms.

3. Mathematical model formulation:
- HC distribution among phases
- HC biodegradation

4. Model predictions.



***Highlights (for review)**

- 1 A mathematical model is proposed to describe a soil bioremediation process
- 2 The model couples mass transfer phenomena among phases with biodegradation
- 3 Model predictions were validated with previous data reported by the authors
- 4 A correct fit and correlation coefficients were observed

Kinetic modelling of a diesel-polluted clayey soil bioremediation process

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Abstract

A mathematical model is proposed to describe a diesel-polluted clayey soil bioremediation process. The reaction system under study was considered a completely mixed closed batch reactor, which initially contacted a soil matrix polluted with diesel hydrocarbons, an aqueous liquid-specific culture medium and a microbial inoculation. The model coupled the mass transfer phenomena and the distribution of hydrocarbons among four phases (solid, S; water, A; non-aqueous liquid, NAPL; and air, V) with Monod kinetics. In the first step, the model simulating abiotic conditions was used to estimate only the mass transfer coefficients. In the second step, the model including both mass transfer and biodegradation phenomena was used to estimate the biological kinetic and stoichiometric parameters. In both situations, the model predictions were validated with experimental data that corresponded to previous research by the same authors. A correct fit between the model predictions and the experimental data was observed because the modelling curves captured the major trends for the diesel distribution in each phase. The model parameters were compared to different previously reported values found in the literature. Pearson correlation coefficients were used to show the reproducibility level of the model.

Keywords: bioremediation; soil; hydrocarbon; kinetic; modelling

Nomenclature

A	aqueous phase
C_A	dissolved diesel concentration in the aqueous phase ($\text{mg}_{\text{TPH}} \text{L}^{-1}$)
C_{Ae}^*	equilibrium concentration of dissolved diesel in the aqueous phase ($\text{mg}_{\text{TPH}} \text{L}^{-1}$)
C_{AI}	residual diesel concentration contained in the aqueous phase ($\text{mg}_{\text{TPH}} \text{L}^{-1}$)
C_{NAPL}	free diesel concentration as pure organic phase per Kg of dry soil mass ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$)
C_{NAPLI}	residual diesel concentration contained in the NAPL phase ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$)
C_s	diesel concentration adsorbed to the soil ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$)
C_{Sd}	saturation concentration of diesel adsorbed onto the solid phase ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$)
C_{SI}	residual diesel concentration contained in the solid phase ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$)
C_T	total diesel concentration in the system per Kg of dry soil mass ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$)
C_V	volatilized diesel concentration in the gas phase referred to dry soil mass ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$)
C_{Ve}^*	equilibrium concentration of volatilized diesel into the gas phase referred to dry soil mass ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$).
f_A	constant mass ratio between water and dry soil amounts in the system ($\text{L Kg}_{\text{soil}}^{-1}$)
J_N	irreversible flotation flow of part of the diesel present in soil ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1} \text{h}^{-1}$)
K_d	kinetic constant of cell death (h^{-1})
$K_{\text{NAPL-A}}$	mass transfer coefficient between the NAPL phase and the aqueous phase (h^{-1})
$K_{\text{NAPL-V}}$	mass transfer coefficient between NAPL phase and gas phase (h^{-1})
K_S	half-saturation coefficient ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$)
$K_{\text{S-A}}$	mass transfer coefficient between solid phase and aqueous phase (h^{-1})
NAPL	non-aqueous phase liquid
S	solid phase
t	time (h)
TPH	total petroleum hydrocarbons
V	gas phase
X	total biomass concentration able to degrade diesel ($\text{mg}_{\text{cells}} \text{Kg}_{\text{soil}}^{-1}$)
X_0	biostimulation
X_0+X_{0e}	combination of biostimulation with autochthonous bioaugmentation
X_0+X_C	combination of biostimulation with exogenous bioaugmentation
X_C	exogenous bioaugmentation
$Y_{x/s}$	biomass growth yield coefficient ($\text{mg}_{\text{cells}} \text{mg}_{\text{TPH}}^{-1}$)
α	coefficient of the irreversible flotation rate of the non-adsorbed diesel (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})

1. Introduction

Petroleum hydrocarbons are hazardous substances that are released into soils as a consequence of accidental spills in the transport, refining and storage stages. Many alternatives are available for the remediation of hydrocarbon contamination in soils, although bioremediation is one of the most eco-friendly technologies. The efficiency of the bioremediation process for hydrocarbon-polluted soils can be increased using the following methodologies: (1) biostimulation, which involves improving the operating conditions, such as nutrient concentrations, pH level and moisture content, that affect the bioremediation process and thereby improving the potential of autochthonous microorganisms and (2) bioaugmentation, which is based on inoculation with special microbiota (Gentry et al., 2004). Several possibilities for bioaugmentation exist: a single strain or a known mixed microbial consortium can be used; an autochthonous microbial consortium previously isolated from the polluted soil and cultivated with hydrocarbons as the carbon source can be used, or an exogenous consortia previously drawn from a different hydrocarbon polluted site can be used (Ueno et al., 2007). In the literature, some research papers compare the role of bioaugmentation to the role of biostimulation related to the efficiency of the bioremediation of hydrocarbon-polluted soils (Agarry et al., 2013; Fan et al., 2014).

The bioaugmentation is a promising and low-cost bioremediation technology, and its effectiveness can be influenced by abiotic factors such as pH, temperature, soil properties, chemical structure, concentration and availability of pollutants and by biotic factors such as the selection of the proper microorganisms, competition with indigenous microorganisms or predation by protozoa (Mrozik and Piotrowska-Seget, 2010). Different researchers have studied bioaugmentation processes and reported hydrocarbon removal efficiencies between approximately 70 and 96% (Gargouri et al., 2014; Ma et al., 2015; Nasser et al., 2010). Furthermore, other researchers have studied the hydrocarbons biodegradation efficiency in

1 soils using a combined biostimulation-bioaugmentation treatments (Agarry and Latinwo, 2015;
2 Fan et al., 2014; Suja et al., 2014).
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5 The authors of the present work previously studied the bioremediation of a diesel-polluted soil
6 with several strategies that combined biostimulation and bioaugmentation through soil-water
7 suspension batch microcosm experiments (Moliterni et al., 2012a). We found that the efficiency
8 of the process strongly depended on the pollutant availability and the soil properties. The
9 pollutant distribution and transport between the different phases in the soil matrix is a very
10 important aspect to be considered. This aspect depends on the physicochemical properties of
11 the soil (Atlas and Bartha, 1998). In soil-water slurry systems, where the hydrocarbon is partially
12 or strongly adsorbed to the soil particles, hydrocarbon accessibility by the microbial population
13 is presumed to be limited. The studies about mass transfer of pollutants between different
14 phases involved in the process must be mentioned: the soil matrix as solid phase (S), water or
15 aqueous (A) and organic (NAPL) as liquid phases, and air in contact with these phases as the gas
16 phase (V) (Hale and Werner, 2010; Mukherji et al., 1998). Likewise, many other reports are based
17 on studies of hydrocarbon desorption (Juhasz et al., 2014; Spasojevic et al., 2015; Wang and
18 Vipulanandan, 2001) and solubility, as well as the use of surfactants (Fan et al., 2014; Lin et al.,
19 2011; Souza et al., 2014). Furthermore, a controversial point is found in the literature concerning
20 the availability of hydrocarbons for microorganisms. Several studies indicate that the dissolution
21 rate from the NAPL phase determines the biodegradation rate (Alshafie and Ghoshal, 2003;
22 Ghoshal and Luthy, 1998), because microorganisms are not able to directly consume
23 hydrocarbons from this phase (Mukherji et al., 1998). However, other studies have proposed
24 that microorganisms can directly access hydrocarbons in the organic-water interphase
25 (Nakahara et al., 1977; Rosenberg et al., 1989), which led us to understand the high
26 biodegradation rate of many hydrocarbons in the NAPL phase.
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Modelling of the bioremediation process is a very important tool for reactor design and efficiency prediction. Several mathematical models have been developed and published in the literature to describe the hydrocarbon bioremediation process. Some models are based on the premise that microorganisms can use only the hydrocarbons from the dissolved aqueous phase (Johnson et al., 2013; Mulder et al., 2001; Ostendorf et al., 2007; Ramaswami and Luthy, 1997; Song et al., 2014; Wang and Vipulanandan, 2001), although other models suggest that adsorbed compounds are also directly available for microorganisms without the need to desorb them (Mukherji et al., 1998; Park et al., 2001; Woo et al., 2001).

Only few mathematical models that couple mass transfer with Monod or first-order kinetics for hydrocarbon biodegradation are found in the literature (Kosterin and Sofinskaya, 2010; Park et al., 2001; Wang and Vipulanandan, 2001; Woo et al., 2001;), and those models were validated for just one or two bioremediation methodologies; there is no concern about mass transfer of hydrocarbon between NAPL and the gas phases in those models.

Thus, in this context, the objective of this study is to propose a mathematical model that describes a diesel-polluted clayey soil bioremediation process. The model couples mass transfer and distribution of diesel hydrocarbons between four phases (S, A, NAPL and V), with Monod kinetics for the biodegradation process. The model predictions have been validated through the comparison with experimental data that had already been reported in the current literature by the same authors (Moliterni et al., 2012a).

2. Materials and methods

2.1. Experimental data

Experimental data are used in the present work to validate the proposed model. These data correspond to previous research by the same authors, whose objective was to study different biostimulation-bioaugmentation options to remove diesel from clayey and silty polluted soils

1 through batch soil-water slurry experiments. The complete description of the experimental
2 procedure was reported by Moliterni et al. (2012a), and only some important details are
3 described here: The clayey soil used in this study was obtained from an agricultural area in
4 Ciudad Real, Spain, and it contained approximately 10^8 and 10^6 MPN g^{-1} of total heterotrophic
5 bacteria (THB) and total diesel-degrading bacteria (DDB), respectively. The soil was artificially
6 contaminated with commercial diesel from a petrol station to produce a final concentration
7 corresponding to 17,000 mg kg^{-1} soil. The autochthonous DDB microbial consortium (named as
8 X_0) found in the soil was isolated and enriched with diesel fuel for 4 weeks, obtaining an enriched
9 consortium named as X_{0e} . In addition, a previously developed microbial consortium isolated
10 from a hydrocarbon-polluted site at an oil refinery in central Spain and enriched in diesel for 4
11 months was used as the exogenous consortium (X_C) (Moliterni et al., 2012b). Five slurry-phase
12 batch experiments in closed reactors were conducted with clayey soil to evaluate the efficiency
13 of hydrocarbon biodegradation. The experiments were planned to compare the biostimulation
14 and bioaugmentation options, with either the autochthonous consortium, X_0 , or the exogenous
15 consortium, X_C . The experimental conditions included a mixing rate of 130 rpm, a temperature
16 of 25°C and an experimental period of 11 days. To monitor abiotic losses, abiotic control
17 experiments were performed with soils that were autoclaved to inhibit the activity of
18 autochthonous soil microorganisms. The total petroleum hydrocarbon (TPH) concentration in
19 the samples at different retention times was measured by gas chromatography (ISO method
20 9377-2, 2000; EN method 14039, 2005). The biomass concentration was measured by the most
21 probable number (MPN) method (Youssef et al., 2010). The MPN results were converted to dry
22 cell weights (ASTM E method 1755-01, 1991) using a prepared calibration curve.

2.2. System description and mathematical model assumptions

23 The reaction system under study can be considered a completely mixed batch reactor, which
24 initially contacts a soil matrix polluted with diesel hydrocarbons (organic substrate) and an

1 aqueous liquid-specific culture medium (water with inorganic nutrients). Once the solid and
2 water phases have been mixed, a portion of the diesel fuel is transferred from the soil (S) to
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4 water (A) and air (V), in addition to as a pure hydrocarbon phase (NAPL), and the system
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6 progresses until an equilibrium in the diesel fuel distribution is reached. The reaction starts with
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8 the addition and activation of an inoculum from a microbial consortium, which is
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10 homogeneously distributed in the medium to reach the initial concentration. Once the reaction
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12 has been initiated, the microbial activity consumes hydrocarbons and modifies the equilibrium
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14 distribution, and the substrate concentration changes over time due to the transport
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16 phenomena between phases and biodegradation. This biodegradation leads to biomass growth.
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22 The following assumptions have been considered to develop the mathematical model:
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- 25 - The system is assumed to be closed, and the total mass is maintained constant.
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- 28 - The diesel hydrocarbons are assumed to be present in the system in four phases: dissolved in
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30 aqueous phase (A), adsorbed in the soil (S), free in the organic liquid phase (NAPL) or volatilized
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32 in the gas phase (V).
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- 36 - After the inoculation, microorganisms are homogeneously distributed all over the aqueous
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38 phase and also have access to hydrocarbons in the following 3 phases: A, S or NAPL.
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40 Biodegradation in the S and NAPL phases is assumed to be carried out through A-S and A-NAPL
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42 interphases (Guo et al., 1999; Park et al., 2001; Woo et al., 2001) and because of biosurfactant
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44 generation (Souza et al., 2014; Suja et al., 2014).
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- 49 - The microbial consortium responsible for hydrocarbon biodegradation follow growth kinetics
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51 according to the Monod equation. For that reason, the system imposes the requirement that
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53 the growth rate is null only when the diesel concentration is zero. The existence of a residual
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55 substrate concentration in each phase unavailable for growth, even using long incubation time,
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57 is considered (Nocentini et al., 2000). This residual concentration can be a non-biodegradable
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1 fraction or a non-accessible fraction. Thus, the biological reaction is paralysed when the residual
2 concentration is reached.

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5 - During the experiments, hydrocarbon transport phenomena exist among different phases.
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7 Because of the practical point of view, the mass transport is considered to be carried out only
8 among some phases due to the system characteristics and the difference between water and
9 NAPL densities (Figure 1). Under the mixing rate applied, the NAPL phase floats and remains
10 above the A phase, and the transport processes between S-V and A-V phases are assumed to be
11 negligible, and these equilibria have therefore not been included in this model approach. The
12 interaction in the S-NAPL phase is not considered as equilibrium, and it is just considered that
13 the excess of diesel oil is separated from soil and moves up by flotation due to its lower density
14 in comparison with water.
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31 *2.3. Model formulation*

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34 A global mass balance of organic substrate (diesel hydrocarbons) is applied in the system. The
35 time variation of the total substrate concentration is the result of the variation in all phases due
36 to transport phenomena and biodegradation. This overall mass balance is shown in Equation [1].
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$$42 \frac{dC_T}{dt} = \frac{dC_S}{dt} + f_A \cdot \frac{dC_A}{dt} + \frac{dC_{NAPL}}{dt} + \frac{dC_V}{dt} \quad [1]$$

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46 where C_T is the total diesel concentration in the system per Kg of dry soil mass ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$), C_S
47 is the diesel concentration adsorbed in the soil ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$), f_A is the constant mass ratio
48 between water and dry soil amounts in the system ($\text{L Kg}_{\text{soil}}^{-1}$), C_A is the dissolved diesel
49 concentration in the aqueous phase ($\text{mg}_{\text{TPH}} \text{L}^{-1}$), C_{NAPL} is the free diesel concentration as pure
50 organic phase per Kg of dry soil mass ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$), C_V is the volatilized diesel concentration in
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the gas phase per Kg of dry soil mass ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$), and t is time (h). If no biodegradation exists, C_T is constant, and equation [1] is equal to zero.

Next, partial diesel mass balances are proposed in each phase of the soil-slurry system: solid, aqueous liquid, gas and NAPL liquid phases. Equation [2] describes the diesel balance in the solid phase. The first two terms on the right side are related to the diesel transport phenomena, whereas the last term corresponds to the diesel biological consumption according to Monod kinetics:

$$\frac{dC_S}{dt} = J_n - f_A \cdot K_{S-A} \cdot (C_{Ae}^* - C_A) - \frac{\mu_{\max} \cdot (C_S - C_{SI})}{K_S + C_S - C_{SI}} \cdot X \cdot \frac{1}{Y_{x/s}} \quad [2]$$

where K_{S-A} is the mass transfer coefficient between the solid phase and the aqueous phase (h^{-1}), C_{Ae}^* is the equilibrium concentration of dissolved diesel in the aqueous phase ($\text{mg}_{\text{TPH}} \text{L}^{-1}$), μ_{\max} is the maximum specific growth rate (h^{-1}), K_S is the half-saturation coefficient ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$), X is the total biomass concentration that is able to degrade diesel in the system ($\text{mg}_{\text{cells}} \text{Kg}_{\text{soil}}^{-1}$), $Y_{x/s}$ is the biomass growth yield coefficient ($\text{mg}_{\text{cells}} \text{mg}_{\text{TPH}}^{-1}$), C_{SI} is the residual diesel concentration contained in the solid phase ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$), and finally, J_N is the irreversible flotation flow of part of the diesel present in soil ($\text{mg}_{\text{TPH}} \text{kg}_{\text{soil}}^{-1} \text{h}^{-1}$). This fraction of diesel is desorbed from the soil when the soil is suspended with water, and diesel rises to the top of the soil-slurry system due to its lower density. The J_N value has been calculated through equation [3], where α is a coefficient of the irreversible migration rate of the non-adsorbed diesel (h^{-1}), and C_{Sd} is the saturation concentration of diesel adsorbed onto the solid phase ($\text{mg}_{\text{TPH}} \text{Kg}_{\text{soil}}^{-1}$).

$$J_n = \alpha \cdot (C_{Sd} - C_S) \quad [3]$$

Equation [4] describes the diesel balance in the liquid aqueous phase. As in equation [2], the first two terms on the right side are related to the diesel transport phenomena, whereas the last term corresponds to the diesel biological consumption according to Monod kinetics:

$$\frac{dC_A}{dt} = K_{S-A} \cdot (C_{Ae}^* - C_A) + K_{NAPL-A} \cdot (C_{Ae}^* - C_A) - \frac{\mu_{max} \cdot (C_A - C_{AI})}{K_S / f_A + C_A - C_{AI}} \cdot X \cdot \frac{1}{Y_{x/s}} \quad [4]$$

where K_{NAPL-A} is the mass transfer coefficient between the NAPL phase and the aqueous phase (h^{-1}), and C_{AI} is the residual diesel concentration that is contained in the aqueous phase ($mg_{TPH} L^{-1}$).

In the case of the NAPL phase, the partial diesel balance is described in Equation [5], where C_{NAPLI} is the residual diesel concentration contained in the NAPL phase ($mg_{TPH} Kg_{soil}^{-1}$).

$$\frac{dC_{NAPL}}{dt} = -J_N - K_{NAPL-V} \cdot (C_{Ve}^* - C_V) - f_A \cdot K_{NAPL-A} \cdot (C_{Ae}^* - C_A) - \frac{\mu_{max} \cdot (C_{NAPL} - C_{NAPLI})}{K_S + C_{NAPL} - C_{NAPLI}} \cdot X \cdot \frac{1}{Y_{x/s}} \quad [5]$$

where K_{NAPL-V} is the mass transfer coefficient between the NAPL phase and gas phase (h^{-1}), and C_{Ve}^* is the equilibrium concentration of volatilized diesel onto the gas phase referred to the dry soil mass ($mg_{TPH} Kg_{soil}^{-1}$).

In the case of the gas phase, biological reaction does not occur because microorganisms do not exist, then the partial diesel balance is then defined in Equation [6]:

$$\frac{dC_V}{dt} = K_{NAPL-V} \cdot (C_{Ve}^* - C_V) \quad [6]$$

Finally, the global balance of biomass generated in the system is described in Equation [7]. This balance describes the biomass growth due to the diesel consumption in each phase (solid, aqueous and NAPL) and the subtraction of the possible biomass decrease due to cell death processes:

$$\frac{dX}{dt} = \frac{\mu_{max} \cdot (C_A - C_{AI})}{K_S / f_A + C_A - C_{AI}} \cdot X + \frac{\mu_{max} \cdot (C_S - C_{SI})}{K_S + C_S - C_{SI}} \cdot X + \frac{\mu_{max} \cdot (C_{NAPL} - C_{NAPLI})}{K_S + C_{NAPL} - C_{NAPLI}} \cdot X - K_d \cdot X \quad [7]$$

where K_d is the kinetic constant of cell death (h^{-1}).

The overall model considers a unique heterotrophic biomass concentration (X) in the soil-slurry system that is able to degrade diesel in whatever phase diesel may be accessible for microorganisms: dissolved diesel (aqueous phase), adsorbed diesel (soil-water interphase) or free diesel (NAPL-water interphase). Regardless of where the diesel substrate is located, the model considers constant values for μ_{max} , $Y_{X/S}$ and K_S .

2.4. Parameter estimation

2.4.1. Mass transfer coefficients

The experimental data obtained under abiotic conditions (no inoculum and autoclaved soil) reported by Moliterni et al. (2012a) allowed us in the present work to obtain the mass transfer coefficients. Obviously, the biodegradation rate term in the equations previously described is zero under abiotic conditions. Here, the result is a four-equation system ([2], [4], [5] and [6]) with four adjustable parameters: α , K_{S-A} , K_{NAPL-A} and K_{NAPL-V} . Note that the diesel equilibrium concentrations in the different phases were not obtained by mathematical fitting, but were measured by experimentation in that previous work. This equations system has been fitted to the experimental data obtained under abiotic conditions by using the Gauss-Newton algorithm. An initial value was assigned to the adjustable parameters (α , K_{S-A} , K_{NAPL-A} , K_{NAPL-V}), and after several iterations, the values of parameters that produced the minimum at the objective function $\Psi(p)$ (Equation [8]) were chosen.

$$\Psi(p) = \sum_{i=1}^n \left((C_{A \text{ exp},i} - C_{A_i}(p))^2 + (C_{S \text{ exp},i} - C_{S_i}(p))^2 + (C_{NAPL \text{ exp},i} - C_{NAPL_i}(p))^2 + (C_{V \text{ exp},i} - C_{V_i}(p))^2 \right) \quad [8]$$

where n is the number of experimental data; $C_{A \text{ exp},i}$, $C_{S \text{ exp},i}$, $C_{NAPL \text{ exp},i}$, $C_{V \text{ exp},i}$ are the experimental values of diesel concentration in the aqueous liquid, solid, NAPL and gas phases, respectively, when those values were measured at the same t_i time; $C_{A_i}(p)$, $C_{S_i}(p)$, $C_{NAPL_i}(p)$ and $C_{V_i}(p)$ are the predicted values calculated by the model, which correspond to t_i .

2.4.2. Kinetic parameters

Once mass transfer coefficients have been determined by fitting experimental data under abiotic conditions, the stoichiometric and kinetic parameters for the diesel biodegradation were determined by using equations [2], [4], [5] and [7], which were fitted to the experimental data reported by Moliterni et al. (2012a) when both mass transfer phenomena and biological reactions occurred. In this occasion note that the final residual concentrations (C_{Sl} , C_{NAPL} , C_{Al}) were measured by experimentation in that previous work. The four adjustable parameters (μ_{max} , K_s , $Y_{X/S}$ and K_d) were estimated again using a nonlinear regression technique, minimizing the objective function $\Omega(p)$ (equation [9]), during the simultaneous resolution of equations [2], [4], [5] and [7] by the Gauss-Newton algorithm.

$$\Omega(p) = \sum_{i=1}^n \left((X_{exp,i} - X_i(p))^2 + (C_{exp,i} - C_i(p))^2 \right) \quad [9]$$

where n is the number of pieces of experimental data; $X_{exp,i}$ and $C_{exp,i}$ are the experimental values of biomass and diesel concentrations, respectively, in the aqueous liquid phase, solid, NAPL and gas phases when those values were measured at the same t_i time; and $X_i(p)$ and $C_i(p)$ are the predicted values calculated by the model, which correspond to t_i .

3. Results and discussion

3.1. Model validation and parameter estimation for abiotic experiments

Figure 2 shows the diesel distribution (expressed as TPH) among the four phases (S, A, NAPL, and V) considered in the system under abiotic conditions (i.e., no biodegradation is present). Points correspond to experimental data, while lines correspond to the model predictions. The clayey soil quickly transferred part of the diesel into the liquid phase (aqueous and NAPL phases); approximately 55% of the diesel was detected in the liquid phase after the first 48 h, although approximately 40% of the diesel was not desorbed from the clayey soil at the end of the

1 experiments. A correct fit between the model predictions and the experimental data can be
2 observed because the modelling curves capture the major trends for the diesel distribution in
3 each phase.
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7 Moreover, Figure 3 represents the TPH concentration calculated as the sum of TPH in the three
8 phases (S, A and NAPL) and the possible biomass growth in the abiotic experiments.
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10 Approximately 8% of the TPH was reported to be volatilized to the V phase (low molecular
11 weight diesel components) after 11 days, which is in agreement with other values found in the
12 literature such as 5% of the naphthalene losses from control reactors for a period of 60 h (Wang
13 and Vipulanandan, 2001). In addition, biomass growth may be considered negligible during the
14 abiotic experiments.
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25 The experimental data obtained under abiotic conditions were used to estimate mass transfer
26 coefficients according to the steps previously described, and those coefficients are summarized
27 in Table 1. Likewise, these experimental data have been used to validate the model with regard
28 to the diesel distribution among phases, as previously shown in Figure 2.
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35 In the literature, mass transfer models can be found for the description of the transport of
36 organic pollutants: between river sediment to polyethylene passive samplers in the presence
37 and absence of activated carbon (Hale and Werner, 2010); in flowing groundwater between
38 mobile groundwater and stationary biofilms and diffusion within the biofilms (Mendoza-Sanchez
39 and Cunningham, 2012); or between the water column and the sediment layer in a river (Wang
40 et al., 2012). Likewise, Park et al. (2001) investigated the bioavailability of sorbed naphthalene
41 in soil-slurry systems, and these authors obtained desorption rate coefficients from
42 nonequilibrium sites of soils that ranged from 0.23 to 14.0 h⁻¹, which agree with α value of 0.98
43 h⁻¹ estimated for the desorption flux (J_n) by the present mathematical model. Similarly, Woo et
44 al. (2001) studied the mass transfer coefficients for phenanthrene from the abiotic experimental
45 data in soil-slurry systems for a roller-bottle test at 2 rpm, and these authors established that
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1 the overall mass transfer coefficient for phenanthrene sorbed in soil was 0.014 h^{-1} in a standard
2 case of soil content. This last overall mass transfer coefficient value of 0.014 h^{-1} is quite similar
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4 to the K_{S-A} value of 0.03 h^{-1} estimated by the present mathematical model. However, no
5
6 information is available on mass transfer coefficients among the 4 phases (solid, aqueous, NAPL,
7
8 and gas) involved in a remediation technique for soil contaminated by TPH.
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10 11 12 *3.2. Model validation and parameter estimation for bioremediation experiments*

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16 Figure 4 shows the experimental data (points) of different bioremediation experiments and the
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18 model predictions (lines) for treatment of clayey soils artificially polluted with diesel
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20 hydrocarbons. In Figure 4, four pairs of figures (a, b, c, d) are plotted to represent the different
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22 bioremediation strategies used: (a) biostimulation, i.e., using the autochthonous microbial
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24 consortium, X_0 ; (b) exogenous bioaugmentation, using sterile soil and the addition of an
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26 acclimated external consortium, X_C ; (c) a combination of biostimulation with exogenous
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28 bioaugmentation, X_0+X_C ; and (d) a combination of biostimulation with bioaugmentation using
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30 the same autochthonous consortium previously enriched with diesel fuel for 4 weeks, X_0+X_{0e} .
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32 More details about the different strategies were reported by Moliterni et al. (2012a). On the
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34 left, the figures show the time courses of total TPH concentration (C_T) and the biomass evolution,
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36 whereas on the right, the figures show the diesel concentration through time in the phases of
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38 the soil-slurry system (C_A, C_{NAPL}, C_S).
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46 More than 60% of the diesel was observed to be biodegraded in all experiments in the first 48
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48 h, and, regardless of bioremediation strategy used, more than 90% of the initial TPH was
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50 biodegraded after 11 days. The diesel was desorbed from the soil in the first experimental hours,
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52 and consequently, the diesel concentration increased in the NAPL phase. Subsequently, a
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54 decrease in the diesel concentration was observed in the NAPL phase between 30 and 70 h due
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56 to diesel biodegradation. In the NAPL phase, the first increase and later decrease in the diesel
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58 concentration were more or less significant depending on the bioremediation strategy used, and
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1 this scheme would support one of the model assumptions, because it has been supposed that
2 microorganisms could directly access the non-aqueous liquid phase in each bioremediation
3 strategy. This behaviour of diesel in the NAPL phase is the same as in the aqueous phase,
4 although a higher diesel concentration in the aqueous phase exists at the first experimental
5 time. The diesel concentration also decreased in the aqueous phase over time due to the
6 biodegradation process, and this decrease was more significant for a combination of
7 biostimulation and bioaugmentation strategies. The final amount of biomass growth was also
8 reported to be very similar in all bioaugmentation strategies, although the lag time of adaptation
9 for the polluted microcosms in each case is different. Likewise, biomass concentration increased
10 over time, assuming that the consortia are well adapted to the microcosms, and a higher
11 biomass concentration produces an increase in the diesel biodegradation rate. In general, the
12 combined biostimulation/bioaugmentation strategies (X_0+X_c and X_0+X_{0e}) lead the maximum
13 diesel biodegradation efficiencies and rates compared to simple strategies (X_0 and X_c) (Moliterni
14 et al., 2012a).

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34 Once the experimental data were briefly explained, the results shown in Figure 4 were fitted to
35 equations [2], [4], [5] and [7] to predict the behaviour of the bioremediation process in clayey
36 soil-water suspensions. For fitting the model to the experimental data, it is necessary to estimate
37 the model parameters μ_{max} , K_S and $Y_{X/S}$. The final values of these estimated parameters are
38 shown in Table 2. On one hand, the highest values of the maximum specific growth rate (μ_{max})
39 were obtained in the experiments carried out by the combination of biostimulation and
40 bioaugmentation strategies (X_0+X_c and X_0+X_{0e}), whereas the lowest values were observed for
41 simple exogenous bioaugmentation (X_c). Thus, the experiments with the combination of
42 biostimulation and bioaugmentation strategies presented a maximum growth rate of
43 approximately 0.01 h^{-1} , which is one order of magnitude higher than in the experiments with
44 simple exogenous bioaugmentation. On the other hand, the values obtained for the half
45 saturation coefficient (K_S) were in the range of 4162 to 9300 mg Kg_{soil}^{-1} , presenting the same

1 order of magnitude whatever the strategy used. Finally, the values of the biomass yield
2 coefficient ($Y_{x/s}$) were observed to be slightly higher for the combination of biostimulation and
3 bioaugmentation strategies.
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7 Kinetic parameters (μ_{\max} , K_s , $Y_{x/s}$) calculated in the present work are within the same order of
8 magnitude as the values found in the literature. Thus, in the kinetic study of a diesel-degrading
9 bacterium that was isolated from a diesel-contaminated site in Malaysia (Dahalan et al., 2014),
10 the μ_{\max} rate had a value of 0.039 h^{-1} , slightly higher compared to the μ_{\max} rate value of 0.0146
11 h^{-1} estimated by the present mathematical model for the combination of biostimulation with
12 enriched autochthonous bioaugmentation (X_0+X_{0e}). In general, the μ_{\max} rate values shown in
13 Table 2 are also within the range found in the literature that varied between 0.036 h^{-1} for
14 phenanthrene biodegradation and 0.468 h^{-1} for naphthalene biodegradation in soil (Mulder et
15 al., 2001). Otherwise, $Y_{x/s}$ values in Table 2 are lower than those $Y_{x/s}$ values reported in the
16 literature for microorganisms that can use the polycyclic aromatic hydrocarbons as source of
17 energy, which range from 0.25 to 1.2 kg kg^{-1} (Mulder et al., 2001; Song et al., 2014).
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34 In the literature, the bioremediation process of hydrocarbon-polluted soils has been separately
35 modelled by mass transfer or kinetic models. Thus, some researchers have developed mass
36 transfer models to describe, for instance, the mass transfer of hydrocarbons from the non-
37 aqueous phase liquid to the aqueous phase (Mukherji et al., 1998) or from river sediment to
38 activated carbon (Hale and Werner, 2010). Likewise, several kinetic models have been reported
39 because the kinetics in the soil bioremediation processes are of great importance in
40 understanding the hydrocarbon degradation rate and the efficiency. According to the literature
41 (Yadav and Hassanizadeh, 2011), different simplified kinetic models could be used such as zero-
42 order kinetic, first-order or linear kinetic, the hyperbolic equation proposed by the Monod
43 (Monod, 1949), logarithmic model or logistic model. The zero-order kinetic, logarithmic model
44 and logistic model are not generally use to describe hydrocarbon biodegradation in soils, but
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1 the first-order kinetic model has been widely used due to the easier data analysis and simplicity
2 of graphic presentation. Then, the first-order kinetic model has been used to fit several sets of
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4 current experimental data such as the i) biodegradation of a soil polluted with used engine oil
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6 (Abioye et al., 2010); ii) degradation of kerosene in soils by bioattenuation, biostimulation,
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8 bioaugmentation and combined biostimulation and bioaugmentation (Agarry et al., 2010); iii)
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10 biodegradation of diesel fuel in soil enhanced by the addition of tea leaf, soy cake and potato
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12 skin (Dadrasnja and Agamuthu, 2013); iv) bioremediation of a chronically oily sludge-polluted
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14 soil by natural attenuation, biostimulation and bioaugmentation (Soliman et al., 2014); v)
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16 biodegradation of phenanthrene and pyrene in soil-water systems (Yu et al., 2014); and vi)
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18 biodegradation of diesel oil in soils enhanced by bioventing as a biostimulation agent and
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20 amendment with organic waste effluents that could serve as a bioaugmentation agent (Agarry
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22 and Latinwo, 2015). At this point, the mentioned experimental data fitted well to the first-order
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24 kinetic model, with correlation coefficients higher than 0.90. However, the most popular kinetics
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26 for characterizing hydrocarbon biodegradation is the Monod kinetics because it can describe
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28 degradation rates ranging from zero- to first-order kinetics with respect to the target pollutant
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30 concentration; thus, it seems to be the most rigorous of the simplified kinetic models
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32 mentioned. Hence, some researchers have used the Monod kinetic model to estimate both
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34 microbial growth and biodegradation of hydrocarbon pollutants in soils. Chen et al. (2008)
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36 considered the Monod model to fit biodegradation kinetics of phenanthrene in contaminated
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38 sediment slurry and Dahalan et al. (2014) studied the growth of a diesel-degrading bacterium
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40 isolated from a diesel-contaminated soil in Malaysia by using the Monod model. Nevertheless,
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42 these researchers did not obtain high correlation coefficients (r^2) and for that reason, other
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44 researchers combined Monod and logistic kinetics to fit experimental data in the study of the
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46 natural attenuation of hydrocarbon contaminants as observed in a field lysimeter experiment,
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48 and they achieved an r^2 value of 0.91 (Song et al., 2010).
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Furthermore, it is also possible to find models that couple mass transfer phenomena with kinetics to study the bioremediation process of hydrocarbon polluted groundwater in the literature. Thus, some researchers have developed models that couple the transport of hydrocarbons with Monod kinetics for hydrocarbon biodegradation in groundwater (Mendoza-Sanchez and Cunningham, 2012; Song et al., 2014). Song et al. (2014) developed a mathematical model to simulate groundwater flow, naphthalene transport and biokinetic parameters that provides a general description of the experimental trends, although there are some discrepancies between the observed and simulated data. These discrepancies are that naphthalene pulses broke through earlier and the experimental naphthalene spread out wider in the experiment than predicted by the model and that the peak naphthalene breakthrough concentration data from experiments are almost all greater than that simulated by the model.

However, in the literature, there are few mathematical models that couple mass transfer phenomena with kinetics for hydrocarbon biodegradation in soils. At this point, Park et al. (2001) developed a mathematical model to fit experimental data from the degradation of naphthalene by using an exogenous bioaugmentation strategy in sandy soils from forest environments in Michigan. This model assumed that soils have equilibrium, nonequilibrium and non-desorption sites that can be accessed by biomass, and the model fitted both liquid and sorbed data reasonably with $r^2 > 0.988$ for Monod kinetics and $r^2 > 0.990$ for first-order kinetics used. Another mathematical model was found in the literature (Wang and Vipulanandan, 2001) to describe the degradation of 25,000 mg/kg of naphthalene using an exogenous bioaugmentation strategy in soil samples prepared by mixing various amounts of sand, clay and organic matter. Here, the model assumed that biodegradation occurs only in the aqueous phase following first-order kinetics, and then, researchers observed that their predictions were in agreement with experimental data although correlation coefficients were not evaluated. Likewise, Woo et al. (2001) proposed a mathematical model to describe the degradation of phenanthrene in soils by the same bioremediation strategy mentioned above. This model with sorbed-phase

1 biodegradation and the same Monod kinetic parameters, but unique mass transfer coefficients,
2 provided good predictions for both fast and slow mass transfer regimes within 27.7% of relative
3 error. Kosterin and Sofinskaya (2010) developed a mathematical model for the 1 wt% *n*-
4 tridecane degradation by using biostimulation strategy in an oil-polluted soil. This model
5 includes equations for the diffusion transfer of hydrocarbons and microorganisms into the water
6 phase and the Monod equation for the biodegradation process, and the model was expanded
7 by the hypothesis that the hydrocarbon-oxidizing microorganisms, in the course of their life
8 activity, excrete products whose specific concentrations inhibit the utilization of the
9 hydrocarbon. Thus, this model fitted experimental data with deviations no higher than 5%.

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22 In this context, to the best of our knowledge, the mathematical model proposed by the authors
23 is the first model that couples the mass transfer of TPH into four phases (A, S, NAPL and V) with
24 the Monod equation for TPH biodegradation and that studies four different bioremediation
25 strategies in soil-slurry systems. Thus, Table 3 displays Pearson correlation coefficients to
26 validate the model. The Pearson correlation coefficient test is a statistical analysis to determine
27 the fit between experimental and modelled data for every phase studied in the system. The
28 Pearson correlation coefficients shown in Table 3 indicate that the linear relationship between
29 the two sets of data has a high positive correlation as the values of Pearson are above 0.95 in
30 most bioremediation strategies, which is indicative of the correct reproducibility of the model
31 and this finding would validate the assumptions on which the model is based.

32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 **4. Conclusions**

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50 A correct fit between the model predictions and the experimental data was observed because
51 the modelling curves captured the major trends for the diesel distribution in each phase, and
52 high Pearson correlation coefficient values were obtained. This finding would validate the
53 assumptions on which the model is based. When available, the mass transfer coefficients and
54 the kinetic parameters were compared to other values reported in the literature. However, to

1 the best of our knowledge, no similar models were found that couple the diesel hydrocarbon
2 mass transfer among four phases and Monod kinetics to describe the bioremediation process.
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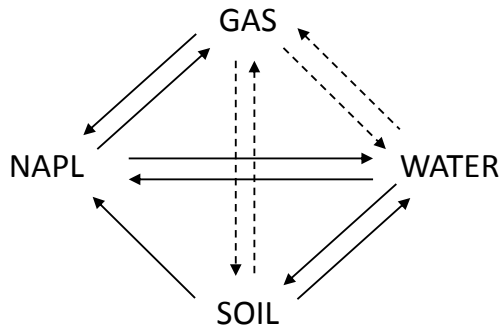
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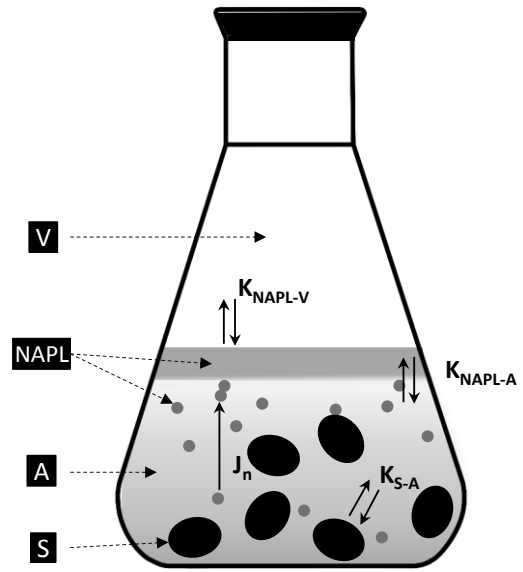
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(a)



(b)

Figure 1. (a) Possible transport phenomena among phases (solid lines indicate mass transfer phenomena considered by the model and dotted lines indicate negligible mass transfer considered by the model). (b) Scheme of transport phenomena involved in the soil-slurry system.

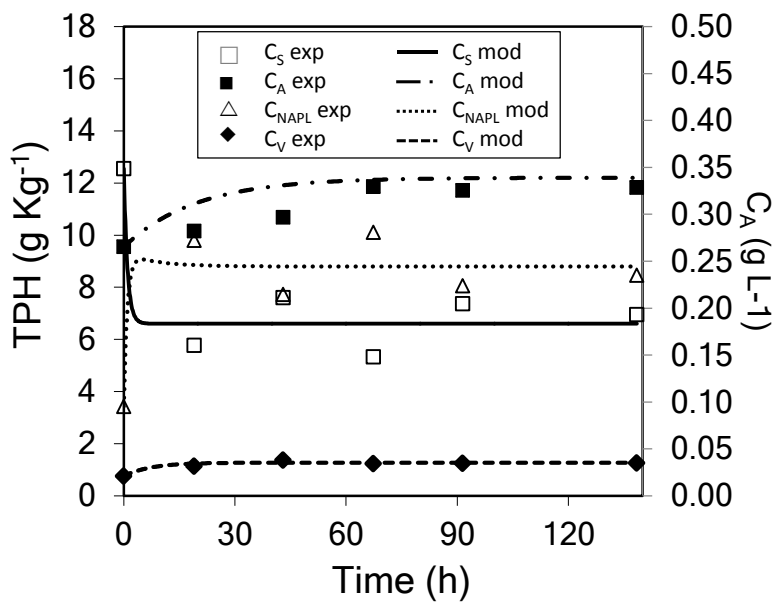


Figure 2. Diesel distribution in the abiotic experiments in soil-slurry systems.

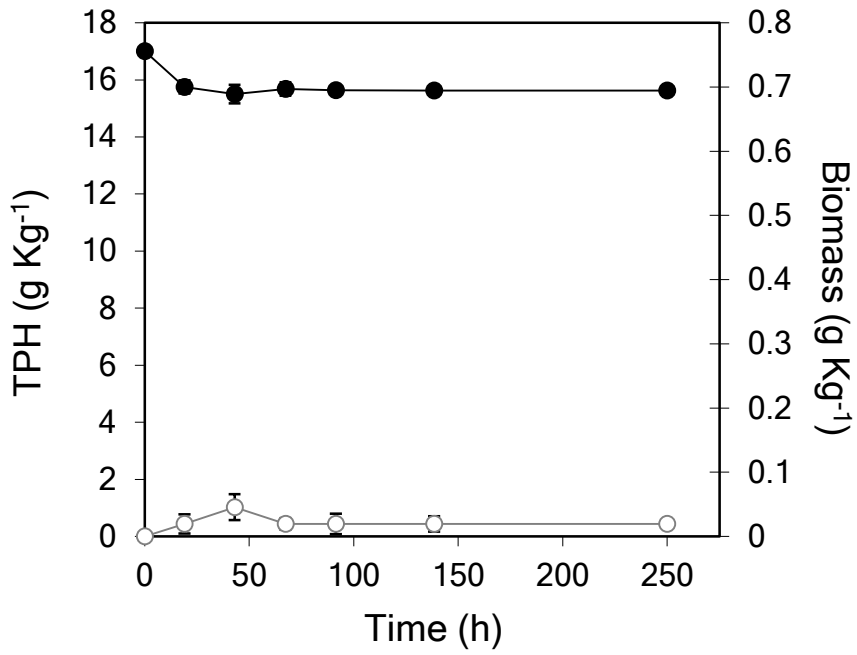
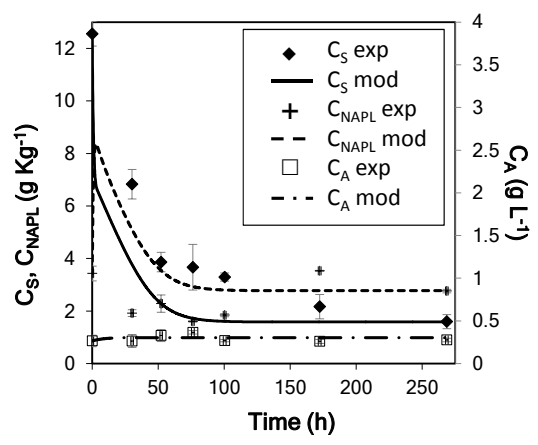
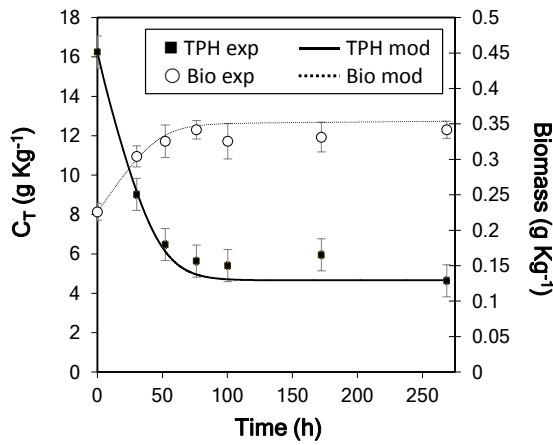
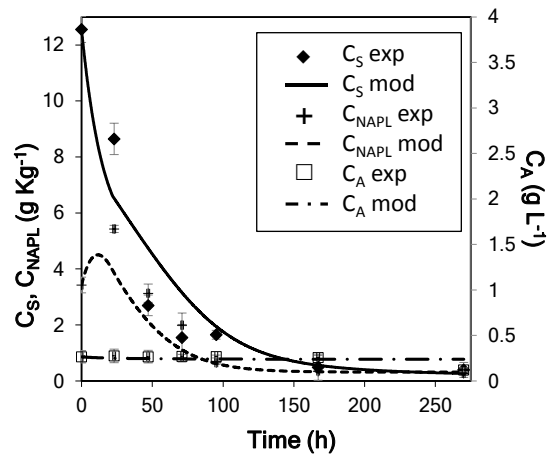
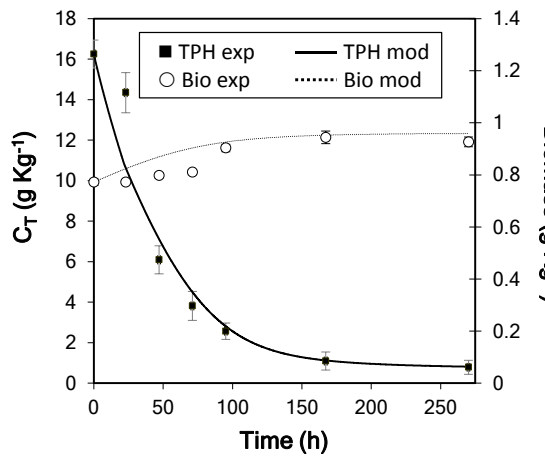


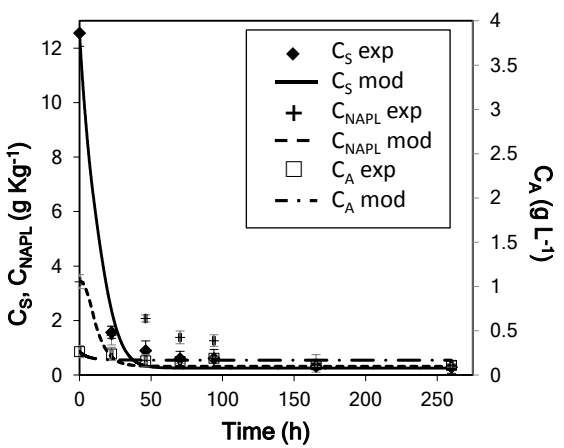
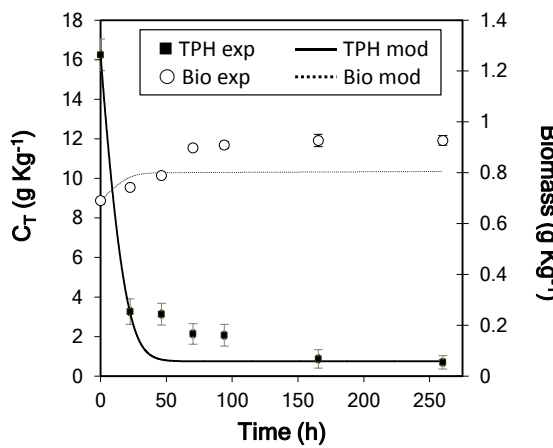
Figure 3. TPH and biomass concentrations in the abiotic experiments. (● TPH and ○ biomass). TPH values include S, A and NAPL phases. Error bars represent the standard deviation of the mean, and lines represent tendencies.



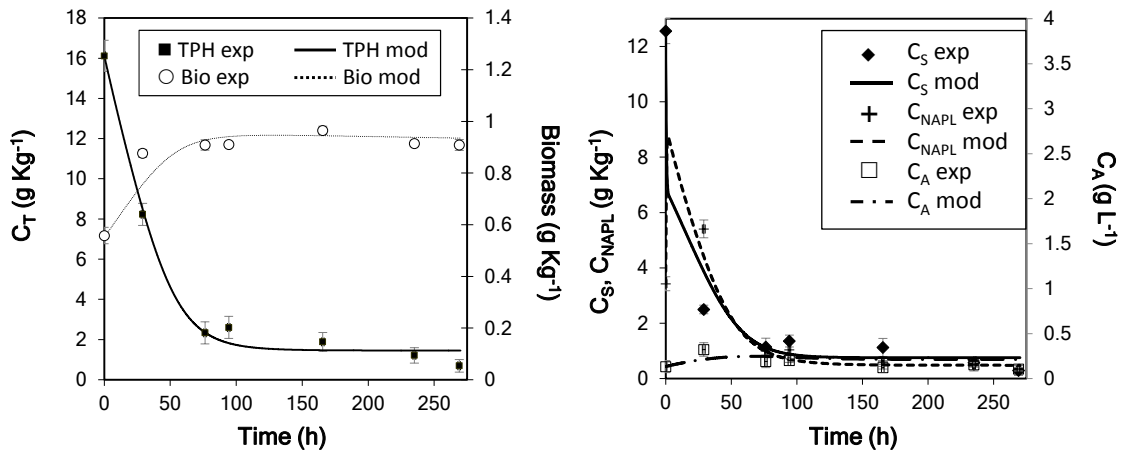
(a)



(b)



(c)



(d)

Figure 4. TPH and biomass concentrations in experiments with clayey soil. Experimental data (points) and model predictions (lines). Four strategies used: (a) biostimulation; (b) bioaugmentation; (c) combined biostimulation and exogenous bioaugmentation; and (d) combined biostimulation and autochthonous bioaugmentation.

Table 1. Estimated parameters for the abiotic experiments.

K_{S-A} (h^{-1})	K_{Napl-A} (h^{-1})	K_{Napl-V} (h^{-1})	α (h^{-1})
0.03	0.02	0.14	0.98

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Table 2. Estimated parameters for the bioremediation model.

Strategy	μ_{max} (h ⁻¹)	K_S (mg Kg _{soil} ⁻¹)	$Y_{X/S}$ (mg _{cell} mg _{TPH} ⁻¹)
X ₀	0.0096	4162.4	0.011
X _c	0.0031	5898.5	0.010
X ₀ +X _c	0.0114	5678.0	0.013
X ₀ +X _{0e}	0.0146	9300.0	0.031

Table 3. Pearson correlation coefficients for the bioremediation model.

Strategy	Pearson Coefficient
X_0	0.9234
X_C	0.9589
X_0+X_C	0.9688
X_0+X_{0e}	0.9665