Adriana Dorado-Martínez

Nora Ruiz-Ordaz

Juvencio Galíndez-Mayer*

Fortunata Santoyo-Tepole

Oswaldo Ramos-Monroy

Instituto Politécnico Nacional,
Escuela Nacional de Ciencias
Biológicas, Mexico City, Mexico

Research Article

Effect of propanil, linuron, and dicamba on the degradation kinetics of 2,4-dichlorophenoxyacetic acid by *Burkholderia* sp. A study by differential analysis of 2,4-dichlorophenoxyacetic acid degradation data

The successive application of distinct pesticides, or mixtures of them, is a frequent practice that could adversely affect the microbial species inhabiting soil and aquatic ecosystems. The ability of soil or aquatic microbiota to degrade a pesticide could be affected by the presence of another. If the degradation rate of the first compound is inhibited, its dissipation half-life in the environment could be hazardously enlarged. Few studies have been made to quantify the impact on the biodegradation rate of pesticides in soils or water by the presence of other pesticides. In this work, a method for assessing the effect of a pesticide on the biodegradation rate of another, measuring its effect on the biodegradation kinetics of a single bacterial strain is presented. The mathematical analysis is a powerful tool to study the stoichiometry and kinetics of microbial processes, which was used to evaluate independently, in detail, the effect of three pesticides (propanil, linuron, and dicamba) on the biodegradation kinetics of 2,4-dichlorophenoxyacetic acid by a strain of *Burkholderia* sp. It was evidenced that linuron and dicamba caused a decay of more than 40% in the top instantaneous degradation rate of 2,4-dichlorophenoxyacetic acid, while propanil showed a minimal effect.

Keywords: 2,4-Dichlorophenoxyacetic acid / Biodegradation kinetics / *Burkholderia* sp. / Dicamba linuron / Propanil

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1 Introduction

The application of pesticides, single or mixed, is a frequent practice affecting microbial species inhabiting soil and aquatic ecosystems, which receive direct and indirect pesticide inputs [1] similar to the insecticides methylpyrimifos and chlorpyrifos that significantly decrease aerobic dinitrogen-fixing bacteria and dinitrogen fixation in agricultural soils [2], or with the mixture of the herbicides (atrazine, glyphosate), and insecticides (malathion, carbaryl, permethrin) that reduced the microbial

diversity and altered the community structure of aquatic ecosystems [3]. In addition, it is known that the combination of pesticides could enhance their adverse effects on soil microbial communities [4] and that the biodegradation rate of some pesticides is affected by the presence of organic coformulants [5]. Although the pesticide effects on microbial species are well documented, the effect and the inhibitory concentrations of pesticides on microbial growth and metabolism are not widely studied. A pioneer in vitro study about the inhibitory effect of methylcarbamate and organophosphorus insecticides on phenylamide hydrolysis by *Bacillus sphaericus* report that linuron degradation was completely inhibited by carbo-insecticides at concentrations ranging

Correspondence: Dr. Nora Ruiz-Ordaz (noraruizordaz@yahoo.com.mx), Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Av. Wilfrido Massieu s/n Zacatenco, Mexico City CP 07738, Mexico

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; BM, basal medium; MSM, minimal salt medium

***Additional correspondence:** Dr. Juvencio Galíndez-Mayer (juvencioigm@yahoo.com), Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Av. Wilfrido Massieu s/n Zacatenco, Mexico City CP 07738, Mexico

from 1×10^{-6} to 1×10^{-3} M [6]. Another in vitro study concerning resistance to various herbicides in soil was made with *Azotobacter chroococcum* strains, finding that linuron did not inhibit the growth of *A. chroococcum* at the tested concentrations [7]. Other studies on phenylurea herbicides report that diuron concentrations greater than 20 $\mu\text{g/mL}$ inhibited the cell growth of *Arthrobacter globiformis* in plates of Luria–Bertani agar and MSM (Minimal Salt Medium) agar [8]. Studies quantifying the impact on the biodegradation rate of pesticides in soils or water by the presence of other pesticides are not frequent [9–11]; anyhow, several possibilities for assessing pesticide effects can be explored. One of them is measuring the impact exerted by some frequently used pesticides on the biodegradation rate of another, studying a single bacterial strain.

The genus *Burkholderia* contains an increasing number of species that are found almost everywhere in the environment [12], which are exploited for biocontrol, bioremediation, and plant growth promotion purposes [13]. The functional diversity and ecological versatility of *Burkholderia* species are attributed to the huge coding capacity of their large multireplicon genomes containing an array of insertion sequences, which promote genomic plasticity and general adaptability [13, 14]. Members of this genus have been used in various biotechnological applications, including bioremediation of a great number of recalcitrant xenobiotic compounds [15].

For these reasons, a strain of the fast-growing bacterium *Burkholderia* sp. was used to investigate the effect of three herbicides, propanil, linuron, and dicamba on 2,4-dichlorophenoxyacetic acid (2,4-D) biodegradation kinetics. It was selected as a model because it is a genus potentially usable in soil and water bioremediation, and by its capability to degrade organochlorine herbicides [16–19].

2,4-D is a common soil contaminant; as occurs with many soil pollutants, the 2,4-D concentrations found in contaminated soils depend on many factors such as weather, physicochemical properties of soil, soil microbiota, soil depth, time elapsed after 2,4-D application, irrigation, rainfall, and applied doses [20]. However, in studies made under real field conditions in Roorkee, India [20] and in soils near Tecolutla River in Veracruz, Mexico [21], the detected levels of 2,4-D varied from 0.3 to 0.11 mg/Kg, respectively.

2,4-D and propanil are ecotoxic compounds; both are suspected endocrine disruptors and potential groundwater contaminants (http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC33440; http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC34265). Linuron and dicamba are also ecotoxic compounds considered developmental or reproductive toxins and potential groundwater contaminants. For these reasons, both pesticides are considered chemicals of special concern by the Pesticide Action Network (http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC32874; http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC32871).

To describe the microbial degradation of organic compounds in soils and bioreactors, the batch experimental data are usually described by first order models [22–24]. Although this approach could be applied to compounds not supporting growth or to abiotic pesticide degradation kinetics, when an organic compound is microbiologically degraded, frequently the kinetic

behavior diverges from first or pseudo-first kinetics, and in many biodegradation experiments, a sigmoidal decay in the compound concentration is observed [25–31]. In this case, the peak degradation rate is reached at a value corresponding to the inflection point of the curve. This sigmoidal degradation behavior can be represented by a logistic model. Visualization of the kinetic response from coarse degradation data is facilitated when this information is carefully processed and mathematically analyzed. Using this approach, in this work, the differentiated effect of some pesticides on the biodegradation of another was clearly observed, indicating that the permanence of a pesticide in the environment can be prolonged when the microbiota is adversely affected by the application of pesticide mixtures.

To study the biodegradation kinetics of an organic compound by pure microbial strains or by microbial consortia the specific biodegradation model is commonly expressed as a function of the reactant concentration ($q_s = f[s]$). This model can be experimentally obtained in batch culture by measuring the specific degradation rate ($q_s = -(ds/dt) (1/x)$) determined at the initial concentration (s_0) of the compound and at a cell concentration value (x). Usually, to evaluate the kinetic behavior of $q_s[s]$, several values (s_0) of the compound are tested to estimate the corresponding q_s values [32, 33].

However, when a sigmoidal decay of the compound occurs, the maximum volumetric degradation rate of the substrate [$(ds/dt)_{\text{peak}}$] is not obtained at the initial concentration s_0 . In this case, the peak degradation rate is reached at a value $s[t]$ corresponding to the inflection point of the curve. This sigmoidal degradation behavior can be represented by several empirical models [34]. The kinetic data obtained from batch cultures were processed and analyzed by fitting the experimental values $s[t]$ to a logistic function and valuing their derivatives to delimit the peak values of (ds/dt) . Using this approach, the influence caused by the herbicides linuron, propanil, and dicamba on 2,4-D biodegradation kinetics of *Burkholderia* sp. was assessed.

2 Materials and methods

2.1 Chemicals

Standards of propanil (N-(3,4-dichlorophenyl) propanamide) purity >95%, linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea) (99.5% purity), 3,6-dichloro-2-methoxybenzoic acid (dicamba), purity >95%, and 2,4-D, purity >95% were from ChemService, PA, USA. The solvents used for HPLC were purchased from J. T. Baker.

2.2 Microorganism

The bacterial strain used in this work was isolated from soil samples treated with herbicides in different areas of farmland in the municipality of Tenampa, located in the mountainous central region of the State of Veracruz, Mexico. This bacterium can grow using 2,4-D as the sole carbon and energy source, meaning that it possesses the genetic information encoding the enzymes involved in the 2,4-D degradation pathway. Using PCR amplification, sequencing, and comparison of its 16S rDNA

Figure 1. Phylogenetic tree of *Burkholderia* sp.

with the NCBI GenBank, the isolated bacteria was identified as *Burkholderia* sp. (accession number CP000150.1, with 96% of similarity). In Fig. 1, the phylogenetic tree of *Burkholderia* sp. is shown.

The plasmids harbored by this strain were isolated; one of about 9.5 Kb and another of about 23 Kb, which resulted positive for the *tfdA* gene, encoding the enzyme responsible for the first step in 2,4-D biodegradation pathway [35]. The presence of this gene was first reported by Bhat et al. [36] for *Pseudomonas cepacia* CSV40 in a 90-Kb plasmid. Frequently, by integration and excision of 2,4-D degradative plasmids the genetic information is acquired by natural intergeneric transfer between bacteria [37]; thus, it could be presumed that the 2,4-D degradation pathway of the strain of *Burkholderia* sp. is similar; as that reported for *P.cepacia* CSV40. In the complete degradation pathway proposed by Bhat et al. [36], 2,4-dichlorophenol is generated as the first catabolic intermediate for the degradation of 2,4-D.

2.3 Culture medium

The basal medium (BM) used through this study consisted of (g/L): KH_2PO_4 (1.36), Na_2HPO_4 (1.4), $(\text{NH}_4)_2\text{SO}_4$ (0.30), and $\text{Mg}(\text{SO}_4) \cdot 7\text{H}_2\text{O}$ (0.050). After autoclaving the medium at 121°C for 15 min, 2,4-D was added to reach the required concentrations. When needed, the herbicides dicamba, propanil, and linuron were added (Tables 1 and 2).

2.4 Determination of cell mass

In the different experiments, an aliquot of a concentrated cell suspension of *Burkholderia* sp. grown on BM plus 2,4-D (100 mg/L) was inoculated in 250 mL flasks containing 80 mL of BM containing 2,4-D alone, or mixed with another herbicide (Tables 1 and 2). Knowing the concentration of the cell suspension, the initial cell concentration x_0 (inoculum) was estimated. At the end of each batch culture, after sample filtering, the cell concentration was determined by weighting the cells retained in Whatman GF/F, 0.7 μm filters.

2.5 Determination of the herbicides 2,4-D, propanil, linuron, and dicamba

The herbicides were evaluated by liquid chromatography, using an HPLC 1260 Infinity, from Agilent Technologies, equipped with a diode arrangement detector and a Zorbax SB C18 column (dimensions 150 $\times$ 4.6 mm, with 5 μm particle size). A method to separate and detect the four herbicides was developed. The mobile phase was a mixture 30:70 of acetic acid (1%) in water and acetic acid (1%) in ACN. The flow rate was 1.0 mL/min. The retention times for the herbicides were: dicamba, 1.94 min; 2,4-D, 2.16 min; propanil, 2.72 min, and linuron, 2.96 min. By UV absorbance at 283 nm, dicamba and 2,4-D were quantified; while propanil and linuron were determined at 250 nm.

Table 1. Experimental conditions and results of 2,4-D biodegradation after 24 h of batch culture of *Burkholderia* sp. grown on basal medium plus 2,4-D

Experiment	Initial 2,4-D concentration (mg/L)	Final 2,4-D concentration (mg _{2,4-D} /L)	2,4-D Removal efficiency (%)	Final cell concentration (mg _{cell} /L)	Overall cell growth yield (mg _{cell} /g _{2,4-D})
D13	13.51 $\pm$ 1.22	4.61 $\pm$ 0.78	65.87 $\pm$ 8.93	6.62 $\pm$ 1.12	0.42
D34	34.36 $\pm$ 1.07	4.34 $\pm$ 0.28	87.40 $\pm$ 1.20	17.54 $\pm$ 1.21	0.48
D70	69.66 $\pm$ 2.02	5.02 $\pm$ 0.57	92.79 $\pm$ 1.03	34.07 $\pm$ 2.82	0.49
D100	98.10 $\pm$ 1.48	6.22 $\pm$ 1.43	93.59 $\pm$ 1.55	46.14 $\pm$ 3.57	0.47
D120	123.08 $\pm$ 1.56	9.84 $\pm$ 1.75	92.00 $\pm$ 1.52	57.03 $\pm$ 3.93	0.48
D150	147.38 $\pm$ 2.16	10.79 $\pm$ 2.45	92.58 $\pm$ 1.77	67.81 $\pm$ 4.60	0.47

In the experimental series, the initial cell concentration (x_0) was 2.75 mg/L.

Table 2. Experimental conditions of batch cultures to evaluate the 2,4-D degradation kinetics in batch culture

Series 2,4-D-L	Linuron L_0 (mg/L)	Series 2,4-D-P	Propanil P_0 (mg/L)	Series 2,4-D-D	Dicamba D_0 (mg/L)
D100-L2	2.29 ± 0.18	D100-P10	8.50 ± 0.88	D100-D5	4.80 ± 1.00
D100-L4	4.45 ± 0.47	D100-P20	17.52 ± 1.63	D100-D10	9.60 ± 1.38
D100-L7	7.77 ± 0.29	D100-P25	24.88 ± 1.39	D100-D15	15.83 ± 1.10
D100-L10	10.86 ± 0.09	D100-P35	35.46 ± 3.15	D100-D20	20.95 ± 2.37
D100-L12	11.37 ± 0.10	D100-P40	40.87 ± 4.37	D100-D25	25.22 ± 1.18

In all experiments, the initial 2,4-D concentration (s_0) was 119.215 ± 7.308 mg/L. In this series, the estimated initial cell concentration (x_0) was 2.75 mg/L. The concentrations of linuron (L_0), propanil (P_0), and dicamba (D_0) varied as shown.

2.6 Biodegradation experiments in batch culture

Since the genetic information for degradation of 2,4-D is contained in a plasmid in *Burkholderia* sp., to ensure that the bacterium did not lose this information it was always cultured in selective medium containing 2,4-D as the sole carbon and energy source. All experiments were done in 250 mL Erlenmeyer flasks containing 80 mL of BM, according to the experimental conditions shown in Tables 1 and 2. All experiments were carried out in intermittent orbital shakers (100 rpm), at $26 \pm 3^\circ\text{C}$ for 24 h. All determinations were made in duplicated flasks.

2.7 Experimental data analysis

The program Mathematica 11.0 (Wolfram Research, USA) was used for the analysis and processing of experimental data. The sigmoidal decay of 2,4-D concentration (s) was represented by a logistic function (Eq. (1)).

$$s[t] = s_0 - \frac{a}{(1 + e^{b-ct})} \quad (1)$$

By fitting the raw data of the temporal variation of (s) to the logistic function $s[t]$, the values of the constants a , b , and c for each batch culture are determined; then, deriving the Eq. (1), the function (ds/dt) is obtained (Eq. (2)).

$$\left(\frac{ds}{dt}\right) = -\frac{ac e^{b-ct}}{(1 + e^{b-ct})^2} \quad (2)$$

The estimated peak value of (ds/dt) obtained at the inflection point (t_i) of the degradation curve corresponds to the particular values of the substrate (s_i) and cell mass (x_i) concentrations found at (t_i).

When the temporal trajectory of the cell mass concentration (x) is unknown, and the only measured values are at the beginning (x_0) and end (x_F) of the batch process, the overall growth yield Y_{XS} of cells grown on 2,4-D can be estimated in batch cultures as $Y_{XS} = \Delta x / \Delta s$. The values of Δx and Δs correspond, respectively, to the change in cell mass ($x_F - x_0$) and compound ($s_0 - s_F$) concentrations. Knowing the values of the constants a , b , and c , and assuming constant values of the cell growth yield Y_{XS} for each batch culture, the theoretical changes of cell concentration $x[t]$ can be easily estimated using the Eq. [3]:

$$\begin{aligned} x[t] &= x_0 + Y_{XS}(s_0 - s[t]) \\ &= x_0 + Y_{XS}\left(s_0 - \left(s_0 - \frac{a}{(1 + e^{b-ct})}\right)\right) \end{aligned} \quad (3)$$

By derivation of Eq. (3), the Eq. (4) is obtained:

$$\left(\frac{dx}{dt}\right) = Y_{XS} \frac{ac e^{b-ct}}{(1 + e^{b-ct})^2} \quad (4)$$

By relating the values (ds/dt) and (dx/dt) to the cell concentration $x[t]$ (Eqs. (5) and (6)), the specific biodegradation rates $q_s[t]$ and cell growth rates $\mu[t] = (q_s)Y_{XS}$ can be estimated; thus the peak values of $q_s[t]$ and $\mu[t]$ can be determined.

$$q_s[t] = -\left(\frac{ds}{dt}\right)\left(\frac{1}{x}\right) = \frac{ace^{b+ct}}{(e^b + e^{ct})(e^b x_0 + e^{ct}(x_0 + aY_{XS}))} \quad (5)$$

$$\mu[t] = \left(\frac{dx}{dt}\right)\left(\frac{1}{x}\right) = \frac{ace^{b+ct}Y_{XS}}{(e^b + e^{ct})(e^b x_0 + e^{ct}(x_0 + aY_{XS}))} \quad (6)$$

The analysis of the parametric plots of $q_s[t] = f_1(s[t])$ and $\mu[t] = f_2(s[t])$, describes an accurate kinetic behavior of the microorganism. The data of the parametric profiles can now be adjusted to the biokinetic models $q_s = f_1(s)$ and $\mu = f_2(s)$. The model profile is indicative of the effect of 2,4-D on the growth and degradation kinetics of *Burkholderia* sp.

3 Results and discussion

3.1 Biodegradation kinetics of 2,4-D in batch culture

In the experimental series, the initial 2,4-D concentrations tested (s_0), varied from 13.51 ± 1.22 to 147.38 ± 2.16 mg/L, with an initial cell concentration (x_0) of 2.8 ± 0.32 mg/L. These values, the 2,4-D and the final cell concentrations obtained after 24 h, together with the estimated cell growth yields (Y_{XS}), are shown in Table 1. The average Y_{XS} value of the experimental series was 0.468 ± 0.024 g_{cd}/(g_{2,4-D}). Figure 2 shows the degradation profiles of 2,4-D in batch cultures at the initial concentrations tested. The values of the constants of the logistic function obtained by fitting the 2,4-D degradation data for batch experiments D13 to D150 are presented in Table 3. These values were used to obtain the curves shown in Fig. 2, the peak values of $q_s[t]$ and $\mu[t]$ at their corresponding values of $s[t]$ and $x[t]$ (Table 4); and finally, to estimate the behavior of $q_s = f_1(s)$ and $\mu = f_2(s)$ using Eqs. (5) and (6) (Fig. 3).

It was evident that at the concentrations tested, 2,4-D was not inhibitory to *Burkholderia* sp.; thus, hyperbolic Monod models

Figure 2. Degradation profiles of 2,4-D in batch culture at various initial concentrations of the herbicide, in milligram per liter. Empty diamonds (13.51 ± 1.22), empty triangles (34.36 ± 1.07), filled squares (69.66 ± 2.02), filled circles (98.10 ± 1.48), empty squares (123.08 ± 1.56), empty circles (147.38 ± 2.16).

Table 3. Initial values of 2,4-D concentrations (s_0), and values of the logistic function constants (a , b , c) and regression coefficient of the batch degradation data of 2,4-D, fitted to the logistic model [1]

Experiment	s_0 (mg/L)	a	b	C	R^2
D13	13.51 ± 1.22	11.59 ± 0.89	2.37 ± 0.42	0.26 ± 0.05	0.99
D34	34.36 ± 1.07	31.90 ± 2.24	2.79 ± 0.38	0.24 ± 0.04	0.99
D70	69.66 ± 2.02	70.23 ± 6.77	3.71 ± 0.57	0.25 ± 0.05	0.99
D100	98.10 ± 3.48	99.96 ± 4.39	4.30 ± 0.28	0.27 ± 0.02	0.99
D120	123.08 ± 3.56	133.75 ± 5.92	3.94 ± 0.16	0.23 ± 0.02	0.99
D150	147.38 ± 2.16	212.48 ± 39.35	4.59 ± 0.16	0.21 ± 0.02	0.99

Table 4. Instantaneous values of $s[t]$ and $x[t]$ corresponding to the peak values of the specific degradation rates $q_s[t]$ of 2,4-D, and the specific growth rates $\mu[t]$ of *Burkholderia* sp. growing on 2,4-D

Experiment	$s[t]$ (mg/L)	$x[t]$ (mg/L)	Peak $q_s[t]$ (mg _{2,4-D} mg _{CEL} ⁻¹ h ⁻¹)	Peak $\mu[t]$ (h ⁻¹)
D13	9.269	4.707	0.148 ± 0.0108	0.070 ± 0.0051
D34	25.717	6.780	0.223 ± 0.0122	0.105 ± 0.0056
D70	55.028	9.601	0.301 ± 0.0110	0.142 ± 0.0050
D100	87.577	7.660	0.331 ± 0.0101	0.156 ± 0.0045
D120	101.35	12.944	0.323 ± 0.0098	0.152 ± 0.0044
D150	118.75	16.190	0.321 ± 0.0095	0.151 ± 0.0042

were assumed for cell growth rate and biodegradation rate of 2,4-D (Eqs. (7) and (8))

$$\mu = \frac{\mu_{\max} s}{K_s + s} \quad (7)$$

$$q_s = \frac{q_{s\max} s}{K_{sb} + s} \quad (8)$$

The estimated values of the kinetic constants $\mu_{\max}$ and $q_{s\max}$ were $0.176 \pm 0.003/\text{h}$ and $0.369 \pm 0.007 \text{ mg}_{2,4\text{-D}} \text{ g}_{\text{cel}}^{-1} \text{ h}^{-1}$, respectively. The values of K_s and K_{sb} were 15.287 ± 1.288 and $14.808 \pm 1.279 \text{ mg}_{2,4\text{-D}}/\text{L}$, respectively. In both cases, the values of the regression coefficients R^2 were about 0.99.

Some information in the literature about the growth or biodegradation rates of *Burkholderia* strains growing on

xenobiotics was found. The value of the term $\mu_{\max}$ reported for free cells of *Burkholderia cepacia* growing in EDTA was comparatively small, $\mu_{\max} = 0.206/\text{d} = 8.56 \times 10^{-3}/\text{h}$, with a relatively high K_s value of 80 mg/L [38]. In the case of biphenyl degradation kinetics by *Burkholderia xenovorans* in a two-phase partitioning bioreactor, the estimated values were $Y_{XS} = 0.48 \text{ g biomass per gram biphenyl}$, $\mu_{\max} = 0.25/\text{h}$ and $K_s = 0.1 \text{ mg/L}$. Although the K_s value was extremely low (hydrophobic substrate), the estimated values were considered characteristic for bacteria [39]. On the other hand, the value reported for *B. cepacia*, grown on LBG-rich medium was $\mu_{\max} = 0.32 \pm 0.005/\text{h}$ [40]. Clearly, the growth rate obtained in rich medium was higher than the value obtained for the strain of *Burkholderia* sp. growing on 2,4-D as its sole carbon source. Additionally, when the physiological and cellular responses of batch-cultivated *B. cepacia* YK-2 on 2,4-D was studied [41], no stress was observed in bacteria grown at 30°C in a salts medium containing 2.25 mM of 2,4-D. Only at higher concentrations ($5\text{--}7 \text{ mM}$, 2,4-D), an effect on cell viability was observed in this strain.

3.2 Biodegradation kinetics of 2,4-D in the presence of linuron, propanil, and dicamba

Since no inhibitory effect of 2,4-D was observed at the tested concentrations, a BM containing an initial 2,4-D concentration (s_0) of $119.215 \pm 7.308 \text{ mg/L}$ was used in the following experiments to measure the effect caused by the herbicides linuron, propanil,

Figure 3. Experimental values of the specific cell growth rate μ (filled circles) and specific consumption rate q_s (empty circles) of *Burkholderia* sp. grown at different concentrations of 2,4-D, corresponding to the instantaneous peak values of μ or q_s in batch cultures. Solid lines represent the profile described by Eqs. [7] and [8], using the estimated values of the kinetic constants $\mu_{\max}$, $q_{s\max}$, K_s , and K_{sb} . Dotted lines represent the models' profiles using their respective SD values.

Table 5. Values of the constants (a , b , c) and regression coefficient (R^2) of the batch degradation data of 2,4-D fitted to the logistic model [1]

Model constants	Linuron ($s_{0_LIN} = 11.37$ mg/L)	Control ($s_{0_LIN} = 0.0$ mg/L)	Dicamba ($s_{0_DIC} = 25.22$ mg/L)	Control ($s_{0_DIC} = 0.0$ mg/L)	Propanil ($s_{0_PRP} = 40.87$ mg/L)	Control ($s_{0_PRP} = 0.0$ mg/L)
a	64.41 ± 17.30	119.21 ± 7.30	61.686 ± 2.92	100.90 ± 2.67	124.15 ± 7.46	119.669 ± 10.19
b	5.08 ± 0.94	4.78 ± 0.17	6.953 ± 1.15	7.60 ± 0.73	3.87 ± 0.47	3.454 ± 0.652
c	0.41 ± 0.11	0.38 ± 0.02	0.546 ± 0.092	0.56 ± 0.05	0.28 ± 0.04	0.299 ± 0.063
R^2	0.998	0.997	0.997	0.994	0.994	0.992

Values obtained in the presence of linuron, dicamba, and propanil. In all cases, the initial 2,4-D concentration was 119.215 ± 7.308 , and the inoculum concentration of *Burkholderia* was $x_0 = 2.75$ mg/L.

and dicamba on 2,4-D biodegradation kinetics of *Burkholderia* sp. in batch cultures. Different concentrations of each herbicide were added to the BM (Table 2). For these cases, and for batch cultures of *Burkholderia* sp. grown on BM containing only 2,4-D (control experiments), the experimental data were fitted to a logistic model (equation (1)), finding the model constants (Table 5). These values were used in Eq. (2) to obtain the profiles of the volumetric biodegradation rates along batch cultures. Figures 4 and 5, and 6 show that the most negative impacts were observed at the higher concentrations of linuron (11.37 ± 0.10 mg/L) and dicamba (25.22 ± 1.18 mg/L) tested. With propanil, even at the highest concentration (40.87 ± 4.37 mg/L) probed, no effect on 2,4-D degradation was observed.

In the context of the available literature, numerous references about the impact of some agents on the microbial activity in soils are reported [42,43]. Also, information about the toxic effect of metals on the biodegradation capability of pesticides can be found [44]. But, evidence about the inhibition mechanisms of pesticides on microorganisms is rarely reported. In the case of the sulfonyleurea herbicides, metsulfuron methyl, chlorsulfuron,

Figure 4. Effect of linuron on the instantaneous biodegradation rate of 2,4-D in batch culture. Growth on 2,4-D (solid line), growth on 2,4-D plus linuron (dotted line). The difference in peak values represents a decay of 42.26% in the top instantaneous degradation rate of 2,4-D.

Figure 5. Effect of dicamba on the instantaneous biodegradation rate of 2,4-D in batch culture. Growth on 2,4-D (solid line), growth on 2,4-D plus dicamba (dotted line). The difference in peak values represents a decay of 40.95% in the top instantaneous degradation rate of 2,4-D.

Figure 6. Effect of propanil on the instantaneous biodegradation rate of 2,4-D in batch culture. Growth on 2,4-D (solid line), growth on 2,4-D plus propanil (dotted line). The difference in peak values represents a decay of 1.8% in the top instantaneous degradation rate of 2,4-D.

and thifensulfuron methyl, it was observed a growth-reducing effect on several *Pseudomonas* strains. The effect was attributed to an inhibition of the enzyme acetolactate synthase, which is involved in the bacterial synthesis of the branched amino acids valine, leucine, and isoleucine [45].

Although reports about the toxic effects of pesticides on the biodegradation capability of microorganisms can be found [46], an approach using the kinetic analysis of experimental results of biodegradation is rarely described. While this is a regular practice in bioprocess modeling, the analysis and handling of kinetic information are not frequent in studies of biodegradation of organic compounds. However, this practice could be advantageous in biodegradation studies because it could render information not easily observed from raw numbers.

4 Concluding remarks

Initially, the possible inhibitory effect of the substrate 2,4-D on *Burkholderia* sp. was assessed. The kinetic analysis reveals that at the 2,4-D concentrations used, no inhibitory effect on this bacterial strain was observed. Using the methodology mentioned above, the impact caused by the herbicides linuron, propanil, and dicamba on 2,4-D biodegradation kinetics of *Burkholderia* sp. was measured in batch cultures. At the concentrations probed, the tested herbicides were not degraded by this strain. However, adverse effects of linuron and dicamba on *Burkholderia* sp. degradation kinetics of 2,4-D were observed in batch cultures.

Because the mathematical methodology is a powerful tool to analyze the stoichiometry and kinetics of microbial processes [47], it was used to study in detail the effect of three pesticides on the biodegradation kinetics of 2,4-D by *Burkholderia* sp. Although data processing involves a bit of work, the mathematical analysis of biokinetic data provides information that is not always perceived by mere observation of the raw data. In this case, it was observed the differentiated effect of some pesticides on the biodegradation of another, indicating that the permanence of a pesticide in the environment can be prolonged when the microbiota is kinetically affected by the application of pesticide mixtures.

Finally, searching for information about inhibition of *Burkholderia* by linuron, dicamba, or propanil, or any known mechanism of inhibition of 2,4-D catabolism by these pesticides, we were unable to find evidence. Thus, to our knowledge, this is the first report about inhibition of linuron and dicamba on the 2,4-D catabolism by the genus *Burkholderia*.

Practical application

This paper presents a methodology for the deep analysis of batch biodegradation data using the program Mathematica 11.0 (Wolfram Research). The method consists of fitting the temporary changes in substrate concentration to sigmoidal models ($s[t]$), and its subsequent derivation to obtain the profiles of the volumetric biodegradation rates $[ds/dt]$ of the compound. The analysis of the biodegradation profiles renders information about the impact of an imposed environmental factor on the physiological state of the microorganism tested.

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