(e.g. SO_4^{2-} from alkyl sulphates), in many instances organic residues remain including butanoic acid-4-sulphate (VII), ethanoic acid-2-sulphate (VIII), ethandiol monosulphate (IX), sulphosuccinate (X) and short-chain sulphophenyl alkanoates such as 2-(*p*-sulphophenyl)-butanoic acid (XI). Further biodegradation of these metabolites, which makes available not only carbon but also sulphur for microbial growth, is usually accomplished by organisms other than those initiating bio-degradation of the original surfactant, i.e. ultimate biodegradation of the more complex anionic surfactants often requires microbial consortia.

Biodegradation pathways of relatively few of these intermediates have been elucidated. The best understood is the biodegradation of substituted benzene sulphonates which in pure culture undergo dioxygenase-dependent desulphonation to catechol derivatives.[6,7] Early removal of the highly polar sulphonate group enables competent organisms to degrade the catechol products using the widely distributed ring-cleavage enzymes which bacteria have evolved to assimilate carbon from the numerous aromatic compounds that occur in nature. However, the ability to accomplish desulphonation is not widely distributed, which is hardly surprising in view of the absence of naturally occurring aryl sulphonates. Interestingly, in mixed cultures there is evidence that ring cleavage precedes desulphonation, and this may be an important contribution in the natural environment.

Although sulphated intermediates of surfactant degradation have not been studied in any detail, bio-degradation pathways of the related short-chain alkyl sulphates reveal interesting trends in relation to carbon-number and the initial point of attack. Thus butyl sulphate is degraded by desulphation in an organism which produces abundant poly β-hydroxy butyric acid, a polymer based on C_4 units. In contrast, propyl-2-sulphate is first oxidised to lactate-2-sulphate so that subsequent desulphation leads directly to lactate.[8] Methyl sulphate is degraded by methylotrophic bacteria, either directly to methanol via a sulphatase (*Hyphomicrobium* sp.) or by mono-oxygenation followed by

spontaneous desulphation to formaldehyde (*Agrobacterium* sp.).[9]

Collectively, these studies indicate that bacteria participating in different phases of surfactant biodegradation make use of metabolic systems evolved for exploiting natural nutrient sources which are structurally similar to, but greatly pre-date the advent of, synthetic anionic surfactants.

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Designing Biodegradable Molecules for the Oleochemical Industries

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When an organic chemical is released into the environment, either accidentally or deliberately, it becomes subjected to a complex set of distribution and transformation processes which will determine its ultimate environmental fate. Its disappearance from the environment is influenced both by physico-chemical processes and by its interaction with the *biota*, i.e. the living organisms in the biosphere with which it comes into contact.

The principle of 'What Nature synthesizes, she can also biodegrade', is based upon the assumption that the plethora of organic compounds produced biosynthetically by plants and animals are the products of 900 million years of gradual evolution, during which time microorganisms have concurrently evolved the appro-

priate catabolic mechanisms to reoxidize these compounds back to CO_2. One estimate of the annual turnover of biogenic material quotes a figure of 2×10^{11} tons, a large proportion of which is in the form of biopolymers and hydrocarbons. Synthetic organic chemicals—*xenobiotics*—comprise no more than 0·1 % of the total organic matter in the biosphere but interest in their pollution potential stems from the fact that population increases and industrial activities have led to much greater use and release of xenobiotics into the environment and from the demonstration that many of them are persistent, even in the most microbially-active environments. Such *biological recalcitrance* implies chronic exposure of the biota to persistent chemicals and the likelihood that such chemicals will *bioaccumulate* in exposed organisms and even *biomagnify* in organisms at higher trophic levels in the food chain which feed exclusively on pollutant-contaminated prey.

In the present aerobic atmosphere on earth, in which the most stable compound of carbon is CO_2, *all* more-reduced organic compounds are thermodynamically unstable and theoretically could serve as putative energy sources for aerobic microorganisms by which they would be oxidized to CO_2. While xenobiotics thus represent a potential nutrient and energy source for catabolically versatile microorganisms, such xenobiotics will be biodegradable only if they are substrates for the suites of enzymes acquired by microorganisms during evolution which enable them to utilize biogenic molecules. The catabolic pathways evoked to degrade natural products usually involve enzymes of high specificity, the synthesis of which is efficiently regulated; microorganisms rarely waste biochemical energy by producing (enzyme) proteins which have no physiological function.[1] Microbial transformations of xenobiotics, in contrast, are fortuitous and random; such molecules were not designed to serve as microbial substrates, so microbial enzymes attack them only if there is some structural similarity to the substrates they meet in nature. Exposure of xenobiotic analogues to one or indeed several such enzymes in a microorganism still does not confer benefit to the organism unless the enzymes are acting as a detoxification agent or are coordinated into metabolic sequences that eventually provide ATP for the biosynthesis of cellular constituents.

Biodegradability then is more likely to be achieved by incorporating into synthetic molecules structural features encountered in their natural counterparts. Thus synthetic lubricants containing ester bonds are good substrates for the esterases (lipases) evolved by microorganisms to deal with their own natural glycerides and they biodegrade rapidly compared with synthetic hydrocarbons containing highly-branched or acetylenic chains which are not substrates for, or may even be inhibitors of, the enzymes of β-oxidation.

The last two decades have witnessed growing public awareness of environmental problems especially in industrialized countries. While oleochemicals form only a small proportion of the total global xenobiotics in the biosphere, some 600000 tons of lubricants alone 'disappear' uncontrolled to the environment in the EEC annually.[2] Contamination of the aquatic environment by water-immiscible pollutants is aesthetically unacceptable as well as a danger to clean water supplies and has already led in western Europe to legislation, enacted by both national and provincial governments, restricting the use of non-biodegradable lubricants on freshwater lakes.

Biodegradability is still not a major consideration in the development of a novel synthetic oleochemical to be used alone or in a new commercial blend formation; the improved performance function remains the primary concern. But if satisfactory performance can be matched by low toxicity, low bioaccumulation and by biodegradability that is both rapid and extensive, the marketability of that oleochemical must be significantly enhanced. The term *biodegradability*, however, extends from a minimal transformation that may alter the physical characteristics of the compound while leaving the molecule largely intact (*primary biodegradation*), to complete mineralization (*ultimate biodegradation*). The term *environmentally-acceptable biodegradation* is used to indicate a molecular cleavage sufficiently extensive to remove the biological, toxicological, chemical and physical properties associated with the use of the original product while not attaining mineralization, and is as far as many xenobiotics will degrade. Indeed the intermediary metabolites of some xenobiotics are more toxic than the original substrate, so mineralization is the preferred aim.

The assessment of a structure–biodegradability relationship for the large number of novel synthetic oleochemicals which may eventually enter the natural environment requires simple and cheap test procedures. Using a hierarchy of tests of increasing complexity and cost, the Degradation/Accumulation Expert Group of the OECD Environment Committee has organized the examination of biodegradability into a sequence which classifies compounds as:

(i) *readily biodegradable*—rapid and complete mineralization.

(ii) *inherently biodegradable*—20–70 % biodegradation in 28 days but requiring further examination with assumed 'worst possible case' estimates of likely environmental concentrations; further simulation tests may be required.

(iii) *non-biodegradable*—negligible biotic removal of the material under test conditions.

Tests which are based on the removal of a soluble substrate by, or on the respiratory activities of, the microbial inoculum depend critically on bringing the test material and microorganisms into consistent and intimate contact. This is much more difficult with water-immiscible oleochemicals and a variety of dispersion techniques and inoculum densities have been examined[3]
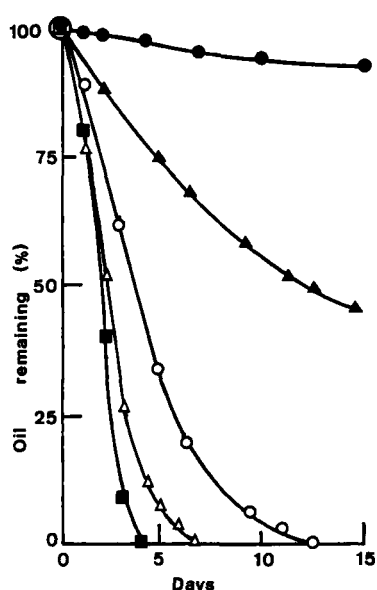
Fig. 1. Biodegradation of several oils in a CEC-type 'die away' test measured in mineral salts medium with an inoculum of lake water bacteria (10^5 cfu cm^{-3}). The oils were: (●), mineral oil; (■), TMP–triester; (▲), TMP–triester with additive package; (○), a commercial fully-formulated biodegradable lubricant; and (△), a natural triglyceride (sunflower oil).
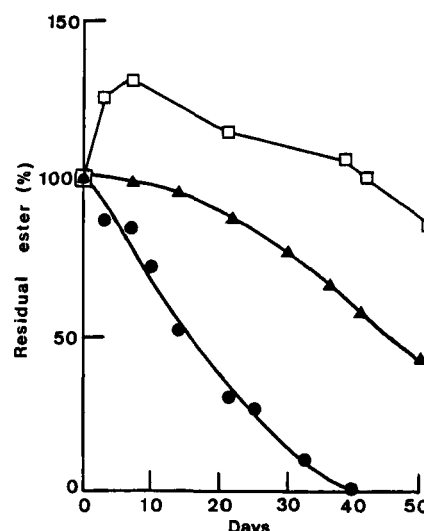

Fig. 2. Disappearance of a trimellitate ester base oil in a CEC-test using inocula derived from different sources; (□), river water; (▲), unadapted sewage; and (●), a mixed culture of D120-degrading bacteria obtained by enrichment. All inocula were adjusted to 10^5 cfu cm^{-3}.

but it is significant that the oleochemical industry's present preferred test (CEC-L-33-T-82) uses no dispersant while maintaining the oleochemical at 0·005% (50 μl dm^{-3}) with shaking on an orbital shaker. The use of the CEC test, and those based on respiratory activity of the microflora, in laboratory tests for assessing biodegradability of oleochemicals (including additives) has shown that:

(a) mineral oils, alkylated benzenes, polyisobutylenes, polyalkylolefines and polyalkyleneglycols show poor biodegradability (0–40%);

(b) vegetable oils (triglycerides), diesters, and polyol esters show good biodegradability (60–100%) (Fig. 1);

(c) aromatic polycarboxylate esters show a range of biodegradabilities from 5% to 80%; this is not necessarily the effect of substitution pattern; the same molecule can show consistently wide variation from laboratory to laboratory, which suggests the competent microflora is restricted in distribution;

(d) biodegradability is retarded by particular alkyl chain lengths ($\leqslant C_4$ and $> C_{25}$) and by degree of branching of the chain;

(e) biodegradability is profoundly influenced by available N and P in the environment and by this and inoculum size if a laboratory test assessment is made after the recommended 21 or 28 day period;

(f) additives almost invariably retard lubricant degradation in proportion to their concentration and many are themselves poorly degraded. Some het-

erocyclic structures (triazine, triazole) are particularly recalcitrant in field and laboratory tests.

The relevance of these laboratory tests to disappearance in the environment, however, needs much more careful study. Figure 2 shows the disappearance of a trimellitate ester D120 exposed to a lakewater inoculum, to sewage and to an enrichment culture previously isolated on this oil (all adjusted to 10^5 cfu cm^{-3}). The profoundly different degradation rates reflect the widely different incidences of the competent bacteria in environmental samples.

Nevertheless, in over 12 years of laboratory, field and model tank experiments, we have consistently found synthetic polyol esters to biodegrade as well as vegetable oils and both much faster than mineral oils; aliphatic diesters are intermediate. This almost certainly reflects the ubiquitous occurrence of esterases in all living organisms compared with the much more restricted distribution of the ω-oxidation complex in hydrocarbon utilizers. Atlas[6] has found, and our own experiments also indicate, that hydrocarbon oxidizers vary between 0·1% and 10% of the total number of heterotrophic bacteria in lake water. The deleterious effect of additives and the benefit of supplementary mineral nutrition is also evident in open lake experiments or in laboratory experiments done with an unmodified lake water inoculum.

We have isolated mixed cultures of bacteria that utilize tricresyl phosphate and zinc dithiodiphosphates (ZDDPs) as carbon and phosphorus sources and are presently examining N-heterocycles as N-sources to examine whether it should be possible to formulate blends in which the additives might supply the necessary N, S and P mineral nutrients for total base oil

degradation especially in oligotrophic lakes where these minerals would be limiting.

There is little evidence in the literature of any extensive study of Quantitative Biodegradability–Structure Relationships (QSBRs) for oleochemicals other than surfactants, probably because no widely acceptable test procedure for biodegradability of all types of compound in all environments has been agreed internationally. While the CEC test may be convenient and easy to perform, it measures by infra-red absorbance only lipophilic molecules extractable into a chloroalkane solvent; it does not measure metabolites that are water-soluble and poorly extractable, i.e. it cannot measure extensive degradation or mineralization, which would require parallel tests measuring O_2 consumption or CO_2 evolution. And what structural criterion is to be adopted that would permit comparison of a diester lubricant, a ZDDP additive or a PEP:PPG copolymer? Several modest studies, usually with a narrow range of compounds, have attempted to correlate 5-day Biological Oxygen Demands (BOD_5) with parameters such as molecular connectivities,[7] lipophilicity,[8] Hammett substituent constant[9] and molecular volume and atomic charges.[10] The latter study showed exceptionally good correlation with BOD_5 for 71 unrelated compounds ($r = 0.993$), and deserves extension to a much wider range of xenobiotics including water-immiscible oleochemicals.

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