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Application of the growth substrate pulsed feeding technique to a process of chloroform aerobic cometabolism in a continuous-flow sand-filled reactor

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ABSTRACT

A process of chloroform (CF) aerobic cometabolic biodegradation by a butane-growing consortium was studied for 354 days in a 2-m continuous-flow sand-filled reactor. The study was aimed at (a) investigating the oxygen/substrate pulsed injection as a tool to control the clogging of the porous medium, to attain a wide bioreactive zone and to reduce substrate inhibition on CF cometabolism; (b) developing a reliable model of CF cometabolism in porous media. While the continuous supply of butane rapidly led to the clogging of the porous medium due to excessive biomass growth, the testing of six types of oxygen/substrate pulsed feeding led to the identification of a feeding schedule capable to prevent aquifer clogging and to lead to the development of a long bioreactive zone and to satisfactory CF degradation performances. The tested model of aerobic cometabolism allowed a suitable interpretation of the experimental data as long as the ratio of CF degraded to butane consumed was $\leq 0.27\ mg_{CF}\ mg_{butane}^{-1}$. A long-term 1-D simulation of the best-performing schedule of pulsed oxygen/substrate supply extended to a 30-m aquifer length resulted in a 20-m bioreactive zone and in a 96% CF removal.

1. Introduction

Chlorinated aliphatic hydrocarbons (CAHs) are widespread subsurface contaminants [1]. CAH-contaminated sites are usually treated by means of physical–chemical methods, such as air-stripping or pump & treat followed by adsorption, which are often limited to contaminant transfer to a different matrix. On the other hand, numerous studies showed that aerobic cometabolism can lead to the complete and rapid dechlorination of a wide range of chlorinated solvents [2–17]. Although numerous studies focused on in situ pilot-scale applications of aerobic CAH cometabolism [3,5,6,8,18–20], full-scale applications of aerobic cometabolic bioremediation of CAH-contaminated sites are still rare [21–23], as several issues still need to be addressed. Of these, two deserve particular mention: (i) there is a risk of completing growth substrate consumption near the injection wells; (ii) aquifer clogging near the injection wells can occur due to excessive biomass growth [3,24–26]. The supply of alternated pulses of growth substrate and oxygen is effective both in the creation of a long bioreactive zone and in controlling aquifer clogging: as a result of hydrodynamic dispersion and substrate sorption, the over-lapping of substrate and oxygen occurs at low concentrations, over a wide aquifer portion and, in each point, in a discontinuous way.

Chloroform (CF) is frequently found in surface and ground waters. Its main sources of release in the environment are manufacturing plants for the production of paper, CAHs, fluorocarbon and pharmaceuticals [27,28]. The U.S. E.P.A. Toxic Release Inventory Program reports 10^6 CF releases in 2007 [27,29].

This work, focused on CF cometabolism by butane-growing bacteria, was conducted in a 2-m continuous-flow sand-filled column simulating a portion of saturated aquifer. In a previous microcosm study we evaluated the CF degradation capacity of the indigenous consortium of the aquifer material utilized in this research [7]. The goals of this work were: (a) to investigate the oxygen/substrate pulsed injection as a tool to control the clogging of the porous medium, to attain a wide bioreactive zone and to reduce substrate inhibition on CF cometabolism; (b) to develop a comprehensive model of aerobic CAH cometabolism utilizable for a model-based optimization of the oxygen/substrate feeding schedule. The main novelty of this work lies in the specific focus on the experimental study of the pulsed substrate injection. Indeed, while some studies of CAH cometabolism included the implementation of this

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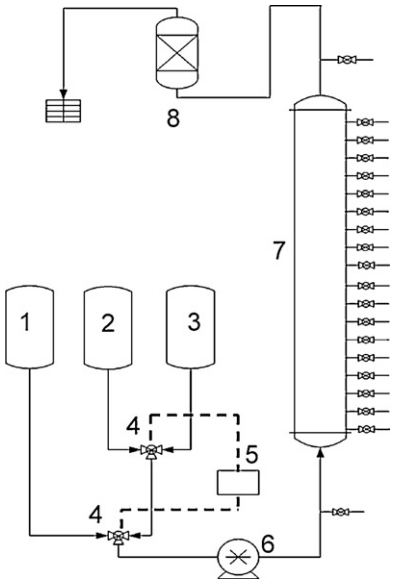

Fig. 1. Schematic representation of the plant: 1, CF-B feeding bag; 2, CF feeding bag; 3, CF-O feeding bag; 4, 3-way valves; 5, timer; 6, peristaltic pump; 7, column reactor with 40 teflon-lined sampling ports; 8, granular carbon filter.

technique [3,5,6,18–20], only one study focused on its model-aided optimal design [25], whereas the experimental optimization of the pulsed substrate feed was never performed.

2. Materials and methods

2.1. Experimental set up and pulsed substrate feed design criteria

The study was conducted in a 35 L glass column (height 2 m, diameter 0.15 m) filled with a non-polluted sandy soil (organic carbon fraction 0.1%, hydraulic conductivity 10^{-4} m s^{-1}) and saturated with tap water. The column, maintained at room temperature (22–26 °C), was provided every 5 cm with a side sampling port sealed with teflon-lined septa. Water was sampled through steel needles ending at the center of each column section. A Masterflex peristaltic pump provided with a Viton tube was utilized to feed the column from the bottom section, to facilitate the exit of possible gas bubbles. In order to operate a pulsed injection of primary substrate (butane) and oxygen, 3 gas-tight 15-L magnetically stirred LDPE bags were connected to the feeding pipe by means of 2 electro-valves controlled by a timer (Fig. 1). The bags were periodically filled with tap water. Besides, one bag (CF-B) was nitrogen-stripped to remove dissolved oxygen and additioned with gaseous butane; the second (CF–O) was additioned with gaseous oxygen; the third (CF) was just nitrogen-stripped. All the bags were supplied with 0.38–0.82 mL of a CF-saturated aqueous solution (8200 g m^{-3}) so as to attain the desired CF concentration, reported in Table 1 for each experimental phase. The need of the third feeding bag derives from the choice to introduce two oxygen-free and butane-free periods, one before and one after each butane pulse. This choice was aimed at avoiding an excessive biomass growth in the very first portion of the column. Although no significant CF losses occurred through the feeding system, the inlet concentrations utilized for the model simulations were measured at the column entrance, after the feeding system.

The reactor operation was articulated into 4 phases, whose operational details are reported in Table 1. After an initial phase of continuous substrate supply in the absence of CF, the reactor was fed for 193 days with alternated butane and oxygen pulses, at CF inlet concentrations in the 0.21–0.45 mg L^{-1} range (phases B, C, D). These phases were characterized by different values of the ratio of CF mass to butane mass supplied to the reactor ($\dot{m}_{CF,in}/\dot{m}_{B,in}$) and by different durations of the pulsing cycle. Indeed, several studies of aerobic cometabolism highlighted the importance of the ratio of the CAH mass degraded to the growth substrate mass utilized, or transformation yield (T_y), equal to $(\dot{m}_{CF,in}/\dot{m}_{B,in}) \cdot (\eta_{CF}/\eta_B)$ [11,30,31]. In the microcosm study conducted with the same aquifer material used in this work [7], we showed that an experimental T_y of 0.15 mg_{CF} mg_{butane}^{-1} ensured a CF cometabolism sustainable over time, whereas a T_y of 1.3 mg_{CF} mg_{butane}^{-1} was not sustainable. We also provided a theoretical evaluation of the maximum sustainable T_y, equal to 0.62 mg_{CF} mg_{butane}^{-1}. As T_y can be evaluated only at the end of each assay, the injection schedules of phases B, C and D were designed so as to test different values of the $\dot{m}_{CF,in}/\dot{m}_{B,in}$ ratio. The duration of the pulsing cycle represents an important design parameter too, as it is inversely proportional to the frequency of the passage of the bioreactive substrate/oxygen interface in each point, and therefore to the frequency with which biomass growth occurs. In particular phase B was characterized by a 1–2-day pulsing cycle and by a 0.04–0.07 mg_{CF} mg_{butane}^{-1} $\dot{m}_{CF,in}/\dot{m}_{B,in}$, largely lower than both the experimental and the theoretical maximum sustainable T_y [7]. Thus, phase B was maintained in a "safe" $\dot{m}_{CF,in}/\dot{m}_{B,in}$ zone, in order to make an initial process optimization and model calibration without any lack of butane. Conversely, phase C was operated in a less safe condition, by raising the duration of the pulsing cycle to 7 days and $\dot{m}_{CF,in}/\dot{m}_{B,in}$ to 0.35 mg_{CF} mg_{butane}^{-1}. Finally in phase D the duration of the pulsing cycle was reduced to 3.5 days, whereas $\dot{m}_{CF,in}/\dot{m}_{B,in}$ was maintained about equal to that of phase C.

2.2. Modeling

The experimental data were interpreted by means of the following mass-balance, based on a plug flow model with axial dispersion and equilibrium adsorption:

$$\delta_i \frac{\partial c_i}{\partial t} = -v\frac{\partial c_i}{\partial z} + D_{L,i}\frac{\partial^2 c_i}{\partial z^2} + R_i \quad (1)$$

The reaction terms R_B (butane), R_{CF} and R_O (oxygen) were expressed on the basis of a Michaelis–Menten-type model with competitive inhibition [11]:

$$R_B = q_B \cdot c_X = -\frac{q_{max,B} \cdot c_B}{K_{s,B} \cdot (1 + c_{CF}/K_{i,CF}) + c_B}\frac{c_O}{K_{s,O} + c_O}c_X \quad (2)$$

$$R_{CF} = q_{CF} \cdot f_a \cdot c_X = -\frac{q_{max,CF} \cdot c_{CF}}{K_{s,CF} \cdot (1 + c_B/K_{i,B}) + c_{CF}}\frac{c_O}{K_{s,O} + c_O}f_a \cdot c_X \quad (3)$$

$$R_O = 2.2 \cdot R_B + 0.34 \cdot R_{CF} - k_{O,abio} \cdot c_O \quad (4)$$

In Eq. (3) f_a indicates the fraction of the total biomass active on CF degradation. Indeed, preliminary pure-culture batch tests showed that, among the culturable strains of the consortium, only one (*Rhodococcus aetherovorans* BCP1) showed a CF degradation capacity. Although non-culturable CF-degrading strains might be present in the consortium, the above-mentioned result indicates the presence of a non-CF-degrading biomass fraction. Frascari et al. [2] showed that the above-described kinetic model can satisfactorily simulate CF aerobic cometabolism by resting cells of BCP1. The mass ratio of oxygen consumed to butane utilized, set equal to 2.2 in Eq. (4), was evaluated in preliminary microcosm tests. The oxygen/CF mass ratio was set in Eq. (4) to 0.34, corresponding to the stoichiometry of the complete CF oxidation to CO_2, H_2O and Cl^- [2]. $k_{O,abio}$ accounts for the abiotic oxidation of the reduced species present in the sand and groundwater.

As for the biomass, our model includes both a suspended fraction (c_{Xs}, $mg_{protein}$ $L_{pore\ volume}^{-1}$), subject to advection and dispersion, and a soil-attached fraction (c_{Xa}, $mg_{protein}$ $kg_{dry\ soil}^{-1}$). The biomass mass balance was developed by assuming that biomass follows an equilibrium adsorption ($c_{Xa} = k_X c_{Xs}$). The total biomass concentration (c_X, $mg_{protein}$ $L_{pore\ volume}^{-1}$) is thus given by $c_{Xs} + c_{Xa}\rho_b/\varepsilon$. The resulting biomass mass balance is:

$$\left(1 + \frac{\rho_b k_X}{\varepsilon}\right)\frac{\partial c_{Xs}}{\partial t} = -v\frac{\partial c_{Xs}}{\partial z} + D_{L,X}\frac{\partial^2 c_{Xs}}{\partial z^2} + R_X \quad (5)$$

The Monod-based expression of R_X (sum of the growth/death rates relative to the CF-degrading and to the non CF-degrading bacteria) was corrected by the introduction of a term of biomass death or inactivation proportional to the rate of CF degradation:

$$R_X = -Y_B \cdot R_B - b \cdot c_X + \frac{R_{CF}}{T_{c,CF}} \quad (6)$$

The CF transformation capacity $T_{c,CF}$ is defined as the CF transformed per unit amount of cells inactivated or killed by the toxic transformation products [32–35]. The kinetic model defined by Eqs. (2)–(4) and (6), characterized by competitive inhibition and product toxicity, was selected by several authors as the most appropriate one for aerobic cometabolism both for lab-scale studies [2,4,33,34,36,37] and in situ applications [5,18,25,38,39], although a smaller number of studies obtained better results with non-competitive or mixed inhibition [40–42]. Alternative approaches to simulate transformation product toxicity, such as the one proposed by Ely et al. [43,44] were not considered here, given the higher number of parameters in the kinetic equations. While several models of in situ CAH biodegradation do not include a suspended biomass fraction [5,18,25,38,39], we distinguished between c_{Xa} and c_{Xs}, in order to account for the loss of suspended cells in the reactor outlet, to be able to compare the experimental and simulated values of c_{Xs} and to develop a tool capable to predict in situ microbial transport in bioaugmentation treatments. Our assumption of equilibrium between c_{Xa} and c_{Xs} can be found in similar studies [45] and represents a simplification of more complex models of microbial adhesion and detachment [19,46–49], aimed at limiting the number of model parameters.

2.3. Parameter estimation strategy and model application

The integration of the above-illustrated differential equations was performed with a finite elements solver of partial differential equations (Comsol Multiphysics). The solution was obtained with a direct Paradiso linear system solver, a 0.002 m