Modeling the bioremediation of a diesel-contaminated soil using an enriched hydrocarbon-degrading inoculant

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Abstract

A model was developed to simulate the bioremediation of a diesel-contaminated soil slurry in a mixed closed batch system, using an enriched diesel-degrading bacterial inoculant. Mass transfer processes of desorption of diesel from soil to water and volatilization from water, and biodegradation by bacterial inoculant were included in the model, using complex Weibull sigmoid, first-order, and logistic/Monod kinetics, respectively. Long-term model predictions indicated that, in the given conditions of biomass concentration, soil characteristics and diesel concentration, the contaminants could be practically eliminated from the system in less than 70 days. Sensitivity analysis of the model revealed the importance of maintaining a high biomass concentration in the system to improve bioremediation efficiency. Model was short-term validated with a biodegradation experiment for 360 h, during which near the 30% of diesel present in the system was eliminated. The developed model could be a useful tool to predict bioremediation efficiencies of different soil scenarios, by adapting the needed parameters to each system.

Keywords: bioremediation; diesel-contaminated soil; sigmoid desorption kinetics; Monod biodegradation kinetics

Introduction

Conventional soil remediation methods are based on removal or containment of hydrocarbons, but they often present high costs and environmental risks, due to soil excavation and removal, application of chemicals, such as solvents or surfactants, application of hot water or air at high pressure, *etc.* These disadvantages encouraged researchers to develop "environmentally-friendly" remediation technologies, cheaper and with less impact on the soil environment. Bioremediation is a clean-up strategy with a lower impact on the soil environment (Pilon-Smits, 2005), as it uses soils organisms (including, plants, bacteria, or fungi) to degrade the hydrocarbons present in soil.

Modeling of bioremediation processes can be a very useful tool for the design of remediation experiments, and may be used to determine the requirements of microbial biomass inputs, the time to achieve remediation objectives, or the influence of soil, and other environmental conditions on remediation efficiency. This efficiency is largely evaluated based on the time necessary to achieve an acceptable contaminant concentration in soil, for which System Dynamics-based models can play an important role. Several bioremediation models have already been described (Borsi and Fasano, 2009; Fernández *et al.*, 2016). These models generally assumed simple first-order desorption kinetics, which may not predict accurately complex desorption from soils. This process is usually retarded at first stages, following a sigmoid distribution (Skrdla, 2007), due to sorption forces exerted by soils. Furthermore, biodegradation is in the majority of cases only modeled following Monod function, which may not precisely predict logistic bacterial growth and substrate utilization in slow desorption and bioavailability-limited systems as soil.

In this context, the objective of the present work was to formulate a model based on system dynamics to simulate a bioremediation process of a diesel-contaminated soil in a batch system in the presence of an enriched diesel-degrading inoculum.

Materials and methods

Description of the modeling scenario and model assumptions

The system under study could be considered as a static batch reactor containing a slurry of a diesel-contaminated soil and an aqueous media, and four identified phases: (a) a solid phase (soil), contaminated with diesel; (b) a non-aqueous phase liquid (NAPL), corresponding to diesel adsorbed and/or retained in soil pores, and desorbed (but not dissolved) in water; (c) an aqueous phase; (d) a gaseous phase (head-space of the batch tubes). In addition, a pure culture of a diesel-degrading strain was added to the system. This batch system was completely mixed and several mass-transfer and degradation processes are taking place: (a) diesel dissolution and desorption from solid to liquid phase, striving towards equilibrium; (b) microbial degradation (both in soil and liquid phases), which will reduce hydrocarbon concentration and modify soil-water equilibrium distribution; (c) and microbial growth, driven by hydrocarbon consumption.

Several assumptions were considered to formulate the model:

- The system is closed, and mass conservation is assumed.
- Microbes were considered homogeneously distributed in the system, so they accessed diesel in both soil and water phases. Degradation was not considered in the air compartment.
- Microbial growth or biomass (*BM*) was modeled following Verhulst logistic Equation (Kargi, 2009) and assumed constant once asymptotic growth was reached (Equation 1):

$$\frac{dBM}{dt} = BM_0 + k_l \cdot BM \cdot \left(1 - \frac{BM}{BM_{max}}\right)$$

Equation 1

where *BM* is the concentration of microbial biomass in the system (colony forming units per kg of dry soil, CFU kg^{-1}), BM_0 is the initial concentration of biomass inoculated, BM_{max} is the maximum concentration of biomass reached under the batch conditions, and k_l is a constant parameter from logistic function (h^{-1}).

- Diesel biodegradation from the solid and liquid phase was modeled according to Monod kinetics of substrate (*S*) uptake (Equation 2):

$$\frac{dS}{dt} = \left(\frac{\mu_{max} \cdot S}{K_s + S} \cdot \frac{BM}{\gamma}\right)$$

Equation 2

where S is the substrate concentration per gram of dry soil (mg kg^{-1}) or per liter of aqueous phase (mg L^{-1}), μ_{max} is the maximum specific growth rate (h^{-1}), K_s is the saturation or half-rate constant (mg L^{-1}), and γ is the growth yield coefficient (CFU mg of substrate^{-1}). Biomass (BM) was included as Equation 1.

- Diesel desorption from soil to aqueous phase followed a sigmoid distribution with time, in which diesel water concentration followed Weibull function (Skrdla, 2007) (Equation 3):

$$C_w = C_{wmax} \cdot \left(1 - e^{-(k_{wb} \cdot t)^n}\right)$$

Equation 3

where C_w is the concentration of diesel in the aqueous phase (either dissolved or as dispersed NAPL phase) (mg L^{-1}), C_{wmax} is the maximum concentration of diesel in the aqueous phase (mg L^{-1}), and k_{wb} and n are kinetic the parameters from Weibull adjustment.

- Volatilization was only considered from water phase and followed a first-order kinetics (Equation 4):

$$\frac{dC_v}{dt} = -k_{vol} \cdot C_w \cdot f$$

Equation 4

where C_v is the concentration of volatilized diesel per kg of dry soil (mg kg^{-1}), k_{vol} is the first-order kinetic constant of volatilization of diesel from aqueous phase (h^{-1}), C_w is the concentration of diesel in the aqueous phase (either dissolved or as dispersed NAPL phase) (mg L^{-1}) and f is the constant mass ratio between water and dry soil (L kg^{-1}).

Model formulation and Vensim stock-and-flow diagram

The total variation of diesel concentration in the system can be expressed as the sum of the variations of diesel concentration in the phases considered (Equation 5):

$$\frac{dC}{dt} = f\frac{dC_w}{dt} + \frac{dC_s}{dt} + \frac{dC_v}{dt}$$

Equation 5

where C is the total concentration of diesel in the batch system per kg of dry soil (mg kg^{-1}), and C_s is the concentration of diesel in soil per kg of dry soil (either adsorbed or as free NAPL trapped in pores) (mg kg^{-1}).

Diesel concentration variation in the aqueous phase (C_w) corresponds to the sum of mass transfer flows between phases (desorption and volatilization) and biodegradation process by inoculated bacteria. The concentration in the liquid phase will increase due to desorption from soil phase, and decrease due to volatilization and biodegradation. These last processes will stimulate desorption which will tend to equilibrium with soil (Equation 6):

$$\frac{dC_w}{dt} = \frac{C_{wmax} \cdot n \cdot (k_{wb} \cdot t)^{n-1}}{e^{(k_{wb} \cdot t)^n}} - k_{vol} \cdot C_w - \frac{\mu_{maxw} \cdot C_w}{K_{sw} + C_w} \cdot \frac{BM}{\gamma_w}$$

Equation 6

where first term corresponds to desorption flow, second term to volatilization flow and third term to biodegradation flow; and μ_{maxw} is the maximum specific growth rate in water (h^{-1}), K_{sw} is the saturation or half-rate constant of bacteria colonizing the aqueous phase (mg L^{-1}), and γ_w is the growth yield coefficient of bacteria colonizing the aqueous phase (CFU mg of $diesel^{-1}$). C_{wmax} is not a constant parameter in biodegradation experiments (it was only constant in desorption abiotic experiments), since concentration in soil and water is continuously changing due to volatilization and biodegradation flows. Therefore, C_{wmax} was calculated as a function of soil concentration (C_s), using the distribution coefficient between soil and water, k_d (C_s/C_w), of desorption experiments.

Diesel concentration decrease in the soil phase (C_s) was due to desorption and biodegradation flows (Equation 7):

$$\frac{dC_s}{dt} = load - f\frac{C_{wmax} \cdot k_{wb} \cdot n \cdot t^{n-1} \cdot e^{k_{wb} \cdot t^n}}{e^{k_{wb} \cdot t^n}} - \frac{\mu_{maxs} \cdot C_s}{K_{ss} + C_s} \cdot \frac{BM}{\gamma_s}$$

Equation 7

where *load* corresponds to initial concentration of diesel in soil (mg kg^{-1}), second term to desorption flow, and third term to biodegradation flow; and μ_{maxs} is the maximum specific growth rate in soil (h^{-1}), K_{ss} is the saturation or half-rate constant of bacteria colonizing the soil phase (mg kg^{-1}), and γ_s is the growth yield coefficient of bacteria colonizing the soil phase (CFU mg of $diesel^{-1}$).

A biodegradation model for diesel was developed in Vensim® software (Ventana Systems, Inc.) based on the previous assumptions and equations (Figure 1).

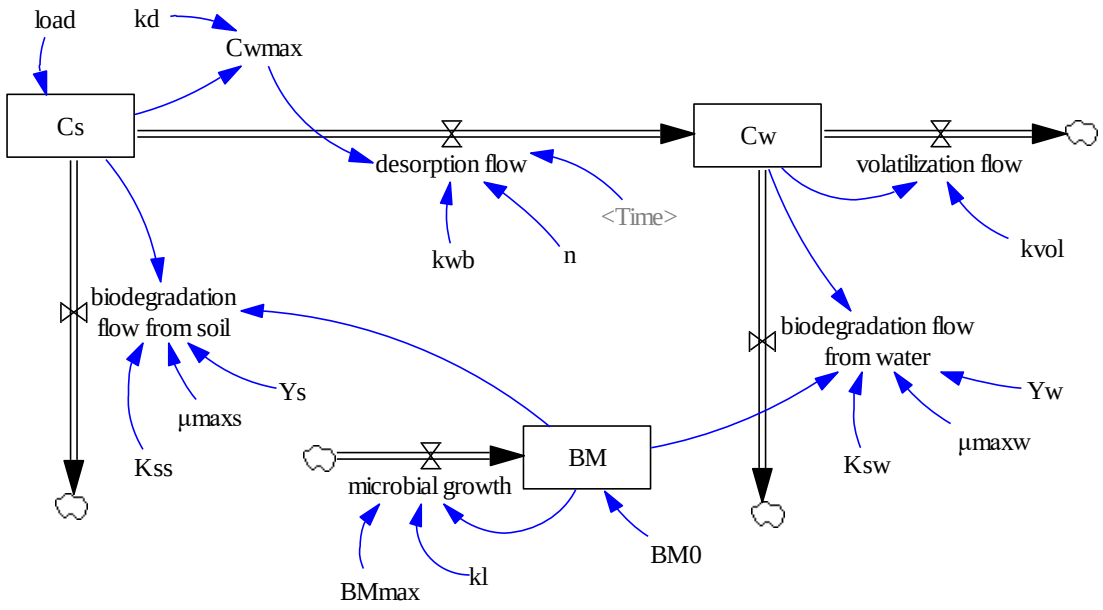

Figure 1. Configuration of the model by Vensim® software.

Description of desorption and bioremediation batch experiments

A sample from the A horizon from a cambic Umbrisol profile collected in the surroundings of Santiago de Compostela (Galicia, NW Spain) was used for the desorption and bioremediation experiments. The soil sample was sterilized (3 times, each 24 h) and contaminated with 1.5 % (*w*/*w*) of diesel, which was previously filter-sterilized (PTFE 0.22 µm filter; Millipore). Soil was kept in closed recipients and stabilised at 4 °C for at least 2 weeks before setting up the experiments.

For desorption experiments (abiotic) a slurry of 2 g of contaminated soil and 8 mL of Bushnell Haas modified mineral medium (BH2) (Bushnell and Haas, 1941). For biodegradation experiments, slurry of 2 g of contaminated soil and 8 mL of BH2 mineral medium comprising 10% (*v*/*v*) of bacterial inoculum was set up, in sterile conditions (*n*=*5*). The inoculum used was a diesel-degrading strain

(*Acinetobacter calcoaceticus* GK2; NCBI GenBank accession GCA_001510805.1) and it was selected due to the high degrading capacity when assessed *in vitro*: 80-90 % of diesel range organics present in the liquid media were degraded after 10 days of incubation (Balseiro-Romero *et al.*, 2017). The inoculum was adjusted to an optical density around 1 at 660 nm, corresponding to an initial concentration of biomass of $4.4 \cdot 10^9$ colony forming units -CFU- per kg of dry soil or $1.8 \cdot 10^{10}$ CFU per L. In both experiments, tubes were incubated at 30 °C and agitated at 150 rpm, and 3 or 5 replicates for each experiment were sacrificed at 1, 2, 4, 6, 8, 11, 13 and 15 days to monitor the desorption and biodegradation processes.

At each monitoring time in bioremediation experiments, an aliquot of soil slurry was used to determine bacterial densities (*n=5*). 100 µL of serial ten-fold dilutions were plated in 1:10 diluted 869 agar medium. After 7 days of incubation at 28 °C, CFU were counted and calculated per gram of dry soil or mL of water in the slurry.

The concentration of diesel in the slurry was determined by analysing diesel range organics (DRO, alkanes from C_{10} to C_{25}) by gas chromatography (Model 450 GC, Agilent Technologies) coupled to mass spectrometry (Model 220 MS, Agilent Technologies) (GC/MS). Diesel was extracted from soil and liquid phases using solvent-based techniques, respectively accelerated solvent extraction (ASE, Dionex) and ultrasonic-assisted extraction (Balseiro-Romero, 2015).

Results and discussion

Estimation of model parameters

Model parameters required to formulate the model of diesel bioremediation (using the sum of C_{10} to C_{25} concentrations, ∑DRO) are specified in Table 1. This table includes the numerical values and the estimation methods (and experiments from which they were obtained) for each of the parameters used in the model.

Table 1. Values and estimation methods of parameters used for model resolution in Vensim.

Process	Parameter	Value	Estimation method
Artificial contamination	*load*	1367.7 mg DRO kg^{-1}	Determined in soil at t=0 by ASE extraction and GC/MS analysis
Desorption from soil to water	Soil-water partition coefficient (k_d)	2.8 (dimensionless)	Estimated from desorption experiments ($k_d=C_s/C_w$)
	Weibull function parameter (k_{wb})	0.007 h^{-1}	Weibull sigmoid adjustment of desorption kinetics in desorption experiments (Equation 3)
	Weibull function parameter (n)	7.4 (dimensionless)	As k_{wb} (Equation 3)
Microbial growth	Initial biomass concentration (BM_0)	$4.40 \cdot 10^9$ CFU kg^{-1}	Determined by CFU counting using serial dilutions and plating in rich agar media
	Maximum biomass concentration (BM_{max})	$2.75 \cdot 10^{10}$ CFU kg^{-1}	Verhulst logistic adjustments of biomass growth kinetics (Equation 1)
	Logistic function constant (k_l)	0.045 h^{-1}	As BM_{max} (Equation 1)
Biodegradation from water	Maximum specific growth rate in water (μ_{maxw})	0.001 h^{-1}	Estimated from previous biodegradation experiments in liquid media *in vitro* (Balseiro-Romero *et al.*, 2017)
	Half-rate constant of bacteria in water (K_{sw})	55.8 mg L^{-1} (223.48 mg kg^{-1})	As μ_{maxw} (Balseiro-Romero *et al.*, 2017)
	Growth yield coefficient of bacteria in water (γ_w)	$1.58 \cdot 10^7$ CFU mg DRO^{-1}	As μ_{maxw} (Balseiro-Romero *et al.*, 2017)
Biodegradation from soil	Maximum specific growth rate of bacteria in soil (μ_{maxs})	0.06 h^{-1}	Estimated from biodegradation experiments
	Half-rate constant of bacteria in soil (K_{ss})	1123.2 mg kg^{-1}	Estimated from biodegradation experiments
	Growth yield coefficient of bacteria in soil (γ_s)	$7.67 \cdot 10^8$ CFU mg DRO^{-1}	Estimated from biodegradation experiments
Volatilization	First-order kinetic constant of volatilization from aqueous phase (k_{vol})	$5 \cdot 10^{-5}$ h^{-1}	First-order kinetics adjustment of volatilization in desorption experiments (Equation 4)

Simulation of long-term bioremediation procedures: Sensitivity analysis

Figure 2 represents the decrease of total concentration of diesel (ΣDRO) (corresponding to the sum of C_s+C_w) in the system, using 0 to 1600 hours as model boundaries to predict bioremediation efficiencies with time. Assuming a constant concentration of the degrading inoculant, 1.5% (*w*/*w*) concentration of diesel, and a completely mixed batch reactor, 50 % of diesel may be degraded in 22-23 days, 75% of diesel in 43-44 days, and complete elimination could be practically achieved in less than 70 days.

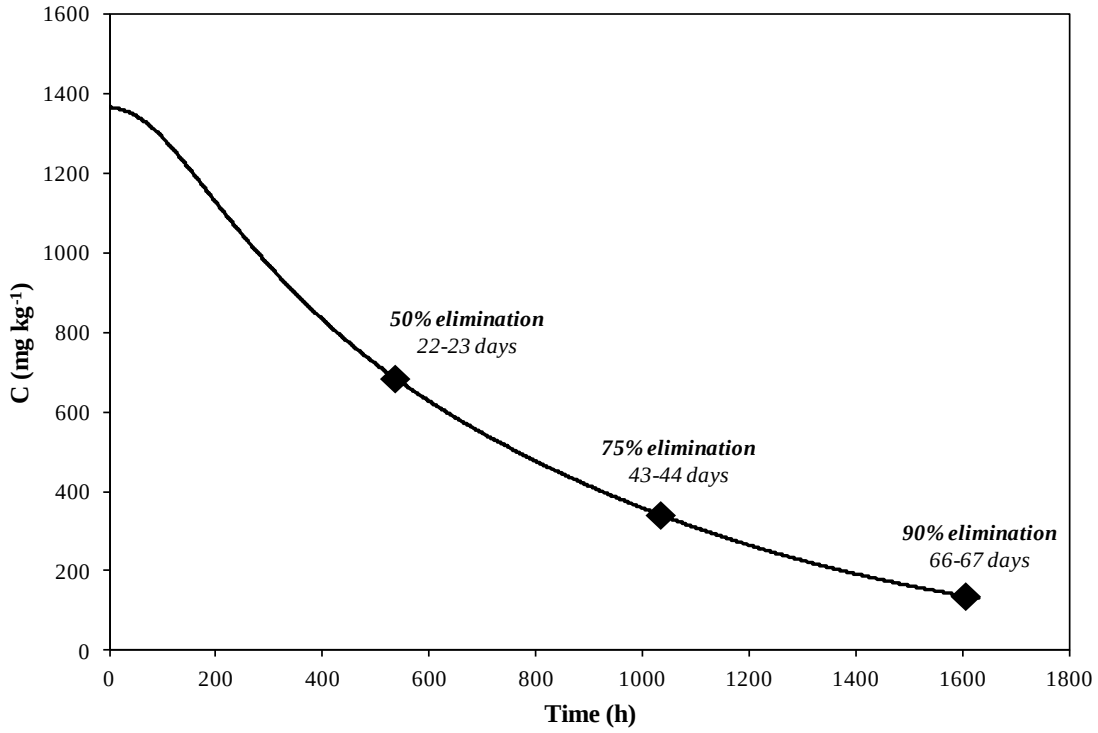

Figure 2. Long-term simulation of total concentration of diesel (C) in the system from 0 to 1600 hours. Time to achieve 50, 75 and 90% of elimination of diesel was also indicated.

Sensitivity of the model to selected process parameters was also evaluated. In order to test model sensitivity to the modification of diesel initial concentration in soil (*load*) (Figure 3a), three contamination scenarios were considered (maintaining the rest of parameters as specified in Table 1): *i.e.* 1.5% (*w*/*w*), which is the concentration used experimentally, and two other concentrations, 0.5% and 2.5% (*w*/*w*). On the other hand, the influence of microbial concentration (BM_{max}) on diesel elimination was also evaluated (Figure 3b), using the value determined experimentally ($2.75 \cdot 10^{10}$ CFU kg^{-1}), and two higher values, *i.e.* $5.50 \cdot 10^{10}$ CFU kg^{-1} (twice the experimental value) and $2.75 \cdot 10^{11}$ CFU kg^{-1} (an order of magnitude higher).

Model predictions indicated that, regardless of initial concentration, diesel elimination presented an initial phase characterized by a high degradation rate, which slowed down with time (Figure 3a). As expected, the highest the initial concentration, the more time was needed to reduce diesel concentration in the system: *i.e.* elimination of 50% of diesel in the system was reached in 16-17, 22-23 or 27-28 days, considering respectively 0.5, 1.5 or 2.5% (*w*/*w*) of diesel as soil initial concentration.

On the other hand, sensitivity analysis of the model reflected that microbial biomass has an extremely significant influence on bioremediation efficiency (Figure 3b), since the increase of this variable involved a dramatic decrease on the time necessary to achieve diesel elimination from the system.

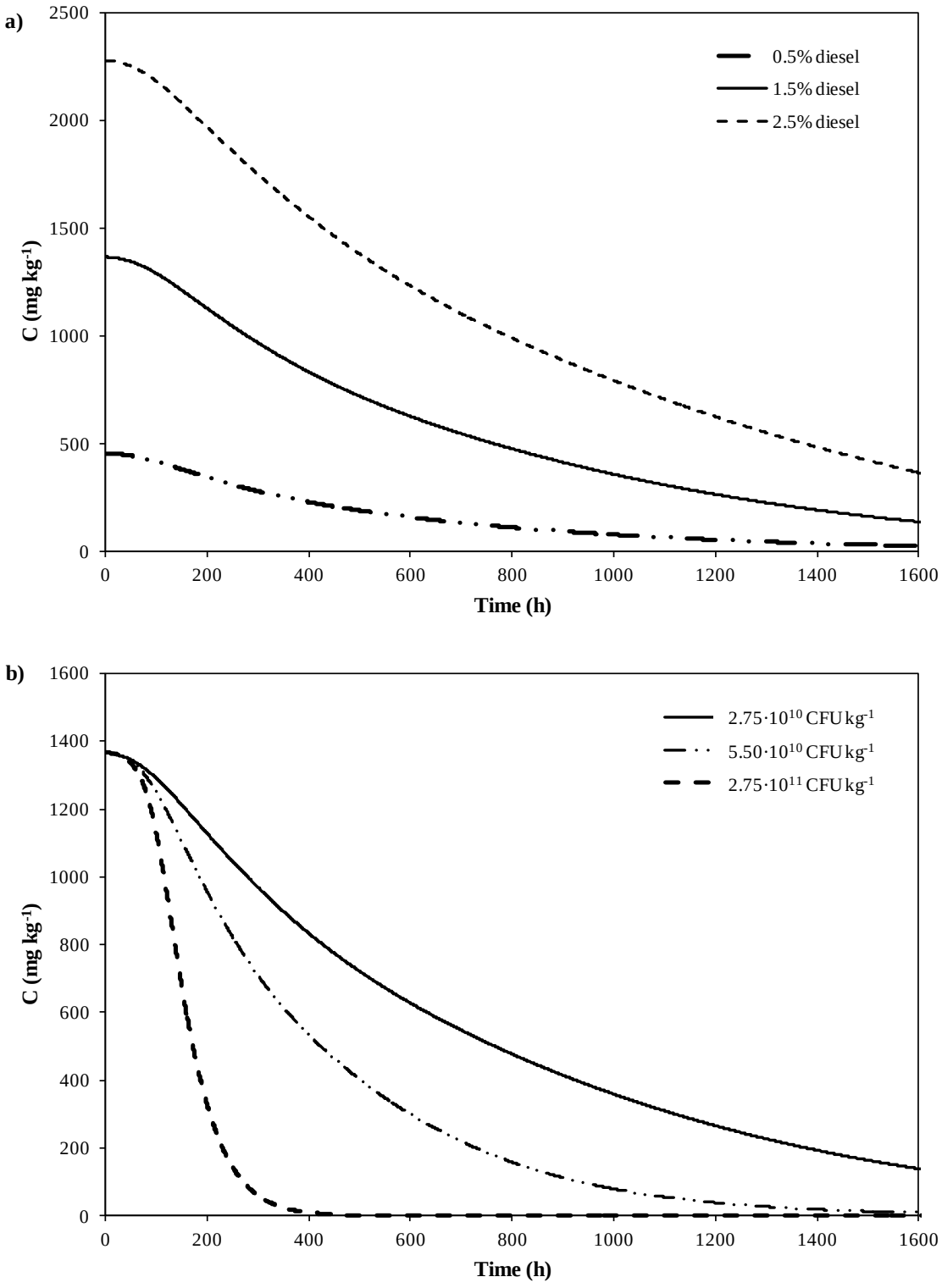

Figure 3. Sensitivity analysis of model predictions on diesel total concentration (*C*) (from 0 to 1600 hours), modifying, a) diesel initial concentration in soil (*load*) and b) biomass maximum concentration reached in the system (BM_{max}).

Model validation and biodegradation potential of bacterial inocula in short-term bioremediation experiments

Figure 4 represents the kinetics of the bioremediation batch experiment up to 360 h, as the total concentration of ∑DRO in the system (corresponding to the sum of C_s+C_w of ΣDRO, in mg kg^{-1}), including experimental data (dots) and model predictions (lines).

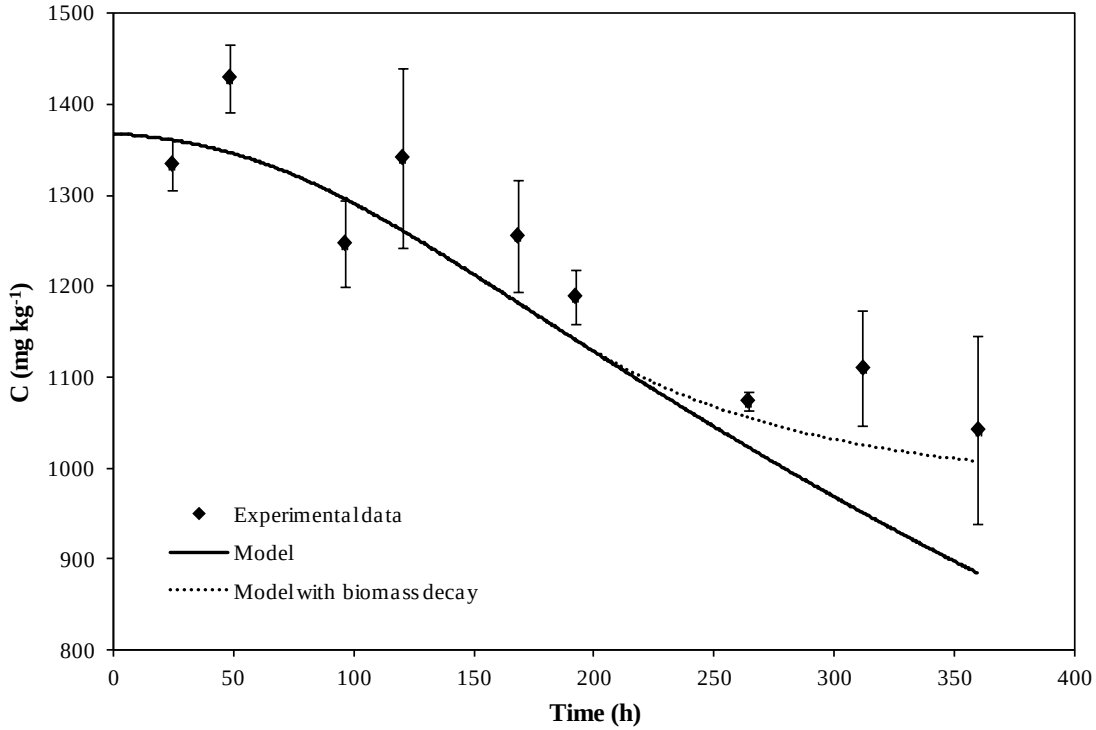

Figure 4. Variation of ∑DRO total concentration in the system (*C*) with time. Dots correspond to experimental data (mean ± standard error; *n=5*) and lines to model predictions.

Experimental data fitted with the predictions of the developed model, only up to 192 hours, from when experimental data tended to equilibrate, but the predicted concentration continued dropping. This difference was due to the detected decrease in biomass concentration from 192 h (data not shown), probably caused by a limitation in oxygen in the batch reactors (closed tubes). This indicates that at the given conditions, bioremediation was only efficient during the first week. On the other hand, experimental data fitted more accurately to the dotted line (Figure 4), which represents the model predictions when biomass concentration was modeled as Equation 1 before 192 h, and as first-order decay after 192 h (Equation 8):

$$\frac{dBM}{dt} = \begin{cases} k_l \cdot BM \cdot \left(1 - \frac{BM}{BM_{max}}\right), & time \leq 192\ h \\ -k_{decline} \cdot BM\ , & time > 192\ h \end{cases}$$

Equation 8

where $k_{decline}$ is the first order kinetic constant of cell death (h^{-1}).

These experimental results reflect the importance of maintaining microbial biomass at an appropriate concentration in the system, in order to ensure high bioremediation efficiencies.

Conclusions

Modeling of a soil bioremediation strategy in a mixed batch system was developed and short-term validated for specific soil conditions and model assumptions. Short-term validation experiments (360 h) reached a 30% elimination of total diesel, and long-term simulations predicted that remediation of soils could be achieved in less than 70 days, in the case of 1.5% (*w*/*w*) of diesel concentration in soil. Sensitivity analysis reflected the significance of biomass concentration on biodegradation kinetics and the importance of maintaining this parameter at its highest possible value (through periodical inoculations, or the maintenance of oxygen and nutrient concentrations under non-limiting conditions) to reduce bioremediation times. Besides, this model could be a base to develop other models in a wide variety of soil systems with different properties.

Acknowledgments

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References

Balseiro-Romero, M., 2015. Behaviour of fuel organic compounds in contaminated soils and development of a phytoremediation procedure. University of Santiago de Compostela.

Balseiro-Romero, M., Gkorezis, P., Kidd, P.S., Van Hamme, J., Weyens, N., Monterroso, C., Vangronsveld, J., 2017. Characterization and degradation potential of diesel-degrading bacterial strains for application in bioremediation. Int. J. Phytoremediation. doi:10.1080/15226514.2017.1337065

Borsi, I., Fasano, A., 2009. A general model for bioremediation processes of contaminated soils. Int. J. Adv. Eng. Sci. Appl. Math. 1, 33. doi:10.1007/s12572-009-0003-x

Bushnell, L.D., Haas, H.F., 1941. The Utilization of Certain Hydrocarbons by Microorganisms . J. Bacteriol. 41, 653–673.

Fernández, E.L., Merlo, E.M., Mayor, L.R., Camacho, J.V., 2016. Kinetic modelling of a diesel-polluted clayey soil bioremediation process. Sci. Total Environ. 557–558, 276–284. doi:10.1016/j.scitotenv.2016.03.074

Kargi, F., 2009. Re-interpretation of the logistic equation for batch microbial growth in relation to Monod kinetics. Lett. Appl. Microbiol. 48, 398–401.

doi:10.1111/j.1472-765X.2008.02537.x
Pilon-Smits, E., 2005. Phytoremediation. Annu. Rev. Plant Biol. 56, 15–39. doi:10.1146/annurev.arplant.56.032604.144214
Skrdla, P.J., 2007. A simple model for complex dissolution kinetics: A case study of norfloxacin. J. Pharm. Biomed. Anal. 45, 251–256. doi:10.1016/j.jpba.2007.06.012