GERBAM — Deuxième Colloque International de Bactériologie marine — CNRS, Brest, 1-5 octobre 1984
IFREMER, Actes de Colloques, 3, 1986, pp. 579-587

69

CHITIN/TOXICANT INTERACTIONS AND MICROBIAL DEGRADATION WITHIN ESTUARINE ECOSYSTEMS

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ABSTRACT - The environmental effect and fate of several agricultural and industrial source toxicants were evaluated in controlled laboratory microcosm systems in the presence of the aminopolysaccharide polymer, chitin. Toxicants examined included methyl parathion, phenol, pentachlorophenol, 2,4 dichlorophenol, 1,2 dichloroethane and Kepone.
Microcosm operating parameters, including pH/Eh, salinity, temperature, flow rate and sediment/water interface conditions, were based on comparable *in situ* measurements. Fate analysis predictions were based on mineralization of the parent toxicant compound as measured by chromatographic analysis or $^{14}CO_2$ expiration. Chitin, amended to continuous flow microcosms, promoted either cometabolic or cooxidative biotransformation for each toxicant as a result of epiphytic cross-coupling of toxicant and aminopolysaccharide. In the absence of chitin, toxicant/substrate biotransformation profiles were significantly altered. A ranking profile of relative postulated toxicity to the aquatic food web can be determined for each chemical class using this microcosm approach.

Key words : chitin, toxicant, estuaries, aquatic microcosms, biotransformation, prediction.

RÉSUMÉ - L'effet sur l'environnement et le devenir de quelques substances toxiques d'origine agricole et industrielle ont été évalués dans des systèmes microcosmiques contrôlés en laboratoire, en présence d'un aminopolysaccharide polymèrique, la chitine. Les substances toxiques examinées sont le méthylaparathion, le phénol, le pentachlorophénol, le 2,4 dichlorophénol, le 1,2 dichloroéthane et le kepone. Les paramètres utilisés au niveau du microcosme sont comparables à ceux mesurés *in situ*. Ils concernent le rapport pH/Eh, la salinité, la température, le taux d'écoulement et les conditions à l'interface eau-sédiment. Les analyses prévisionnelles de leur devenir sont basées sur la minéralisation des composés toxiques d'origine par analyse chromatographique ou par mesure du taux de $^{14}CO_2$ expiré. La chitine, mêlée au flux continu du microcosme, favorise la transformation soit cometabolique, soit cooxydaire de chaque substance toxique et l'aminopolysaccharide. En l'absence de chitine, les profils de biotransformation sont significativement altérés. Par cette approche microcosmique, une bonne estimation de la toxicité relative de chaque espèce chimique peut être déterminée au niveau de la chaîne alimentaire aquatique.

Mots clés : chitine, substance toxique, estuaires, microcosmes aquatiques, biotransformation, prévision.

INTRODUCTION

Chitin, or poly-N-acetyl D-glucosamine, is distributed widely in aquatic and marine environments, comprising 12 % of freshwater crayfish meal, 13 % of crab meal and 8 % of shrimp meal (Patton and Chandler, 1975). An excellent summary on chitin preparation and its adsorptive/desorptive properties has been compiled by Muzzarelli (1977). Lord (1948) first reported that DDT is sorbed by chitin. However, few articles have been

published on chitin/toxicant interactions in wastewater, aquatic or marine environments. Davar and Wightman (1981) presented uptake isotherms of 2,4 D, Dicamba, 2,4,5, T and MCPA on chitosan, the deacetylated derivative, at room temperature. Wolfville (1978) used chitin and chitosan for decolorization of paper mill effluents. Hung *et al.* (1977) used shellfish-shell powders as absorbents for heavy metal ions from aqueous solutions.

In our earlier studies with the organophosphate, azinphosmethyl (Portier and Meyers, 1981), we reported on pesticide-induced microbial population changes and increases in substrate utilization of chitin. Rapid uptake and utilization of chitin and its derivatives, enhanced by controlled azinphosmethyl additions, was examined using a tagged chitin polymer unit, N-acetyl D-glucosamine (glucosamine-1-^{14}C). The work reported here is a summarization of subsequent research with other toxicant classes using similar analytical methods combining *in situ* and laboratory microcosm approaches. The objective of this work is to characterize the influences of naturally-occurring substrates in toxicant biotransformation and biodegradation processes in estuarine ecosystems.

MATERIALS AND METHODS

Collection, Enumeration and Microcosm Test Protocol

Soil and sediment collections were made in the Terrebonne-Timbalier Bay and Barataria Bay drainage basins in Louisiana. These watershed regions are comprised of distinct vegetation zones and areas of contrasting salinity, all affected by a Gulfward movement of water. Brackish and freshwater regions, located adjacent to intensively cultivated agricultural fields, are most directly affected by runoff.

Aluminium cylinder cores, 60 cm tall x 7.6 cm diameter, were inserted into the sediment to the water level, capped and sealed, and stored on ice for transportation to the laboratory for microbial analysis. All samples were processed within 12 h. Surface sediments from the particular sites, i.e. the top 15 cm, were used to inoculate individual microcosms and to categorize the major microbial groups present.

Several major toxicant classes were analyzed for environmental fate. Toxicants examined included the organophosphate methyl parathion, phenol, pentachlorophenol, 2,4 dichlorophenol, 1,2 dichloroethane, and the organochlorine, Kepone (Chem Service). Radiolabelled versions of each toxicant were used when available (Pathfinder Laboratories).

Chitinoclasts were enumerated using a chitin medium containing 2.5 % precipitated chitin (Sigma) plus mineral salts (Hsu and Lockwood, 1975). Preparation of the precipitated chitin followed the methods of Okutani (1966) and Hood (1973). Pure chitin, ball-milled for 72 h at 4°C, was dissolved in concentrated HCl in quantities of 10 g, 150 ml. The dissolved chitin was added to a large vol of distilled water. The milky white precipate was washed repeatedly to remove acid, and the solution was adjusted to give a value of 10 mg/ml at pH 7.2. The plates incubated at 30°C were inspected after 3 d. Colonies exhibiting clearing zones were diagnostic of chitin utilization.

The microcosm systems used for this study have been discussed in detail elsewhere (Portier, 1982; Portier, 1984; and Portier and Meyers, 1984). Water/sediment phase tests were conducted in which toxicants were introduced into continuous flow and carbon metabolism microcosms with sediment/water interfaces analogous to that of the natural environment. Replicate microcosm units were established for each toxicant with (N=6) and without (N=6) chitin-amended sediments. An equal battery of microcosms served as controls for both chitin-amended (N=6) and non-amended (N=6) tests, respectively. The total number of all microcosms used for each toxicant test was N = 24. Microcosm capacity was 2 L.

Freshwater sedimentary materials were amended with 1.0 g of chitin from the freshwater crayfish, *Procambarus clarkii.* Saline sedimentary materials were amended with a commercially available chitin (Sigma Chemical) from blue crab waste.

Temperature, pH, and flow rate parameters and controls reflected *in situ* conditions and are summarized in Table 1. Chitin additions represented *in situ* measurements of chitin in freshwater environments (Portier, 1984) and marine environments (Hood, 1973).

An additional microcosm series employed the use of a radiolabelled polymer unit of chitin, $^{14}CO_2$ expiration rates using methods described earlier (Portier, 1984).

Chemical	Salinity (ppt)	Flow Rate	pH	Temperature	Toxicant Concentration
Methyl parathion	0	0.4 ml/min	6.7	28°C	5 mg/L
(MP)	10	0.4 ml/min	6.9	28°C	5 mg/L
	20	0.4 ml/min	7.4	28°C	5 mg/L
	24	0.4 ml/min	7.5	28°C	5 mg/L
Phenol	0	0.4 ml/min	6.7	28°C	5 mg/L
(PH)	10	0.4 ml/min	6.9	28°C	5 mg/L
	20	0.4 ml/min	7.4	28°C	5 mg/L
	24	0.4 ml/min	7.5	28°C	5 mg/L
Pentachlorophenol	0	0.4 ml/min	6.7	28°C	5 mg/L
(PCP)	10	0.4 ml/min	6.9	28°C	5 mg/L
	20	0.4 ml/min	7.4	28°C	5 mg/L
	24	0.4 ml/min	7.5	28°C	5 mg/L
2,4 Dichlorophenol	0	0.4 ml/min	6.7	28°C	5 mg/L
(2,4 DPH)	10	0.4 ml/min	6.9	28°C	5 mg/L
	20	0.4 ml/min	7.4	28°C	5 mg/L
	24	0.4 ml/min	7.5	28°C	5 mg/L
1,2 Dichloroethane	0	0.4 ml/min	6.7	28°C	5 mg/L
(1,2 DCE)	10	0.4 ml/min	6.9	28°C	5 mg/L
	20	0.4 ml/min	7.4	28°C	5 mg/L
	24	0.4 ml/min	7.5	28°C	5 mg/L
Kepone	0	0.4 ml/min	6.7	28°C	5 mg/L
(KP)	10	0.4 ml/min	6.9	28°C	5 mg/L
	20	0.4 ml/min	7.4	28°C	5 mg/L
	24	0.4 ml/min	7.5	28°C	5 mg/L

Table 1 : Operating parameters for microcosm systems.

Residue Analysis

Residual levels of all toxicants were analyzed using gas chromatography methods as outlined by Gambrell *et al.* (1982) or liquid chromatography methods by Shoup *et al.* (1982). Compound identification was based on comparisons to known concentrations of standards.

Data Analyses, Models

Statistical analyses included multivariate analyses for microcosm correlation, together with SAS and CSMP programming approaches. Biotransformation kinetics interpretation used pseudo-first order and second order kinetics models as outlined by Larson (1980), Lee (1982), and presented in Portier and Meyers (1984) for microcosm systems.

Scanning Electron Microscopy Sample Preparation

Chitin pieces were cemented with silicon rubber (Dow Corning) to 2.2 x 5.0 cm glass cover

slips, cut into 1.0 x 2.0 cm pieces, rinsed in ethanol and suspended in microcosms at the sediment/water interface. Upon removal from the microcosms, the chitin pieces were prepared using methods of Postek *et al.* (1980) and described by Scherbarth (1984). An Hitachi S-500 scanning electron microscope was used for examination of each specimen.

RESULTS

Toxicant/Substrate Biotransformation Profiles

Toxicant biotransformation profiles for chitin amended (chitin) and non-amended (control) sediment/water microcosms for a salinity gradient are shown in Figures 1 and 2. Both phenol (PH) and methyl parathion (MP) demonstrated rapid biotransformation at 48 h for freshwater conditions (Fig. 1). With increasing salinities, these rates (k) decreased noticeably. At 24 ppt salinity, simulating that a salt marsh, the rate of phenol biotransformation dropped from 1.91 % h^{-1} ± 0.08 at 0 ppt to 1.33 % h^{-1} ± 0.11 at 24 ppt. Similarly, methyl parathion (MP) biotransformation rates ranged from 1.77 % h^{-1} ± 0.10 at 0 ppt to 1.20 % h^{-1} ± 0.09 at 24 ppt. Pentachlorophenol (PCP) biotransformation rates were nominal, with a 0.25 % h^{-1} ± 0.06 value at 0 ppt and no detectable biotransformation at 24 ppt after 48 h.

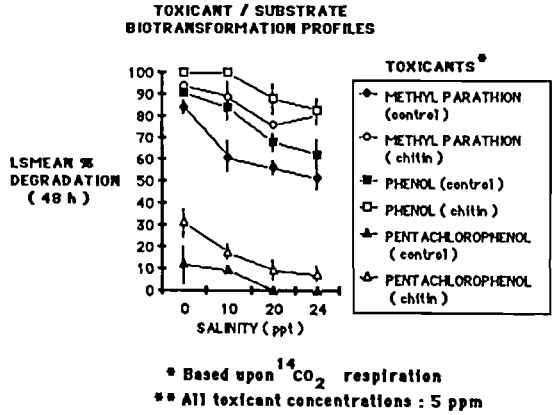

Figure 1 : Toxicant/substrate profiles for MP, PH, and PCP.

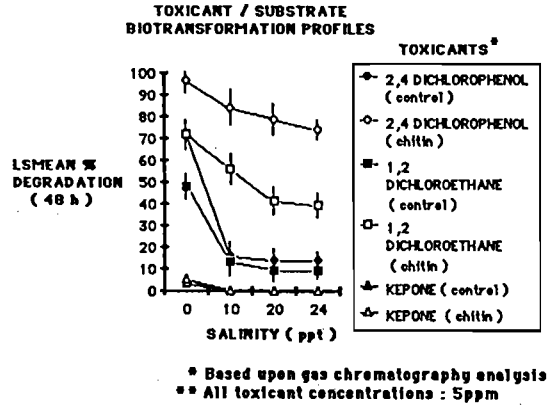

Figure 2 : Toxicant/substrate profiles for 2,4 DPH, 1, 2 DCE, and KP.

In chitin-amended microcosms, these toxicant/substrate biotransformation rates were higher across the salinity gradient, particularly at higher salinities, for all three toxicants. Values for PH increased to 2.08 % $h^{1-} \pm 0.04$ at 0 ppt and 1.875 ± 0.07 % h^{-1} at 24 ppt. The most significant rate increase was at 20 ppt. Methyl parathion chitin-amended biotransformation rates increased slightly to 1.83 ± 0.11 % h^{-1} at 0 ppt and to 1.60 ± 0.12 % h^{-1} at 24 ppt, a 26 % increase in toxicant biotransformation. PCP values were highly significant at 0 ppt exhibiting a biotransformation rate of 0.66 ± 0.14 % h^{-1}. This is roughly twice as rapid a turnover of PCP than in non-amended microcosms. At higher salinities, PCP biotransformation rate changes were equally significant, 0.26 ± 0.08 % h^{-1}, demonstrating toxicant turnover, in the presence of chitin. In non-amended microcosms, biotransformation was not rated. Interestingly, this k value for PCP at 24 ppt was equal to non-amended k value for PCP at 0 ppt.

In Figure 2, noticeable increases in toxicant biotransformation rates for chitin-amended microcosms over non-amended was seen for both 2,4 dichlorophenol (2,4 DPH) and 1,2 dichloroethane (1,2 DE) for salinity levels shown. Values for 2,4 DPH ranged from $2{,}04 \pm 0.16$ % h^{-1} at 0 ppt to 1.65 ± 0.19 % h^{-1} at 24 ppt for chitin-amended microcosms. This compared with k values of 1.58 ± 0.12 % h^{-1} in non-amended microcosms for 0 ppt and 24 ppt, respectively. The rate increase at 24 ppt for 2,4 DPH was highly significant. Values for 1,2 DE were quite similar with the most significant increases in k noted for chitin-amended saline microcosms. At 24 ppt, k values increased from 0.23 ± 0.04 % h^{-1} to 0.885 ± 0.10 % h^{-1}.

Figure 2 also shows toxicant/substrate biotransformation profiles for Kepone (KP), an organochlorine. Turnover of KP was not detectable for all non-amended microcosms. For chitin-amended microcosms, only a slight biotransformation of KP was noted, $k = 0.008 \pm 0.0004$ % h^{-1}. This is approximately a 0.2 % removal rate of KP per day or $T_{1/2} = 262$ days. Subsequent radiotracer studies with ^{14}C-UL-Kepone (Portier and Meyers, 1984) genereated values of 266 d for non-amended and 272 d for chitin amended microcosms.

Toxicant Effects on Substrate Colonization

As shown earlier in Figures 1 and 2, significant changes in toxicant biotransformation was noted for several toxicants in the presence of chitin. In subsequent microcosm investigations using a radiolabelled chitin polymer unit, N-acetyl-D-glucosamine (glucosamine ^{14}C), toxicant effects on substrate biodegradation was examined. Manuscript limitations prevent presentation of mineralization profiles for all toxicants for each salinity range. Figure 3 presents information on ^{14}C-NAG mineralization in the presence of 2,4 DPH and 1,2 DCE in saline microcosms at 24 ppt. These two toxicants, however, exhibited the greatest increases in toxicant biotransformation. Total chitinoclasts, based on colony forming units CFU/mL, were used to follow microbial population dynamics.

The toxicant 2,4 DPH had no significant effect on the chitinoclastic population with levels of CFU/ml similar to control microcosms. However, at 12 h, CFU levels were somewhat higher in 2,4 DPH microcosms. In 1,2 DCE microcosms, total chitinoclasts were reduced through 12 h, with increases above initial CFU levels noted at 24 h. The toxicant 1,2 DCE induced an incubation period of about 8 h to 12 h. Analysis of $^{14}CO_2$ expiration data confirmed this delay in NAG mineralization. A delay in substrate turnover for 1-4 h was noted for 2,4 DPH. Thus, one could postulate that while chitin addition significantly increased toxicant biotransformation over a time frame of days, at the period of initial exposure, chitinoclastic populations are affected by the presence of these toxicants.

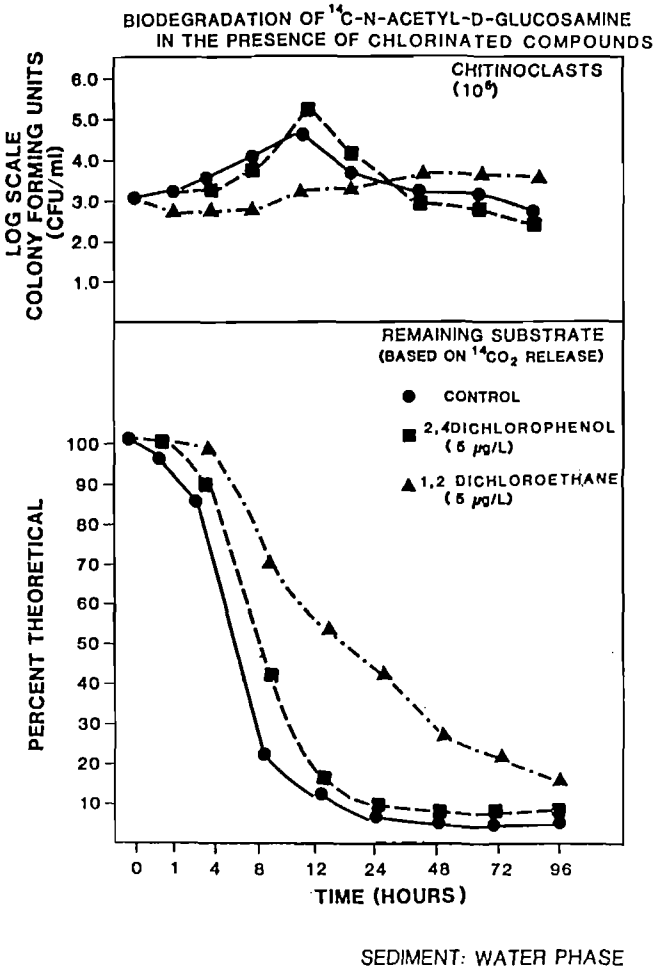

Figure 3 : Biodegradation of ^{14}C-N-acetyl D-glucosamine in the presence of chlorinated compounds.

This phenomena was subsequently confirmed by scanning electron microscopy in microcosm tests with chitin suspended above the water/sediment interface. Figures 4 and 5 depict chitin colonization at 4 h and 12 h following 1,2 DCE toxicant addition. A more homogeneous epiphytic population can be seen at 12 h. This ecological transition toward a more specific, dominant microbiota was demonstrated in SEM studies with other toxicant classes.

Discussion

Toxicant-mediated activities can be described and partially modeled in bench-scale laboratory microcosm units. Toxicant residence time and half-lifes are affected by the relative heterotrophic activity and available naturally-occurring substrate concentration (biotic factors), as well as by sportive phenomena under diverse salinity and pH states (abiotic factors). Flow rate is an additional physical factor to be considered.

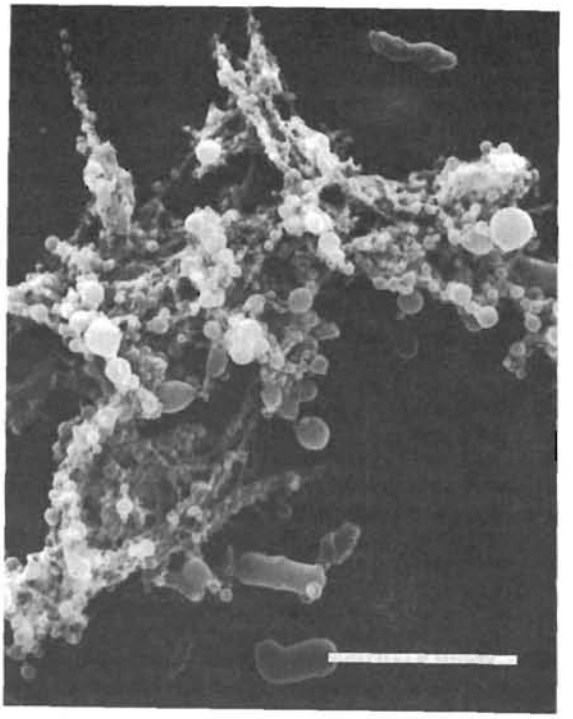

Figure 4 : Scanning electron micrograph of colonized chitin in the presence of 1,2 DCE. Time = 4 h. Bar = 5 microns.

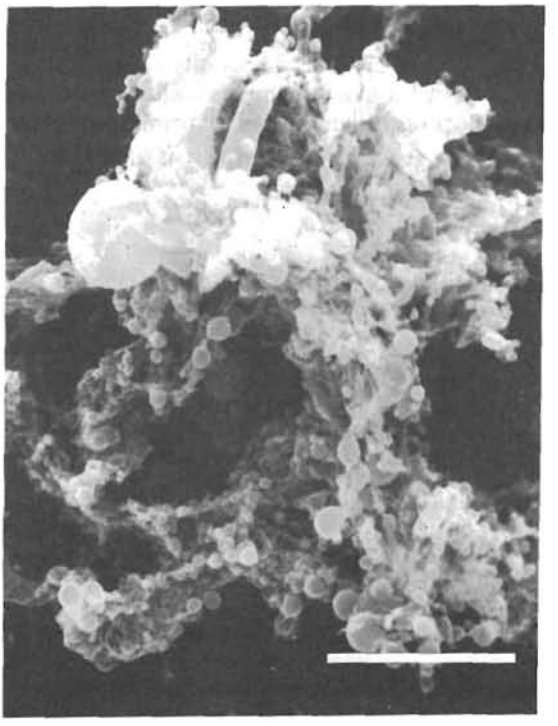

Figure 5 : Scanning electron micrography of colonized chitin in the presence of DCE. Time = 12 h. Bar = 5 microns.

This is particularly true with toxicants in the presence of an adsorptive substrate such as chitin. For all toxicants presented, with the exception of Kepone, residence time in aquatic and marine environments was reduced. As shown with 1,2 DCE, toxicants, upon adsorption to a chitinous surface, select for those microorganisms which are non-sensitive and, perhaps, pre-adapted for toxicant biotransformation.

The removal of competing heterotrophic microorganisms by the toxicant facilitates rapid colonization of the chitinous surface by the remaining epiphytic population and may induce subsequent cooxidative or cometabolic phenomena. Furthermore, this chitin/-toxicant interaction may be more pronounced in saline microenvironments than in fresh water, particularly where readily available energy substrates are abundant.

The organochlorine, Kepone, failed to appreciably degrade in both freshwater and saline microcosms despite chitin amendment. Subsequent microcosm tests confirmed the adsorption of Kepone to chitin and selection of non-sensitive strains on these surfaces. However, microbial degradation by the selected population was minimal. Thus, an additional factor in toxicant biotransformation is evident, namely the contribution of functional groups, i.e. chlorine, attached to a parent molecule structure. Despite toxicant adsorption/concentration and epiphytic population selection, toxicant biotransformation also is determined by enzyme orientation to the toxicant molecule stoichiometry.

CONCLUSION

Microcosm approaches have been used to develop a correlated interpretative analysis of fate and effect of a variety of toxicants in aquatic environments. Fate estimates for important toxic chemical classes such as organophosphates, organochlorines, phenolics and dichloroethanes may be biased when the availability and influence of naturally-occurring substrates such as chitin, within the aquatic/marine environment, are not considered. A more complete discussion on this point is presented in Portier and Meyers, 1984. Thus, examination of changes to the epiphytic microbial community can serve as a basis for modeling the environmental fate of a toxicant in a dynamic estuarine system as well as forming the basis of interpreting the impact of the compound on primary trophic levels in estuarine food webs.

ACKNOWLEDGEMENTS

Portions of the work reported in this paper were funded from grant NA81AA-D-0046, NOAA, Office of Sea Grant Development. Additional support was obtained from a research and development program funded by the Louisiana Board of Regents, Contract No. B22.

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