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A new mathematical model to evaluate simazine removal in three different immobilized-biomass reactors

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ABSTRACT

A new mathematical model based on the cinetical Langmuir equation is developed to interpret and predict the effectiveness of simazine (SZ) removal in immobilized-biomass reactor (IBR), to consider herbicide-support affinity (Cx), and herbicide-cell affinity (Cy). Three solid supports: sepiolite monolith, granular sepiolite, and alginate were used in pilot-scale reactors that were inoculated with *Klebsiella planticola* DSZ. The abiotic process was analysed by measuring the SZ sorption capacity of the reactor supports. Sepiolite monolith showed the maximum value for herbicide-support affinity ($28.02 \pm 0.9\%$). The effectiveness of the biotic process was estimated considering the formation of biomass and SZ biodegradation. Granular sepiolite showed either higher affinity with SZ and viability rate (0.90) throughout the process, and SZ removal rate was 3.39 ± 0.06 mg/h. The mathematical model presented in this paper provides useful insights into the interpretation of experimental data as well as prediction for the implementation of biological reactors.

1. Introduction

s-Triazine herbicides have been used in a variety of weed-control programs with major crops, but herbicides containing an s-triazine ring are relatively persistent in the environment. Simazine (SZ) is a synthetic chemical that is widely used as a selective triazine herbicide to control the growth of broad-leaved weeds and annual grasses in field, berry fruit, nuts, vegetable and ornamental crops, turfgrass, orchards, and vineyards. At higher concentrations, it is used for non-selective weed control in industrial areas, and before 1992, it was used to control submerged weeds and algae in large aquariums, farm ponds, fish hatcheries, swimming pools, ornamental ponds, and cooling towers. The increasing awareness of the harmful effects of environmental pollution has led to a notable increase in research on various strategies that may be employed to clean up the environment (Vandecasteele et al., 2000; Wackett et al., 2002). It is now accepted

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that microbial metabolism provides a safer, more efficient, and less expensive alternative to physicochemical methods for pollution abatement (Pandey and Jain, 2002; Boudreau and Daugulis, 2006; Zheng et al., 2006).

The natural tendency of many microorganisms to bind to solid surfaces (Chen et al., 2005; Jefferson, 2004; Zacarias et al., 2005) has been utilized in a number of biotechnological processes. For example, immobilized microorganisms are used for the decontamination of wastewater in fixed/film treatment plants with systems such as trickling filters, rotating biological contactors, or fluidised beds (Costerton et al., 1995; Garbi et al., 2006; Nicolella et al., 2000). In all these systems, the bacteria responsible for biodegradation are present in a microbial biofilm (Branda et al., 2005). Physiological cooperativity is a major factor in shaping the structure and in establishing the eventual juxtapositions that make mature biofilms very efficient microbial communities adhering to surfaces (Cogan et al., 2005; Heipieper and Bont, 1994; Panikov, 1995) and immobilized cells are known to show a greater tolerance of various antibiotics and xenobiotic compounds.

Bioremediation systems, whether with free or with immobilized cells as in biofilm reactors, must be based on a sound knowledge of the processes and factors involved in the system. For the technology to be effective it must be shown to bring about a higher rate of biodegradation of the contaminant without any harmful environmental effects on the final products. The optimization of the system requires expert knowledge at the cellular level and an understanding of the macroscopic processes that affect the persistence and the possible elimination of the contaminants. The quest for this knowledge which underlies both the design of bioreactors and the modelling of the systems at cellular and/or macroscopic levels can now count on the recent advances in molecular technology and on mathematical modelling (Alonso-Sanz and Martin, 2005a,b, 2006; Laspidou and Rittmann, 2004) to predict the behaviour of a system from such variables as the dynamics of the contaminants, of the fluids, and of the biomass.

The control and understanding of processes catalysed by biofilms are important from both industrial and ecological perspectives. Mathematical models represent one end of a spectrum of activities designed to investigate natural phenomena. They attempt to simplify systems to uncover relationships that yield a consistent pattern when compared with in situ behaviour (Garbi et al., 2006; Alonso-Sanz and Martin, 2006).

The aim of this work is to develop a mathematical model to evaluate the effectiveness of herbicide removal in three different immobilized-biomass reactors (IBRs) by calculating the coefficients of herbicide-support affinity (C_x), and herbicide-cell affinity (C_Y). SZ biodegradation data from bioreactor experiments and simulations are compared, and the capacity of the model to predict the process is evaluated in this study.

2. Materials and methods

2.1. Pilot-scale immobilized-biomass reactor (IBR)

To evaluate the effectiveness of decontamination of the IBR, the experiments were performed in a bioreactor with an internal diameter of the reactor glass column of 8 cm, and a total height of 38 cm (Martin et al., 2000). Sterile monolith (500 g), granular sepiolite (500 g), or alginate beads (495 g) were set up in the reactor, and liquid samples were distributed over the packing material through a microsprinkler. Dissolved oxygen, pH, and temperature were monitored each minute by specific sensors connected to a Biocontroller ADI 1030 (Applikon). The experiments were performed at room temperature ($20 \pm 2\,^{\circ}C$) by circulation of MB medium (Sanchez et al., 2005) and 0.025 mM SZ. The circulation flow was of 50 ml/min, and the reactor was operated over a cycle of 100 days. Samples were taken periodically to monitor the state of the chlorinated herbicide and microorganisms.

2.2. Biomass immobilization

Klebsiella planticola strain DSZ was isolated from agricultural fields in Alcala de Henares (SE-Madrid, Spain), and it grows on a wide range of *s*-triazine and aromatic compounds (Martin-Montalvo et al., 1997; Sanchez et al., 2005). Cells were grown aerobically at 30 °C in MB medium and SZ was added to make up 0.025 mM. Cell immobilization by attachment was done by using sepiolite (Tolsa SA, Spain), a porous carrier material, with two different structures: monolith (Ferrer et al., 1996) and granular sepiolite (3–5 mm ∅) (Martin et al., 2000). The immobilization method is described elsewhere (Ferrer et al., 1996), we used the pure culture of DSZ strain grown on 0.025 mM SZ as the cell source, harvesting cells at the exponential phase for use as the inoculum in the reactor experiments. Cell immobilization by entrapment was carried out with alginate beads (Gibello et al., 2005). DSZ strain cells grown on 0.025 mM SZ were suspended in a 3% alginate suspension by stirring for 20 min, and the mixture was extruded through a needle into a 1% $CaCl_2$ solution. The beads were collected in 0.9% NaCl and washed twice with saline solution. For the immobilized-cell assays an inoculum of 1.15×10^{10} cells per gram of alginate beads was used.

Cell quantification in the immobilized-cell samples from the reactors was carried out by using 4′,6′-diamidino-2-phenylindole (DAPI) for DNA staining. DAPI solution (0.55 μM) in distilled water was added to each sample and left in contact for 10–15 min at room temperature in the dark. The remaining DAPI solution was removed by rinsing twice with distilled water and finally air-dried. Each sample was mounted with drops of Vecta-Shield on a slide and the preparations were examined by confocal laser scanning microscopy (CLSM).

2.3. Chemicals

SZ (99% purity), and [U-ring ^{14}C] SZ (5mCi $mmol^{-1}$, 95% radiochemical purity) were purchased from Sigma-Aldrich (St. Louis, Mo). All the chemical compounds were of the highest purity commercially available.

2.4. Chemical analyses

SZ was measured by HPLC as previously reported by Sanchez et al. (2005), and the analyses were performed with a Waters model 616PDA996 photodiode array detector equipped with a