Microbial degradation of xenobiotic compounds

from Philip R. Lehrbach

MOST organic compounds released into the environment are rapidly broken down by the activities of fungi and bacteria. However, certain man-made compounds (xenobiotics) are not quickly degraded. Public concern over the persistence of such compounds stems from their accumulation in food chains and their toxicity to living organisms. Xenobiotics in the form of pesticides, dye stuffs, surfactants and the waste products of industrial processes require detoxification before they are allowed to enter the environment and new techniques are needed to bring this about.

At a recent meeting* convened to discuss the capabilities of microbes and microbial communities to degrade exotic compounds, several strategies were considered from the viewpoints of industrial chemists, microbiologists and molecular biologists. H. Bretscher (Ciba-Geigy, Basel) discussed various methods for the total chemical and physical destruction of many xenobiotic compounds. Thermal degradation at incineration temperatures of 1,100°C, and the less developed but lower energy-consuming wet air oxidation (300°C) process were mentioned. But these processes have the disadvantages of high-energy expenditure and the total detruction of a potentially useful resource. In the future, economic constraints may dictate the use of biotechnical methods for the treatment of effluent. Such methods would be aimed at eliminating the environmental dangers while providing the basis for recovery of useful organic compounds used in the production of chemicals or microbial proteins.

Procedures are available (batch or continuous culture) for the isolation of bacteria or fungi able to grow on a particular organic compound. However, it is difficult to adapt these isolates so that they perform the same degradative function in the complex mixture of organic chemicals and salts present in industrial effluent. Reviewing the topic, W. Harder (Rijksuniversiteit Groningen, Haren) said that failure to isolate a pure culture able to utilize a particular compound may indicate that metabolism involves a community of bacteria and/or fungi. Important parameters such as culture conditions, interfaces, salt or acid gradients may have been overlooked. One example among many which illustrates the complexity of degradative processes is dichlorodiphenyltrichloroethane (DDT) metabolism. DDT is slowly broken down anaerobically to *o*-dichlorophenylmethane by *Aerobacter aerogenes*. This compound is then co-metabolised by *Hydrogenomonas* to other less-toxic, chlorinated compounds.

One approach to the problem of waste disposal (D. Munnecke, University of Oklahoma), which applies a detailed knowledge of enzyme technology but eliminates the frailty of the living organism, is to supply the appropriate inactivating enzyme to a contaminated area. Such an approach has been taken to detoxify contaminating amounts of the insecticide parathion from liquid waste effluent and industrial or domestic containers. In this case parathion is detoxified to *p*-nitrophenol in the presence of the enzyme hydroxylase. This enzyme can be used successfully in soluble (whole cell or partially purified) or immobilized (glass matrix) form. This example serves as a useful model for the development of other enzyme systems. However, several limitations are placed on this method of waste disposal. The enzymes used must be readily obtainable at relatively low cost, simple in their application particularly for domestic uses, stable, and not require an expensive cofactor to function. To justify this approach the chemicals to be inactivated must be of considerable environmental and public hazard (as was the case with parathion). The quantity and dispersal of the compound must be sufficient to require a concerted effort for its eradication.

U. Gasche (Cellulose Attishelz, Luterbach) and H. Kern (Institut für Botanik und Microbiologie, Jülich) focused on the complex problems of dealing with effluents from cellulose manufacturers. To achieve high yields of cellulose fibres, mechanical and chemical treatment of wood is necessary to convert lignin into physically and chemically altered derivatives which become a predominant by-product in the effluents. Lignosulphonates are lignin products formed in the acidic sulphite pulping process and are more resistant to microbial degradation than lignin itself. H. Kern discussed the possibility of enzymatic removal of the sulphonic acid groups and the subsequent bio-oxidation of the desulphonated products. He quoted data of Ban *et al. (Biotechnol. Bioengng* **21** 1917; 1979) on mixed cultures containing yeasts and bacteria as an approach to economically feasible processing systems.

An understanding of the numerous biochemical pathways necessary for the bacterial degradation of various organic compounds is well advanced. Several participants suggested that this knowledge could be used to develop bacteria with new metabolic capabilities. The reasoning is that bacteria able to degrade organic compounds that are structurally related to xenobiotics may be 'modified' to metabolize these new compounds. Modification using established mutation/-selection procedures or the specific genetic manipulation of appropriate genes determining catabolic enzymes to form new biochemical pathways were suggested. Such an approach has already been taken in the construction of haloaromatic-utilizing bacteria (H.-J. Knackmuss, Institut für Mikrobiologie, Göttingen, see *Nature* **277**, 385; 1979). A similar approach was suggested from an appreciation of the metabolic diversity of methane-utilizing bacteria (methanotrophs) (I. J. Higgins, D. J. Best and R. C. Hammond *Nature* **286**, 561; 1980).

Several important recent developments in molecular biology make this approach attractive to biotechnologists and may aid in the industrial exploitation of these organisms. First, the discovery that many catabolic pathways, including the degradation of substituted aromatic compounds (toluene, xylenes, naphthalene and styrene), are determined by the presence of transmissible plasmids. This makes possible the transfer of metabolic capabilities between various Gram-negative bacteria. Second, powerful new *in vivo* and *in vitro* techniques for the analysis and manipulation of genetic material permit rapid characterization of the structure and regulation of important degradative pathways, precise manipulation of the expression and the construction of multipathway organisms. The recent development of suitable vector systems in *Escherichia coli and Pseudomonas* (M. Bagdasarion and K. Timmis, Max-Planck Institut für Molekulare Genetik, Berlin) make this approach feasible.

The molecular analysis of the plasmid involved in the degradation of toluene and xylenes (the TOL plasmid) is probably the most advanced. F. H. C. Franklin and K. Timmis (Max-Planck Institut für Molekulare Genetik, Berlin) presented a functional map of the catabolic enzymes of the TOL plasmid based on the phenotypic properties of Tn5 (kanamycin resistance) insertion mutants. In this manner three genes, including the *xylE* gene encoding 2, 3-oxygenase, were located. A subsequently cloned *XhoI* restriction fragment (XhoI-I) verified these data and showed expression of catechol 2, 3-oxygenase activity in the *Pseudomonas* host strain. These and similar studies on various catabolic plasmids will provide important information for the future construction of organisms capable of the specific degradation of xenobiotic compounds. □

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