TIBTECH – JULY 1988 [Vol. 6]

Biodegradation of xenobiotic and other persistent compounds: the causes of recalcitrance

Charles A. Fewson

There is enormous scope for increasing the range and extent of biodegradative procedures that can be used to reduce problems of hazardous and unpleasant wastes and to exploit renewable feedstocks. Success will largely depend on a greatly improved understanding of the chemical, biochemical and ecological principles that lead to biological recalcitrance. The other side of the coin is that the same principles are important in understanding and attempting to control biodeterioration.

More than 1000 new compounds are marketed every year. The total annual world production of synthetic organic chemicals is over 300 million tonnes. Nobody knows how many or how much of these substances are dangerous. However, it is estimated that in eleven OECD countries production of hazardous wastes ranges from 12 to 600 kg per person each year[1,2]. The US Environmental Protection Agency's list of priority pollutants includes pesticides, halogenated aliphatics, aromatics, nitroaromatics, chloroaromatics, polychlorinated biphenyls, phthalate esters, polycyclic aromatic hydrocarbons and nitrosamines. Surveys of industrial waste waters have shown the presence of many compounds that are resistant to biodegradation in laboratory tests and so may well persist in the environment[3]. In the UK, public expenditure on waste collection and disposal amounts to over £600 million per year. Similar problems face the disposal of non-hazardous bulk wastes. In England and Wales alone, about 15 million tonnes of straw have to be disposed of each year. This is just a tiny fraction of the 50 billion tonnes of lignocelluloses produced annually worldwide, by far our most important renewable feedstock and energy source[4].

In all these cases, the fundamental problem is recalcitrance (see Glossary). Many xenobiotic (see Glossary) and naturally occurring compounds are resistant to biodegradation. For biotechnology to provide solutions to these problems, much greater understanding of the reasons for recalcitrance – and how it can be circumvented – is needed. Indeed, McCormick[2] concluded that:

> In the very near term, biodegradation will take a back seat to quicker, less lasting fixes – gathering, storing, leaching, incinerating... point source treatment of industrial waste water and solid waste streams will probably come first. Pretreatment will slow the buildup of environmental poisons, and careful experience with seeding spills will bolster public confidence – and both will increase the fund of information and the toolbox of techniques. Then biotechnologists and society can move to defuse the toxic-waste time bomb ticking in our back yards.

For less dramatic but bulky wastes that are found at scattered locations, biodegradation is the only realistic alternative to burning (which is socially unacceptable). The US market for biotechnology in the treatment of toxic and hazardous wastes is probably now of the order of $2–3 billion per year[5]. Bioaugmentation, the use of genetically engineered and other cultivated organisms to accelerate biodegradation, could soon produce total annual sales of up to $500 million, including bioreactors[5].

Glossary

Combustion – Extracellular, non-specific oxidative enzymic attack on lignin and other compounds, apparently evolved to cope with the large size, heterogeneity, non-hydrolysability and molecular complexity of the substrate.

Co-metabolism (co-oxidation, analogue metabolism) – The process in which a substrate is modified (often by stoichiometric conversion to a single product) but is not utilized for growth, by an organism that is grown on or metabolizing another substrate.

Communities (consortia, syntrophic associations, synergistic associations) – Different species of microorganisms that exist together and jointly carry out reactions that could not be achieved by any of the single component species.

Recalcitrance (persistence) – Ability of a substance to remain in a particular environment in an unchanged form.

Xenobiotics – Man-made compounds with chemical structures to which microorganisms have not been exposed in the course of evolution.

Biodegradation is morally neutral

Sometimes biotechnology is concerned with enhancing biodegradation, sometimes with limiting it (Fig. 1). At one extreme is the attempt to produce saleable commodities from potentially or actually hazardous or unpleasant materials, or from feedstocks that are cheap and readily available[4,6]. At the other is the biological production of mutagenic, carcinogenic and teratogenic derivatives of polycyclic aromatic hydrocarbons[7], or the decreased effectiveness of pesticides after repeated applications as a result of their enhanced biodegradation[8].

Charles A. Fewson is at the Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, UK.

Fig. 1

Beneficial effects

Detoxication, decontamination, bioremediation: biodegradation of hazardous or unpleasant materials (polychlorinated biphenyls, pentachlorophenol, pesticide residues, dyes, solvents)

Defusing: conversion to nontoxic products of compounds that might otherwise give rise to harmful substances (removal of phenols from water before chlorination)

Formation of useful products (plastics, fibres, fuels)

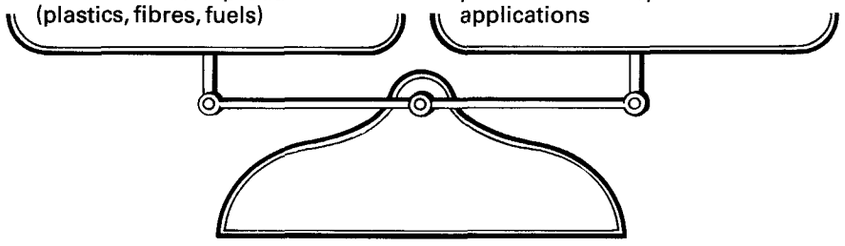
Deleterious effects

Biodeterioration of useful materials: spoilage

Activation: production of toxic compounds from innocuous precursors (oxidation of polycyclic aromatic hydrocarbons, *N*-nitrosation of secondary amines)

Conversion to more recalcitrant forms (humus-bound residues, polymerized catechols)

Enhanced biodegradation of pesticides after repeated applications



The balance of biodegradation.

What is special about the biodegradation of xenobiotic compounds?

There is probably nothing that distinguishes biodegradation of xenobiotic compounds from that of naturally occurring material. In any event, unless the source of a particular compound can be traced directly, it can be surprisingly difficult to discover to what extent it was derived from natural sources and to what extent it was produced by manufacturing industry, agriculture or other human activities. Methyl chloride, for instance, is industrially important and has been classified by the US Environmental Protection Agency as a priority pollutant, yet it occurs naturally.

Some xenobiotics are rapidly degraded, whereas there are naturally occurring materials such as lignin that are extremely recalcitrant. In some sediments *p*-chlorophenol has a half-life of only three days, but certain classes of soil organic matter have half-lives of thousands of years. Samples of wood have been discovered that were virtually unchanged after over 3000 years submersion in sea water. Some xenobiotics are extremely recalcitrant – often deliberately so. Most plastics would be useless if they were readily biodegraded, although this causes problems with their disposal, as a walk along a lonely beach can testify. In other cases recalcitrance is accidental and can have disastrous consequences, as with DDT and polychlorinated biphenyls which are lipid soluble and accumulate in food chains.

What stages are there in biodegradation?

A great deal of laboratory work focuses on details of the metabolism of individual compounds, often emphasizing batch cultures of single organisms supplied with high concentrations of pure substrates. This is important, but it ignores much of the story in nature (Fig. 2).

Proximity to substrate

Organisms need to be close to a substrate if they are to degrade it. Presumably 'close' means within the diffusional distances of the compound, or of extracellular enzymes to reach the substrate, or of the products of extracellular digestion. In this respect there is a profound difference between well mixed aqueous environments (lakes, rivers, seas) and largely unmixed environments (soil, sediments) where there are barriers to movement and diffusion. In soil, a distance of a few centimetres can mean the difference between a material being or not being metabolized. Some bacteria and other microorganisms can display chemotaxis towards substrates and this is undoubtedly important in microenvironments. Many soil microorganisms are filamentous and can show what amounts to directed growth towards substrates. For instance basidiomycete fungi such as *Hypholoma* and *Phanaerochaete* appear to be able to 'explore' the environment, locate uninoculated blocks of wood and then colonize them[9].

Adsorption to solid surfaces

Adsorption may be vital to allow compounds to be metabolized. Physical attachment seems necessary for cellulose digestion[10]. Similarly, in attempts to isolate bitumen-degrading organisms, a very close association between bacteria and the solid substratum could be observed[11].

Extracellular enzymes

Insoluble polymeric materials, whether natural (such as lignin) or xenobiotic (such as plastics) are generally recalcitrant. This seems to be a consequence of their size because the corresponding monomers, dimers and even trimers can often be metabolized. The biological answer to this problem is to excrete extracellular enzymes which diffuse to the substrate and carry out an initial attack to provide soluble low molecular weight products. This process is prone to inefficiency: the enzymes may be subject to adsorption, denaturation and biodegradation, and the products can be captured by competing organisms[10].

Fig. 2

Chemotaxis or directed growth

Adsorption to solid substrates

Excretion of extracellular enzymes

Uptake of diffusible compounds

Metabolism by peripheral metabolic pathways

Utilization of amphibolic metabolites

Possible stages in the metabolism of substrates in natural environments.

Table 1

Possible reasons for recalcitrance

Concentration of substrate is too high (toxic) or too low
The substrate is adsorbed or covalently attached to clays, humus, etc., or is physically inaccessible
The temperature, pH or pO_2, are too low or too high; ionic conditions are unsuitable
Appropriate organisms do not exist or are not present in the environment, perhaps because of predation, parasitism or poor viability
There are inadequate nutrients or co-metabolites
The substrate is not susceptible to initial attack because it is too large and insoluble and there are no suitable extracellular enzymes
The substrate – is not transported into the cell – is not a substrate for the available enzymes – is not an inducer for appropriate enzymes or transport factors – does not give rise to products that can integrate into the cell's overall metabolism – is converted into products that are toxic or interfere with the cell's metabolism

Substrate transport

Substrates may often be taken into the cell by specific, inducible transport systems. This may be particularly important in those natural environments (probably the majority) where the concentrations of nutrients and biodegradable materials are very low – perhaps μM rather than the mM concentrations usually used by microbial physiologists in batch cultures. Accumulation mechanisms may be required at these low concentrations that would be unnecessary, or even harmful, at higher extracellular concentrations.

Intracellular metabolism

Once in the cell, substrates can be degraded by peripheral metabolic pathways. These are generally inducible and sometimes plasmid encoded. The products of initial metabolism are often funnelled into a limited number of intermediate pathways, such as the β-ketoadipate pathway in the case of aromatic metabolism[7,12].

The amphibolic compounds that are formed enter the central metabolic pathways. If it is true, as it is often thought, that peripheral pathways are subject to control only at the level of gene expression, then this throws an added burden on to the regulatory mechanisms that govern the central amphibolic pathways and perhaps makes the production of overflow metabolites more likely.

Biodegradation may be complete or only partial

Complete mineralization may be a lengthy process involving many generations of organisms. Even if an organism can grow aerobically on a xenobiotic compound as sole source of C and energy, only about two thirds of the C will be oxidized to CO_2 whilst the remaining one third is converted into new cell material. Less biomass is formed during anaerobic growth because of the lower growth yields that are achieved, but organic products are likely to accumulate before they are finally converted into methane etc. Other atoms of the substrates (such as N, P or S) may also be incorporated into cell material and so are sequestered into organic matter.

Co-metabolism (see Glossary) can certainly be important in artificial conditions and may be so in nature, although there is little proof of its occurrence. Organisms must usually encounter mixtures of nutrients, rather than the single substrates provided in laboratory growth flasks. It is a great pity that semantic controversy has partly obscured the potential significance of this process – both for direct biotechnological exploitation and in peripheral respects, such as attempts to isolate valuable organisms. The quality and quantity of the primary substrate, for instance whether it supports good yields and induces the appropriate enzyme, may affect the rate of co-metabolism[13,14].

Overflow metabolites could be utilized by other organisms as substrates or co-metabolites. Even *Escherichia coli* shows a transient accumulation of acetate when growing on glucose in batch culture, so it is difficult to see why metabolism of xenobiotics should be expected to proceed stoichiometrically at all times.

Dead-end products such as methanol formed from methoxylated aromatic compounds may accumulate only transiently before they are used by other organisms present in the same environment. In other cases, the dead-end products may themselves be recalcitrant or may give rise to recalcitrant products such as unsightly polymerized catechols.

Side reactions can be beneficial in giving products that are used by other organisms. On the other hand, microbial *O*-methylation of halogenated phenols can give neutral products that have a high potential for bioconcentration and may be toxic[15].

The classic example of a *lethal metabolite* is fluoracetate which can be formed enzymically from fluoro-substituted substrates but then inhibits the tricarboxylic acid cycle. The suicidal consequences can be avoided by mutation so that the organisms do not form, or are resistant to, lethal metabolites. Unfortunately, this type of mutation may give rise to organisms that cannot metabolize that particular compound and so its recalcitrance is enhanced[16].

What factors affect recalcitrance?

The reasons for recalcitrance (Table 1) are a mirror image of the factors that affect the rate and extent of biodegradation in any particular environment (Table 2)[3,5,17,18].

Concentration and solubility

The concentration of available substrate is a particularly important feature. Solid, water-insoluble materials such as anthracene, naphthalene and phenanthrene appear to be used only in the soluble form. The rate of dissolution limits the rate of mineralization. The microbial degradation of crystalline hydrocarbons and highly crystalline cellulose may be difficult partly because of the large amount of energy needed to disperse the solid[19]. Nevertheless, many water-insoluble compounds can be

utilized at rates faster than simple solution.

Organisms have various devices for speeding up the utilization of insoluble substrates. In the case of lipid-soluble liquids such as toluene, they may be dissolved in cell-membranes. This may give high concentrations in the neighbourhood of appropriate membrane-bound enzymes and so may be advantageous, provided that the concentration is not so high as to disrupt the membrane structure[20].

The production of emulsifying agents may be more common than sometimes imagined. The ecological roles of these bioemulsifiers are not clear; some of them may have evolved to aid uptake but some are, perhaps, protective devices; in other cases, emulsifying activity may be a fortuitous consequence. In any event, they may have important applications, for instance in dealing with residues or spillages. Bacterial emulsifiers for pesticides such as 2,4,5-trichlorophenoxyacetate and certain organo-phosphorus agents appear to be substrate specific and are produced only by a limited range of organisms during growth under precisely defined conditions[21].

Alexander has published a long series of papers examining the dependence of biodegradation on substrate concentration (e.g. Refs 13 and 22). At treatment plants and spillage sites concentrations can be extremely high, whereas in the open sea concentrations may be miniscule but the total mass of substrate enormous. There may be large and sudden temporal fluctuations at any given place as a result of dosing, pulsing or spillage. Sometimes mineralization shows close to pseudo-first-order dependence on concentration over several orders of magnitude. However there are many exceptions to this rule. At the lower end of the scale, there is convincing evidence of 'threshold concentrations' below which degradation does not occur[23]. These may be due to inadequate uptake mechanisms, failure to induce sufficient rates of enzyme activities, or problems in providing sufficient energy even for maintenance. At the other end of the scale, high concentrations of xenobiotics can often be toxic, necessitating dilution before treatment and/or the protection of the microorganisms (e.g. by entrapment, adsorption and immobilization). Many of these questions concerning the concentration dependence of biodegradation could be best resolved by the appropriate application of continuous culture techniques, which are less used in biodegradation studies than they deserve to be.

Table 2

Factors that affect biodegradation

The molecule
- Molecular size, shape, charge and functional groups (see Table 3)
- Concentration
- Solubility in water; lipid/water partition coefficient
- Solid/liquid/gas; volatilization
- Toxicity
- Possibility of spontaneous non-enzymic reactions

The environment
- Mechanical accessibility
- pH, pO_2, temperature, redox potential
- Presence of interfaces
- Ionic composition and concentration
- Water and wind speed
- Light quality and intensity
- Presence of co-metabolites, essential nutrients, reactive radicals, other organic and inorganic compounds
- Presence of appropriate organisms and plasmids

Accessibility

Sometimes an otherwise biodegradable material may be physically inaccessible. Cellulose in wood may be protected by lignin from microbial attack. Polysaccharides may become bound within the interlamellar spaces of smectite clays[10].

Chemical structure

There is no single feature that determines the degree of recalcitrance. Rather, a set of features (Table 3) contributes to the ease or difficulty of biodegradation. Their relative importance depends on the organisms and on the environmental conditions. Some of the properties (size, degree of branching, hydrophobicity) affect the concentration that can be achieved in solution, the accessibility to enzyme attack and the chances of transport into the cell. Others (substitution, types of bond, size, shape, charge) affect the ability of the compound to serve as either an inducer or a substrate (or indeed repressor or inhibitor) for various enzymes and transport proteins.

Availability of oxygen

The degree of aeration is one of the chief determinants of recalcitrance. There has been an undue concentration of research effort on aerobic degradation. It is usually faster, more extensive, more obvious and easier to study than anaerobic degradation, and it brought into prominence the importance of oxygenative reactions (for instance, in opening aromatic rings) which are much rarer in other areas of biochemistry[14]. However, the significance of anaerobic degradation is being increasingly recognized. For instance, aromatic compounds can be degraded in the absence of oxygen by photometabolism by purple non-sulphur bacteria, by nitrate respiration (in cultures of single organisms or pairs or mixtures), by sulphate

Table 3

Molecular features that increase recalcitrance

Polymerization and branching
Stable components that are linked by bonds which are not subject to facile hydrolysis or other cleavage; heterocyclic, aromatic and polycyclic residues; ether linkages
Presence of chlorine, nitro and sulphonate groups (especially when they are present at the *meta* position of benzene rings or when there is multiple substitution)

respiration involving *Desulfovibrio* spp., by methanogenic consortia, or by fermentation (e.g. *Pelobacter acidigallici*)[3]. It may be a dangerous generalization, but one can speculate that any compound that can be degraded aerobically can also be degraded anaerobically [with the important exception of lignin and any other compounds that are subject to 'biological combustion'[24] (see Glossary)]. Some xenobiotics, such as chloroform and tetrachloroethylene, may be preferentially degraded in the absence of molecular oxygen at low redox potentials[25].

Presence of other molecular species

The importance of ionic composition and concentration can be illustrated by two examples. Firstly, experiments in a multistage microcosm system indicated that nitrilotriacetic acid may persist when discharged into an estuarine environment, but not into river water[26]. Secondly, nitrilotriacetic acid is mineralized more rapidly in hard water than in soft water, and its mineralization in sewage is aided by the addition of Ca^{2+} (Ref. 27). This is probably because calcium nitrilotriacetate is taken up more readily by bacteria than is the free acid. Similar speciation effects may well apply to other organic chelating agents.

The other compounds present in any given environment may often have important consequences for biodegradation. Organisms may require certain nutrients, not just C, N, P and S sources but also essential vitamins, amino acids, etc. Suitable co-metabolites may or may not be present. In addition, binding to organic (e.g. humus) or inorganic (e.g. clay) material may be important. Dipyridylium herbicides become strongly attached to humus by the formation of charge transfer complexes. Compounds containing nitrogen may form Schiff bases, or the nitrogen atom may be incorporated into heterocyclic ring structures. Phenolic compounds may be polymerized, sometimes by fungal phenol oxidases (laccases)[28].

Non-biological processes

Photolysis reactions may be important, for instance with polychlorinated phenols. Other non-biological processes may also be involved. Dagley[14] wrote:

> '... an organism's success in degrading a pollutant may depend upon whether or not a catabolite is formed that has a chemical structure that permits degradation to proceed, e.g. a structure that can cyclize spontaneously, expelling a halide.'

Most biochemists and microbiologists steer clear of non-enzymic processes. However, in some cases it may be inappropriate to look for entirely biological degradation and better to seek a mosaic of enzymic and non-enzymic steps. Hence the suggestion that 'Cyclear' (ICI Bio-Product's enzyme preparation for the breakdown of cyanide) should be used in conjunction with a chemical oxidant in order to achieve the target of 0.2 p.p.m. total cyanide discharge level set in certain parts of the USA.

Mixed cultures

Similarly, it is dangerous always to focus attention on biodegradation by cultures of single pure strains. This no doubt simplifies experiments and is a vital component step in unravelling metabolic details. However, in natural ecosystems and in various types of waste treatment procedures, mixtures of organisms may well be the norm. These mixed cultures, communities, or consortia (see Glossary) may be more or less stable and clearly defined[29]. The composition may change with time depending on the presence of xenobiotic and other compounds. Organisms that are not directly involved in the biodegradation may also be important; for instance, predation by protozoa may partly account for the failure of some inoculants to accelerate the rate of degradation when added to natural environments. Exchange of genetic information (for instance by plasmid transfer), mutation and fluctuation of species composition will all contribute to metabolic fluidity. This may be important in understanding acclimation processes and in the design of effective bioremediation procedures by using genetically engineered organisms[12,18,30].

What are the immediate priorities?

Research and development in biodegradation of recalcitrant compounds is likely to continue on much the same lines as it has done. However, there is a need for greatly enhanced cooperation amongst disciplines. The focus of this cooperation should be on:

- Isolation of many more useful organisms, and consideration of possible merits of photoheterotrophs, fungi, anaerobes, extremophiles and consortia.
- Genetic modification, directed evolution and patchwork assembly of organisms with novel properties by the application of mutation, plasmid transfer, genetic recombination *in vivo* and *in vitro* and site-directed mutagenesis. There will be ethical and legal questions to be faced in both the construction and the application of engineered organisms.
- Elucidation of metabolic pathways, including transport mechanisms, control and integration into the central pathways; relationship of isolated biochemical processes to the overall physiology. Studies should be extended from model substrates to environmentally or economically important materials, even if such studies are more difficult.
- Exploration of the possibility of combining biological, chemical and photochemical reactions and exploitation of nonspecific enzymic processes.
- Physiological studies under ecologically/industrially relevant conditions, examining the effects of, for instance, acclimation, addition of inocula, concentration effects, predation, competition, flocs.
- Immobilized cells and enzymes.
- Non-aqueous systems.
- Reactor design.
- Development of analytical techniques.
- Testing under real-life conditions; interaction amongst science, engineering, economics and social realities.

References

1 Bentley, J. (1987) *Chem. Ind.* 16 November, pp. 775–779

2 McCormick, D. (1985) *Bio/Technology* 3, 429–435
3 Leisinger, T. and Brunner, W. (1986) in *Biotechnology* (Vol. 8) (Schönborn, W., ed.), pp. 475–513, VCH
4 Lynch, J. M. (1987) *J. Appl. Bact.* 63 (Suppl.) 71S–83S
5 Peyton, T. O. (1985) *Hazardous Waste Treatment: Impact of Biotechnology,* OMEC International
6 Griffin, M. and Magor, A. M. (1987) *Microbiol. Sci.* 4, 357–361
7 Fewson, C. A. (1981) in *Microbial Degradation of Xenobiotics and Recalcitrant Compounds* (Leisinger, T., Cook, A. M., Hütter, R. and Nüesch, J., eds), pp. 141–179, Academic Press
8 Walker, A. and Suett, D. L. (1986) *Aspects Appl. Biol.* 12, 95–103
9 Raynor, A. D. M., Boddy, L. and Dowson, C. G. (1987) in *Ecology of Microbial Communities* (Fletcher, M., Gray, T. R. G. and Jones, J. G., eds) (Society for General Microbiology Symposium, Vol. 41), pp. 83–123, Cambridge University Press
10 Burns, R. G. (1983) in *Microbes in their Natural Environments* (Slater, J. H., Whittenbury, R. and Wimpenny, J. W. T., eds) (Society for General Microbiology Symposium, Vol. 34), pp. 249–298, Cambridge University Press
11 Brunner, C., Wolf, M. and Bachofen, R. (1987) *FEMS Microbiol. Lett.* 43, 337–344
12 Ramos, J. L. and Timmis, K. N. (1987) *Microbiol. Sci.* 4, 228–237
13 Alexander, M. (1981) *Science* 211, 132–138
14 Dagley, S. (1987) *Annu. Rev. Microbiol.* 41, 1–23
15 Neilson, A. H., Lindgren, C., Hynning, P-A. and Remberger, M. (1988) *Appl. Environ. Microbiol.* 54, 524–530
16 Slater, J. H. and Bull, A. T. (1982) *Phil. Trans. R. Soc. London Ser. B* 297, 575–597
17 Guthrie, R. K. and Davis, E. M. (1985) *Adv. Biotech. Processes* 5, 149–192
18 Johnston, J. B. and Robinson, S. G. (1984) *Genetic Engineering and New Pollution Control Technologies,* Noyes Publications
19 Thomas, J. M., Yordy, J. R., Amador, J. A. and Alexander, M. (1986) *Appl. Environ. Microbiol.* 52, 290–296
20 Button, D. K. (1985) *Microbiol. Rev.* 49, 270–297
21 Patel, M. N. and Gopinathan, K. P. (1986) *Appl. Environ. Microbiol.* 52, 1224–1226
22 Scow, K. M., Simkins, S. and Alexander, M. (1986) *Appl. Environ. Microbiol.* 51, 1028–1035
23 van der Meer, J. R., Roelofsen, W., Schraa, G. and Zehnder, A. J. B. (1987) *FEMS Microbiol. Ecol.* 45, 333–341
24 Kirk, T. K. and Farrell, R. L. (1987) *Annu. Rev. Microbiol.* 41, 465–505
25 Kuhn, E. P., Zeyer, J., Eicher, P. and Schwarzenbach, R. P. (1988) *Appl. Environ. Microbiol.* 54, 490–496
26 Hunter, M., Stephenson, T., Kirk, P. W. W., Perry, R. and Lester, J. N. (1986) *Appl. Environ. Microbiol.* 51, 919–925
27 Madsen, E. L. and Alexander, M. (1985) *Appl. Environ. Microbiol.* 50, 342–349
28 Bollag, J-M. and Loll, M. J. (1983) *Experientia* 39, 1221–1231
29 Grady, C. P. L. (1985) *Biotechnol. Bioeng.* 27, 660–674
30 Jain, R. K. and Sayler, G. S. (1987) *Microbiol. Sci.* 4, 59–63

□ □ □ □ □ □ □ □ □ □ □ □

Fusing plant protoplasts

Michael Jones

As a result of improvements in regeneration of whole plants from isolated protoplasts and improvements in protoplast fusion technology, more new somatic hybrids plants are being produced. Both bulk electrofusions and microfusion of selected protoplast pairs are being applied to produce new combinations of genetic material. Practical application of this technology is leading to field testing of somatic hybrid plants.

Plant cells are normally surrounded by a rigid cell wall which, with certain exceptions, prevents contact of plasma membranes and therefore cell fusion. With the development of suitable wall-degrading enzymes and the production of wall-free plant cells (protoplasts) this limitation to plant cell fusion can be overcome. Fusing protoplasts of different origins together to produce novel somatic hybrids provides both new opportunities to study somatic cell genetics and, currently of more practical interest, new approaches to genetic manipulation of plants[1].

Protoplasts can be isolated from a wide range of model and crop plant species. The protoplasts may be cultured in suitable media so that they will re-form cell walls, divide to form colonies and then regenerate shoots and roots, so producing intact plants. Those species and genotypes that can be regenerated to plants in this way are amenable to manipulation by protoplast fusion.

Michael Jones is at the Biochemistry Department, Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ, UK.

The application of protoplast fusion to somatic cell genetics can lead to genetic analyses that cannot be achieved by conventional sexual means. Protoplast fusion is an additive process that allows investigation of combined genomes. In comparison, in sexual crosses, gene segregation occurs and only part of each parental genome is studied. However, the emphasis of current interest lies with the potential application of fusion to the genetic manipulation of crop plants. Although rapid progress is being made in techniques of genetic engineering of plants by the introduction of specific genes using recombinant DNA technology, many characters of agricultural interest are polygenic or ill defined. Despite the less precise nature of cell fusion, it is possible to transfer or combine useful characters by fusion, without detailed genetic or molecular knowledge of the genes underlying such characters[1]. Applications of plant protoplast fusion include:

- combination of two complete genomes;
- partial genome transfer from a