ON-LINE MONITORING OF BIODEGRADATION IN SOILS: MICROCOSM AND COLUMN EXPERIMENTS

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ABSTRACT : The objective of the study described in the present paper was to develop a model-based estimator (software-sensor) of biodegradation in unsaturated soil. This would allow real-time assessment of the efficiency of treatment bioprocesses, such as bioventilation and biopile, and eventually permit optimization through the implementation of control strategies. Based on a reduced-order model, an asymptotic observer has been designed to estimate on-line the contaminant concentration, using carbon dioxide measurement. The software-sensor has been confronted with the experimental results of biodegradation in microcosms and in column. Hexadecane was used as the model compound, representing petroleum hydrocarbons. The asymptotic observer was able to predict hexadecane depletion with an error on the overall time trajectories of less than 5% and 8% for the experiment in microcosm and in column, respectively. These results are very encouraging with respect to the potential for on-line assessment of the performance of treatment bioprocesses in unsaturated soils.

INTRODUCTION

On-line monitoring of contaminant biodegradation in unsaturated soils could provide a valuable tool for real-time assessment of the efficiency of treatment bioprocesses, such as bioventilation and biopile, and to optimize them by implementing control strategies. However, the real-time evaluation of biodegradation in unsaturated soils must overcome major difficulties in both measurement and modeling.

With respect to measurement, no analytical tool is available for on-line measurement of the soil contaminant concentration, and only a few on-line measurements give reliable information on biodegradation. Oxygen and carbon dioxide concentrations in the gas phase are the main variables that can indirectly characterize biodegradation in unsaturated soils. However, the transformation of oxygen consumption or carbon dioxide production into biodegradation rates is a difficult task, as confounding phenomena can occur, such as mineralization of soil organic matter and microorganism mortality. As a consequence, knowledge of these competitive reactions with respect to oxygen consumption and carbon dioxide production is necessary in order to deduce the biodegradation rate from the on-line measurements (Alexander, 1994; Baker and Herson, 1994).

With respect to modeling, there is a lack of reliable dynamical models for the prediction of biodegradation. This is largely due to the complexity of the

phenomena, requiring identification of a large number of parameters. For instance, Fu et al. (1996) have proposed a detailed model of biodegradation in unsaturated soils involving 20 parameters, of which several must be fitted to the experimental data. Although such models provide accurate descriptions of biodegradation under controlled environmental conditions and are useful tools to generate understanding of phenomena, they cannot be used for prediction or on-line monitoring of biodegradation in unsaturated soils. Moreover, the selection of the appropriate model structure, in particular with respect to the kinetics, is typically a heuristic task (Bastin and Dochain, 1990).

Given that some process variables are measured, parameter estimators and state observers (also called software sensors) can be developed to estimate unknown parameters and to observe non-measured variables (Bastin and Docahin, 1990). Such techniques have been successfully implemented on bioreactors in most biotechnological fields, including wastewater treatment and biomolecule production. However, to our knowledge, no estimator of biodegradation in unsaturated soils has been developed.

Schoefs (2002) proposed a two-compartment reduced-order model, with biodegradation assumed to be governed by two rates: the intrinsic contaminant biodegradation by indigenous soil microorganisms and a biocontact kinetics representing the soil resistance to biodegradation (contaminant adsorption, oxygen limitation, microbial mobility, etc). The yield coefficients and the specific microbial growth kinetics were identified from a preliminary biodegradation test in liquid phase, and the parameters involved in the biocontact kinetics were determined by fitting the carbon dioxide curve provided by a biodegradation experiment using a soil matrix. Although curve fitting was necessary to predict contaminant depletion, this work revealed that carbon dioxide measurement in the gas phase could be sufficient to build a state observer of the biodegradation in unsaturated soil. Development of state observers and parameter estimators is the purpose of the present study.

This paper is organized as follows. The two-kinetics reduced-order model developed in Schoefs (2002) will be first presented. The biodegradation tests that were designed to provide data for the validation of the software-sensor will be then described. The mathematical development involved in the estimation scheme will follow. Finally, the performance of the estimators and observers applied to the experimental data will be described and discussed.

THE MATHEMATICAL MODEL

The mathematical model of biodegradation used in this study was introduced in Schoefs (2002). It is conceptually based on a two-compartment scheme corresponding to two contaminant location sites. This conceptual approach depends on knowledge of whether or not the contaminant is in direct contact with the degrader. The portion of the contaminant that is directly in contact with the degrader population can be degraded at a rate that depends only on the intrinsic microbial growth kinetics. The portion of the contaminant that is not directly in contact with the degrader population cannot be degraded. The two contaminant concentrations are linked with a biocontact kinetic model that

integrates the physico-chemical and biological phenomena that enhance the contact between contaminant and degraders, such as contaminant desorption and diffusion, specific microbial attachment extra-cellular activity, and microbial and contaminant mobility to aerobic zones.

Based on this conceptual scheme, biodegradation of hexadecane in unsaturated soil can be described by the following set of differential equations:

$$\frac{dX}{dt} = \mu_g \cdot X - \mu_d \cdot X \quad (1)$$

$$\frac{dS_b}{dt} = \varphi - Y_S \cdot \mu_g \cdot X \quad (2)$$

$$\frac{dS_{nb}}{dt} = -\varphi \quad (3)$$

$$\frac{dP}{dt} = Y_P^g \cdot \mu_g \cdot X + Y_P^d \cdot \mu_d \cdot X \quad (4)$$

where X, S_b, S_{nb} and P refer to the masses of degraders, contaminant in biocontact, contaminant not in biocontact, and produced carbon dioxide, respectively; μ_g and μ_d are the specific growth and decay rates; Y_S, Y_P^g and Y_P^d are the contaminant, growth- and decay-based carbon dioxide yield coefficients, respectively; and φ is the biocontact kinetic that quantifies the contaminant transfer to a state where it is in contact with the degrader. Function φ can be represented by a number of mathematical expressions, depending on the phenomena under consideration.

A Haldane-based kinetic model was used for the specific growth rate:

$$\mu_g = \frac{\mu_S \cdot S_b}{K_S + S_b + \frac{S_b^2}{K_I}} \quad (5)$$

where μ_S is a constant, and K_S and K_I are the affinity and the inhibitory constants. The specific decay rate μ_d was specified as a constant.

MATERIALS AND METHODS

Three series of biodegradation experiments were performed: 1) biodegradation tests in aqueous-phase and soil-free microcosms, 2) biodegradation tests in soil-containing microcosms, and 3) biodegradation tests in a soil-containing column. The second one soil-free experiment in aqueous-phase microcosms (soil-free system), one soil-containing experiment and the other in a three-phase system (soil, water, and air). Data from the liquid-phase experiments were used to identify microbial growth parameters and yield coefficients, and data from the three-phase experiments were utilized to validate the observers. Materials and methods are summarized in the following subsections and details are available in Schoefs (2002). Soil used for this study was a loamy sand. Hexadecane was chosen as the representative compound for petroleum hydrocarbon contamination.

Biodegradation Tests in Aqueous-Phase and Soil-Free Microcosms. The liquid-phase experiment consisted of biodegradation tests performed in 120 mL biometric flasks containing 10 mL of a soil biomass extract. Two levels of contamination were tested and radioactive hexadecane was introduced. A CO_2 trap, composed of a KOH solution, allowed monitoring of total mineralization and hexadecane mineralization. Similar biometric flasks were regularly sacrificed to monitor non-consumed hexadecane and biomass evolution, using a new experimental protocol described in Schoefs (2002). Other biometric flasks containing no radioactive hexadecane were sacrificed to monitor biomass evolution using the Most Probable Number (MPN) technique.

Biodegradation Tests in Soil-Containing Microcosms. The three-phase experiment consisted of biodegradation tests performed in 250 mL biometric flasks containing 20 g of contaminated soil. The soil was artificially contaminated with hexadecane at 10,000 mg/kg dry soil, and radioactive hexadecane was also introduced along with a CO_2 trap. Similar biometric flasks containing no radioactive hexadecane were sacrificed to monitor hexadecane consumption (Soxtec extraction + GC/FID analysis) and biomass evolution using the Most Probable Number (MPN) technique. Water content remained constant during the experiment, and the mean values were equal to 0.2378±0.0019 g water/g dry soil.

Biodegradation Tests in a Soil-Containing Column. A 1.5m high column of contaminated soil was equipped with an irrigation and aeration system. TDR probes for water content measurement and soil interstitial gas extractors (connected to an oxygen/carbon dioxide analyzer) were located at five different depths. A computer has collected data given by the sensors and the analyzer, and was controlling airflow and soil water content using an irrigation system at the top of the column.

MATHEMATICAL DEVELOPMENT

Considering the biodegradation model represented by Equations (1) to (4), the equation for total hexadecane depletion can be obtained by summing Equations (2) and (3):

$$\frac{dS_t}{dt} = -Y_S \cdot \mu_g \cdot X \tag{6}$$

where S_t refers to the mass of total hexadecane, with $S_t = S_b + S_{nb}$.

Under the assumption that yield coefficients Y_S, Y_P^g, Y_P^d and the specific microbial decay rate μ_d are known, and if produced CO_2 concentration P is measured, it is possible to build an asymptotic observer for biomass and total hexadecane (Bastin and Dochain, 1990).

Then the asymptotic observer is given by the following equations:

$$\frac{dZ_1}{dt} = -\frac{Y_S}{Y_P^g} \cdot \mu_d \cdot (P - Z_2) \tag{7}$$

$$\frac{dZ_2}{dt} = \frac{(Y_P^g + Y_P^d)}{Y_P^g} \cdot \mu_d \cdot (P - Z_2) \tag{8}$$

$$\hat{X} = \frac{1}{Y_P^g} \cdot (P - Z_2) \tag{9}$$

$$\hat{S}_t = Z_1 - \frac{Y_S}{Y_P^g} \cdot (P - Z_2) \tag{10}$$

where $\hat{X}$ and $\hat{S}_t$ denote on-line observations of X and S_t, respectively, and Z_1 and Z_2 are two auxiliary variables.

RESULTS AND DISCUSSION

Identification of the Biokinetic Parameters and the Yield Coefficients. The experimental liquid-phase data allows for the identification of the biokinetic model parameters. As biomass was removed from soil particles, all hexadecane that lies on the water surface was in contact with the degraders and a constant shaking ensured oxygen non-limitation. It follows that the function φ can be equaled to zero. Consequently, in the liquid-phase experiment, seven parameters have to be identified: μ_S, K_S, K_I, μ_d, Y_S, Y_P^g and Y_P^d.

Parameter identification was performed using the two sets of data corresponding to the injections of 9.663 mg and 19.325 mg of hexadecane. Values of parameters are given in Table 1 and will be used in the modeling of biodegradation in the soil-containing system.

TABLE 1. Haldane kinetics parameters and yield coefficients

Parameter (unit)	Value
μ_S (d^{-1})	1.1285
K_S (mg)	2.5032
K_I (mg)	8.4615
μ_d (d^{-1})	$4.3 \cdot 10^{-3}$
Y_S (mg/mg)	1.0604
Y_p^g (mg/mg)	0.9382
Y_p^d (mg/mg)	5.5858

Estimation of Contaminant Biodegradation in the Soil-Containing Microcosms. The performance of the asymptotic observer is illustrated in Figure 1. Figure 1a shows the cumulative CO_2 production that was used as the on-line measurement for the asymptotic observer. Figure 1b shows the off-line measured and observed values of the hexadecane mass. Figure 1c shows the off-line measured and observed values of degrader mass.

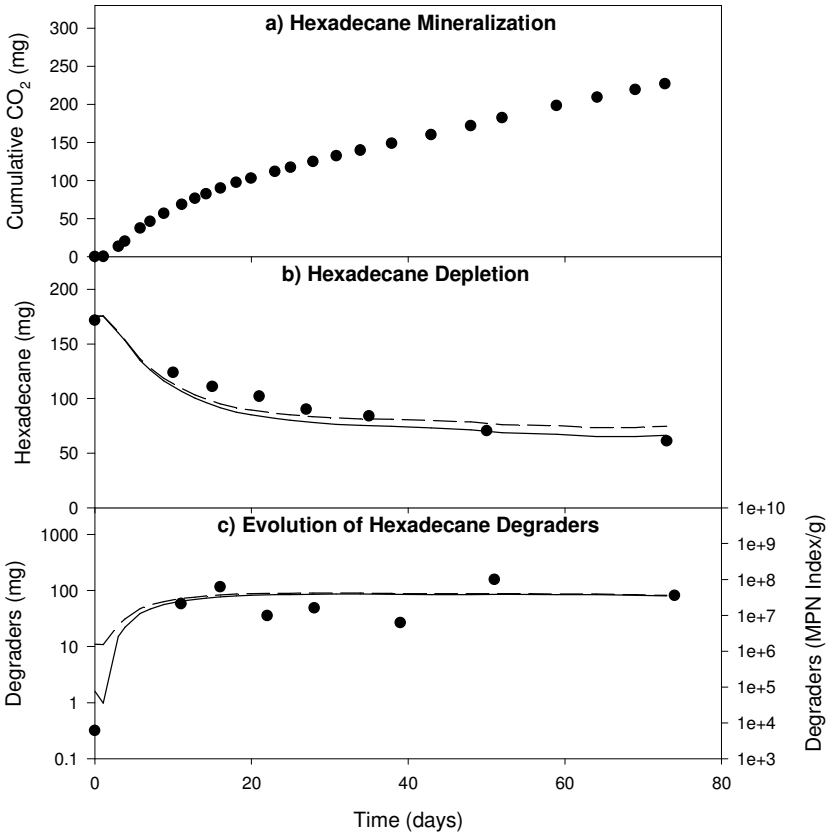

FIGURE 1. Asymptotic observer for the experiment in microcosm (● Experimental data; —— with the default value of X_0, 1.6 mg; — — — with the optimal value of X_0, 11 mg).

To take into account the error on the overall time trajectory, the following non-dimensional criterion, based on the difference between curve surfaces and used in previous studies for sensitivity analysis, was chosen:

$$\sigma_y = \frac{1}{t_f}\int_0^{t_f} \frac{\hat{y}(\tau) - y(\tau)}{y(\tau)}\, d\tau \qquad (11)$$

where $\hat{y}(\tau)$ and $y(\tau)$ denotes the observed and the actual value of the variable y at time τ.

Using this criterion, the relative error based on the entire time trajectories was equal to 4.67%. Profile of hexadecane degraders was satisfactorily observed. A rapid growth of hexadecane degraders occurred during the first 20 days, after which the microbial population remained constant.

As the convergence speed of the asymptotic observer is imposed by the intrinsic biodegradation kinetics, no parameter needs to be tuned other than the initial conditions. Although initial hexadecane concentration can be easily estimated, it is difficult to evaluate the initial degrader quantity. Hexadecane degraders were monitored using the MPN method, based on liquid-phase culture of a soil biomass extract. The biomass extraction from soil is a critical step, and biomass quantity can easily be underestimated. In order to evaluate the sensitivity of the observer to the initial biomass concentration, an optimization procedure

based on a non-linear least squares regression was performed. The best simulation curves, reported in Figure 1, were obtained for initial biomass quantities equal to 11 mg. The error on the whole time trajectory was reduced from 4.67% to 2.08%. The improvement in the observation curves is small compared to the change in the initial biomass quantity (about one order of magnitude), so that observation results using the default (non-optimized) values for the initial biomass quantity are acceptable. Note that the optimized value of the initial biomass quantity is about one order of magnitude over the default value, which is in accordance with the underestimation tendency of the MPN method.

Estimation of Biodegradation in the Soil-Containing Column.

The performance of the asymptotic observer is illustrated in Figure 2. The estimation algorithm was applied from day 20 after the system reached a hydrodynamic and biological pseudo-equilibrium. Although the observer tends to underestimate the hexadecane depletion, the relative error on the overall time trajectory is only 7.82, which is acceptable given that biokinetic parameters were identified from liquid-phase experiment, and only CO_2 on-line measurements were used. Profile of hexadecane degraders was also satisfactorily observed.

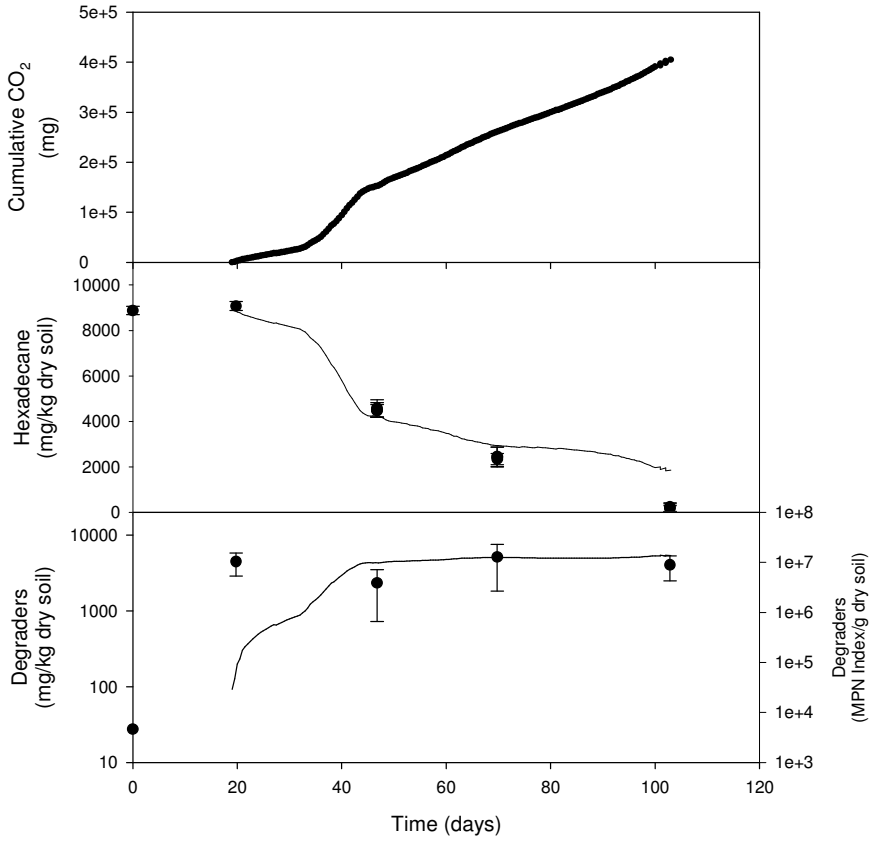

FIGURE 2. Asymptotic observer for the experiment in column (● Experimental data; —— observation data).

CONCLUSION

The asymptotic observer of the contaminant concentration has allowed estimating the hexadecane depletion with a relative error below 5% for the microcosm experiment and below 8% for the column experiment. These results are significant, as it indicates the potential for real-time monitoring of the performance of treatment bioprocesses in unsaturated soils, with a minimum of costly sampling and chemical analysis.

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