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Opportunities for Biotechnology in Food Processing

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Introduction

Well-established sectors of the food and drink industries are based on biotechnology: cheese, bread, alcoholic drinks and oriental fermented foods have been manufactured using traditional biotechnological practices for thousands of years. This review will not discuss traditional methodology, nor established uses of enzymes, micro-organisms and functional constituents of foods, but will consider the opportunities to introduce some novel processes as a result of the recent explosion of knowledge in the biological and biochemical sciences. In some cases biotechnology offers the possibility that traditional methods may be replaced by more effective and economic ones; in others the application of biotechnology is allowing entirely novel events to take place and new foods to be produced.

The food industry is a very large one and, in the developed world, the value added by food processing is of the same order as that produced by the growth of the crops and animals that are the raw materials of food. In the United Kingdom, the value of the food consumed in 1983 was about £35 million (Anonymous, 1982; Dunnill and Rudd, 1984; Harris, Swinbank and Wilkinson, 1984).

In the developing world, the situation is very different, in that industrial food processing is of minor importance compared with agriculture, and the opportunities to utilize biotechnology in these countries are thus relatively few at present. This review is therefore concerned with the opportunities that arise in the developed countries to apply biotechnology to food processing. Although it is health care that is currently the lead market in biotechnology, as a result of the high value of the products sold on this market, the magnitude of the food market is substantially greater. That the food market will eventually present major opportunities for profit from biotechnology is generally agreed, but estimates of its value, both absolutely and in relation to other opportunities, vary greatly from one assessor to another.

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Nutritional aspects of food quality have not commonly been a matter of concern to the general public in developed countries, even though it is the need for nutrition which enforces food consumption. This situation is changing gradually: nevertheless, to the average consumer, foods and beverages are characterized by their texture and their flavour rather than their nutritional value and it is variation in these former respects which lead to the consumption of one food rather than another. It is essential to recognize, when considering the application of biotechnology to food processing, that it is successfully introduced only if the consumer continues to buy the new product. The latter may be 'new' in the sense that a novel procedure has been used which nevertheless gives rise to a well-established 'normal' product, as when plant hormones and enzymes are exploited to produce malt and beer more effectively. Alternatively, it may be a new product such as the flavoured yoghurts which today form a major commodity. Although the underlying situation is different in these two cases, in both the product must be acceptable to the consumer who is using ill-defined but sensitive sensory criteria to make judgements. The situation in food contrasts with that in the health-care field, where objective definitions of product quality are usually possible.

Food manufacture involves a number of unit operations such as heating, drying, grinding, emulsification and mixing to convert raw materials into food. Raw materials may be those used to provide the bulk of the food or they may be minor components with special properties. The opportunities for producing raw material and ingredients using a biotechnological approach will first be considered. The opportunity to modify raw materials enzymically will then be described. Some opportunities to improve food-processing operations by genetic alteration of micro-organisms which are involved in food processes will be discussed and, finally, the role of biotechnology in treatment of waste arising from food processing will be considered.

Raw materials

It seems likely that the growth of plants in soil, using the energy of sunlight mediated by photosynthesis, will always be more economic as a source of bulk materials than the use of fermenters, although special cases may exist where products with particular functional or other properties may be of sufficient value to warrant utilization of a biotechnological route. The application of genetic manipulation to plants may nevertheless have profound effects on food processing, for the compositional aspects of crop plants and animals that are important determinants of food quality can be desirably altered in new cultivars. Recently, the possibilities for improving the quality of wheat using molecular genetics have been reviewed (Flavell *et al.*, 1984) and there are clearly real opportunities. Additionally, it may be possible to increase the nutritional value of cereal proteins, for example with regard to lysine content, by bringing about genetic change. Opportunities clearly exist in barley and maize (Munck, 1972, 1981; Rexen and Munck, 1984). In relation to beer production, the possibilities of producing barley with lower content of

β-glucan (Aastrup, 1983) or of proanthocyanidins (Von Wettstein *et al.*, 1977) suggest that very substantial economies in production are possible.

In contrast to production of bulk components of foods, the production of minor food ingredients by biotechnological processes offers attractive possibilities to food processers. Essentially, these components are secondary metabolites of plant origin used to impart flavour or colour to food. They are usually of moderately high value and used in relatively low concentration in food. They are also characteristically produced in developing countries and are imported in crude form into the countries of the developed world, where they are processed into high-value components. Important examples are bitter compounds, such as quinine, and flavouring materials such as pepper, vanilla, cloves and saffron.

There are important political and climatic factors which influence the supply of the raw materials and lead to uncertainties. The production of flavours and dyes by using cultured plant cells in fermenters offers the prospect of improved stability and is attracting much research effort, paralleling (although not yet matching) that directed at the production of pharmaceutical products (Kurtz and Constabel, 1979; Nickell, 1980; Fowler, 1981; Rhodes and Kirsop, 1982).

The general technology of growing plant cells in culture is described by Seabrook (1980) and in a number of texts (e.g. Evans *et al.*, 1983). Although with many species there are difficulties in establishing cultures, and although there is an undesirable degree of empiricism involved, it is normally possible to establish cultures. The major problems arise subsequently and must be solved before viable processes can be developed. Principally, means must be found to ensure that the genes responsible for the production of the compounds of interest are expressed in the cultured cells. Although every cell may be regarded as totipotent, in that it contains the genetic information for all the reactions that the plant is able to carry out, the reality is that many pathways are not expressed in normal circumstances, even in the intact plant. Secondary metabolites of industrial interest are found primarily in specialized tissues or organs such as scent glands (mint), stamens (saffron) or bark (quinine). They may be synthesized elsewhere in the plant, but accumulate in such specialized areas. The problem of inducing cultured cells to form the particular metabolite of interest in the conditions of a fermenting vessel is one that cannot be addressed other than empirically at present.

Plant cells are also slow growing and the growth media used are relatively complex and expensive in comparison with those used for microbial cultivation (Dougal, 1980; Seabrook, 1980; Fowler, 1984). Both these factors mean that secondary metabolites produced by plant cells will be more expensive to produce than those from micro-organisms. In addition, plant cells are relatively fragile (Martin, 1980b; Fowler, 1984; Scragg and Fowler, 1984) so that special fermentation systems are required for their cultivation. All these considerations mean that the target compounds for the biotechnological production of food ingredients must be of relatively high value if they are to be produced economically. Judgement of the precise level is difficult at present and depends on many factors: it is my own view that compounds must

be valued at >$50 per kilogram before they are of interest. Others expect the economic limit to be higher, particularly if cells are grown and harvested to extract products contained within them, but this view takes little account of the foreseeable improvements in technology.

Cultured cells contain the genes necessary to induce the pathways leading to the product of interest but some means of expressing the genes must be found. Little is yet known about the control of gene expression, and empirically derived information about the factors known to influence the production of secondary metabolites must be used. It is known that gene expression can be affected by hormonal factors. There is much evidence that the level of plant hormones such as gibberellic acid, indoleacetic acid (IAA), the phenoxy acetic acids and the cytokinins will alter production of secondary metabolites. There is, unfortunately, no general principle to follow which allows other than empirical development of effective procedures. Thus Zenk, El-Shagi and Schulte (1975), Tabata *et al.* (1975) and Furuya, Kojima and Syono (1971) showed that 2,4-D (dichlorophenoxyacetic acid) suppressed anthraquinone, shikonin and nicotine synthesis, respectively. IAA and α-naphthyleneacetic acid (NAA) stimulated alkaloid production from *Papaver bracteatum* (Kamimura and Nishikawa, 1976), and alkaloid production from *Catharanthus roseus* (Zenk *et al.*, 1977) and from a number of other species in callus culture (Khanna, 1978). Many other instances are known in the literature. Kinetin has shown stimulatory (Constabel, Shyluk and Gamborg, 1971) or inhibitory (Shiio and Ohta, 1973) effects on secondary metabolite production.

Physical factors influence growth and secondary metabolite production by cultured plant cells. Light has marked effects on secondary-product formation with very different effects noted with individual species. The topic has been reviewed by Seibert and Kadkade (1980). Little information is available about the influence of temperature on secondary-product formation (Martin, 1980a).

Nutrient supply is an important factor, particularly because many secondary metabolites are not produced by growing cells but are formed in the non-growing or stationary phase. Although a phase of exponential growth followed by a stationary phase is less readily discerned in cultured plant cells than in microbial cultures, the concept of two media, one of which encourages biomass production while the other is more suitable for secondary-product formation, is widely adopted. It is also held with some species that a degree of morphogenesis may be useful in inducing product formation. Methods of inducing morphogenesis are particularly important in developing new plants for agricultural or horticultural purposes, but the techniques used may be of interest if a limited degree of tissue differentiation is useful for product formation (Kohlenbach, 1977; Fowler, 1983).

Statements about the relationship between environment and secondary metabolite production are based only on observations that the product is produced in particular circumstances: there is no evidence that processes such as translation and transcription of genes on a particular pathway are affected. A given secondary metabolite may be formed because the genes affecting a quite different pathway are switched on. Provision of tryptophan to cultures

may allow alkaloid biosynthesis to be stimulated (Zenk *et al.*, 1977), suggesting clearly that the environmental stimulation of alkaloid production may arise because a key 'substrate' for the alkaloid pathway becomes available. The absence of detailed information about the metabolic pathways leading to important secondary metabolites is a major stumbling block in the study of the factors controlling gene expression in cultured plant cells.

The possibility of obtaining, by selection, cell lines which produce large amounts of a particular compound, attracts attention because of the genetic heterogeneity of plant-cell cultures. Examination of cultured beetroot cells, which reveals that red, yellow and colourless cells are present, is a convincing demonstration of heterogeneity, as is the wide variety of cell morphology which can be seen in many species in culture. The extent to which this is a reflection of genetic variation in the cells before isolation, and/or of variability introduced during the process of isolating cells from tissue, is not known; it is probable that both factors are important. There is little information about the extent of genetic variation in cells of species producing important secondary metabolites, but the fact that high-yielding lines can readily be obtained from, for example, cultures of carrot (Eichenberger, 1951), maize (Strauss, 1958), *Happlococcus* (Blakely and Steward, 1964) and *Beta* (Constabel, 1967) suggests that distinct lines are present in suspension cultures, varying in their response to the environmental features of the culture. The possibilities are discussed by Zenk *et al.* (1977). The situation clearly differs substantially from that in microbial cultures, where constancy of genetic make-up is commonly assumed.

Cultured plant cells may be used as callus culture, suspension culture or as immobilized cells. It seems unlikely that callus will be widely used industrially, for the problems of handling these fragile lumps of plant tissue on the large scale are very great. There has been considerable study of the technology of large-scale suspension culture. The dominating factor is that the cells are relatively fragile and liable to destruction by high shear forces generated by conventional stirrers and aeration devices. The preferred fermenter configuration may be that of the air-lift reactor (Wagner and Vogelmann, 1977; Martin, 1980b; Fowler, 1982; Smart and Fowler, 1984a,b; Scragg and Fowler, 1985), where stresses are substantially reduced in comparison with conventional reactors. Other air-stirred reactors may also be useful. Because the oxygen requirement of plant cells is relatively low, there is less need for very efficient oxygen transfer systems than in microbial cultures.

Reactors may be operated in batch or continuous mode. The relative ease of operation of a batch unit, which has led to its predominance in production of antibiotics and alcoholic beverages, encourages its use. Batch units are also well suited to the production of substances which are formed in the stationary phase following growth, as is the case with many plant-cell metabolites. However, because the cost of growing plant cells is high, the use of batch culture systems seems likely to be viable only for the production of particularly valuable substances which are retained within the cells so that the culture must be harvested. In this circumstance, the ability to grow cells to a high concentration may be advantageous in reducing the capital cost of plants.

It seems unlikely that conventional, chemostat-type continuous reactors

will be commercially attractive for use with slow-growing plant cells. On the other hand, continuous systems in which cells are immobilized at high concentration are attractive, particularly as it will be possible to use a culture medium that supports growth until a suitable concentration of cells is present in the reactor and then to change its composition so that cells are in an environment supporting the production of secondary metabolites rather than of cells. A number of methods of immobilization have been proposed, including the use of alginate beads, nylon mesh and reticulated foams. The reviews include those by Rhodes (1985), Rosevere and Lambe (1985), Brodelius and Mosbach (1982) and Lindsey and Yeoman (1983). The use of cells immobilized on foam particles in continuous reactors may be the system of choice for the production of less expensive secondary metabolites, provided that cells produced in the growth phase can subsequently be maintained in the producing mode for very long periods and that the product is excreted into the media, from whence it may be collected by an appropriate downstream processing operation. Whether this is achieved by permeabilization treatment, as suggested for use with *Catharanthus* cells (Brodelius and Nilsson, 1983), or more readily by the use of cell lines which are spontaneously 'leaky' with regard to the target compound, remains to be established.

Enzyme modification of raw materials

The bulk, plant-derived, raw materials of food are polymeric carbohydrates, lipids and proteins. Although these give most of the bulk to food, other components needed in food processing are smaller molecules derived from these polymers by chemical or, more commonly, enzymic reaction. The latter is generally preferred in food manufacturing because of the lesser danger of toxicity and because yield and quality are commonly improved thereby. In line with the wish to produce smaller molecules, the enzymes used have usually been hydrolytic ones, as have the enzymes used to remove undesirable constituents from raw materials.

In traditional processes, such enzymes were introduced as components of the materials used. Thus, malt contains enzymes which hydrolyse starch during later processing to give the fermentable sugars necessary in beer fermentations. The application of genetic engineering techniques may facilitate the production of raw materials containing additional or altered enzymes which improve its quality; a higher content of β-glucanase in malt is such an example. The present account is, however, concerned with novel opportunities for the the deliberate utilization of exogenous enzymes in food processing. This already occurs, to a substantial extent, and well-established hydrolytic processes are discussed in detail by Schwimmer (1981).

There are also newer opportunities to alter the properties of food components in more subtle and sophisticated ways than those listed. Already there is a major market for glucose isomerase, which isomerizes glucose to fructose and so produces a substance with an increased sweetness per unit of weight. The isomerase is used in conjunction with enzymes which hydrolyse starch to

glucose, so that the process is a multienzyme one. Immobilized enzymes are important in the process and it is quite clear that they will be the preferred method of treatment of liquid substrate in the future, because of the efficiency with which hydrolytic enzymes can be used and also because of their absence from the product (Taylor and Richardson, 1979; Cheetham, 1980; Poulson, 1984; Powell, 1984). Lipases may be used to produce new triglycerides, for in certain conditions the enzyme brings about transesterification. The process is particularly interesting because it uses essentially non-aqueous systems and clearly requires a completely new technology to allow enzyme action to be exploited (*see* Chapter 6 of this volume).

FUNCTIONALITY

Food and drink have bulk which is sensed in the mouth and gives rise to a variety of sensory responses which may be collectively described as texture recognition. It is, for example, everyday experience that solid foods may be crunchy, soft, chewy, etc. and that drinks may be viscous or thin. The sensations arise as a response to components of the food which possess functional properties. Functionality is also significant in food manufacture at stages before the product is consumed, for the physical properties of components influence mixing, gas retention, solubility and foam production, all of which are important operations in food production. It is easier to recognize the existence of functionality, which is a broad term used to embrace a number of characteristics, than to define it in any exact way and the absence of adequate definitions is a barrier to progress. However, functionality has a physical basis and there are a number of physical attributes of food components which are associated with texture (Cherry, 1981).

It is important to control functionality so that food with particular properties may be designed. In current practice, individual components of foods are used because they are known, on an empirical basis, to have particular functional effects. A biotechnological approach offers the possibility of altering the chemical structure of present-day ingredients in ways that change the physical properties, and thus functionality. The classic case of such a change being brought about is the addition of rennet (chymosin) to milk, which results in the highly specific hydrolysis of a peptide bond between phenylalanine and methionine in the molecules of κ-casein present in the milk (MacKinlay and Wake, 1971). Because κ-casein is necessary for the maintenance of the colloidal stability of milk, its hydrolysis to give two smaller molecules which lack the necessary properties results in the precipitation of the major proteins of milk—α- and β-casein. As a result the curd is formed and this, after further processing, gives rise to cheese. This dramatic consequence of very specific enzyme action was discovered empirically, but similar opportunities exist. In the case of rennet, enzymes produced by micro-organisms—*Mucor* in particular—broadly duplicate its action but, because of slightly different specificities, differ from rennet in stability to heat and pH, and are more readily controlled.

In order to alter functionality in a beneficial way it is important to know the

relationship between chemical structure and physical attributes of molecules and this information is becoming available in the areas of gelation, the physical properties of gums and mucilages and in relation to foam-stabilizing materials.

Thus the functional properties and particularly the gel-forming ability of various preparations of alginates vary and this can be related to the proportion of mannuronic and guluronic acid residues in the molecule. Gel formation depends on the ability to bind calcium and this is enhanced in material containing a higher content of guluronic acid. It was shown by Skjak-Braek (1984) that an enzyme which he isolated from *Azotobacter vinelandii* and termed mannuronan *C*-5 epimerase can convert mannuronic acid residues into guluronic acid ones and that its use gave substances with increased ability to form gels. Attempts to alter beneficially the properties of galactomannan gums (McCleary, Dea and Clark, 1984) and of pectins (Kirsop, 1981) are in progress. It is interesting that the application of controlled enzymolysis is itself giving insight into the relationship between structure and functionality.

There have been many empirical investigations of the effect of proteolytic enzymes on the functionality of proteins in preparations from major crop plants, such as soya. They have been carried out using industrial enzymes—as must be the case in any major application of enzymes in the food industry.

Proteolytic enzymes modify protein functionality by cleaving peptide bonds, which decreases the size of the molecule, increases the number of polar groups by the formation of NH_4^+ and CO_2 groups and may alter molecular configuration. While increase in polar groups will decrease hydrophobicity, changes in configuration may increase it, particularly if a globular molecule is opened to expose the interior. Increase in the water solubility of soy protein isolate, of proteins from peanut and rapeseed, and of sesame, legume, leaf, seed and fish proteins has been reported to follow treatment with proteolytic enzymes. The emulsifying capacity and the stability of the emulsions formed were improved by treatment of protein from soya, whey, rapeseed, peanut and fish with proteases. Useful improvements in the foaming capacity and water-binding capacity of a similar range of proteins have been reported. Texts by Pour-El (1979) and Cherry (1981) give useful summaries of present knowledge. One of the major problems encountered in the field is the undesirable taste of some proteolysed products, with the production of bitter peptides being a particular problem.

The controlled improvement of the functional properties of proteins used in food is hindered because the background of knowledge of the primary structure of the proteins that are important in food processing is inadequate: accordingly, the relationship between primary structure, the physical form of the molecule and the functional properties of the protein is unknown. Attempts are being made to increase knowledge, particularly with gluten, but a very considerable amount of work is needed before a reliable, scientifically based set of procedures can be established. Indeed, it is likely that complete precision is unattainable because of the multiplicity of interfering substances present in food—including a range of proteins other than the one of special

interest—and the need on grounds of cost to use commercially available mixtures of enzymes rather than the pure ones used to establish principles.

The plastein reaction contrasts with proteolysis in that it involves the protease-catalysed synthesis of peptide bonds. Low-molecular-weight peptides are required—4 to 6 units of amino acid—and pepsin and papain are commonly used as the enzyme. There is now no doubt that peptide bonds are formed, although the relative importance of condensation and transpeptidization reactions is not known. Moreover, there is no agreement as to the size of the molecules formed in the reaction; Phillips and Beuchat (1981) discuss the differing claims. The plastein reaction has been used to remove bitter peptides formed during proteolytic treatment carried out to improve functionality (Arai, Yamashita and Fujimaki, 1975).

The application of genetic engineering to the synthesis of food polymers with improved properties is attracting attention. The primary opportunities arise with proteins, where the structure of the molecule is determined by genome and where genetic adjustments will alter protein structure. With polysaccharides, which are polydisperse (i.e. vary in molecular weight for a given substance), detailed structure is primarily determined by the extent of enzyme activity rather than by genetic 'template' and the genetic possibilities for construction of a specific molecular structure texture are less. With regard to protein engineering, site-specific mutagenesis is becoming well established as a technique and, although there is as yet no X-ray crystallography information at a sufficiently advanced stage to give detailed information about the conformation of proteins which are used in foods, such studies are beginning. Knowledge of the structure of genes coding for the storage proteins of wheat is increasing (Flavell *et al.*, 1984) and programmes aimed at the genetic engineering of cereal proteins are under way in a number of countries. Similar opportunities are concerned with peas and other legumes. There must eventually be opportunities to synthesize plant polysaccharides in micro-organisms but there is, as yet, little activity in this field.

MICROBIAL PROCESSES

Yeasts and lactic acid bacteria have been essential to the manufacture of many foods and beverages for centuries; there are large numbers of specialized strains of both groups of organisms which possess suitable properties for particular processes and particular specific products. These strains differ particularly in their technological suitability while possessing the same basic biochemistry. Individual strains have normally been obtained by selection from a large wild-type pool and then maintained for many years by repeated use in industry and by preservation in culture collections. Because process technology changes or because customer preferences alter, new strains are frequently required today which need to possess essentially all the properties of an existing strain but to differ in one respect. While it may be possible to adjust the details of processing to alter the effect of an existing strain, particularly where flavour characteristics of the product are concerned, it is also often the case that the required strain needs to possess a property which

is absent in the existing one. For example, if a new fermentation system is introduced in which a yeast which previously has been collected by flotation must subsequently be removed by sedimentation, a new strain with the property of aggregating into flocs at an appropriate stage in the process is required.

Classic methods of genetics may be used, but these normally lead to organisms which are changed in a number of respects so that hybrids are commonly unsuitable; the use of classic procedures of sporulation and mating in yeast, for example, lead to large numbers of differing hybrids and to major problems of selection of any suitable hybrids that may be present. The development of genetic engineering and the opportunity it gives to introduce new properties by transfer of single genes is clearly attractive in some circumstances and many groups are studying the possibilities. Yeast DNA plasmids, which are particularly suitable vectors for new genetic material, have been reviewed (Gunge, 1983). The general opportunities for improving industrial strains of yeast have been reviewed (Spencer and Spencer, 1983; Stewart *et al.*, 1984; Tubb, 1984). Major interest attaches to transfer of the genes which allow the direct fermentation of dextrins, and of the genes conferring flocculation. The possibility of using a plasmid present in *Zygosaccharomyces* spp. has been discussed (Painting and Kirsop, 1984), as the selection of yeasts for use in wine fermentation has been described (Kunkee, 1984). Kunkee also discussed the possibility of modifying the genetic nature of lactic acid bacteria used in wine fermentation, where the property of interest is the improvement of the performance of malolactic acid bacteria in fermentation. Other lactic acid bacteria, and in particular the lactic streptococci, are important in the manufacture of dairy products, where their use as starter cultures is widespread; the genetic adjustment of these strains is being studied. It has been shown that lactic streptococci contain more than one plasmid and these clearly offer the possibilities of introducing novel characteristics. The possibility of modifying the lactic acid bacteria that are important in vegetable fermentations, such as those used in pickling cucumbers and sauerkraut, has been discussed (Daeschel and Fleming, 1984).

Much of the food consumed in the developing world is produced by microbial modification of raw materials using processes developed in antiquity and which utilize a wide range of fungi and bacteria. The processes impart flavour, improve texture, increase nutritional value and in some cases remove toxic substances. The very wide range of products has been described (Hesseltine, 1983) and it is characteristic of many of them that the details of microbiological events are unknown. Some information is known about products such as soy sauce (Sugiyama, 1984) and other soy products such as miso (Abiose, Allen and Wood, 1982). Developments in the biotechnology of fermented foods in the next decade seem likely in two directions. First, there must be an increased knowledge of the microbial and chemical events occurring during the production of fermented foods, so that a logical basis for industrialization of the process can be developed. In addition, expertise in solid-state fermentation will be required and this is only now developing (Aidoo, Hendry and Wood, 1982). Very considerable developments are still

needed before it will be possible to contemplate the production of solid and semi-solid fermented foods on a commercial scale.

Waste treatment and utilization

A substantial proportion of the raw materials and ingredients used in food processing are wasted during manufacturing. The waste may be the surface layers of fruit and vegetables and the other unwanted parts of the starting materials necessarily removed during processing, together with other material accidentally lost. The total proportion of the initial material which is wasted is quite high, approaching 50% in some cases, and it is very varied in composition. Because large quantities of water are commonly used in food and drink manufacture and eventually appear as a component of the waste stream, food-processing wastes are usually relatively dilute and produced in large volume relative to the quantity of product. There are significant exceptions to this generalization: many distilleries produce concentrated waste containing up to 10% of dissolved material, and vegetable-processing plants may create wastes consisting of the tissues of the plants used, with little added water.

Whatever the origin and composition of the waste, its disposal is costly, particularly since legislative constraints introduced in many countries in recent years have prevented its disposal by simple discharge into rivers and estuaries. There have, accordingly, been many discussions of methods which would allow the waste to be used in some way or disposed of at a lesser cost than that commonly charged by municipal authorities (Herzka and Booth, 1981; Ledward, Taylor and Lawrie, 1983). Waste may be converted to other substances of value but there are very few processes which are suitable for use on a production scale at lower cost than that of disposal via municipalities. This is because products produced for sale to the public must meet stringent safety regulations which cannot be met without costly sterilization, cleaning and possibly detoxification of the waste. The production of microbial biomass is not usually economically viable for these reasons, although special circumstances may offer opportunities, as in the case of confectionery waste (Forage and Righelato, 1979) and whey (Meyrath and Bayer, 1979; Moulin and Galzy, 1984). Producing bulk chemicals is not attractive because of their relatively low value and the high costs of downstream processing when the product concentration is relatively low, as it is when dilute waste streams are used as the feedstock. This prevents the economic manufacture of ethanol from waste containing low concentrations of sugar, except in unusual circumstances.

The manufacture of high-value compounds is superficially attractive but is not generally useful as a technique of waste utilization because high-value substances are normally required only in small quantities: their manufacture is not therefore an option when large quantities of waste require treatment. There is a growing consensus that the process of choice is the anaerobic conversion of the organic matter in the waste to methane, using the process of anaerobic digestion. The advantages are that the product separates spon-

taneously as a gas from the waste material, so that it is free from the contaminant chemicals and micro-organisms present, and that it has a value as a fuel which can be used in conventional furnaces.

The biotechnology of anaerobic digestion is now the subject of study in a number of laboratories and there is substantial literature on the topic and useful reviews and conference reports are available (e.g. Stafford, Wheatley and Hughes, 1980; Wentworth, 1983; Kirsop, 1984; Kirsop *et al.*, 1984; Wheatley, 1984; Lettinga *et al.*, 1984). Essentially, the problem is to develop the technology to the point at which the process can be carried out rapidly and reliably under industrial conditions where waste load varies considerably; at present neither objective can be guaranteed. Viable rapid processes seem likely to follow the adoption of systems of continuous fermentation in which the concentration of the organisms can be raised to high levels by some mechanism which retains them in the reactor while allowing the treated effluent stream to leave. Both attached-film reactors, in which the micro-organisms which convert organic matter to methane and carbon dioxide are present as a coating attached to the surface of solid particles in the reactor, and sludge blanket reactors, in which the microbes are retained because they associate in granules which sediment in the reactor and do not flow out with the waste stream, achieve this. Alternatively, the contact system, in which organisms are allowed to leave the reactor with the waste stream but are then removed and recycled, may be used. Lettinga *et al.* (1984) give references to the types of reactor of major interest; fluidized-bed systems are discussed in Cooper and Atkinson (1981).

The problem of reliability will be solved only when the appropriate control points have been recognized and methods for monitoring methanogenesis have been developed, so that process controls may be applied. It is becoming clear that the concluding stages in the process, namely the formation of methane from either hydrogen and CO_2 or from acetate, are also the ones which regulate the complex series of conversions involved. If the methane-producing steps occur at diminished rate, the concentrations of hydrogen and acetate build up and the preceding events are restricted, either because they become thermodynamically impossible or because of feedback events (Archer, 1983). The outcome in either case is that the concentration of the immediate products of breakdown of organic matter, namely short-chain fatty acids, increases and the pH falls, killing the methanogens and rendering the reactor ineffective. The recognition of these facts clearly suggests that the appropriate monitors of performance are instruments to measure the concentration of hydrogen and that of acids; the latter could presumably be measured indirectly by a pH meter but it would be helpful to have fatty-acid specific electrodes available for this purpose. Hydrogen meters are becoming available and preliminary reports of their use have appeared (Mosey and Fernandes, 1984). Archer (1983) has summarized control strategies.

Adequate monitoring will give early warning of problems. Although pH fall can be prevented by addition of alkali, it will also be necessary to take action to stimulate methanogenesis, so that the rate of methane generation becomes adequate to minimize the production of proton-generating acids,

e.g. propionic and butyric acids. In an operating reactor treating industrial waste, the option of reducing flow rate and thus substrate supply will be possible to only a limited degree; other steps, as yet ill-defined, will be required. The options are to increase the content of methanogenic bacteria or to increase the activity of those already present. An essential preliminary is to quantify the methanogenic bacteria, and improved methods to enable this to be done are being developed (Delafontaine, Naveau and Nyns, 1979; Van Beeden, Dijkstra and Vogels, 1983; Archer, 1984). Definition of the problem will also be facilitated as methods become available to allow the total biomass to be measured, where there are difficult problems to overcome in reactors containing mixed populations of relatively undefined mixtures of micro-organisms, which are usually associated in flocs and films.

Turning to the optimization of digester operation, the stages of start-up and of steady-state operation must be considered separately. In start-up, the objective is to attain as high a concentration of relevant micro-organisms as rapidly as possible. Usually, the initial inoculum is taken from an environment somewhat different from that of the waste stream to be treated and it must be modified to suit the new circumstances. Little information about this is available; in general, however, it seems likely that the events occurring in start-up will determine in broad outline the general level of performance of the digester in steady-state operation, for the population of biofilms and flocs, once established, is likely to be stable. One may postulate, for example, that the exclusion of sulphate-reducing bacteria from the population developed during start-up will greatly reduce the tendency for hydrogen sulphide formation during later operations, even when sulphate is present in the water stream.

With regard to steady-state operation, information is accumulating about the nutritional requirements of methanogens (Kirsop, 1984). Perhaps the most significant observation in recent years is that energy metabolism is not necessarily coupled to growth in methanogens (Powell *et al.*, 1983; Kirsop *et al.*, 1984). There is good evidence that, in some circumstances, a period of exponentially increasing methanogenesis is followed by a phase in which methane production continues for lengthy periods, in the presence of substrate, at the maximum rate established at the end of exponential growth. This implies that cells produced earlier continue to metabolize without growing further and suggests that, by control of the content of a regulating nutrient, it would be possible to ensure rapid increase of population during start-up and the absence of growth during subsequent operation. The latter will reduce the need for sludge disposal; some surplus biomass will still be formed during the reactions which lead to the production of substrates for methanogenesis.

Conclusions

It seems likely that these new opportunities for utilizing biotechnology in food processing will be implemented. The impact of biotechnology will thus be extended beyond the traditional areas in which it is already dominant, namely

in the dairy, baking and alcoholic drinks industries. The major markets are probably in the modification of raw materials to improve food quality; biotechnologists will be applying their knowledge to processes involving very high volumes and relatively low profits, where efficiency of operation will be all-important.

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