C.Ü. Fen-Edebiyat Fakültesi
Fen Bilimleri Dergisi (2002)Cilt 23 Sayı 1

Dicamba ((3,6-dichloro-2-metoxybenzoic acid) Degradation By *Pseudomonas maltophilia*

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Received;15.04.2003, Accepted;25.04.2003

Summary

Dicamba (3,6-dichloro-2-metoxybenzoic acid) is a herbicide which is used to treat broadleaf vegetation. Dicamba is used by *Pseudomonas maltophilia* as a sole source of carbon and energy. The goal of this study was to quantitate dicamba degrading activity in concentrated whole cells of *P. maltophilia*. *Pseudomonas maltophilia.* can degrade dicamba at 30 ^{o}C. It was found that concentrated exponentionally grown cells degrade dicamba at a great rate. Dicamba degradation is measured in washed cells harvested from exponential phases of growth. Dicamba degradation was shown to occur in three kinetic phases.

Key Words: dicamba, herbicide degradation, aromatic compounds.

ÖZET

Dicamba (3,6-dikloro-2-metoksibenzoik asit) geniş yapraklı bitkileri kontrol etmek için kullanılan bir herbisittir. Dicamba, *Pseudomonas maltophilia* tarafından bir enerji kaynağı olarak kullanılır. Bu çalışmanın amacı, *P. maltophilia* tarafından metabolize edilen dicamba yı ölçmekti. *Pseudommonas maltophilia* 30 C de dicambayi metabolize edebilmektedir. Exponent olarak büyüyen *P. maltophilia* hücrelerinin dicamba yı büyük bir oranda metabolize ettikleri saptandı. Dicamba metabolize hızı exponenet olarak büyütülen hücreler kullanılarak ölçüldü. Dicamba nın metaboloize olması 3 kinetik faz tarafından sağlandığı saptandı.

Anahtar Kelimeler: dicamba, herbisit metabolizması, aromatik bileşikler.

INTRODUCTION

Chlorinated aromatic compounds have been extensively utilized in agriculture and industry for many years and used as solvents, lubricants, plasticizers, insulators as well as for use as pesticides and herbicides. Although, commercial utility of such products offers substantial benefits, some of these compounds, such as polychlorinated biphenyls, possess inherently inimical properties rendering them harmful to animals and humans [1]. In this situation, they become environmental pollutants. Microorganisms may develop new pathways for degrading chloroaromatic compounds.This result from genetic rearrangements in microbial genomes or plasmid exchanges. Plasmids are self-replicating extrachromosomal genetic molecules that containing genes cabable of degrading some chloroaromatic compounds. The catabolism of dicamba (3,6-dichloro-2-metoxybenzoic acid) and some of its derivative metabolic intermediates correlates with the presence of a large unstable plasmid[2].

Studies show that *Pseudomonas* and related species also have a large biodegradative plasmid [3, 4]. Many microorganisms can degrade chlorinated aromatic compounds. *Pseudomonas* are involved in many cases of microbial degradation of xenobiotic chloroaromatics.

Chlorinated aromatic compounds are more stable than non-halogenated aromatic compounds. Therefore, these chlorinated compounds are major environmental pollutants. Halogen and alkyl substitions make the molecule more resistant to biodegradation [6]. Boidegradation of chlorinated aromatic compounds depend upon position of the functional group and degree of substition [7]. In general, the more chlorine substitution, the less biodegradation. Compounds having a high degree of chlorine substition have greater reduced water solubility and compounds with less solubility show more resistance to microbial degradation in the soil [8].

MATERIAL AND METHODS

Dicamba Purification

Crude dicamba powder (technical grade, 89.2%) was purified to >99% by triple extraction with toluene (Fisher) in a ratio of 1 gram dicamba/ 1 ml toluene. After final

extraction, dicamba was allowed to dry completeley and was then pulverized to a powder. HPLC analysis confirmed the purity of dicamba to be more than 99%.

Stock Solutions

Stock solutions contained 10,000 ppm (μg/ml) of dicamba. It was prepared as follows:

1. 5 grams of dicamba was added to 450 ml deionized distilled H_2O.
2. The solution was stirred for 1-2 hours and pH adjusted to 7.0 by using 1 M NaOH.
3. The total volume was adjusted to 500 ml. Before adding to the culture medium dicamba was filtered by using 0.2 μm Millipore filter.

Dicamba Solid Medium

Chlorine free medium with 2000 ppm dicamba was solidified by using gelrite at the concentration of 0.8% magnezium sulfate.

Preparation and Analysis of Submerged Cultures

The following conditions were used for growth of *P. maltophilia*

1. Culture vessel: 500 ml Erlenmeyer flask with cotton plug.
2. Working volume: 100-200 ml.
3. Temperature: 30^oC
4. Agitation: 200 rpm (rotary shaker)
5. Medium: Dicamba and reduced chlorine added.

The reduced chlorine medium at pH 7.0 was first autoclaved in the growth flask.

Growth Curve of *P. maltophilia*

The growth of *P. maltophilia* was studied by performing absorbance measurements at 600 nm. The absorbance of the cultures was measured in every 8 hours and results were plotted.

Purity of *P. maltophilia* culture

P. maltophilia was maintained on dicamba selective pressure in liquid and solid medium gelrite plates containing 2,000 ppm dicamba. The pure colonies appeared smooth and round on the plates.

Preparation of Cell Stock Solution in Stationary Phase:

After 3 days growth of *P. maltophilia* in the flasks (OD=0.7) aliquots were centrifuged at 6,000 rpm for 20 minutes using Servall superspeed RC2-B automatic centrifuge. Supernatant was discarded and pellet collected. During the experiment 3.5

grams of wet weight of the cells was transfered to 50 ml of chlorine reduced medium (3.5 gm/50ml). The composition of the reduced chlorine free medium was as follows: 1.39 g $KHPO_4$, 0.87 g KH_2PO_4, 0.66 g $(NH_4)_2PO_4$, 0.097 g $MgSO_4$, 0.025 g $MnSO_4$, 0.005 g $FeSO_4.6H_2O$ and 0.001 g $CaSO_4$ per 1000 ml deionized distilled water with the pH adjusted to 7.0 with 5 M NaOH. Before reaction with dicamba, cells were washed three times with chlorine reduced medium at pH 7.0.

Preparation of Wash Cells from Exponentially Grown Cultures

After 24 hours of *P. maltophilia* growth (OD=0.5) on 4 mM dicamba, cells aliquots were harvested and centrifuged at 6,000 rpm for 20 minutes. Supernatants were discarded and pellets collected. A total of 3.5 gms wet weight cells were used and washed in a total volume of 50 ml reduced chlorine medium at pH 7.0.

Dicamba Reaction with Washed Whole Cells

For this experiment 2 sets of tubes were prepared. Each set consists of 10 tubes. Each of the 10 tubes contain 1.67 ml chlorine reduced medium, 1 ml of cell stock solution, and 0.33 ml dicamba. A second set of 10 tubes contains 1 ml of cell stock solutions and 1 ml of chlorine reduced medium. Each tube was centrifuged for 5 min and the supernatant was added to cuvete with 2 ml of 0.5% $FeCl_3.6H_2O$ (4 ml total volume), After adding 2 ml of $FeCl_3.6H_2O$, each tube was mixed and the absorbance of each tube was measured at 515 nm. The absorbance was measured at 30 min. time interval. A second set of tubes was used as the blanks. All tubes were wrapped with aluminium foil to avoid exposure to light.

Other Reagents for the Dicamba Assay:

For dicamba assay 0.5 gm of $FeCl_3.6H_2O$ was dissolved into 100 ml of deionized water (0.5 gm / 100 ml). This reagent was used to chelate the DCSA and find chromophore has a purple color.

RESULTS AND DISCUSSIONS

Degradation of dicamba by whole incubated cells *of Pseudomonas maltophilia* was investigated. Several qualitative and quantitative results were obtained. Figure 2 shows growth curve of *P. maltophilia* is reached stationary phase around 33 hours. As can be seen in figure 2. *P. maltophilia* cells are in exponential phase from around 8 to 33 nd hours of growth . Dicamba can also be degraded by *P. maltophilia* as the source of carbon for growth [8]. Degradation of dicamba was measured at the logarithmic

phases of growth. In logaritmic phase cells were expected to degrade more dicamba because of perceived greater metabolic activity. As described in material and methods, absorbance of these cells preparations supernatant were measured at 30 minutes time intervals. As time of incubation increased the absorbance of the supernatants decrased. Typicals results are shown in figure 3. A standard curve was obtained for dicamba in range of 1 mM to 5 mM. Standard curve of the dicamba is shown in figure 1. In all cells used in incubations with dicamba were obtained from batch reactors, contained dicamba as the sole source of carbon for growth. Figure 3 depict an increase in dicamba degradation as a function of the time of incubation. The results, shown in figure 3 indicate that, during the first 30 min. of the reaction there was a higher rate of dicamba degradation for the exponentially harvested and washed cells. The total amount of degraded dicamba was 0.016 mM/ml-min. during first 30 min.

A total of 2.74 mM dicamba was degraded for the entire reaction time with exponential phase harvested cells. As is shown in figure 3, there are 3 phases of degradation.

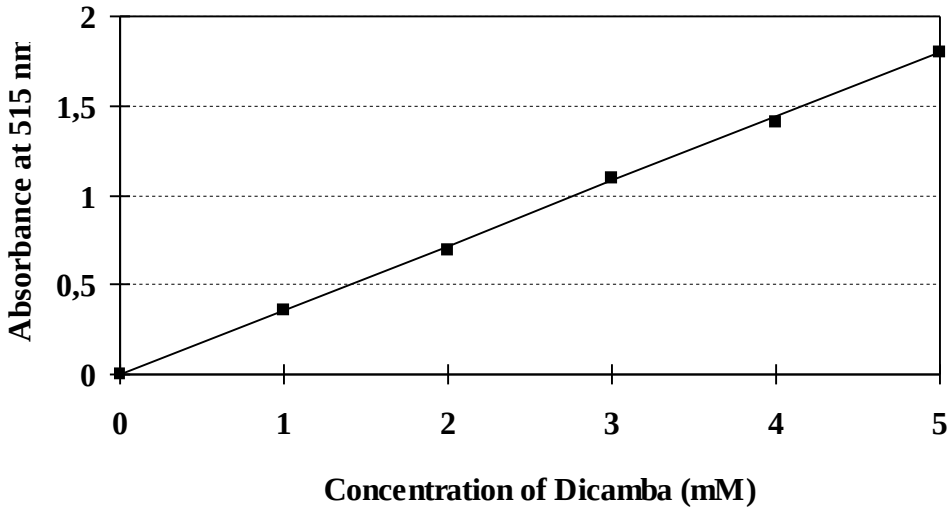

Figure 1. Dicamba Standard Curve.
Each Point is the Average Result of Three Trials

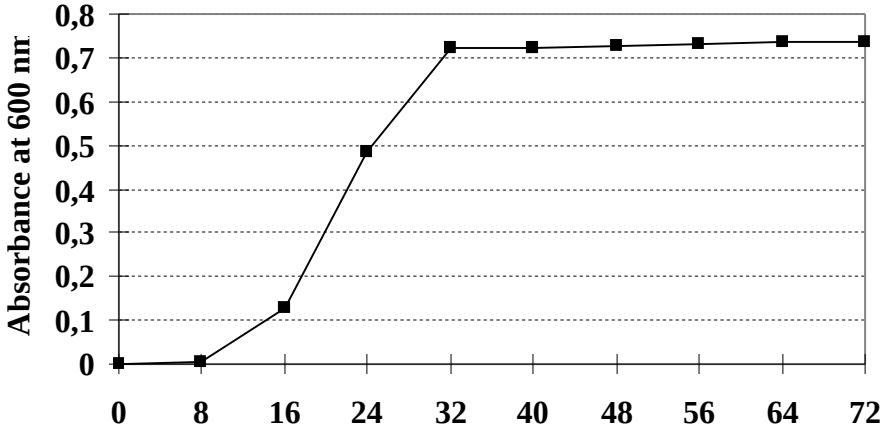

Figure 2. Growth Curve of P. maltophilia on 9 mM Dicamba
Each Point is the Average Result of Two Trials

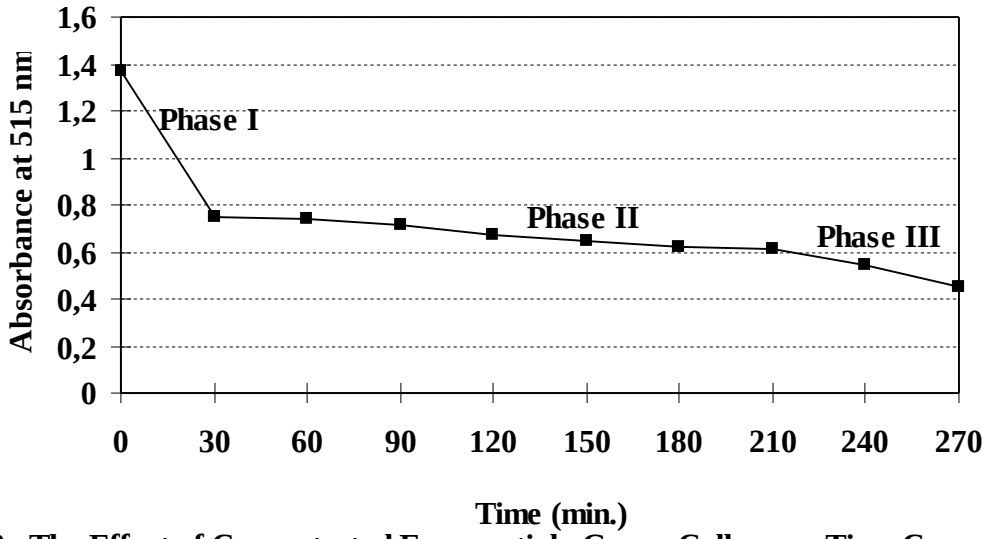

Figure 3. The Effect of Concentrated Exponentialy Grown Cells on a Time Course Absorbance of dicamba
Each Point is the Average Result of Three Trials

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