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The inhibitory biodegradation effects of the pesticide 2,4-D when chemically anchored on silica gel

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Abstract

The pesticide 2,4-D (2,4-dichlolorophenoxyacetic acid) was immobilized on a silica gel surface to yield a new compound designed SiD. The free and anchored pesticide was applied to typical Brazilian agricultural soils and the biodegradation caused by microbial activity was followed by microcalorimetry. The activity of the microorganisms on soil samples under 34.8% of controlled humidity at 298.15 ± 0.02 K was determined through power–time curves. The recorded curves for increasing amounts of the 2,4-D active principle varied from zero to 6.67 mg per gram of soil. The increased amounts of both free and immobilized pesticide caused an enhancement of the original thermal effect. The calorimetric data demonstrated that the anchored pesticide (SiD) presents a much lower biodegradation with microbial activity, when compared with the free pesticide (2,4-D).

Keywords: 2,4-D; Silica; Calorimetry; Biodegradation; Immobilization

1. Introduction

Some pesticides have been shown to be suitable carbon sources for microorganism development. In such process, the rapid biodegradation induces its use in much larger amounts than those expected due to partial degradation do. Then, an additional consumption often occurs prior to reaching the final step [1,2]. Depending on the method of application and climatic conditions, more than 90% of conventionally applied doses are consumed without attaining the desirable biological response at the precise time, indicating the requirement for increased amounts to be applied [3].

The results of these inefficiencies cause an excessive non-specific reactions and induce periodic applications of such agents. In the normal course of social development, many areas are affected by indiscriminate application and much attention is now given to reduce the pollution pressure on the environment. Meanwhile, the appearance of a certain degree of pollution is directly related to imperfect application of chemicals to an ecosystem [4]. In attempting to minimize such condition, controlled release pesticide formulations have been developed [1–3]. Thus, one of the promising methods consists in anchoring the pesticide onto a silica gel surface, to explore the new surface as a useful source and to take advantage of this controlled release properties. Some pesticide compounds presenting this tendency have previously been studied [4–6].

The development of the new modified surface containing a pesticide is an important feature to be explored due to the fact that this class of compounds becomes an integral component of modern agricultural systems [7]. Among the pesticides, 2,4-D (2,4-dichlolorophenoxyacetic acid) is a herbicide that

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presents a rapid biodegradation and is extensively used in Brazilian agriculture, being mainly applied on sugarcane plantations and other important crops [8].

The biodegradation of free and immobilized pesticide on soil can be followed through microcalorimetry, which is a useful tool to extract information about such systems. This method has the advantage of being specific and demands only a knowledge of the initial and final energetic states of the system, and is also independent of the organisms and their reaction pathways [9,10].

The present investigation reports the synthetic methodology applied to the pesticide 2,4-D anchored on silica gel surface. The biodegradation of both free and immobilized 2,4-D by microorganisms on red Latosol soil was followed microcalorimetrically.

2. Materials and methods

2.1. Reagents

All chemicals used such as glucose and ammonium sulfate were reagent grade. The pesticide 2,4-D (Sigma) and the silylant agent 3-trimethoxysilylpropylamine (APTS) (Fluka) were used without purification. Silica gel (Merck) was activated in a stream of dry nitrogen and the specific area of $387.1 \pm 22.9\,m^2\,g^{-1}$ was determined by the BET method [11].

2.2. Soil samples

The origin, sampling and soil storage used a previously described procedure [12,13]. For organic matter determination, the dry soil was placed in a muffle furnace to follow the decrease in mass at a temperature of 823 K for 24 h, as recommended [14]. Under these conditions organic matter is combusted, to leave only the inorganic component of the soil. These results, as well as those from other determinations, represent the mean of triplicate runs.

Carbon, nitrogen, hydrogen and sulfur contents of soil were determined by elemental analysis on a Fisons Instruments CHNS-O model 1110 Elemental Analyzer.

Measurements involving pH were obtained by means of a Digimed DMPH-2 instrument, by using 2.0 g of soil sample suspended in $5.0\,cm^3$ of $1.0\,mol\,dm^{-3}$ calcium chloride in a proportion of 1:2.5 for soil:solution [12,13].

Microbial activity is related to the inherent physical chemical properties of the soil such as pH, organic matter content and elemental constitution. The soil assayed contained $5.50 \pm 0.22\%$ of organic matter, 5.22 ± 0.11, 0.92 ± 0.04 and $2.73 \pm 0.14\%$ of carbon, nitrogen and hydrogen, respectively, and gave a pH value of 5.8 ± 0.1 [12].

2.3. Organofunctionalization

A sample of 45.0 g of activated silica gel, suspended in $100.0\,cm^3$ of dry xylene was refluxed and mechanically stirred under dry nitrogen for 1 h. To this suspension, $15.0\,cm^3$ of APTS was added dropwise. The mixture was refluxed for another 72 h and the solid was filtered and washed with water and ethanol [11,15]. This immobilized surface designated SiAPTS was dried in vacuum at room temperature for several hours.

2.4. Immobilization of 2,4-D

A sample of 5.0 g of the previously organofunctionalized silica was suspended in $100.0\,cm^3$ of dry xylene, refluxed as before, while being mechanically stirred, with 2.0 g of 2,4-D for 72 h under dry nitrogen [11,16]. The solid containing the immobilized pesticide, named SiD, was filtered, the excess of diuron was eliminated by washing with water and ethanol and the solid was dried in vacuum at room temperature.

2.5. Characterization

Elemental analysis, surface area, infrared spectra and nuclear magnetic resonance spectra of ^{29}Si and ^{13}C nuclei characterized the silica surfaces, as before [11].

2.6. Microcalorimetry

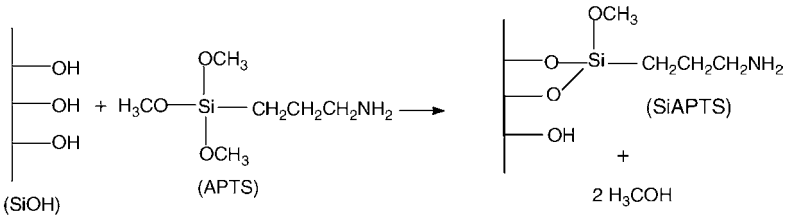
A heat-flow microcalorimeter, LKB 2277 Thermal Activity Monitor, was used for all measurements, as previously described [12,13]. The thermal effect was obtained using $5.0\,cm^3$ stainless steel ampoules, with Teflon sealing discs to avoid evaporation inside the apparatus, at 298.15 ± 0.02 K [8,12]. All thermal

effect determinations were performed by triplicate in ampoules charged with 1.50 g of soil and 0.80 cm^3 of water without nutrient [8]. The toxic effect of 2,4-D on microbiota was followed by using nutrient solutions with increasing amounts of the herbicide and SiD, varying from zero to 6.67 mg per gram of soil of the active principle, 2,4-D [8]. Under these conditions, the moisture in the experimental system was controlled at 34.8%, which induces the highest microbial activity, as reported before [17]. The thermal effect associated with nutrient degradation was recorded as a function of time. The final value was calculated by comparing the integrated area of the power–time curve for each experimental determination [12].

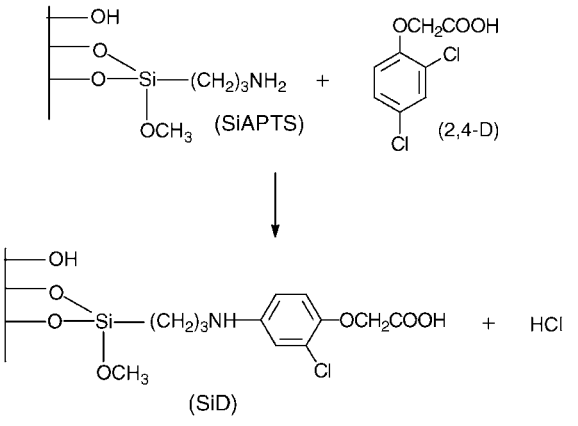
3. Results and discussion

The degree of the precursor SiAPTS immobilization was calculated from the nitrogen analysis of 1.44%, which gave 1.16 mmol of this element per gram of silica. This value confirmed that the silylant agent APTS reacts with the activated silica, to yield the organic molecule covalently bonded to the surface. A qualitative test gave a clear evidence of the presence of unreacted chlorine atoms on the immobilized herbicide molecule, which is in agreement with the success of immobilization. This observed result strongly suggested that the reaction of 2,4-D with SiAPTS occurred through the reaction of only one chloro atom of the pesticide bonded on the unsaturated ring structure, with an amine group of the silylant agent. On the other hand, based on the nitrogen to carbon ratio obtained from the elemental analysis for the SiD surface, 1.05 mmol of 2,4-D was anchored per gram of silica gel, as observed before [11]. For this final immobilized compound, the specific area was determined as $225.5 \pm 18.4\ m^2\ g^{-1}$ [11].

The synthetic procedure to describe the new anchored compound can be summarized in reactions (1) and (2). The first stage of this preparation consisted in reacting the activated silica gel (SiOH) with APTS to form the SiAPTS product, as seen in reaction (1). After the precursor, the herbicide 2,4-D was immobilized onto the surface to yield the final product, SiD, as represented in reaction (2).



(1)



(2)

As observed before, for any stable system the equilibrium stage can be physically or chemically disturbed and the response reflected in a thermal effect, which may be measured calorimetrically. Thus, considering a system formed by soil sample, stimulation can be forced on the microorganism metabolism that is directly promoted by the addition of a given amount of the desired nutrients to the ecosystem [9]. Unequivocal evidence of the degradation of free and anchored pesticide by microbial metabolism can be observed through the thermal effect evolved from a sequence of assays, when 2,4-D or SiD are added to the system. Previous reports on 2,4-D showed that this pesticide is quickly biodegraded by microorganisms of red Latosol soil [12]. The previous results are very important to evaluate the biodegradation of the pesticide

immobilized onto silica gel (SiD) and to discuss its action due to its inhibitory effect on biodegradation.

The metabolism caused by microbial population in red Latosol soil in the absence and the presence of distinct amounts of the free and immobilized pesticide is collected from power–time calorimetric curves. From these data, the total thermal effect of the system, Q, was calculated by integration of the curve. From this outlined curve, the peak time values, PT, can be obtained when the maximum activity is displayed and the microbial growth rate constant, μ, is calculated from the slope of the semi-logarithm of exponential phase of the recorded curves, involving the total thermal effect, as a function of time [8]. Thus, the evolution of the thermal effect results from biodegradation with time for SiD, as shown in Fig. 1.

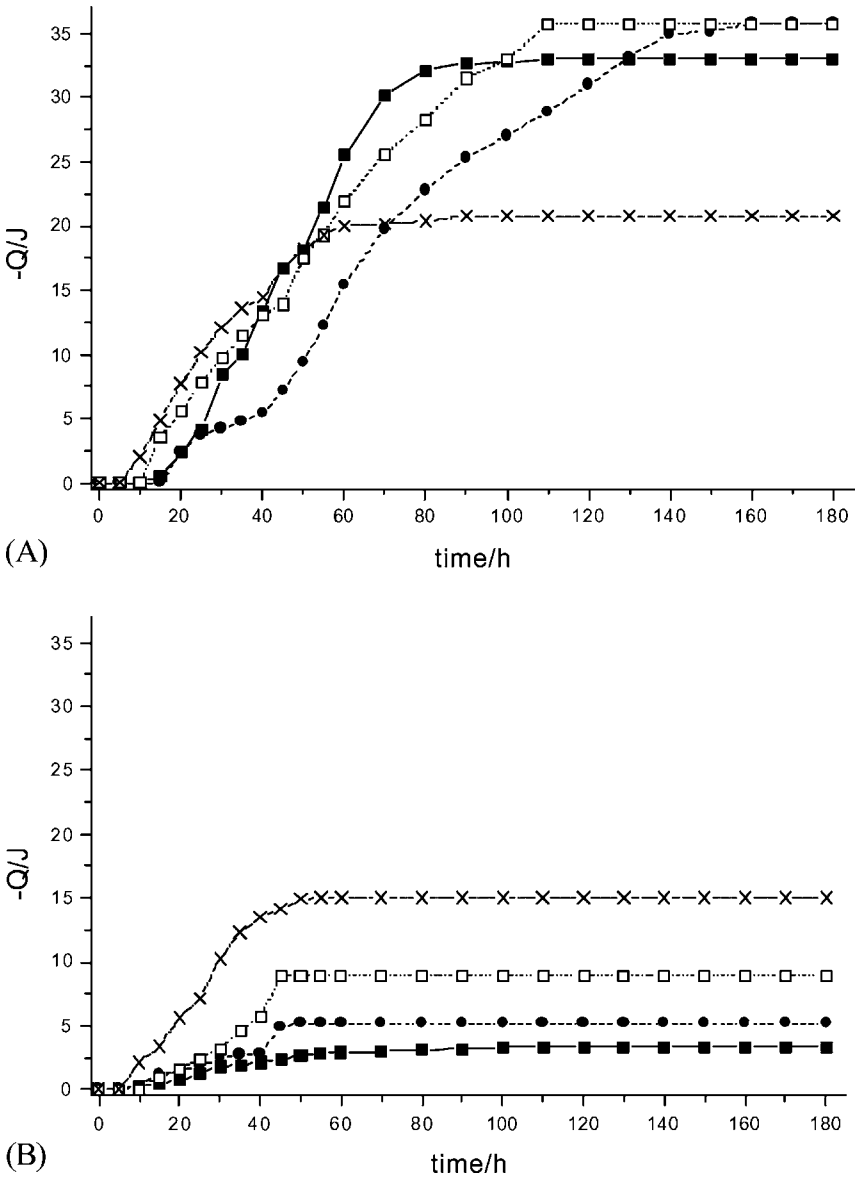


Fig. 1. Total thermal effect, Q, as a function of time, for different doses applied to soil: 0.67 (■), 1.33 (●), 2.67 (□) and 6.67 (×) of 2,4-D (A) and of SiD (B).

The direct effect caused on microbial metabolism was detected by applying increasing amounts of the immobilized pesticide SiD. However, no thermal effect was detectable from the curve without pesticide. Then, the signal displayed is only caused from SiD metabolism, wherein a unique carbon source is added to the ampoule [12]. On the other hand, the data associated with the free pesticide were previously reported [12].

The thermal effect evolved by the immobilized xenobiotic is much lower than for the free one for all doses investigated, as shown in Fig. 2. This fact shows that the anchored pesticide presented a protection against microbial attacks. Another interesting feature in Fig. 2 is related to the evolved thermal effect of SiD biodegradation, which always increases in value. However, in comparing this to the thermal effect generated by free 2,4-D, an increase up to 1.33 mg g^{-1} was observed. Above this dose, the thermal effect is nearly constant up to 4.00 mg g^{-1} and then decreases as the maximum dose was applied. This behavior can be explained by considering the toxic metabolites formed during the pesticide degradation. Thus, it is expected that the metabolite concentration promoted a toxic effect for microbial soil population, which contrasted with the lowest concentration on SiD. The lowest biodegradation in the system is a consequence of the amount of the toxic metabolites formed. These data corroborate with the fact that immobilization reduces the degradation of the anchored pesticide. On the other hand, addition of these compounds on soil dramatically affected the PT values, as shown in Fig. 3.

The PT values obtained from the metabolism of the free pesticide are higher than those of the anchored xenobiotic for all doses applied and previous results demonstrated that the increased amount of nutrients caused a delay of the metabolism [9]. Considering that the silica support protects the access of the microorganisms to the pesticide, then the available amount is lower than for free 2,4-D. Consequently, the data obtained in this experiment series is in agreement with previously reported results [9].

The change in μ values caused by different doses of free and anchored 2,4-D is illustrated in Fig. 4. These values were plotted as a function of the dose applied, which showed that μ values obtained on SiD metabolism increase with the applied doses. However, μ values related to 2,4-D metabolism present a small random change in behavior. This fact occurs due to

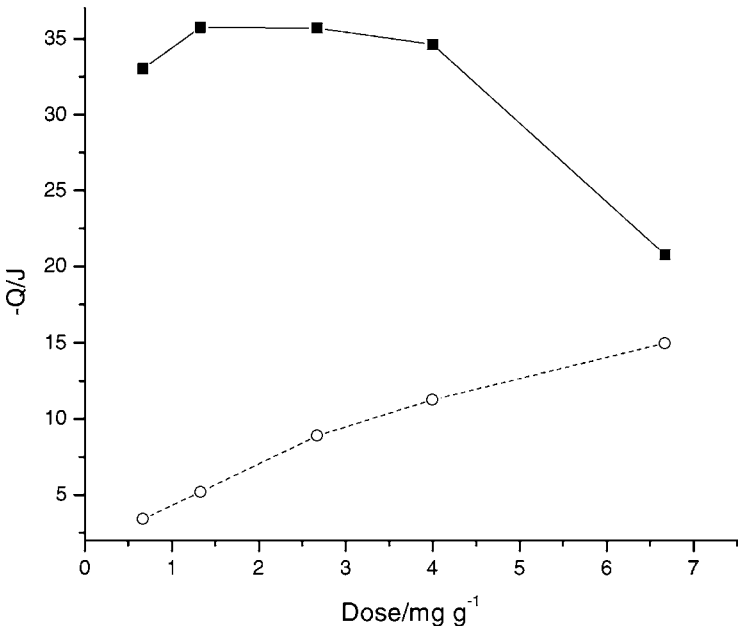


Fig. 2. The total thermal effect, Q, calculated from the metabolism of soil as a function of the 2,4-D (■) and SiD (○) applied.

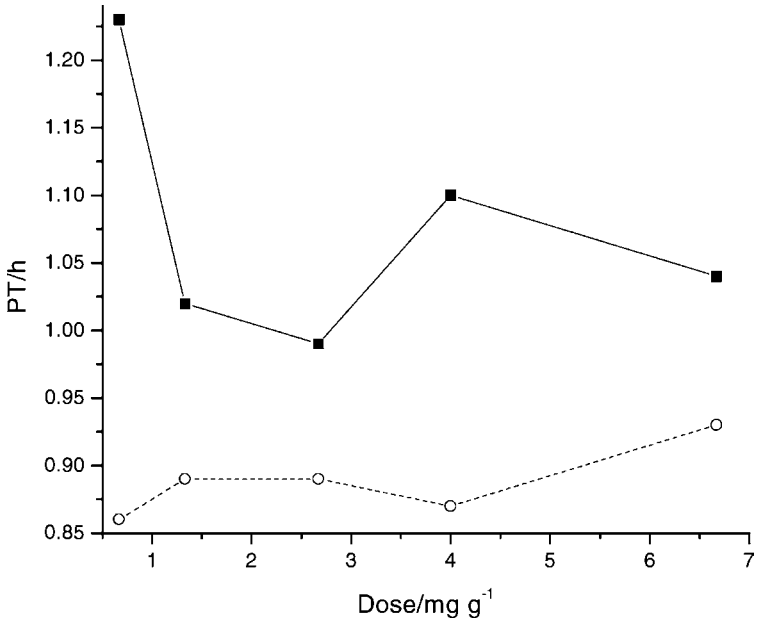


Fig. 3. The change in peak time, PT, affected by doses of 2,4-D (■) and SiD (○).

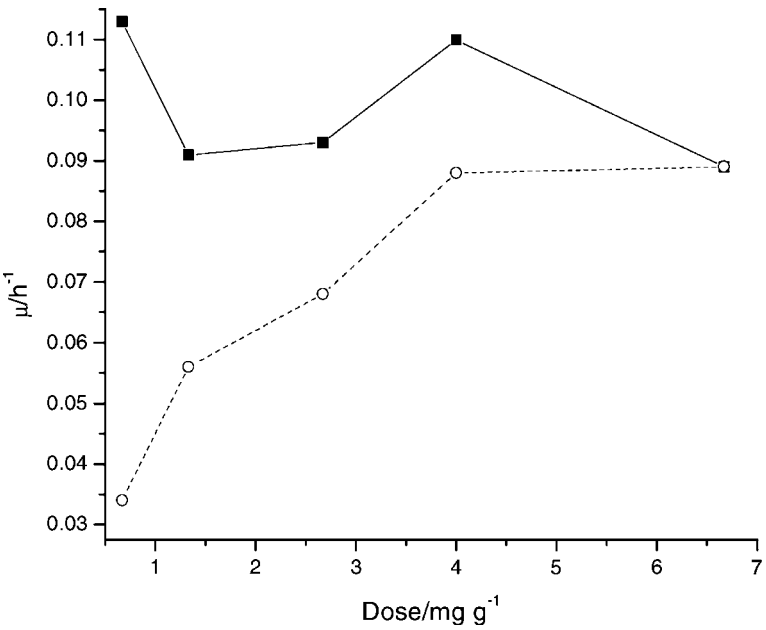


Fig. 4. The microbial growth rate, μ, as a function of 2,4-D (■) and SiD (○) dose applied.

simultaneous effects, part connected to degradation of 2,4-D and part to formation of toxic metabolites that alter the usual biodegradation process [8,12].

The immobilized pesticide SiD in the presence of red Latosol soil shows a slower degradation, when compared with free 2,4-D, demonstrating that this pesticide retains activity when immobilized. The decrease of the biodegradation rate, which has fast degradation characteristics, is an important result for the environment of the ecosystem. The decrease of pesticide biodegradation by soil microflora, may result in use of lower quantities of pesticide to obtain the desired weed elimination in crops, which is the expected action of any herbicide. Remember that the reduction of the xenobiotic amounts on the environment is a significant research priority for the 21st century [18]. Another advantage observed on SiD metabolism is the increase of the exothermic thermal effect, wherein the 2,4-D metabolism presents a decrease of the exothermic thermal effect caused by toxic effects of the metabolites formed. This result corroborates with low degradation of 2,4-D on a SiD surface, due to the fact that its metabolism does not present a decrease caused by the toxic metabolites formed in course of degradation.

4. Conclusion

The immobilization of the pesticide 2,4-D onto a silica gel surface was successfully obtained. Microcalorimetry applied to this system permits following the biodegradation of the pesticides by soil microbial population. Such immobilization onto silica gel surface protects the pesticide from microbial attacks, which causes a decrease in the biodegradation of this pesticide. This property indicates that the immobilization can be successfully applied to the development of controlled release pesticides.

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