Bull. Environ. Contam. Toxicol. 31, 203–207 (1983)

Fulvic-Acid-enhanced Biodegradation of Aquatic Contaminants

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Humic substances are widely distributed in the environment and have been known to enhance the photochemical degradation of synthetic organic contaminants in the aquatic environment (KHAN 1980, KHAN 1980a, WOLFE et al. 1976). Little is known about their role in other degradation pathways. We now report that naturally occurring fulvic acid (FA) can enhance the biodegradation of synthetic organics.

Since a large portion of dissolved organic matter in fresh water is present as fulvic acid (KHAN & SCHINTZER 1978), we speculate that this enhanced biodegradation may play an important role in determining the fate of aquatic contaminants.

MATERIALS AND METHODS

Chemicals and Growth Medium: The biodegradation experiments were carried out in cyclone fermentors at 21°C (LIU et al. 1981), with a mineral basal growth medium which contained 0.5 g L^{-1} each of $NaNO_3$ and K_2HPO_4, 0.2 g L^{-1} of $MgSO_4 \cdot 7H_2O$ and 0.01 g L^{-1} of $FeSO_4 \cdot 7H_2O$. The FA (C 42.3%, H 6.5%, N 1.3%, S 2.0%, ash 5.0%) was extracted from water of the Grand River, Ontario, using a resin adsorption technique (MANTOURA & RILEY 1975). As a model contaminant, we chose 2-(methylthio)benzothiazole (MMBT). Benzothiazole and several of its derivatives are used as antioxidants in rubber products and MMBT has been observed in the water of a Grand River tributary near a firm manufacturing chemicals for the rubber industry. For use as the inoculum, a MMBT degrading bacterial culture was derived from the activated sludge of a sewage treatment plant receiving wastewater from this company. Concentrations of FA and MMBT in the growth medium were 50 and 5 mg L^{-1}.

Experimental Procedures: MMBT degradation was measured in terms of primary degradation, i.e., by following the disappearance of MMBT in the fermentor broth. The presence of $HgCl_2$ and cyanide completely inhibited loss of MMBT, thus confirming that MMBT disappearance in the fermentors was the result of biological rather than abiotic activity. Half-lives were determined graphically from plots of log concentration versus time. For the determination of MMBT in the growth medium, 20 mL of culture broth were centrifuged at 10,000 x g for 15 min. The resultant supernatant was extracted three times with 4 mL of methylene chloride and the

cell pellet was extracted twice with 6 mL of the same solvent. The combined extracts were evaporated to 2.0 mL and analyzed by gas chromatography. The chromatograph, which was operated isothermally at 150°C, was equiped with flame ionization detectors and a 180 cm x 2 mm i.d. stainless steel column packed with 10% OV-1 on 80-100 mesh Chromosorb W (AW-DCMS).

RESULTS AND DISCUSSION

The ability of FA to stimulate biodegradation is clearly demonstrated in Figure 1. The initial lag phase shown in the figure is probably due to our use of a very low bacterial concentration (viable plate count approximately 200 CFU mL^{-1}) at the start of the experiments. MMBT was degraded faster in the fermentor containing FA ($T_{\frac{1}{2}}$ = 60 h) than in the one without FA addition ($T_{\frac{1}{2}}$ = 104 h). As the MMBT was degraded, a brightly yellow

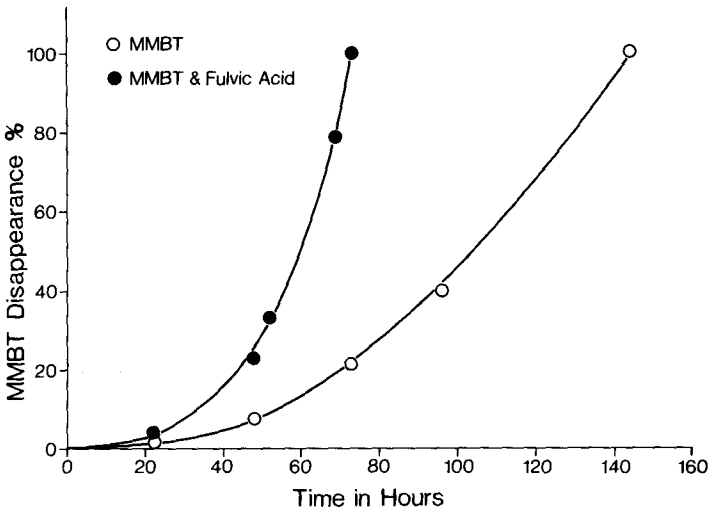

Figure 1. Enhanced MMBT biodegradation by FA in fermentor.

coloured compound was formed (A_{max} 460 nm). As shown in Figure 2, the rate of formation of this compound was enhanced by the presence of FA. Unfortunately, this compound was not amenable to analysis by gas chromatography so that an attempt to obtain its mass spectrum by GC/MS failed. The compound may be a phenol or quinone since its absorption spectrum shifts with changing pH. It is important to note that this compound is formed as an intermediate in both fermentors since it indicates that the addition of FA has not changed the route of degradation.

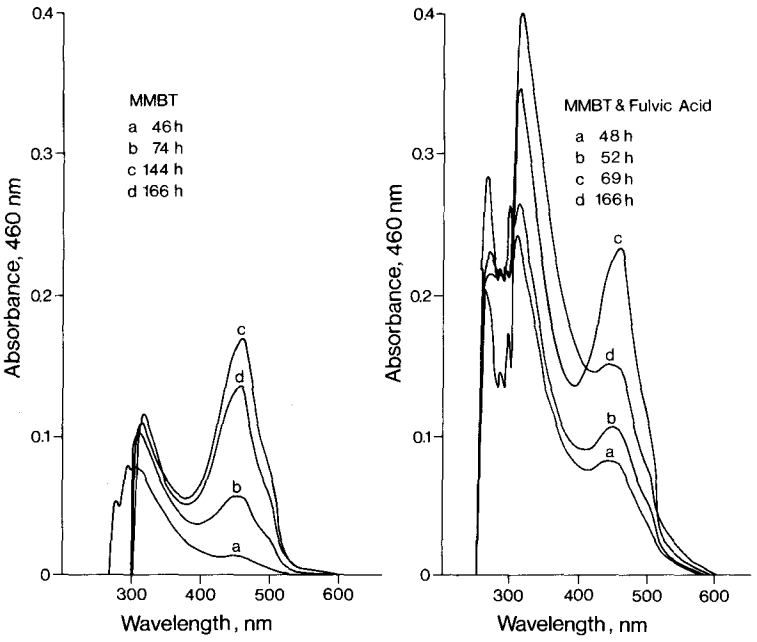

Figure 2. Stimulation of yellow colour compound production by FA in culture broth.

Table 1. Biodegradation of MMBT in fermentors. In experiments when MMBT was added, the concentration of added MMBT was 5 mg L^{-1}; FA and glucose were at 50 mg L^{-1} each.

Combinations	MMBT	MMBT FA	MMBT Glucose	MMBT Glucose FA
$T_{\frac{1}{2}}$ (h)	110	62	69	28

To evaluate whether co-metabolism was involved in the biodegradation of MMBT in the presence of FA, experiments were performed in the presence of 50 mg L^{-1} glucose. The results of these experiments are listed in Table 1. The half-life of MMBT in the presence of glucose was slightly longer than that with FA.

Viable bacterial counts of a second experiment also revealed that the fermentor with glucose addition had a significantly higher cell concentration (approximately 65x) than the ones without, while the one containing FA plus MMBT did not differ significantly from the control MMBT fermentor. Since FA is much less readily metabolized than glucose, these results suggest that the observed enhancement by FA is not simply co-metabolism. Further support for this conclusion is given by the extraordinary degree of stimulation of MMBT degradation in the mixture of glucose and FA.

The observed enhancement was not limited to MMBT but also occurred with 2,4-dichlorophenol which had a half-life of 145 h in the fermentor with FA addition and half-life of 235 h in the fermentor without FA.

At present, the mechanism of the observed enhancement is not clear. Previous studies have reported that the presence of lignin derivatives can considerably increase the biodegradation rate of PCBs and hydrocarbons (LIU 1980, LIU & TOWNSLEY 1970). In these studies, which involved relatively insoluble substances, it appeared that the lignin derivatives formed micro-emulsions into which the substrates dissolved, increasing their availability to the microorganisms. This cannot be the explanation in the present case since the level of MMBT used was well below the solubility limit of this compound in water (approx. 75 mg L^{-1}) and there was no indication from the spectra that micro-emulsions had formed.

Two potential mechanisms for the observed enhancement are worthy of mention. It has recently been demonstrated that humic substances are capable of functioning as electron acceptors from both biotic and abiotic sources (ZIMMERMAN 1981). It may be that the observed enhancement is due to the ability of FA to catalyze extra cellular electron transport or function as a terminal electron acceptor. Alternately, it is of interest to note that many surfactants dissolved in water at levels below their critical micelle concentrations are capable of increasing the bioavailability of certain substrates by increasing the rate of transfer of those substrates across cell walls (WOODHAM et al. 1974, LIU & DUTKA 1973). Whether FA can also increase the permeability of cell walls to these substances has yet to be determined.

Our results show that naturally occurring humic substances can enhance the biodegradation of aquatic contaminants. The mechanism of this enhancement and its environmental significance require further investigation.

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Accepted April 28, 1983