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A kinetics study on the biodegradation of synthetic wastewater simulating effluent from an advanced oxidation process using *Pseudomonas putida* CECT 324

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Abstract

Bacterial growth on mixed substrates is employed for wastewater treatment. Biodegradation kinetics of *Pseudomonas putida* CECT 324 growth on formic acid, vanillin, phenol and oxalic acid mixtures is described. The experiments were carried out in a stirred-tank fermentor in batch mode at different temperatures (25, 30 and 35 °C) and pH (5, 6 and 7). The four compounds selected are typical intermediates in pesticide-contaminated water treated by advanced oxidation processes (AOPs). The toxicity of intermediates was investigated for a combined AOP-biological treatment, and the minimum DOC inhibitory concentration of the intermediate mixture was 175 ppm. The resulting biodegradation and growth kinetics were best described by the sum kinetics with interaction parameters (SKIP) model. Phenol and oxalic acid inhibit *P. putida* growth, and formic acid consumption strongly affects the biodegradation of oxalic acid. At all the temperatures tested and at pH between 5 and 7, *P. putida* CECT 324 was able to degrade the four substrates after culture times of 30 h at 30 °C and pH 7, which were the best conditions, and after 70 h, under the worst, at 35 °C.

Keywords: *Pseudomonas putida* CECT 324; Biodegradation kinetics; Advanced oxidation process; Aromatic compounds; Carboxylic acids

1. Introduction

One of the most alarming phenomena affecting water sources is the growing accumulation of anthropogenic substances that are barely biodegradable due to a lack of water treatment systems capable of diminishing the concentration of toxic substances representing an acute or chronic chemical risk. The inability of conventional biological wastewater treatments to effectively remove many toxic pollutants shows that new treatment systems are needed. Rigorous pollution control and legislation in many countries has resulted in an intensive search for new and more efficient water treatment technologies. In the European Union, water policy is presently undergoing considerable change. The Framework Directive on Water [1] provides a policy tool that enables this essential resource to be sustainably protected. Among other measures, surface water deterioration must be prevented, and bodies of water protected and restored, good chemical and ecological condition of such water achieved and pollution from discharges and emissions of hazardous substances reduced by 2015. It has recently been shown that partial oxidation of toxic compounds by advanced oxidation processes (APOs) (that involve generation and subsequent reaction of hydroxyl radicals, $^{\bullet}OH$) may substantially increase wastewater biodegradability [2–7]. Even though wastewater treatments using AOPs have been shown to be highly efficient, their operation is still quite expensive (tens of € m^{-3}) [7–9]. Therefore, combination of an AOP as a preliminary treatment, followed by an inexpensive biotreatment, would seem to be an economically attractive option.

There are many publications dealing with integrated chemical and biological oxidation processes for wastewater for a variety of single compounds (e.g., aromatics, pesticides, antibiotics,

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Nomenclature

I_{ij}	interaction constant ($L\,mg^{-1}$)
J	objective function (dimensionless)
k_d	Decay coefficient (h^{-1})
$K_{S,i}$	saturation constant for substrate i ($mg\,L^{-1}$)
$K_{S\,ap,i}$	apparent saturation constant for substrate i ($mg\,L^{-1}$)
$K_{I,i}$	inhibition constant for substrate i ($mg\,L^{-1}$)
$K_{S\,Th,i}$	Theoretic saturation constant for substrate i ($mg\,L^{-1}$)
S_i	i substrate concentration ($mg\,L^{-1}$)
S_j	j substrate concentration ($mg\,L^{-1}$)
X	biomass concentration ($mg\,L^{-1}$)
$X(i)_{mod}$	simulated state space vector ($mg\,L^{-1}$)
$X(i)_{exp}$	experimental state space vector ($mg\,L^{-1}$)
Y_{X/S_i}	yield coefficient on substrate ($g\,g^{-1}$)

Greek letters

μ_i	specific growth rate for substrate i (h^{-1})
$\mu_{max,i}$	maximum specific growth rate for substrate i (h^{-1})
$\mu_{max\,ap,i}$	apparent maximum specific growth rate for substrate i (h^{-1})
$\mu_{max\,Th,i}$	theoretic maximum specific growth rate for substrate i (h^{-1})

synthetic dyes, etc.) [10,11] and multicomponent feed streams (effluents from textile mills, paper mills, tannery, vinasse and olive mills, etc.) [3,12]. However, it must be kept in mind that some intermediates of the chemical reaction could be as toxic as the parent substance or even more so [3,13–15]. Therefore, toxicity of the intermediates mixture formed as the chemical reaction proceeds must be evaluated. A recent review of the current technologies for integrated chemical and biological processes in the literature by Gogate and Pandit [16] reported that for quantification of performance with a mixed culture, mainly activated sludge, most papers consider global variables such as BOD_5, COD and TOC. For microbial activity, biomass production and respiration rates are commonly reported, and gas phase composition has also been analysed, especially in small scale bioreactors [15,17]. Little has been written about the kinetics of the integrated oxidation processes or the toxic or inhibitory properties of chemical oxidation intermediates [18]. In wastewater, the occurrence of organic chemical mixtures makes the degradation of one component strongly affected or inhibited by other compounds [19–21].

This work is aimed at studying the biodegradation of a mixture of compounds using the bacterial strain *Pseudomonas putida* CECT 324, a model microorganism for biological treatment. In this research, the growth medium contains two aromatic compounds, vanillin and phenol, and two carboxylic acids, formic acid and oxalic acid, as these substances are frequently found only partly oxidized in the mixture of pesticide residues during the photocatalytic process [22–24]. The aqueous solution of these compounds forms a synthetic wastewater simulating effluents from an AOP. Phenol degradation by *P. putida* is well documented in the literature [25–27]. However, as far as we know, the *P. putida* CECT 324 potential for degrading vanillin and carboxylic acids has not yet been explored. While this pure culture system lacks the complexity of activated sludge, it is a reasonable starting point for the development and validation of mathematical models for integrating AOP and biological treatment units.

In view of the acidic conditions at the pre-treatment (AOP) outlet and the strong effect that will have on the biological state of the culture, two controlled pH conditions were assayed (5 and 7). In a combined treatment, an intermediate neutralization step will be necessary, so these experiments are conceived as a first check of the extent of neutralization. A third experiment was carried out without pH control during culturing, starting out at pH 6. The temperature selected for the pH study was 30 °C, reported by Hill and Robinson [28] and Yang and Humphrey [29] as optimum for phenol biodegradation by *P. putida*. At the best pH, the temperature effect was tested at 25 and 35 °C, covering a 10-degree interval around the reported optimum of 30 °C.

Kinetics models taking several substrates into account were tested, mainly those that capture the strong interaction between chemically similar species and the substrate inhibition effect. The sum kinetics with interaction parameters (SKIP) model [30], was found to be a suitable starting point.

2. Materials and methods

2.1. Microorganism and culture media

P. putida CECT 324 was acquired from the Spanish Type Culture Collection (Colección Española de Cultivos Tipo, Valencia, Spain). Cultures were grown in $1\,g\,L^{-1}$ beef extract at pH 7.2, $2\,g\,L^{-1}$ yeast extract, $5\,g\,L^{-1}$ peptone, $5\,g\,L^{-1}$ NaCl, and $15\,g\,L^{-1}$ agar powder, and were kept in glycerol at −70 °C.

2.2. Flask cultures

The effect of DOC concentration on the growth of *P. putida* was studied in triplicate in shake flasks. Cultures were incubated for 72 h at 30 °C on a rotary platform shaker (150 rpm, 2.6 cm stroke) in 100-mL Erlenmeyer flasks filled with 20 mL of synthetic wastewater. Before addition of the carbon source, the composition of the medium was: $0.5\,g\,L^{-1}$ NH_4Cl, $0.5\,g\,L^{-1}$ K_2HPO_4, $0.5\,g\,L^{-1}$ KH_2PO_4, $0.5\,g\,L^{-1}$ $MgSO_4{\cdot}7H_2O$, and $10\,mL\,L^{-1}$ of trace mineral solution [21]. Completing the synthetic wastewater, the carbon source was a 1:1:1:1 molar mixture of vanillin, phenol, oxalic acid and formic acid. This ratio was maintained throughout the experiments. For instance, to start at 150 ppm DOC concentration, individual species concentrations were: $111.84\,mg\,L^{-1}$ vanillin, $69.18\,mg\,L^{-1}$ phenol, $66.18\,mg\,L^{-1}$ oxalic acid and $33.84\,mg\,L^{-1}$ formic acid, yielding 70.56 ppm DOC from vanillin, 52.92 ppm DOC from phenol, 17.64 ppm DOC from oxalic acid and 8.82 ppm DOC from formic acid. Initial DOC ranged from 25 to 500 ppm.