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# BIODEGRADATION OF XENOBIOTIC COMPOUNDS: PROBLEMS OF ENZYME SPECIFICITY AND WIDE SUBSTRATE SPECTRUM IN REAL WASTES CONTAINING s-TRIAZINES

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## SUMMARY

Wastewater from syntheses of s-triazines was analyzed. This real waste differed from the model wastes for which a triazine-degrading pseudomonad had been isolated. The pseudomonad, while able to grow, did not degrade a significant portion of the s-triazines.

## INTRODUCTION

The biological treatment of xenobiotic wastes in the conventional activated sludge process is known to be successful for some compounds and unsuccessful for others (Thom and Agg, 1975). Attempts to attain or improve the biodegradation of xenobiotics have led in part to commercial preparations claimed to degrade wide varieties of wastes (see Cook et al., 1983c) and in part to systems designed to treat wastes from a single process. Thus Munnecke (1981) has a functioning system proven to detoxify parathion in real production and formulation wastes, and Knackmuss (1981, 1983) has, and is further developing, organisms which degrade individual

haloaromatics in model wastes.

There is an acute shortage of unambiguous data on the treatment of real wastes containing a multiplicity of xenobiotic compounds, largely because of the complexity of the analytical chemistry coupled to the need for comparison with the standard global parameters of Biological Oxygen Demand, Total Organic Carbon or Total Nitrogen. Our studies of the biodegradation of the s-triazines, as a possible competitor to the existing and effective physico-chemical technologies, are designed to help relieve this shortage.

Real industrial wastes, obtained by admixing the effluents from the syntheses of several s-triazines, are available to us. Adequate analytical chemistry for these wastes has been developed (Cook et al., 1983a) and we have isolated bacteria that are able to degrade the major components of model wastes from these syntheses (Cook and Hütter, 1981). This paper describes problems involved in applying the model studies to real wastes.

## METHODS

Wastewater from industrial syntheses was provided by Ciba-Geigy AG, Basel, Switzerland. Pseudomonas sp. strain D (Cook and Hütter, 1981) was used, as well as municipal sewage sludge (Werdhölzli, Zürich).

s-Triazines in filtered solutions were quantified by HPLC with a reversed-phase packing (Beilstein et al., 1981; Cook et al., 1983a). Unknowns were identified by complementary data from HPLC, UV-spectra and mass spectra (Cook et al., 1983a). Total nitrogen was determined by Kjeldahl (Greenberg et al., 1981). Disappearance of total nitrogen from solution correlated directly with loss of s-triazines determined by HPLC.

Biological experiments were done in 1-liter fermenters (500 series; LH Engineering, Slough, Bucks, UK). The nitrogen-limited (i.e. triazine-limited) enrichment medium of Cook and Hütter (1981) was used and it contained 0 to 50 % (v/v) wastewater.

## RESULTS AND DISCUSSION

The wastewater samples always contained the same components but in varying proportions depending on the production schedule. Commercial products were essentially absent from the wastes ( 0.1 %). Most of the components were stable for at least several months, in agreement with our earlier experience (Beilstein et al., 1981), but the chlorotriazinones (Figure 1, VIII) hydrolyzed steadily over this period to the corresponding triazinediones (Figure 1, VI).

The composition of the real wastewater partially overlapped with that of the model (Zeyer, 1979) but additional components (e.g. II, VIII; Figure 1) were observed, which we have now conclusively identified. Our HPLC method superseded the simpler but multiple, limited-range methodologies of s-triazine analysis (cf. Beilstein et al., 1981) by discovering unsuspected components in the wastes. Further, the components common to model and real wastes were present in very different proportions. The model waste was thus qualitatively and quantitatively different from real waste, and we were requested to degrade the compounds shown in Figure 1 in order to obtain sufficient wastewater purification.

Our previous work to isolate organisms able to degrade s-triazines quantitatively (Cook and Hütter, 1981) was done with major components of the model waste, but only components VI, X and XI (Figure 1) of the new requirement were known to be quantitatively degraded (Cook and Hütter, 1981, 1982). We did not know the substrate spectrum of the isolates or their compatibility with the new wastes.

Wastewater was inoculated with Pseudomonas sp. strain D (0.01 mg of protein/ml). The organism grew and components VI and XI disappeared: no

other s-triazine was affected, so only about 10 % of the s-triazine was removed by this treatment. This indicates a high substrate specificity in the biodegradative enzymes.

In a similar experiment, wastewater was inoculated with municipal sewage sludge (at about 0.5 mg of protein/ml). Again only about 10 % of the total s-triazine disappeared, but no individual substance was completely removed, all being reduced by about 10 %: we assume this loss to be due to non-specific binding, which has been observed frequently (Geller, 1980; Schocken and Speedie, 1982), rather than biodegradation.

Biological catalysts (enzymes) can have very wide or very narrow substrate specificities (e.g. Lehninger, 1975). The narrow specificity is essential for e.g. analytical purposes (e.g. Bergmeyer, 1977) but can be seen here to be a disadvantage in waste disposal, where large numbers of enzymes are apparently necessary to degrade the many components. On the other hand, the real wastes were non-toxic for Pseudomonas sp. strain D, which could grow in the wastewater and quantitatively utilize specific components as nutrients. So, using standard microbial methods (Cook et al., 1983b) it is conceiveable that enough novel degradative activities may be developed to allow a practicable biological treatment of these multi-component wastes.

This work shows the need for extensive analytical chemistry of the real wastes before initiating studies on the biodegradation of xenobiotics in wastes, so that the correct activities are looked for. It is a disadvantage of biodegradation that such specific analyses are necessary, whereas simple global parameters such as TOC may be used in other systems: a further disadvantage of the biodegradative removal of xenobiotics is its specificity, compared to such non-specific methods as sorption. The

advantage of biodegradation, when available, is the conversion of the xenobiotic to natural products whose use and disposal are understood.

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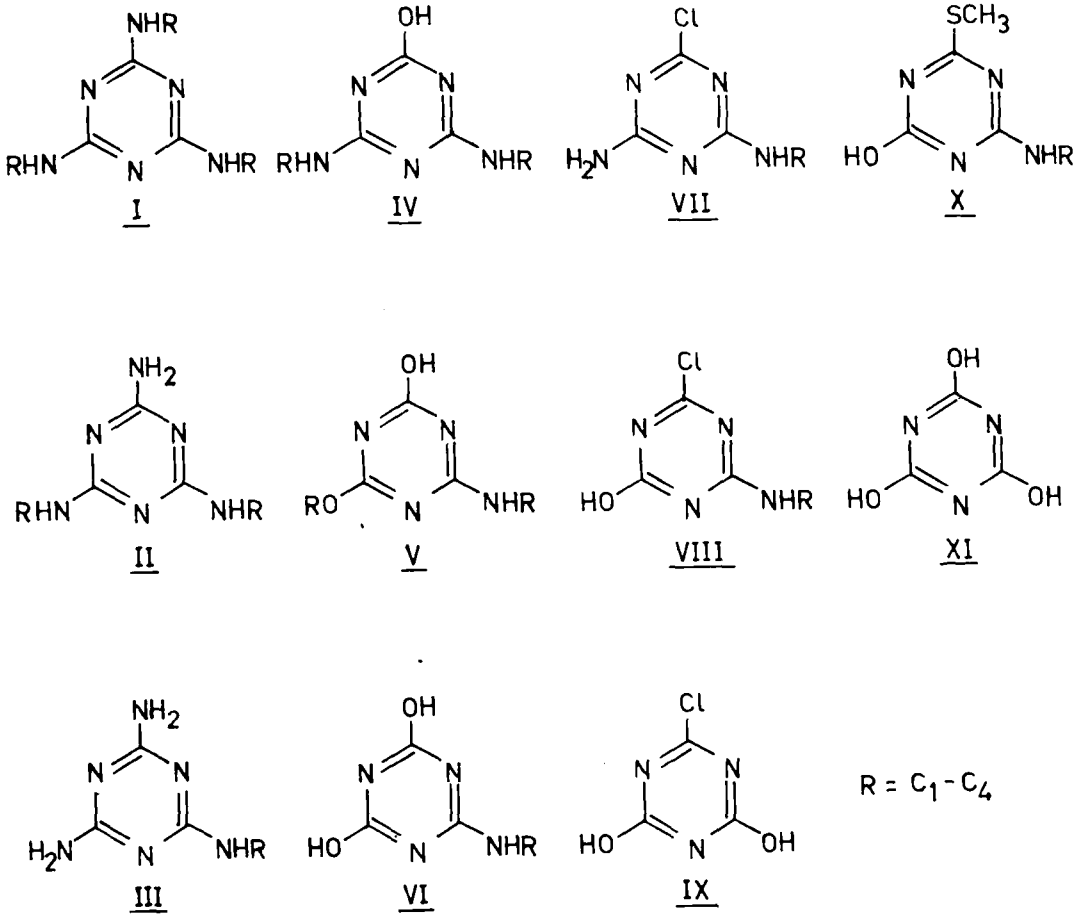


Figure 1. Some s-triazines whose degradation in wastewater is requested.