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Bioprocessing of Lignocelluloses [and Discussion]

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Bioprocessing of lignocelluloses

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Lignocelluloses represent a major source of renewable organic matter. Development of biological processing strategies normally must consider some form of pretreatment, hydrolysis of the polymers and bioutilization or bioconversion of these molecules to useful products. Bioprocessing technologies will usually involve low-moisture solid-substrate fermentations. Landfill techniques are now widely practised and gas abstraction methods developed. Aerobic composting methods have gained increasing importance recently and set out to achieve brevity of process with low energy consumption, safe standard products for agricultural use and hygienic operation. 'Open' and 'closed' composting systems are compared and evaluated. Single-cell protein has been produced from many lignocellulosic wastes and used for ruminant animal feeding. Current practices use pure-culture or mixed-culture bioprocesses. Mushroom cultivation is the most economically successful method for bioprocessing lignocelluloses and many commercial systems operate throughout the world.

Introduction

In current industrial practice, products made by biotechnology are, by volume, dominated by ethanol production, which consumes almost 90% of all carbohydrate feedstocks, with baker's yeast consuming 5%, whereas other processes make up the remainder (Hepner *et al.* 1984). Maize, sugar cane and molasses are the main raw materials for ethanol production whereas the other bioprocesses predominantly use molasses, glucose sugars, potato and corn starches and lactose. Selection of a raw material for bioprocessing will be dictated by price, availability, composition and form and oxidation state of the carbon source (Hacking 1986).

The future raw-material needs of large-scale biotechnology, which must compete with petroleum and coal to supply the primary sources of organic compounds for fuels and high-tonnage chemical commodities, could initially be supplied by cereal products. However, in the long term it is obvious that only the abundantly available lignocelluloses can supply the scale of need required for economic success (Hacking 1986).

In terms of gross tonnage, traditional biotechnological substrates such as sugar and starch are dwarfed by available wood. Wood makes up about 10% of global biomass production and of the remainder (straw, stems, bagasse etc.) most of it is lignocellulose. It has been calculated that as much as 2.2×10^{10} t of cellulose are produced annually and of that as much as 4×10^{9} t could be made available for processing (Phillips & Humphrey 1983).

For renewable raw materials to serve economically for industrial processing, including bioprocessing, the following criteria must be satisfied (Bull *et al.* 1982): namely a sufficiently large resource base to meet market demands; competitively priced products; appropriate technologies and high process efficiency.

Lignocelluloses derived from higher land plants represent a major source of renewable

organic matter. In form and availability they occur in whole trees, forestry processing wastes, agricultural wastes such as straw and bagasse and in many forms of domestic, municipal and industrial wastes. However, the boundary between wastes and by-products is arbitrary and should utility increase a waste can become a by-product and achieve a higher market value.

Bioprocessing technologies are expensive and must be analysed not only against one another but also against competing technologies, such as combustion, pyrolysis and gasification, to determine the most beneficial route to a given end product (Rolz 1984; Hacking 1986).

Bioconversion of lignocelluloses: the strategies

In the biosphere, catabolism of natural polymers such as the lignocelluloses represent mineralization rate-limiting steps with a multiplicity of reactions required to effect conversions to metabolic intermediates and end products. In the presence of oxygen dissimilations proceed comparatively slowly, although under anoxic conditions heterogeneous macromolecules are effectively biologically inert. According to Alexander (1981) persistence of any chemical, natural or xenobiotic, in the environment may be attributed to the inability of microorganisms to derive energy and cellular constituents from the oxidative metabolism of the compound although the supply of appropriate ancillary nutrients is also crucial.

Development of specific biological processing strategies must therefore eliminate or reduce these potential rate limitations, and consequently has led to the consideration of many forms of pretreatment. In addition, sophisticated genetic-engineering techniques have increasingly been considered, although conventional approaches for recruitment of microorganisms (Higgins *et al.* 1984) and, more particularly, interacting microbial associations (Senior & Balba 1984) are likely to remain important for some time, and in some instances may obviate the need for genetic engineering.

All microbial conversions of lignocellulosic materials to useful higher-value products will normally be multistep processes that will include all or some of the following (O.T.A. 1984):

(1) pretreatment (mechanical, chemical or biological);

(2) hydrolysis of the polymers to produce readily metabolizable molecules, e.g. hexose or pentose sugars;

(3) bioutilization of these molecules to support microbial growth (SCP), or to produce chemical products (ethanol, methane *etc.*); and

(4) separation and purification of the end products if required.

Biodelignification by microorganisms or isolated enzymes (ligninases) could well become an

Table 1. Barriers to efficient bioconversion of lignocelluloses

(Wood (1985).)

crystallinity of part of the cellulose fraction
lignin encrustation of the cellulose fraction
surface area available for enzyme attack
differential recalcitrances of the major polymers
pore-size distribution with the cell-wall structure
degree of polymerization of the major polymers
presence and/or toxicity of extractives of low molecular mass
moisture content
morphological heterogeneity at molecular and cellular level

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important pretreatment for lignocelluloses, removing or reducing the lignin and exposing the crystalline cellulose to biological activity. At present, biodelignification is expensive and not yet effective enough for realistic industrial use. However, ligninases from basidiomycete fungi, in particular *Phanerochaete chrysosporium* have now been identified, isolated and characterised (Kirk 1985; Buswell *et al.* 1984). Some of the many barriers to the effective bioconversion of lignocelluloses are listed in table 1.

Bioconversion of lignocelluloses into higher value products: the practices

Extant and potential uses of lignocelluloses for bioprocessing are listed in table 2. Although mushrooms are in essence the only truly economically viable products produced from lignocellulosics by bioprocessing (Wood 1985; Hayes 1985), other processes are now beginning to show economic possibilities (Wood 1985).

Because most lignocellulose substrates are in the solid form, bioprocessing will usually involve various types of solid or semisolid substrate fermentations or bioprocesses (Moo-Young *et al.*

Table 2. Existing and potential applications for bioconversion of lignocelluloses into products

(Wood (1985).)

products	substrates	technology available	technology profitable	constraints
mushrooms	composted: straw sawdust whole wood	yes	yes	consumer resistance to greater consumption
feeds or foods single-cell protein (SCP)	solid substrate: straw wood	no	no	product cost uniformity of scale-up rate of treatment
feeds or foods SCP	hydrolysed: straw sawdust wood	yes	no	price of pre-treatment costs of product
N_2-fixation soil conditioner biological control	straw	no	?	scale up to farm level treatment costs
pulp	wood straw	yes	no	(see feeds) product quality product cost
bulk chemicals e.g. ethanol glucose butanediol	wood straw food by-products	yes	no	other feedstocks more competitive nature of feedstock (see feeds)
speciality chemicals e.g. polymers phenolics	wood straw	yes	?	product price (see feeds)
waste treatment	pulping by-products	yes	no	process cost
enzymes e.g. cellulases hemicellulases	wood straw sawdust	yes	no	product cost compared with liquid fermentation sources

1983; Rolz 1984; Wood 1984). These are primarily low-moisture fermentations and basically lack the sophisticated control systems normally achieved in liquid fermentations. However, there is increasing interest and awareness of solid-substrate fermentations and the expanding role they will play in future biotechnology (Moo-Young *et al.* 1983).

(*a*) *Anaerobic treatment of lignocellulosics*

Although anaerobic treatment or digestion of lignocellulose-containing materials has received attention, the major bioreactor to be widely considered and practically used is the landfill bioreactor.

As a result of the increasing presence of lignocellulosic materials in refuse, the C:N organic-fraction ratios are often in excess of 55:1 and this may prove ultimately limiting to aerobic (composting) and, to a lesser extent, anaerobic catabolism without provision of nitrogen supplementation by, for example, addition of sewage sludge (Diaz *et al.* 1982).

Composting (see later), which is characterized by a period of intense microbial activity, does normally represent a key preliminary stage in the overall treatment of lignocellulosic materials by landfilling.

Although refuse emplacement strategies vary considerably from country to country, the trend in industrialized nations is towards covering the material at the end of the working day thus creating, with time, a multitude of solid-state closed cultures throughout the landfill bioreactor. The actual length of the composting stage varies considerably and is, in part, directed by primary refuse processing, with milling, pulverizing and shredding introducing oxygen, and baling eliminating this gas, and by site practices, because, for example, compaction diminishes trapped air. The degree of decomposition reached during this stage, by the intervention of invertebrates (mites, millipedes, isopods, nematodes and euchytraeids) and microorganisms (fungi, bacteria and actinomycetes) may be assessed by consideration of the cellulose:lignin ratio because estimates of 4.0, 0.9–1.2 and 0.2 have been reported for unfermented refuse, active or partly stabilized landfill and well-stabilized landfill respectively (Jones & Grainger 1983).

The major products of aerobic catabolism operating during the initial rapid phase of landfill technology have been described (Senior & Balba 1987). Depletion of molecular oxygen is accompanied by dramatic reductions in the rates of polymer dissimilation by microbial intervention, although incorporation of leachate recycle in the landfill-treatment strategy results in significant release of aromatic monomers by acid hydrolysis and is facilitated by the solubilization of carbon dioxide.

In addition to the chemical products detailed above, significant temperature increases result that, together with the presence of abiotic antimicrobial molecules (Kuster & Schmitten 1981), partly sanitize the refuse (Wigh 1984).

Oxygen depletion within the refuse mass that results in a slowing of heat production and, as a consequence, no longer facilitates oxygen entry by convection (Dilaj & Lenard 1975), results in reduced redox conditions and the utilization of electron acceptors, such as nitrate, sulphate and carbon dioxide; the actual sequence of which may be predicted on thermodynamic grounds according to the increment of energy liberated from a common electron donor as it is oxidized by each acceptor. In the absence of oxygen, complete mineralization of organic compounds requires the intervention of interacting microbial associations, each species of which contributes a part oxidation of the specific electron donor. Although this phase is arguably the most interesting from the microbiological viewpoint, only limited data (Jones & Grainger

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1983; Coutts 1986) of the catabolic sequences and interspecies interactions are, as yet, available although hypothetical schemes (Senior & Balba 1987; Senior 1987) have been developed.

Although interest in landfill gas recovery has increased considerably over the last decade (Wentworth 1984), this resource still represents tremendous untapped potential, particularly when one considers that 0.047 m^3 of CH_4 per kilogram could be realized annually during the active period of generation (Ham 1979). The landfill ecosystem, however, represents an extremely competitive and hostile environment for the methanogenic species (Senior 1984); therefore implementation of fully effective control strategies must be made in the light of these factors. Manipulation of direct and indirect environmental variables can, in fact, only be made by control of gross-site factors such as refuse composition, pretreatment, emplaced density and moisture content, temperature and pH–alkalinity. Manipulation of these, however, must not interfere with the day-to-day running of the site and must also account for the fact that perturbation of one may cause uncontrolled changes in others (Mouton 1984). Despite these possible eventualities, landfill-site practices to promote methanogenesis within a refuse mass have been described and gas abstraction techniques developed (Mouton *et al.* 1985).

As an alternative to gas generation in the landfill bioreactor, methane could be produced from leachate in an anaerobic digester, which would afford effective production control and optimization. Inhibition of methanogenesis in the refuse mass would, however, be necessitated and could possibly be effected by rapid water elution or leachate recycling to produce high concentrations of fatty acids to the exclusion of methane. By use of bioreactors in this way, loading rates could be controlled to match energy production with industrial demands, both in terms of quantity and time periods, and this would be reflected in the bioreactor configurations.

Despite its obvious attractions, methane generation from landfill leachate must be regarded as a short-term research and development objective because the product has a relatively low market value per unit mass in comparison with other bioreactor alternatives such as reduced organic chemicals. However, although landfill represents a reservoir of untapped potential for the production of added-value chemicals and chemical feedstocks, the motivation for their realization will still not be forthcoming until rising costs in the petrochemical industry identify the attractiveness of this resource.

(*b*) *Composting*

Composting, which is essentially a natural and traditionally used biological method of dealing with lignocellulosic waste material, has seen a remarkable revival of interest in recent years. The process involves the decomposition of heterogeneous organic waste material by a mixed microbial population in a moist, warm aerobic environment. To form compost, the organic matter must have lost its original identity and be stabilized to a humus-like product with an earthy odour. In comparison with anaerobic processes, composting produces less odour and develops a thermophilic stage that decreases the concentration of animal and plant pathogens.

(i) *The composting process*

The microbiological and biochemical changes which take place during composting have been extensively studied (see, for example, De Bertoldi *et al.* 1983) and will not be detailed here except in brief outline. The essential features are the initiation of the process in the organic

mass by a heterogeneous population of mesophilic bacteria, fungi and actinomycetes that are responsible for degrading a large proportion of compounds of low molecular mass that are present. The exothermic reactions associated with this metabolic activity cause the temperature to increase, and when it rises to about 40 °C the mesophiles become inactivated and are replaced by various thermophilic microorganisms including certain thermophilic fungi, which are particularly active in decomposing the cellulose fraction. If the temperature is allowed to reach 70 °C or higher, almost all microbial activity is inhibited and does not restart until the temperature falls and reinvasion can occur.

For the most part, composting processes are started with the existing microbial populations present in the organic wastes. Although various compost 'starters' are commercially available, recent studies with bark composting (Solbraa 1984) and with sewage-sludge composting (Nakasaki *et al.* 1985) have shown no beneficial effect of seeding. In some circumstances, however, inoculum technology may be appropriate, and the work of Lynch & Wood (1985) will subsequently be referred to.

Spontaneous composting of organic materials occurs in Nature, although these processes are slow, discontinuous and heterogeneous. Positive intervention is required to optimize the process and produce conditions that will enhance microbial activity. To make composting an efficient waste disposal process, three fundamental points must be met (De Bertoldi *et al.* 1985), namely: brevity of the process and low energy consumption; guarantee of a standard end product, which is not only safe for agricultural use but also has satisfactory fertilizer value; and, finally, hygienic safety of plant operation and compost end product.

For industrial composting of organic residues, the composting process must be controlled to obtain optimum efficiency, and this can be achieved by maximising microbial growth and activity. The main factors that must be optimized are oxygen supply, particle size and structure, moisture and temperature control, C:N ratio, and balance of nutrients and pH. Of these, the main factor that can be most influenced by technology and around which most designs have been developed is the availability of oxygen. A wide range of systems have emerged that can be classified according to the degree of containment, system configuration and method of aeration and/or agitation (table 3).

In the 'open' systems (windrow and static pile), the solid waste is stacked outside (although it can be under roof) and aeration is achieved in the former by periodic turning and in the latter by forced ventilation. Considerable research has been carried out in the U.S.A. to

TABLE 3. SUMMARY OF COMPOSTING SYSTEMS FOR WASTE TREATMENT

(From De Bertoldi *et al.* (1985).)

open systems		
turned pile		
static pile	– air suction	
	– air blowing	
	– alternating ventilation (blowing and suction)	
	– air blowing in conjunction with temperature control	
closed systems		
vertical reactors	– continuous	
	– discontinuous	
horizontal reactors	– static	
	– with movement of material	

optimize ventilation in static piles. In the so-called Rutgers Process, developed as an alternative to the Beltsville Process, which can suffer from inhibitively high temperatures, the strategy is to manage ventilation as a means of heat removal to prevent excessive accumulation (above 60°C). The strategy is implemented via a temperature-feedback control system, which communicates demand for heat removal from the composting mass to a blower. This constitutes a time-variable interactive control system that appears to provide the best means of temperature control and ensures continuity of the decomposition process at an efficient and predictable rate (Finstein & Miller 1985).

In the 'closed' composting systems, the process takes place within a vessel or bioreactor. Advantages claimed for these systems are that environmental factors do not affect the process, less land is required, and that better odour and operational control are possible. Disadvantages are that these systems have high equipment, maintenance and energy costs.

Aeration in composting bioreactors is accomplished by tumbling or stirring the material with or without forced aeration. Silo systems, of which there are at least five successfully operated designs in Europe (Easter 1982), are vertical plug-flow reactors. Horizontal plug-flow systems include the Dano Biostabilizer and the Tunnel bioreactor. There are also various agitated solid-bed composting systems, which are horizontal flow-reactor systems that intermittently mix the material (see, for example, Anderson *et al.* 1984).

Regardless of configuration, the same ecosystem dynamics are at play and the control strategies designed to maximize decomposition rate can be implemented in most of the 'open' and 'closed' composting systems. It has been argued (Finstein & Miller 1985) that the additional capital expenditure for a bioreactor or enclosure-based system might be justified only if superior performance is demonstrable, based on objective criteria, relative to rationally controlled unenclosed composting.

(ii) *Composting substrates in different regions*

In recent years there has been a worldwide upsurge of interest in the use of composting as a method of solid waste disposal. In the U.S.A., an appreciation of the pollution problems associated with incineration and dumping have led to the introduction of new legislative measures that have promoted composting as an environmentally acceptable means of waste treatment. More than 100 plants, mostly concerned with sewage-sludge composting, are already in operation in the U.S.A. (Willson & Dalmat 1983). In many of these plants considerable use is made of lignocellulosic materials such as woodchips and bark as bulking agents for the composting process.

There has been a longstanding interest in composting in many European countries, and recently the pace of research and development has intensified (Lutz 1984). Within the EEC, coordination of composting projects is being carried out by the Commission of the European Communities (Ferrero & L'Hermite 1985). The quantities of various wastes which arise each year in the member countries of the EEC are shown in table 4. Although sewage sludge, municipal solid waste, manure, straw and tree bark have been the most common substrates for composting, interesting work has been carried out recently that has experimented with a whole range of new or unexploited substrates. Also, several types of waste can be mixed to improve the properties of the substrate. The co-composting of municipal solid waste and sewage-sludge is a widely applied process in which the organic fraction of refuse is used as a bulking agent in the refuse and sludge mixture (Diaz & Golueke 1984).

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TABLE 4. QUANTITIES OF WASTE ARISING IN THE MEMBER STATES OF THE EUROPEAN ECONOMIC COMMUNITY

(From Ferrero & L'Hermite (1985).)

waste	amount of waste / Mt a^{-1}
domestic waste	150
agricultural waste	950
industrial waste	160
sewage sludge	300
waste from extractive industries	250
demolition waste and debris	170
consumer waste (discarded cars, used tyres, etc.)	120
other waste (street litter, dead leaves, etc.)	200

Composting mixtures of lignocellulosic residues and agro-industrial wastes is receiving attention in a number of European countries. The potential for composting waste in Europe is enormous considering the vast quantities of agricultural waste which arise each year (table 4). In Italy, for example, studies have been conducted on composting mixtures of poplar bark and poultry manure (Jodice *et al.* 1984). Also in Italy, Vallini *et al.* (1984) have studied the feasibility of composting wastes from several agricultural industries including tomato-processing wastes, cork-processing residues, olive husks and vegetable-tannery sludge. Satisfactory composts were obtained by co-composting various admixtures of these materials or by co-composting with sewage sludge or refuse. In Belgium, tobacco waste has been successfully composted with bark to produce a horticultural substrate (Verdonck *et al.* 1985).

One of the most innovative recent applications of composting has been its use to treat wool-industry wastes in France (Plat *et al.* 1984). Pollution from the wool-skinning industry could be effectively lowered by concentrating the effluents into sludges and then composting them with suitable agricultural wastes such as grape marcs, corn cobs, wood fibres and sawdust.

In the U.K., large amounts of organic waste are produced each year (table 5). In the past, most interest has been shown in the composting of domestic waste, and although plants have been in existence since 1947 only three of these remain operational. A major obstacle to the use of refuse composting in the U.K. is the very low cost of landfilling operations in many parts of the country. However, current problems associated with leachate and methane production from certain landfill sites together with increasing transport costs associated with dumping could enhance the prospects for composting in some regions (Stentiford *et al.* 1985). There has also been little incentive in the past to develop composting processes for sewage-sludge disposal in the U.K. because approximately half of the sewage sludge produced is spread directly on agricultural land. The forthcoming EEC directive on the disposal of sewage sludge to land, together with problems associated with the field burning of cereal straw at harvest and smell complaints from disposal of animal slurries on land close to housing, are current major issues facing U.K. agriculture (Biddlestone & Gray 1985).

Some of the straw produced by agriculture has traditionally been composted with horse manure to produce a substrate suitable for the production of mushrooms. Each year the mushroom industry in the U.K. consumes about 200–300 kt of straw in producing mushroom compost but this is only a small proportion of the straw from arable croppings (table 5). Consequently, the problems surrounding the disposal of straw in the U.K. and elsewhere

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TABLE 5. ORGANIC WASTES IN THE U.K.

(From Biddlestone & Gray (1985).)

waste	fresh mass 10^6 t a^{-1}
wood shavings, sawdust, and bark	> 1
food processing wastes, brewers grains	> 1
potato haulms, sugar beet tops	1.6
garden and nursery wastes	possibly 5
cereal straws, surplus	5–7
municipal refuse (about 50% organic matter)	18
sewage sludge, (dry solids)	35, (1.2)
farm manures	120

provide a considerable incentive to devise low-cost technologies for the useful treatment of this substrate. The use of straw as a bulking agent in the composting of manure slurries and other wastes has been advocated by Biddlestone & Gray (1985). Another approach has been the development of a novel process based on the inoculation of straw with cultures of lignocellulolytic fungi and nitrogen-fixing bacteria to produce a material with fertilizer, soil conditioning and plant-protecting values (Lynch & Wood 1985).

Japan has also seen a major revival of interest in composting, and many facilities for the treatment of sewage sludge, refuse, manure, bark and food industry wastes have been constructed (Kubota *et al.* 1984). The main impetus has come from efforts to reduce the excessive reliance by Japanese farmers on chemical fertilizers. There have also been recent reports on the value and applicability of composting in Korea (Hyung *et al.* 1984), in Southeast Asia (Gaur 1984) and in certain other developing countries (Dalmat *et al.* 1982). The particular values of composting in these countries centre around the restoration of soil fertility and productivity and, when dealing with sewage solids, the provision of a simple and efficient method of pathogen destruction that is of particular importance when sewage is used as an organic fertilizer.

Economic appraisals of composting as an alternative waste management strategy in different regions raises the problem of taking into account the cost of decisions made on political and environmental grounds. Changes in environmental control regulations such as have occurred in the U.S.A., can greatly alter the viability of composting compared with other forms of waste treatment. Economic appraisals of specific systems may only be relevant for particular types of waste material in particular locations and caution must be shown when drawing conclusions about the viability of similar systems where conditions differ (Gasser 1985).

(*c*) *Production of single-cell protein*

Single-cell protein (SCP) has been produced from a range of forest and agricultural by-products such as sulphite waste liquor, paper and pulp-mill wastes, sawdust, corn cobs, rice hulls, straw, bagasse, cotton hulls, etc. Large-scale production of SCP is feasible on such materials by using bacteria or fungi singly or in combination (table 6).

Successful development of SCP processes from lignocellulosic wastes has been achieved where the waste material has been partly hydrolysed or pretreated before microbial intervention.

Enzymatic degradation of some or all of the lignocellulose polymers is an essential feature of SCP processes. Cellulase, hemicellulase and ligninase enzymes can degrade the lignocellulose

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TABLE 6. PRODUCTION OF SINGLE-CELL PROTEIN (SCP) ON LIGNOCELLULOSIC WASTES

substrate	substrate pretreatment	microorganism(s)	fermentation process and scale	crude protein % dry mass
rice hull	4% NaOH	mixed culture of 4 *Bacillus* spp., 2 *Cellulomonas* and 1 *Pseudomonas*	pilot-plant fermenter with operating volume of 35 l	38.4
wheat straw		mixed culture of *Chaetomium cellulolyticum* and *Candida lipolytica*	3 l fermenter	16.0
wood	10% NaOH	*C. cellulolyticum*	14 l fermenter	16.2
wheat straw & swine manure	1% NaOH	*C. cellulolyticum*	10 l fermenter	25–30
waste water from paper and fibre-board mills		*Sporotrichum pulverulentum*	laboratory scale: 14 l feed trials; 25 m^3 fermenter	42
bagasse	acid/alkali	*Cellulomonas* spp.	Louisiana State University Process; estimated 100 t a^{-1} of SCP	12
paper- and pulp-mill waste		*C. cellulolyticum*	Envirocon Process: 10 m^3 fermenter producing 1 t of SCP from 2 t of pulp sludge	40

polymers to their monomeric moieties. The fungus *Trichoderma reesei* produces cellulase enzymes, and improvements in the strain have contributed significantly to increased cellulase formation (Warzywoda *et al.* 1983). Although studies with *T. reesei* are most advanced, the relatively low glucosidase activity in this strain led to further investigation of cellulases from other microorganisms such as *Pleurotus sajor-caju*, *Aspergillus wentii*, *Pichia etchellsii*, *Sporotrichum thermophile*, *Fusarium moniliforme*, *A. terreus*, *Clostridium thermocellum*, *Streptomyces* spp., *Actinomycetes* spp. and many others (Doelle 1984). Wase & Vaid (1983) isolated a strain of *A. fumigatus* from sawdust with widespread ability to utilize lignocellulosic materials, and then developed a mutant with improved cellulase-degrading abilities. Mishra *et al.* (1984) also reported a high cellulase activity in *Penicillium funiculosum* and noted that the presence of high levels of β-glucosidase, which is low in *T. reesei*, facilitated the yielding of glucose as the major end-product of hydrolysis. Rao *et al.* (1985) used mycelial biomass of *P. funiculosum* to hydrolyse alkali-treated bagasse. *Sporotrichum thermophile*, *Candida utilis*, *Fusarium oxysporum*, *Bacillus subtilis* and *A. niger* are some of the microorganisms that have been used to degrade hemicelluloses.

The lignin-degrading enzymes of the basidiomycete fungus *Phanerochaete chrysosporium*, which causes white-rot decay of wood, have been extensively studied. According to Kirk (1985), some of the characteristics that make *P. chrysosporium* more attractive for research in lignin degradation are high optimum temperature for growth (40°C), rapid degradation of lignin, lack of laccase activity and production of copious asexual spores and sexual fruit bodies. Cellulase-less mutants of the white-rot fungus, *Sporotrichum pulverulentum* (synonym: *Phanerochaete chrysosporium*) produced by Professor K.-E. Eriksson, degrades lignin and hemicellulose in wood leaving the cellulose. The cellulase-less mutants did not display worthwhile activity with bagasse (Al-Ani & Smith 1986).

Bioconversion of acid-treated peanut hulls by the lignin-degrading bacterium, *Arthrobacter* (KB-1) significantly reduced the lignin content and increased digestibility and nutritional value of the waste material (Kerr *et al.* 1984). The successful incorporation of bacterial degradation products from lignocellulose materials into microbial protein has been reported (Kern 1984). Glanser & Ben (1984) have reported a high degradation of lignin extract from corn stover by using a yeast, *Trichosporon* sp.

Microbial systems for upgrading wastes can involve aseptic pure cultures or non-sterile mixed cultures (Bellamy 1983). *Chaetomium cellulolyticum*, *Trichoderma reesei*, *Phanerochaete chrysosporium* and *Candida* spp., have been used extensively for large-scale production of SCP from lignocellulosics. Production of SCP from peat has been highly successful in the U.S.S.R. where a full-scale factory is being constructed with a target of some 1 965 000 t of protein per annum (Rimmington 1985). The specific organism(s) has not been named.

(i) *Pure-culture bioprocessing*

Ek & Eriksson (1980) demonstrated the direct conversion of waste water from paper and pulp mills into combined fungal biomass and water purification by using the thermotolerant white-rot fungus, *Sporotrichum pulverulentum*.

Chahal & Moo-Young (1981) used solid-substrate fermentation techniques to convert lignocellulose waste into animal feed with *Chaetomium cellulolyticum* and obtained a product with 20% crude protein and 33% *in vitro* rumen digestibility (IVRD). Steam pretreatment followed by solid-substrate fermentation was the most economic method for their purpose. A biomass containing 30% protein was obtained from alkali-treated straw fermented with *P. janthinellum* (Rao *et al.* 1985).

Tautorus & Chalmers (1984) have described a three-component system for the production of SCP from pulp-mill wastes with *C. cellulolyticum*. The components are: (i) pretreatment of pulp sludge with nutrients and sterilization; (ii) aerobic fermentation at 37 °C with the fungus; and (iii) product recovery. In this Envirocon process, the pilot-plant stage (fermentation volume 10 000 l) is capable of producing up to 1 t of high-quality SCP daily from 2 t of pulp sludge, and can be used to upgrade other wastes such as sawdust, straw, corn stover, bagasse, coffee grounds, animal manures and canning-industry wastes into high-protein products. The Envirocon process is reported to have advantages such as cost-effective performance, little nutrient requirement and easy plant construction over other SCP processes.

Recent studies on the ligninolytic activities of *Aspergillus japonicus*, *T. harzianum*, *Polyporus verisicolor* and *Pleurotus ostraetus* on hot-water treated wheat straw showed that *A. japonicus* and *P. versicolor* appreciably degraded lignin with increased yield of protein (Milstein *et al.* 1986). Bioconversion of straw to protein was more successful with solid-substrate fermentation than liquid culture. The simplicity of this fermentation technique, absence of organic ingredients, low energy input and absence of polluting by-products make this process more attractive as a low-technology device for on-the-farm processes to upgrade the nutritional value of straw.

(ii) *Mixed-culture bioprocessing*

Various studies have shown that the use of mixed cultures on lignocellulosics has resulted in increased substrate utilization and biomass production. Viesturs *et al.* (1981) showed that a mixed culture of *C. cellulolyticum* or *T. lignorum* and *Candida lipolyticum* on wheat straw gave a feed product containing 16–18% protein with 50% IVRD. *T. lignorum* alone gave 11% protein.

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Veal & Lynch (1984) also reported that the cellulolytic (cellulase) and nitrogen-fixing (nitrogenase) functions by co-cultures of *T. harzianium* and *Clostridium butyricum* resulted in a substantial increase in the rate of substrate decomposition compared with the fungus alone. They concluded that such associations offer an alternative to the genetic manipulation of single species for waste decomposition.

Laukevics *et al.* (1984) obtained high fungal protein from wheat straw with a mixed culture of *T. reesei* and *Endomycopsis fibuliger*.

(*d*) *Mushroom cultivations*

Mushroom cultivation is essentially a solid-substrate fermentation in which the objective is to maintain the dominance of the inoculated mushroom species over other organisms (Hayes 1985). The technology involved is basically concerned with the application of microbiology, fermentation technology and biochemical engineering to the propagation of microorganisms in a large-scale agricultural-type process. The cultivation of edible mushrooms is one of the few examples of a microbial culture in which the microorganisms cultured can be used directly for human food. Mushroom bioprocessing, although being recognized as an example of total biotechnology, is still a somewhat neglected area of 'new' biotechnological research (Flegg *et al.* 1985).

For the complete mushroom growth cycle, several distinct procedures generally associated with traditional fermentation processes such as antibiotic production will be required, namely: (*a*) medium preparation; (*b*) inoculum scale-up; (*c*) biomass production; (*d*) product production and harvesting; and (*e*) equipment sterilization (Tautorus & Townsley 1984; Wood 1984). Purity and stability of the mushroom culture is the foundation on which the industry survives.

Mushroom cultivation may be practised as extensive or intensive bioprocessing. In extensive methods, the cultivation procedures are subject to the local ecosystem and will be strongly influenced by seasonal climate. In contrast, intensive cultivation methods are based on artificially composed and pretreated (fermented, pasteurized) substrate under complete environmental control. By these methods it is possible to optimize fungal growth and regulate and stimulate fruit body formation at any time (Chang & Hayes 1978; Hayes 1985).

Intensive cultivation methods separate broadly into two groups. In the first group, the main feature is the requirement for a composted substrate and a second substrate, a capping or casing soil, which overlays the compost substrate and without which fruit bodies will not develop. In these systems other microorganisms play an essential role in successful cultivation. This is to all intents a mixed-culture fermentation. The main examples of this type of production include *Agaricus bisporus*, *Coprinus comatus* and *Stropharia raguso-annulata*.

The second form of intensive cultivation, while also requiring specific medium preparation, has a unique light requirement to induce fruit-body formation. The main organisms are *Lentinus edodes*, *Volvariella volvaceae* and *Pleurotus* spp.

A large number of edible mushrooms are now artificially cultivated throughout the world (table 7) (Zadrazil & Grabbe 1983). *A. bisporus* is the most widely cultivated and most universally consumed of all edible fungi.

The future role of mushrooms in global biotechnology will be governed by the economics of production methods and costs relative to other animal and vegetable foods. Substrates assume the greatest share of production costs. Compost manufacture and materials have been considered to cost 20–25% of total mushroom expenditure (Royce & Schisler 1980). The

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TABLE 7. PRODUCTION OF EDIBLE FUNGI BY ARTIFICIAL CULTIVATION METHODS

(Zadrazil & Grabbe (1983).)

species	common name	distribution	quantity/kt
Agaricus bisporus	white mushroom	worldwide	750
Lentinus edodes	shii-ta-ke	Japan, Far East	180
Volvariella volvaceae	Chinese or straw mushroom	tropical countries	65
Flammulina velutipes	winter mushroom	Japan, Taiwan	65
Pleurotus spp.	oyster mushroom	worldwide	40
Philiota nameko	nameko	Japan	20
Auricularia polytricha	Jewish ear	Japan, Taiwan	12
Auricularia auricula-judae			
Tremella spp. and some other species		Taiwan and worldwide	3
total			1135

mushroom industry will require continued biotechnological inputs to the whole system to stay competitive with other comparable processes. In particular, there will need to be dramatic improvements in the biological efficiency of solid-substrate fermentation procedures including the use of organic supplements, together with better understanding of mushroom physiology and biochemistry and the use of genetically improved strains. The principles of substrate recycling are now gaining greater importance, and experimental studies with hydroponic mushroom culture with inert support systems and liquid perfusions (derived from traditional solid composts) could well lead to the development of a continuous production process (Tautorus & Townsley 1984).

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Discussion

D. A. Wood (*Glasshouse Crops Research Institute, Littlehampton, West Sussex, U.K.*). I thank Professor Smith for his kind acknowledgement of my recent article on lignocellulose utilization and of our recent book *The biology and technology of the cultivated mushroom* from our Institute.

I would like to emphasize that, as he pointed out, mushroom cultivation represents the only economic system for lignocellulose utilization. At GCRI we have been able to produce significant advances in the study of the physiology, biochemistry and genetics of the cultivated mushroom *Agaricus bisporus*. We now have available a partial library of genes, protoplasts, purified enzymes and enzyme antibodies and work on enzyme mutants. This work should lead to the ability to improve bioconversion and crop yield and cropping strategies in this organism.

It is important, however, that studies of lignocellulose utilization are actively pursued at the fundamental level so that we have model systems to draw on to improve the performance of mushroom strains.

J. M. Lynch (*Glasshouse Crops Research Institute, Littlehampton, West Sussex, U.K.*). The greatest opportunity for mixed-culture fermentations is in non-axenic systems, such as mushroom cultivation and anaerobic digestion. In Ohio, Professor H. Hoitinck has demonstrated that composted barks can be inoculated with *Trichoderma* spp. after a primary degradation stage, and these have the potential to suppress other cellulolytic species such as *Aspergillus* and *Fusarium*. The analysis of the interaction between these species requires an approach at the population biology level. Other non-axenic mixed-culture lignocellulosic fermentations would benefit from such analyses such that the degradations might be manipulated by inoculation.

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