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Biodegradation of acetanilide herbicides acetochlor and butachlor in soil

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Abstract: The biodegradation of two acetanilide herbicides, acetochlor and butachlor in soil after other environmental organic matters addition were measured during 35 days laboratory incubations. The herbicides were applied to soil alone, soil-SDBS (sodium dodecylbenzene sulfonate) mixtures and soil-HA (humic acid) mixtures. Herbicide biodegradation kinetics were compared in the different treatment. Biodegradation products of herbicides in soil alone samples were identified by GC/MS at the end of incubation. Addition of SDBS and HA to soil decreased acetochlor biodegradation, but increased butachlor biodegradation. The biodegradation half-life of acetochlor and butachlor in soil alone, soil-SDBS mixtures and soil-HA mixtures were 4.6d, 6.1d and 5.4d and 5.3d, 4.9d and 5.3d respectively. The biodegradation products were hydroxyacetochlor and 2-methyl-6-ethylaniline for acetochlor, and hydroxybutachlor and 2,6-diethylaniline for butachlor.

Keywords: acetochlor; butachlor; soil; biodegradation kinetics; degradation products

Introduction

The herbicides acetochlor [2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl) acetamide] and butachlor [2-chloro-2′, 5′-diethyl-N-(2-butaoxymethyl) acetanilide] are two important of acetanilide herbicides widely applied for corn and paddy fields respectively in China. The total amount of them has been increasing and already reach above 1.5×10^7 kg (Wang, 1999). Acetochlor was conditionally registered in March 1994 (USEPA, 1994) and has been classified as a B-2 carcinogen.

Although little is known about the behavior of acetochlor and butachlor in the ambient environment, the compounds are structurally similar to several other widely used herbicides, alachlor and metalochlor. Therefore, somebody often indirectly understands the behavior of acetochlor and butachlor through study results of other acetanilide herbicides alachlor and metachlor (Capel, 1992). These indirect understanding can not provide accurate scientific basis for environmental management of acetochlor and butachlor.

The biodegradation behavior of a pesticide in soil is a better factor in understanding of its environmental fate. There are various methods of pesticide biodegradation in literatures. These methods mainly included direct measurement of pesticide parent compounds and indirect measurement of CO_2 produced during the pesticides biodegradation under incubation (Swieten, 1995; Miller, 1997). However few information on the study of their biodegradation in soil have been conducted in China (Liu, 1990; Wang, 1999), even if at abroad such studies are only a few (Mills, 1999). Variation in soil water content, organic matter content, soil temperature (Walker, 1992) and microbial composition (Sun, 1990) may alter the biodegradation rate considerably, sometimes by as much as an order of magnitude. Such as the microbial degradation half-life of acetochlor ranges from 2 to 32d (Vaughan, 1999; Ma, 2000).

The objectives of this study were to (1) compare the biodegradation rates for acetochlor with butachlor in the same soil; (2) compare the influence of sodium dodecylbenzene sulfonate (SDBS) and humic acid (HA) on biodegradation rates of acetochlor and butachlor in soils and (3) identify the biodegradation products of acetochlor and butachlor by GC/MS analysis.

1 Materials and methods

1.1 Soil

The soil samples were taken from the surface 10 cm layer at the agricultural field of Northwestern Beijing, China that had not received previous application of the studied herbicids. The bulk soil was

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slightly air-dried, thoroughly mixed, passed through a 0.15 mm sieve. The selected soil properties analyzed included soil pH, organic carbon content(OC), cation exchange capacity(CEC) and particle-size distribution(Table 1). Soil pH was measured in a 1:1 soil-H_2O suspension with a glass pH electrode at 30 min after mixing. CEC was measured by following the procedure reported by Hendershot and Duquette (Hendershot, 1996). OC and particle-size distribution were determined by potassium dichromate oxidation method and pipette method respectively(NSICAS, 1978).

1.2 Herbicides and other chemicals

Acetochlor (85%) and butachlor (83%) were kindly supplied by Kunshan Chemical Plant of Jiangsu Province, China which were used to conduct biodegradation experiments. Acetochlor and butachlor of 99.5% produced by Monsanto Company were used as analytical reference standard. Sodium dodecylbenzene sulfonate (SDBS) of 83% was purchased from Beijing Chemical Reagents Company, China. All organic solvents were of analytical-reagent grade and purified by redistillation.

Table 1 The physical and chemical properties of studied soil

pH	OC, %	CEC, mmol/100g	Silt, %	Sandy, %	Clay, %
8.4	1.5	84.0	59	38	3

1.3 Analytical method of acetochlor and butachlor in soil

The procedures of extraction of acetochlor and butachlor from soil samples were following: 20g of soil sample from a 100 ml ground-in Erlenmeyer flask and 40 ml acetone solution of 80% were placed into 500 ml of centrifuge tube, and then extracted on ultrasonic oscillator for 10 min. The suspended solution in centrifuge tube was separated with 3000 r/min of centrifuge. The above extraction procedure was repeated 3 times and three extracts were combined. The combined extract was concentrated on rotatory evaporator to remove most of the acetone. The concentration residue was transferred into 100 ml separated funnel with 40 ml water and 3g NaCl, and extracted two times with 30 ml petroleum ether. The two extracts were combined and concentrated near dry on rotatory evaporator, and then evaporated under a stream of N_2 to dryness for HPLC analysis.

LC-6A High Performance Liquid Chromatography (HPLC) equipped with Shimadzu Spectrophotometric Detector (SPD-3) was used to determine the acetochlor and butachlor in soil samples. The stainless steel column (25 cm × 4.6 mm I.D.) was packed with DUPON ODS chemically bonded phase. The particle size of ODS was 10 μm and pre-tested by the manufacture. The detection wavelength of 210 nm was selected for both acetochlor and butachlor measurements. The mobile phase was methanol-water (80/20, v/v) with a flow rate of 1.0 ml/min.

1.4 Procedures of biodegradation experiment

The experimental soil samples which particle size was 0.15 mm sieve were divided into 6 sets and each of them included 24 soil samples. Each soil sample (20g) was placed into a 100 ml Erlenmeyer. The acetochlor and butachlor were added in soil samples in set 1 and set 2 respectively, and mixed with soil to let the concentrations of both acetochlor and butachlor in each sample as 10 mg/kg. Besides the acetochlor and butachlor in set 2 and in set 4 respectively, SDBS of 40 μg was added in each sample and mixed with soil. In each sample of set 3 and set 6, besides acetochlor and butachlor respectively, 40 μg of HA was added and mixed with soil. All samples were kept certain moisture which closes to moisture of soil in the field with asepsis distilled water and top of each Erlenmeyer was sealed with asepsis plastic membrane.

All samples were placed into incubator at 35(±1)℃, three samples of each set were taken out at regular intervals, and extracted for acetochlor and butachlor analysis. The samples were taken from set 1 and set 4 on the 35th day for GC/MS analysis of acetochlor and butachlor degradation products respectively. In order to deduct effect of non-biodegradation, the degradation experiment of sterilized samples corresponding to samples above mentioned was conducted under the same conditions.

2 Results and discussion

2.1 The experimental results

2.1.1 The experimental data

On the basis of dissipation rate of herbicides, the three samples of each set soils were taken out from incubator at 0, 3, 7, 11, 22 and 35 days respectively for analysis of acetochlor and butachlor. The determination results are shown in Table 2 where each concentration value was a mean value of three parallel samples. The values in Table 2 were obtained from non-sterilized samples results minus sterilized samples results. During the 35-day incubation period for acetochlor and butachlor in soil alone, 98.97% of applied acetochlor and 99.57% of applied butachlor were degraded respectively. These results indicated that acetochlor and butachlor were more easily degraded in soil by microorganisms to compare with alachlor which had a biodegradation half-life of 8 – 40d (Walker, 1991).

As a result of very low hydrolysis rate (acetochlor and butachlor have the half-life values of 2310 d and 1155 d respectively) and photolysis (the experiments were under dark condition), the concentration reduction of herbicides in Table 2 were mainly due to biodegradation process. Thus, we can consider the dissipation process as a biodegradation and neglect non-biodegradation in data analysis.

Table 2 The degradation experiment data of acetochlor and butachlor in soils

Time, d	Concentrations of herbicides, mg/kg					
	Acet.	Acet. + SDBS	Acet. + HA	But.	But. + SDBS	But. + HA
0	10.000	10.000	10.000	10.000	10.000	10.000
3	5.960	5.380	4.990	4.060	4.100	3.690
7	2.890	2.600	2.530	3.060	3.240	3.010
11	2.070	1.360	1.400	2.120	1.060	2.120
22	0.401	0.400	0.388	0.352	0.388	0.333
35	0.103	0.059	0.090	0.043	0.160	0.102

Notes: Acet. = acetochlor; But. = butachlor

2.1.2 Biodegradation kinetics

The biodegradation experimental data in Table 2 were fitted to a first-order rate equation using nonlinear curve fitting

$$\ln C = \ln C_0 - kt, \quad (1)$$

where C is the observed concentration of herbicides (mg/kg); C_0 is the initial concentration of herbicides (mg/kg); k is the first-order rate constant (d^{-1}); and t is the time (d). The biodegradation half-life $t_{1/2}$ (d) values were calculated from the respective rate constants as

$$t_{1/2} = \ln 2 \cdot k^{-1}. \quad (2)$$

The fitting kinetics curves of acetochlor and butachlor biodegradation during soil incubation without SDBS and HA are shown in a and d of Fig.1 respectively. The fitting kinetics curves of acetochlor and butachlor biodegradation during soil incubation with SDBS and HA are shown in b, c and e, f of Fig.1 respectively.

The values of k and $t_{1/2}$ calculated using Eq.(1) and Eq.(2) are summed in Table 3. The correlation coefficient has a mean value of 0.9901, the highest value of 0.9963 and the lowest value of 0.9702, the first-order rate equation should be suitable for describing the biodegradation kinetics of acetochlor, and butachlor in soil both with HA and SDBS and without HA and SDBS. Under no HA and SDBS conditions, acetochlor biodegradation rate in soil was faster than butachlor (the half-life = 4.6d for acetochlor and 5.3d for butachlor), because the latter has more complex molecular structure and larger molecular weight than the former.

2.2 Influence of HA and SDBS

2.2.1 Influence on acetochlor biodegradation

From Table 3, it can be found that the rate constants have the following order: $k_{Acet} > k_{Acet+HA} >$

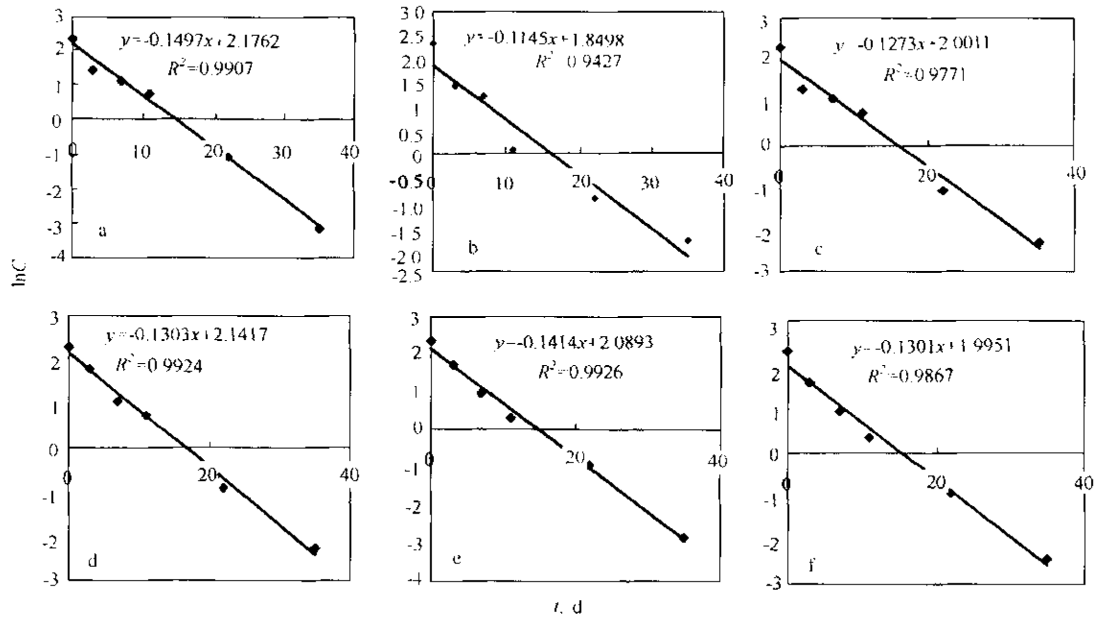

Fig. 1 The data fitting results and kinetics constants of acetanilide herbicides in soil
a. acetochlor alone; b. acetochlor with SDBS; c. acetochlor with HA; d. butachlor alone; e. butachlor with SDBS; f. butachlor with HA

Table 3 Degradation rate constants and half-life of acetochlor and butachlor under different treatment

	Acet.	Acet. + SDBS	Acet. + HA	But.	But. + SDBS	But. + HA
k, d^{-1}	0.1497	0.1145	0.1273	0.1303	0.1414	0.1301
$t_{1/2}$, d	4.6	6.1	5.4	5.3	4.9	5.3
R^2	0.9907	0.9427	0.9771	0.9924	0.9926	0.9867

$k_{ACET+SDBS}$. The results showed that during incubation of the soil-SDBS and soil-HA mixtures, the acetochlor biodegradation rate decreased as compared to the incubation in soil alone. It was indicated that the additives of HA and SDBS can inhibit acetochlor biodegradation, the half-life increase by 17% and 33% for SDBS and HA respectively

2.2.2 Influence on butachlor biodegradation

During incubation of soil-SDBS mixtures and soil-HA mixtures, the butachlor biodegradation rates increased and non-exchanged respectively as compared to the incubation in soil alone. The rate constants have the following order: $k_{But+SDBS} > k_{But} \approx k_{But+HA}$. The results indicated that the additives of HA and SDBS can promote butachlor biodegradation, but unremarkable, the biodegradation half-life of butachlor in soil alone, soil with HA and soil with SDBS were 5.3d, 5.3d and 4.9d respectively.

2.2.3 Influence difference

The different influence of SDBS on acetochlor and butachlor biodegradation rates may be due to the competition between two different mechanisms, solubilization capacity of SDBS to herbicides and inhibition of SDBS on biosurfactant formation in soil.

Aqueous solubility of acetochlor (223 mg/L, 25℃) is greater than butachlor (23 mg/L, 24℃). So, the inhibition mechanism was dominant in influence of SDBS on acetochlor biodegradation. The biosurfactant can be produced during organic matter biodegradation in soil. In the biodegradation reaction equilibrium, SDBS can inhibit formation of biosurfactant as one of the reaction products, thereby decrease acetochlor biodegradation. The solubilization capacity of SDBS was dominant in influence of SDBS on butachlor biodegradation because the herbicide has a very low aqueous solubility. The solubilization capacity of SDBS made more butachlor left soil particles for soil water, thus increased biodegradation of butachlor.

The butaoxymethy group in butachlor molecule has greater steric effect on proton-transfer from HA to butachlor than acetochlor, with the principal proton donor being carboxyl groups in HA can easily form a complex with acetochlor through proton-transfer mechanism, and difficult to form the complex with butachlor.

Acetochlor-HA complex has a much greater molecular weight and easier to be adsorbed into soil particles, and thus more difficult to be degraded by microorganism in soil than acetochlor alone. The additive of HA in soil, thereby, led to decrease of acetochlor biodegradation rate. HA can hardly form complex with butachlor, and had nearly no influence on butachlor biodegradation rate in soil.

2.3 Products of biodegradation

The biodegradation products of acetochlor and butachlor in soil are very complex in fact. Only two compounds can be identified by GC/MS for acetochlor and butachlor respectively due to limitation of spectrum base data and standard reference. The four compounds are hydroxyacetochlor [2-hydroxy-N-(ethoxymethyl)-6′-ethylacet-o-toluidide] and 2-methyl-6-ethylaniline for acetochlor, and hydroxybutachlor[2-hydroxy-2′,6′-diethyl-N-(2-butaoxymethyl) acetanilide] and 2,6-diethylaniline for butachlor. The GC/MS spectra and presumed structures of four degradation products are shown in Fig. 2. It was presumed that the first step of both acetochlor and butachlor biodegradations is dechlorination which was followed by hydroxylation and produced hydroxyacetochlor and hydroxybutachlor. The bonds between N and C in ether and carbonyl groups were broken down to form other two compounds for acetochlor and butachlor respectively.

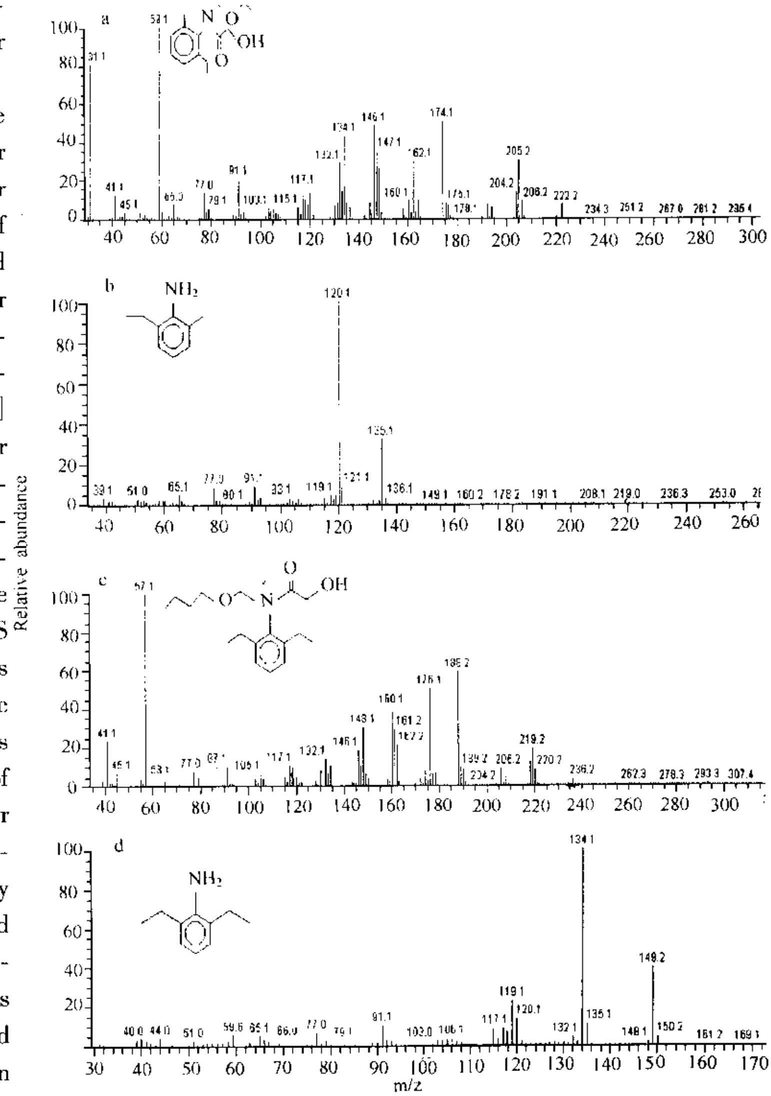

Fig.2 GC/MS spectra and presented structures of biodegradation products
a. hydroxyacetochlor; b. 2-methyl-6-ethylaniline;
c. hydroxybutachlor; d. 2,6-diethylaniline

3 Conclusion

The experimental results showed that the kinetics of acetochlor and butachlor biodegradation in soil can be described by the first-order rate equation. Acetochlor and butachlor are more easily degraded in soil by microorganisms to compare with alachlor.

During incubation of the soil-SDBS and soil-HA mixtures, the acetochlor biodegradation rate decreased as compared to the incubation in soil alone. The rate constants have the following order: $k_{Acet} > k_{Acet+HA} > k_{Acet+SDBS}$. The additives of HA and SDBS can inhibit acetochlor biodegradation. The rate constants of butachlor have the following order: $k_{But+SDBS} > k_{But} \approx k_{But+HA}$. The additives of HA and SDBS can promote butachlor biodegradation, but unremarkable. GC/MS analysis results found that acetochlor degradation products were hydroxyacetochlor and 2-methyl-6-ethylaniline, and the degradation products of butachlor were hydroxybutachlor and 2,6-diethylaniline. Due to the degradation rate constants were calculated on the basis of parent compound concentration decrease of the herbicides, biodegradation rates were dependence on the dechlorination-hydroxylation as the first step in reaction pathway. The dechlorination-hydroxylation step has a little difference between them in the same condition because acetochlor and butachlor have the similar chemical structures. Although acetochlor has a greater degradation rate than butachlor, but much the same (the half-life of 4.6d for acetochlor and 5.3d for butachlor, only the difference of 0.7d).

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