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Degradation of Phenylurea Diuron Herbicide by Coral Bacterium

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Abstrak

Bakteri yang diisolasi dari permukaan karang mampu menggunakan senyawa herbisida fenilurea diuron sebagai sumber karbon dan energi. Organisme ini mampu menggunakan diuron hingga 125 mg/l. Namun fase lag dan waktu untuk mendegradasi mengalami kelambatan bila berada pada konsentrasi diatas 100 mg/l diuron. Kinetika pertumbuhan bakteri ini dilakukan secara kultur batch. Estimasi laju pertumbuhan maksimum (μ_{max}) sebesar 0,46 j^{-1} diperoleh dari pengukuran turbiditas dan nilai konstanta kejenuhan pertumbuhan (Ks) sebesar 49,5 mg/l diuron. Pengaruh konsentrasi diuron paling tinggi pada laju penggunaan substrat spesifik (ó) adalah 0,0195 j^{-1} yang diperoleh pada perlakuan konsentrasi 125 mg/l diuron.

Kata kunci: *bakteri karang, diuron, kinetika pertumbuhan*

Abstract

A bacterium which utilizes phenylurea diuron as a sole source of carbon and energy was isolated from coral surface. The organism utilized diuron up to 125 mg per liter. The lag phase and time for degradation, however, were severely prolonged at diuron concentrations above 100 mg/liter. The growth kinetics of coral bacterium was studied in batch culture. Estimation of maximum growth rates (μ_{max}), obtained from turbidity measurements, was 0.46 h^{-1} and half-saturation growth constant (C_s) was 49.5 mg/l diuron. The highest effect of diuron concentration on the specific substrate removal rate (ó) is 0.0195 h^{-1} obtained from 125 mg/l diuron concentration.

Key words : *coral bacterium , diuron, growth kinetics*

Introduction

The important role of micro-organisms in the degradation of organic pollutant is clearly understand. Eventhough the microorganisms capable of degradation of organic pollutants and their catabolic pathways have been studied intensively, information on the microbial degradation of xenobiotics in marine environments is still very limited.The contamination of marine ecosystems with halogenated organic compounds from agricultural and industrial sources has fulfilled researchs into these frequently toxic compounds. 2,4-Dichlorophenoxyacetate (2,4-D) and 4-chloro-2-methylphenoxyacetate (MCPA), have been used as models for the study of chloro aromatic degradation (Sabdono *et al*., 2000, Sabdono *et al*., 2003). Concern about the environmental impact of pesticides most frequently arises from their ability to leach from soil and contaminate water resources. Misuse or overuse of pesticide chemicals can have considerable environmental and public health consequences.

The phenyl-urea herbicides are of particular significance in this respect, since several members of the group, including diuron [3-(3,4-dichlorophenyl)-1,1-dimethyl urea], isoproturon [3-(4-isopropylphenyl)-1,1-dimethylurea] and linuron [3-(3,4-dichloro phenyl)-1-methoxy-1-methylurea] are degraded slowly in soil and are susceptible to leaching (Bending et al., 2003).

Diuron is a phenylurea herbicide used widely for long-term pre-emergence weed control in non-crop areas, and also in a range of tree crops. It is relatively persistent in soil, with reported half-lives of 90 to 180 days, resulting in sustained herbicidal activity for periods of 4-8 months (Cullington and Walker, 1999). Given the slow natural decrease rate in various soils with respect to mineralization of the phenyl structure and the potential carcinogenic risk of these herbicides and their potential intermediates such as the chloroanilines (Tixier et al. 2001; 2002), there is an important need to develop remediation processes to eliminate or minimize contamination.

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Microbial degradation has been considered to be the primary mechanism for its dissipation from soil, and is believed to occur by successive demethylation of the urea group, followed by hydrolysis to give the chlorinated aniline (Figure 1). To date, there have been no reports on accelerated biodegradation of diuron. Cullington and Walker (1999) reported that degradation of the phenylurea herbicides did not proceed by demethylation of the urea group. Degradation of diuron and linuron did however result in accumulation of 3,4-dichloroaniline, suggesting that the molecule had been cleaved at the carbonyl group of the urea.

Although many bacterial and fungal isolates that are able to break down some of these compounds have been reported, for examples, *Arthrobacter globiformis* (Cullington and Walker, 1999), *Variovorax* sp. WDL1 (Dejonghe et al., 2003), *Arthrobacter oxidans* (Turnbull et al., 2001), and *Sphingomonas* sp (Sorensen et al., 2001), little is known about the kinetic reaction and the environmental conditions needed for the reaction to occur. Therefore, phenylurea diuron degradation in different systems, for example marine and soil, are difficult to control and predict. The objective of this experiment was to study the growth kinetics and substrate degradation of a coral bacterium when growing on phenylurea diuron.

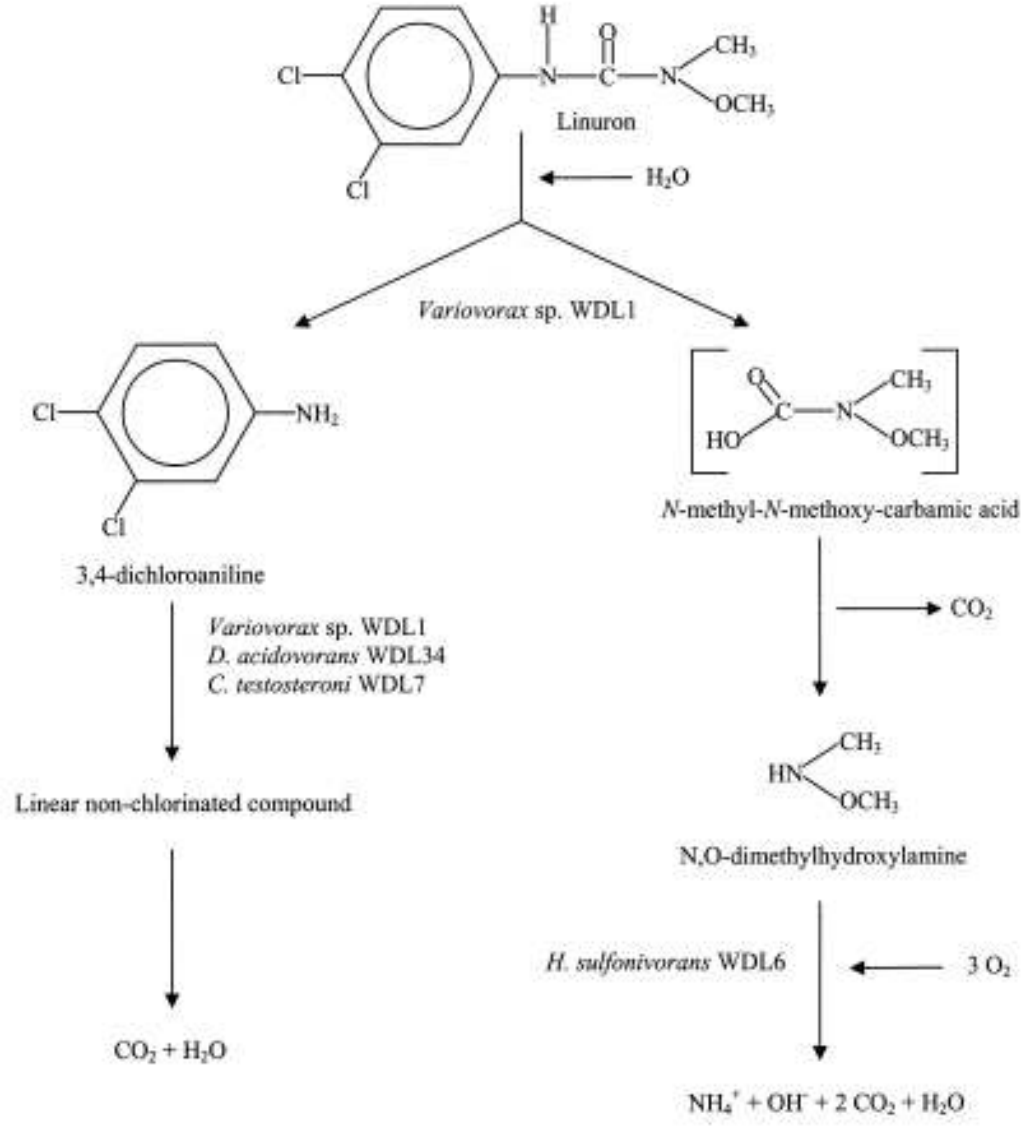

Figure 1. Degradation pathway of phenylurea (Dejonghe et al., 2003)

Materials and Methods

Bacterial strain

A coral bacterium strain MP215 was used in this study. This strain was selected based on the best growth, degradation and sensitivity among coral isolates. This strain was grown at room temperature in Zobell 2216E medium suplemented with 100 ppm diuron.

Experimental protocols

Experiments were conducted by using 100-ml erlenmeyer flasks containing 25 ml of 25, 50, 75, 100, and 125 mg/l diuron in Zobell 2216E medium. Flasks were inoculated with approximately 0.25 ìg (dry weight) of strain and incubated at room temperature on a rotary shaker at 120 rpm. The strain was tested in duplicate. Samples of culture (1 ml) were removed periodically and centrifuged in a microcentrifuge (Microfuge 11; Beckman Instruments, Inc., Fullerton, Calif.) at 12,000 rpm for 2 min, and supernatant was decanted into eppendorf. Samples were analyzed immediately or were fixed with 10 ìl of 40% H_2SO_4 and refrigerated until analysis.

Analysis

Biomass concentrations were measured by spectrophotometry at 600 nm using a dry weight calibration curve. A fluorometer (Turner Model III) with a 10-mm square cuvette was used. Controls were run using medium made with double-distilled water with no carbon source to insure that the growth observed was not due to organic contamination present in the ordinary distilled water. Overall diuron concentrations were measured by a direct UV absorbtion spectrophotometry at 203,2 nm.

Results and Discussion

Diuron degradation

Diuron degradation of the five concentrations showed distinct patterns (Fig. 2). Strain MP215 degraded 75 ppm diuron rapidly. In this concentration approximately 50% of applied diuron was degraded after 18 hours, following which there was a period of rapid loss, with almost constant degradation after 48 hours. Similarly, approximately 50% of the 100 ppm diuron concentration had been degraded in medium after 24 hours. There were a period of slow degradation from three further concentrations, so that complete degradation did not occur. So far, strain MP215 is the first strain of coral bacterium reported to be able to degrade the aromatic ring and to remove the chloride ions of diuron. It assume that this strain converts this phenylurea herbicide at least to a 3-4-dichloroaniline compound (Figure 1). It was not surprisingly that this bacterium could not degrade diuron completely. It seems that cooperative metabolic activities in bacteria of different cultures is needed to degrade diuron herbicide. Dejonghe *et al*. (2003) reported that among five strains, only *Variovorax* sp. strain WDL1 was able to use linuron as the sole source of C, N, and energy. WDL1 first converted linuron to 3,4-dichloroaniline (3,4-DCA). Two strains, *D. acidovorans* WDL34 and *C. testosteroni* WDL7 were found to be responsible for degradation of the intermediate 3,4-DCA. In another study, De Souza *et al*. (1998) determined that in a four-member atrazine-degrading consortium, *Clavibacter michiganese* ATZ1 initiates the degradation of this *s*-triazine herbicide by removing the side chain while *Pseudomonas* sp. strain CN1 subsequently cleaves the ring.

Cell growth and substrate utilization

The relationship of concentration of diuron to growth rate strain MP215 was determined by using batch culture methods. Values of specific growth rate (ì) obtained from turbidity measurements ranged from 0.1599 h^{-1} to 0. 2935 h^{-1} and the rate of substrate utilization (ó) ranged from 0.00001 h^{-1} to 0.01950 h^{-1} (Table 1). Furthermore, the highest growth rate of 0.6501 h^{-1} occurred at 75 mg/liter diuron. Above this level, growth was strongly inhibited. The observation of treshold concentration at 75 ppm diuron and subsequent linear decline in growth rate with increasing concentration was probably more consistent with such general toxicity than with any single-enzyme model. Tyler and Finn (1974) observed linear inhibition curves occurred at 500 ppm 2,4-D, above this concentration, growth was inhibited. The solid line in Fig. 2 is best described by the empirical equation : Y = 0. 1076 X + 2.1722

As shown in Fig. 3, the estimate of half-saturation growth constant (C_s) was 49.5 ppm diuron and the maximum growth rates ($ì_{max}$) was 0.46 h^{-1}. Tyler and Finn (1974) demonstrated that the value of $ì_{max}$ was dependent on the initial inoculum density or initial substrate/biomass ratio. Greer *et al*. (1992) found that the value of $ì_{max}$ was from 0.32 h^{-1} and C_s value was 33.8 ppm.

Lag phase

Batch cultures of the coral bacterium strain MP215 on diuron demonstrated interesting results. The lag growth rate was seen to be reduced by the presence of higher concentration of diuron (Figure 4). Tyler and Finn (1974) stated that the duration of lag phase depended on substrate concentration and on adaption of the inoculum.

In addition, the finite lag phase as predicted by extrapolation of the curves to zero concentration could be due to osmotic shock. Dapaah and Hill (1992) stated that frequently the lag phase is simply modelled by a pure time delay where nothing is assumed to happen. However, microbial cell are fabricating new enzymes and ribosomes to be used in their exponential growth and metabolic pathway (Freifelder, 1987). Furthermore, Esener *et al.* (1982) demonstrated that the RNA content of *Klebsiella pneumoniae* increases with the specific growth rate.

Conclusion

In conclusion, strain MP215 is able to degrade phenylurea diuron. It is necessary to asses the kinetics of growth of inoculant strains over a wide a range of concentrations in order to effectively degrade environmental pollutants and for the successful bioremediation. Molecular identification and characterization of this strain is urgently needed for advanced experiment.

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