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PREFACE

Two complementary review articles over microbial enzymes involved in the degradation of polysaccharides form together half of volume 15 of Progress in Industrial Microbiology. The contribution of Professor Halliwell deals with the β-glucanases and in particular the cellulases. Cellulases have been in the limelight for some years now, due to the fact that cellulose waste materials are so abundant. Cellulose is the most widely available renewable natural resource. When we finally get round to fully utilizing this great potential, microbial cellulases will undoubtedly have a major role to play.

Drs. Fogarty and Kelly have promised a very comprehensive review over microbial α-glucanases and in particular the enzymes involved in starch degradation. In contrast with cellulose degrading enzymes, of which the industrial application is still very much in its infancy, the starch dedrading enzymes are already widely accepted and applied. The starch degrading enzymes are in volume of industrial production second only in importance to the proteases. In Part I of their review Fogarty and Kelly deal with the distribution and characteristics of this group of enzymes. In Part II, which it is hoped will be published in volume 16 of Progress in Industrial Microbiology, the same authors plan to cover the synthesis, regulation and production.

In a short but fascinating article Professor Venkatasubramanian and Professor Vieth deal with one of the most interesting recent developments in industrial microbiology: immobilized microbial cells. The possibilities and prospects for the application of immobilized cells are manifold and exciting. The future will tell us how many of these great expectations were realisitc.

The genetics of yeasts has been the subject of many publications including a number of excellent reviews. The genetics of industrially important yeasts has however been relatively poorly reported. This omission has been rectified in the current review by Dr. Johnston and Professor Oberman. An added dimension is given to this article by the, all to rare, collaboration between experts from West and East Europe. As a result of this collaboration we are given an insight into the large volume of literature on this topic which has been published in Russian and Polish journals which many of us might otherwise have missed.

After a casual glance at the title of the last contribution the reader might be tempted to ask why it has been placed in a publication over industrial microbiology. The economic implications of marine microorganisms in the fouling and corrosion of ships, oil-drilling rigs and power stations are however enormous. Marine transport and energy production and distribution are the life arteries of industry. Drs. Meadows and Anderson give a fascinating insight into this field of study and indicate some of the major problems which are faced by marine microbiologists.

M.J. BULL

CONTENTS

MICROBIAL β-GLUCANASES

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1. INTRODUCTION

Glucans are homopolysaccharides based on the single sugar unit of glucose. Chemically they may be relatively simple consisting of linear macromolecules with the linkages between sugar residues all of the same type, as in cellulose (1, 4-β- linkages) and in laminaran (1,3-β- linkages) or they can be of greater complexity in possessing more than one type of linkage either in linear chains (e.g. lichenan with 1,3-β- and 1,4-β- linkages; nigeran with 1,3-α- and 1,4-α- linkages) or in having branched chains as for example in amylopectin and glycogen, with both 1,4-α- and 1,6-α-linkages (Marshall [1], Sidebotham [2], Aspinall [3], Ward and Seib [4], Manners [5].

Cellulose is found in quantity in plant material along with a related polysaccharide, the 1,4-α-, 1,6-α- glucan, starch, which itself comprises the commonest food reserve material of the plant kingdom, where it is found in seeds, fruits, leaves, bulbs, tubers, rhizomes and in algae. Glycogen, the corresponding 1,4-α-, 1,6-α- glucan constituting the commonest food reserve material of animals is prominent in mammalian liver and found also in brain, muscle, invertebrate tissue and in protozoa. A recent report indicates similar reserve nutrient is synthesised as a highly branched glycogen from glucose in the mycelium of a wood-rotting fungus, Polyporus circinatus [6]. Some of the anaerobic bacteria of ruminants, such as cattle, are able to digest the various forms of cellulose found in plants and, in one such species Ruminococcus albus, the carbohydrate is stored in intracellular granules as a reserve glucan of the glycogen variety [7].

Of considerable interest in recent years has been the role of dextrans in tooth decay, dental caries. Dextrans, extracellular D-glucans containing substantial numbers of 1,6-α- linked-D-glucopyranose residues, are synthesised by certain bacteria, particularly species of the genera Lactobacillus, Leuconostoc and Streptococcus growing on sucrose. The basic skeleton of these dextrans may also be intersected or terminated by isolated 1,3-α- linked glucose residues as well as carrying branch glucose residues attached at carbon 2, carbon 3 or carbon 4 [2]. Leuconostoc species have long been noted for their ability to produce gums and slime

(dextrans) in fermentation media containing sugars. A similar role is apparently played also by Streptococcus mutans and S. sanguis, important human microflora belonging to the largest group of microbes, Streptococci, colonising early dental plaque. Diets with high sucrose levels promote formation of plaques and dextrans, which in turn provide a stable matrix and source of fermentable carbohydrate for oral acidogenic bacteria.

One or two examples will suffice to illustrate the complexity of the problem. In a study of the relationship of extracellular glucans to adherance and cariogenicity, wild type and mutant strains of Streptococcus mutans were used that synthesised such dextrans from sucrose. The mutant produced a non-fibrillar glucan with only one-tenth as many 1,3- linkages as were found in the "wild type polysaccharide" and apparently as a result of this change showed a reduced tendency to adhere to (glass) surfaces [8].

The importance of the secondary 1,3-α- linkages in dextrans is seen in the marked resistance to commonly available dextranase preparations of insoluble glucans synthesised by S. mutans. Enrichment of soil microbes by growth on this glucan(s) provided a Flavobacterium sp. and an enzyme therefrom that acted endo-wise on 1,3-α- glucosidic linkages to solubilise half of the original resistant glucan, 11% appearing as glucose [9]. Glucans containing mainly 1,6-α-, 1,4-α-, 1,3-β-, 1,4-β-, and /or 1,6-β- linkages were unattacked.

Recent work has also recognised a 1,3-α- glucan as a common component of microcyst walls of several Gram negative cyst-forming bacteria where it was thought to contribute to the rigidity of such forms [10]. Polysaccharides can also be found as a characteristic feature outside the cell wall of a number of other microorganisms and consist of polymers of different sugars and/or acid components [11, 12].

In nature, these and associated polysaccharides are subject to breakdown by glucanases and related enzymes, such as the hemicellulases [13], in processes of degradation that may on the one hand be beneficial to man in the recycling of carbon yet detrimental on the other. Saprophytic microbes, phytopathogens and wood-rotting organisms destroy dead and living vegetable matter, timber and similar materials, including valuable crops, by attacking glucans and related polymers occurring as complex mixtures in all plants; dextrans have been implicated in dental decay; extracellular polysaccharides often impede fermentation processes. In contrast rumen microbes assist in converting the same inedible carbohydrates to foodstuffs of value to man and animals.

Recently with the desire for a cleaner environment, more attention has been focussed on some of the superficially objectionable degradative reactions, particularly of glucanases, in attempts to turn these to man's advantage by directing microbes to dispose of wastes of plant and animal origin in the conversion of such products to useful materials including alternative forms of energy (methane and alcohols), sugar and foodstuffs in the form of microbial protein. Earlier reviews or information on such topics can be found in "Cellulases and their applications [14], "Enzymatic hydrolysis of cellulose" [15] and in "Bioconversion of cellulosic substances into energy, chemicals and microbial protein" [16]. "Cellulose as a chemical and energy resource" with particular reference to the substrate, enzyme system, process and product is examined elsewhere [17]. The use of cell-free glucanases provides a unique opportunity to saccharify vast quantities of unwanted but valuable and renewable photosynthate in the form of waste plant polysaccharides. Saccharification technology, the raw materials available and applications have been examined elsewhere [18]. Development of cellulase preparations for similar purposes in the treatment and extraction of foodstuffs [19] and in the saccharification of agricultural wastes has been a particular interest of the Japanese workers [20, 21].

All the processes referred to above depend upon particular microorganisms and the action of the individual glucanases they elaborate and make responsible for hydrolysing one or more of the appropriate polysaccharides. Knowledge of the nature of these enzymes; their mode of action, control, inhibition or promotion of activity, regulation of biosynthesis, their intra- or extra-cellular location, secretion and immobilisation are important pre-requisites in attempting to limit the extent of detrimental processes or to promote them where they might prove useful in the examples given earlier. The present review examines salient features of microbial β-glucanases in the directions outlined above, emphasising recent advances and with particular reference to the cellulase complex. Useful background reading can be found in surveys on the application of glucanases to the structural analysis of polysaccharides [1], cellulases [22], cellulose and cellulolysis [23], formation and degradation of cellulosic fibres [24]. Reviews of more recent vintage are considered in the appropriate section below.

2. THE SUBSTRATES

β-glucans, as current photosynthates, occur as natural macromolecules universally distributed, initially in living organisms where they perform a variety of functions, skeletal in cell walls of higher plants, as a reserve food supply in plants and microbes and as encapsulating substances of miccroorganisms. The most abundant naturally occurring organic substance known is the 1,4-β- glucan cellulose, found as the main structural element and constituent of the walls of higher plants. In a similar form it comprises an increasing fraction, ranging from 45 up to 98% of the dry weight of wood, jute, ramie, flax and cotton. Such sources of cellulose may be treated as fibres themselves, as wood pulp for papermaking, processed to rayon including cellulose acetate for use as a textile, as a plastic, as an ion-exchange material, or employed in the form of soluble derivatives as thickening agents in food, paints and pharmaceuticals.

Structurally, the β-glucans can be conveniently classified for the present purpose according to the predominant linkage and type of chain as illustrated in Table 1. Variations on this scheme with specific references to individual compounds are listed elsewhere [1, 25, 26]. Whereas linear β-glucans containing only one type of linkage such as cellulose, are well known, others may be almost linear or branched with more than one type of linkage in the same polysaccharide. In the 1,3-β-glucan series, only very small amounts of 1,6-β- glucosyl residues are to be found, situated at branch points in laminaran and pachyman but present in the linear chains of curdlan. In general, cell walls of fungi display a varied array of 1,3-β- glucans based on the relatively simple linear pattern above. Thus alkali soluble 1,3-β- glucans from cell walls of Pullularia pullulans and Piricularia oryzae have similar structures with secondary 1,6-β- glucosyl branching more complex than that in laminaran. In the case of a 1,3-β- glucan of yeast cell wall (Saccharomyces sp.) the additional 1,6-β- glucose linkages may be linear or possibly slightly branched [1] (see also below, Manners [28]). Considerable branching is found in the glucan from cell walls of the fungus Phytophthora cinnamoni in which side chains of 1,3-β- linked glucose residues (4 per chain) are linked to a backbone containing 1,3-β- or 1,6-β- glucosidic links or both.

Table 1.

Structure and Distribution of β-glucans

Main linkage	Type of chain	Common name	Source
1,2	linear		Crown gall organism, *Agrobacterium* spp.
1,3	linear (or mainly so)	laminaran	Brown seaweeds, *Laminaria* spp.
		pachyman	*Poria* sp.
		curdlan	*Alcaligenes*, *Bacillus*, and *Agrobacteria* spp
		callose	Woody tissues of higher plants
		various	Cell walls of various algae, fungi, yeast.
1,4	linear	cellulose	Cell walls of higher plants, some algae and fungi; certain bacteria.
1,6	linear	pustulan	lichen (*Umbillicaria* sp.)
		lutean	*Penicillium* spp.
1,3) 1,6)	branched	schizophyllan	*Schizophyllum* spp.
		sclerotan	*Sclerotinia* spp. *Claviceps* spp
1,4) 1,3)	linear	lichenan	Lichen(Iceland Moss)
		β-glucans	cereal grains
1,6) 1,3)		yeast glucan	*Saccharomyces* sp. cell wall

In the case of glucans with mixed linkages (1,3; 1.6 or 1,4; 1.3 or 1.6; 1.3) the first linkage of each pair constitutes the predominant linkage. For further details of fine structure, see text and Marshall [1], Aspinall [3], Barras et al [25], Rogers and Perkins [26].

Extracellular polysaccharides of various organisms can be relatively highly substituted. Those from *Claviceps purpurea* and related glucans such as sclerotan of *Sclerotinia libertiana* and *S. rolfsii* are basically 1,3-β- glucans with single 1,6-β- linked glucose groups on approximately every fourth unit of the backbone. *Pullularia pullulans* secretes an extracellular glucan of the same type but with 70% of the glucose units

in the backbone carrying substituents [1]. Schizophyllan from Schizophyllum commune is similarly structured. Lichenan and the cereal β-glucans are essentially linear with 1,3-β-glucosyl residues (35% and 25% respectively) attached to 1,4-β- glucose units so that lichenan for example appears essentially as repeating cellotriose units (1,4-β-) joined linearly by 1,3-β- glucosidic linkages [1, 4].

Functionally the same β-glucans may be considered as belonging to three main groups: (a) the reserve polysaccharides, of cereals, lichenan of Iceland moss, laminaran of seaweeds, (b) structural components of microbial and plant cell walls as with the 1,3-β- glucans of yeast and Pullularia species examined above, (c) extracellular secretary products including the 1,2-β- glucan of phytopathogenic agrobacteria, cellulose of non-pathogenic microorganisms like Acetobacter xylinum and Rhizobium spp. [27] and the schizophyllan type 1,3-, 1,6- glucan of Claviceps and related species [1]. The site of location of these and related glucans in plant tissues or a microorganism poses problems, in their extraction and identification, that increase in complexity as we pass from extracellular regions to intracellular material to cell wall components.

The complexity of the situation is well illustrated by the difficulties that attended recognition of the mixed 1,3-β and 1,6-β-glucans of yeast cell wall where standard techniques of carbohydrate analysis were less effective with insoluble glucans. Such material from cell walls of the yeast, Saccharomyces cerevisiae is heterogeneous consisting of approximately 85% of an insoluble branched 1,3-β- glucan of degree of polymerisation 1500 with 3% of 1,6-β-glucosidic interchain links; the minor component (15%) is a soluble branched 1,6-β-glucan of degree of polymerisation 135 with 14% of 1,3-β-glucosidic interchain linkages. Glucan preparations from the walls of other yeasts were found to be similarly heterogeneous [28, 29, 30]. Cell walls of the Schizosaccharomyces pombe strain used here also contained a galactomannan and 1,3-α- glucan in addition to a branched 1,3-β-D-glucan, all extractable in cold (4°C) dilute alkali [31]. The picture is further complicated by the effect of temperature on the extraction of glucans. In the case of barley β-glucans their 7:3 ratio of 1,4- and 1,3- linked β-glucosyl residues showed a shift towards material of higher viscosity containing relatively more of the 1,3- sequences at higher temperatures of extraction (100°C) [32].

An interesting qualitative procedure for recognising curdlan and similar 1,3-β- glucans such as pachyman and yeast glucan (but not

laminaran) formed by colonies of microorganisms growing on glucose medium involves staining with Aniline blue. The dye was highly specific for curdlan type polysaccharides and demonstrated their formation by Alcaligenes faecalis, Bacillus spp., strains of Agrobacteria some yeasts but not fungi [33]. One of the strains of Agrobacteria, A. radiobacter which formed distinct blue and white colonies on Aniline blue plates showed a change in ability to produce water soluble and insoluble β-glucans [34]. The white colonies were unstable, and yielded blue colonies on prolonged incubation and produced much water soluble polysaccharide (succinoglucan) but little water insoluble polysaccharide (curdlan type). Blue colonies were stable for two months and produced much curdlan type glucan with little water-soluble polysaccharide.

In a study of the structure and properties of soluble 1,3-β- glucans from the green alga, Caulerpa simpliciuscula, two components were recognised in the soluble β-glucan fraction formed during photosynthesis. One component had a degree of polymerisation of 37, comprised most of the β-glucan fraction and resembled laminaran of other algae and fungi. The second β-glucan, of high degree of polymerisation (270) contained most of the radioactivity accumulated in the β-glucan fraction during short periods of photosynthesis, thus suggesting it might be a precursor glucan of the large amount of low molecular weight glucan which itself acted as the main storage compound within the plant [35].

3. THE ORGANISMS

ACTIVITY AGAINST CELLULOSE AND ITS DERIVATIVES.

As defined above, the bulk of the substrates for β-glucanases are carbon sources that serve in nature as primary foodstuffs for microflora and are found mostly in plant material whether living or dead, subterranean or aerial. Additional supplies of glucans are furnished by microbial cells, fungal and bacterial, extracellularly, intracellularly and as cell wall components. Such material whether attacked in situ by bacterial, fungal, or viral plant pathogens, or after death also by saprophytic microorganisms on entering the soil, present microbes with a variety of substances that are physically and chemically heterogenous. The organic components of plant material serving as nutriment are found in three main forms, cellulose (up to 60% of the dry weight of the plant)

hemicelluloses (up to 30%) and lignin (up to 30%) all closely bound up as part of the cell wall [36, 37]. The greater amount of lignification is characteristic of the walls of mature cells which have become thickened and adopted a definite shape to become secondary cell walls whereas, in contrast, in a growing plant cell the surrounding wall is relatively thin, irregular in shape and as such constitutes the primary cell wall consisting almost wholly (90%) of polysaccharides, cellulose, xyloglucan, arabinogalactan and rhamnogalacturonan, the rest being protein [38. 39]. Details of the fine structure and properties of cellulose itself |40| and of available cellulosic materials [41] are to be found in a recent symposium on the enzymatic hydrolysis of cellulose. An earlier review on the structure and morphology of cellulose is provided by Ranby [42]. Structural, including physical and chemical features of cellulose that influence its susceptibility to enzymic hydrolysis have also been examined in detail [43, 44, 45, 46].

Despite apparent obstacles to cell free enzymic decomposition of cellulose and related glucans, intact microorganisms, particularly in nature, seem to experience relatively little difficulty in this direction. More than 10^{10} tons of the earth's carbon as carbon dioxide are believed to be converted annually into plant matter by photosynthesis [23, 47] whilst cellulose degradation on earth is estimated to consume some 10^{9} tons of carbon per year in forests alone [48]. Since cellulose and associated β-glucans fail to accumulate to any significant extent it is clear that soil microorganisms must play a particularly effective role in recycling carbon. The first agents of biological decomposition of dead organic material are saprophytic 'sugar fungi' mainly Phycomycetes in which the ability to use recalcitrant molecules such as cellulose and lignin is feeble. They are followed by Ascomycetes with or without Basidiomycetes either of which may use cellulose; the final group includes most lignin utilising species with Basidiomycetes predominating [49]. Such general activity is illustrated by the ability of the heterogenous mixture of microbes producing extracellular degradative enzymes from composting leaves and their response to temperature changes over a hundred day test period [50]. Many mesophilic bacteria died as higher temperatures (above 40°C) developed in the interior of the pile whereas the numbers of thermophilic bacteria remained almost constant. In contrast the numbers of mesophilic fungi changed little whilst the thermophilic forms fluctuated considerably, decreasing in numbers when

the pile was hot and increasing as it cooled. Throughout the composting period bacteria and fungi were found to secrete cellulase (carboxymethylcellulase), pectolytic enzymes, protease and lipase. Cellulose in the pile decreased from 40% to 30% in twenty three days during the thermophilic stage whilst bacteria producing carboxymethylcellulase remained constant at about 17% of the total bacteria throughout the fermentation. In somewhat similar circumstances cellulase (carboxymethylcellulase) has been implicated, along with laccase in changes occurring in extracellular enzyme activities during growth and fruiting of mushrooms [51]. Laccase concentration increased during mycelial growth and then declined rapidly at the start of fruiting whereas carboxymethylcellulase was detectable throughout growth and increased on fruiting. No such changes were found with laminaranase.

In such circumstances where, in vivo, mixed substrates of plant or microbial origin, are common one or more organisms may attack β-glucans other than cellulose particularly if these are more susceptible because of their disposition in the plant debris or in their reactivity. A list of such organisms is to be found in an extensive survey of fungi, bacteria and Actinomycetes examined primarily for cellulolytic activity [52]. In this comparative study of eighty microorganisms some (the cellulolytic) were able to degrade cellulose, as illustrated by the reduction in tensile strength of strips of cotton fabric, and also produced an enzyme (carboxymethylcellulase, CM-cellulase) hydrolysing the soluble cellulose derivative carboxymethylcellulose (CMC); many other organisms including Aspergilli and Penicillia and some white rot fungi but more particularly the brown rots, produced just as much CM-cellulase whilst showing relatively feeble activity against cotton. Other microbes (non cellulolytic) possessed neither property though some of these, like Penicillium funiculosum and Rhizopus arrhizus undoubtedly have activity against β-glucans of the 1,2-, 1,3-, or 1,6- series [1, 53, 54, 55]. The cellulolytic forms when grown in agitated cultures provided filtrates with activities that allowed classification into three main groups: the first produced CM-cellulase in absence of cellulose (ground cotton or wood); the second produced CM-cellulase only in presence of cellulosic substrates; the third formed little CM-cellulase in the medium on any cellulosic substrate. Not surprisingly in view of their habitat in soils and the consequent nature of the plant material that constitutes their natural nutriment many of these organisms, like the non-cellulolytic forms referred to above, have been found to share the ability to attack non-cellulosic glucans such

as laminaran [54].

The most effective site of cellulolytic activity is probably that of symbiotic associations, particularly in the rumen of animals such as cattle and sheep [56,57,58,59]. Here [56,57] it was shown that all types of cellulose, from the soluble CM-cellulose through insoluble cellulose powders to native cellulosic fibres, were effectively hydrolysed by mixed rumen bacteria and that agitation retarded the process. Some five pure strains of anaerobic bacteria were isolated from the rumen of cattle of which Bacteroides succinogenes, Ruminococcus albus (two strains), R. flavefaciens (two strains) were highly active against degraded cellulose (ground powder) but only one strain of each organism showed significant activity against fibrous cellulose (native undegraded cotton fibres) [58]. B. succinogenes was by far the most effective cellulolytic organism. The same organisms and related species utilise cellulose more effectively still in mixed culture [56,57] probably because the secondary population, whether weakly cellulolytic or non-cellulolytic, favour cellulolysis by removing metabolic products including excess sugars from the environment thereby relieving possible inhibitory action on the cellulase complex (see below under "The Enzymes"). Factors affecting the cellulolytic activity of rumen bacteria have been re-examined in the last year by Stewart [60]. Although the medium and assay conditions were somewhat different from that of the earlier work the in vitro findings confirmed the reports [56,57] in establishing that a 10% (v/v) inoculum of mixed rumen microbes from sheep was optimal for maximum cellulolysis. This rate was reported as up tc 10 mg/ml rumen contents in 24 hr [60] and was compared with that elsewhere [56]. Because of the nature of the kinetics of enzyme systems where amounts of inoculum and substrate as well as concentrations must be considered particularly in association with the heterogeneity of the cellulose-cellulase assay system a more appropriate comparison is that with Fig.1. of the earlier reference [57] where with only 50 mg of cotton fibres available 10 mg cellulose was used per ml mixed rumen microbes in 24 hr. Stewart [60] observed that the presence of dietary additives (barley) depressed cellulolysis only if the pH fell. Addition of tallow also retarded digestion of grass cellulose and cotton but did not reduce the number of filter-paper degrading bacteria thus suggesting a more specialised effect of the fat on the truly cellulolytic bacteria.

Less obvious symbiotic environments than the rumen must undoubtedly share in the responsibility for continuous and effective removal of the

vast amounts of β-glucans produced each year. None have received the attention afforded to ruminants but evidence is slowly accumulating on beneficial associations to be found between cellulolytic and non-cellulolytic species. An interesting association of anaerobes in the free living state is seen in the fermentation of cellulose in presence of both Clostridium thermocellum and Methanobacterium thermoautotrophicum when the lag period was shortened for growth and cellulolysis, although the final extent of cellulose (powder) decomposition in four days was 15% greater (48% solubilisation) with the Clostridium sp acting unaided [61, Table 1]. On its own, C. thermocellum [62] a thermophilic obligate anaerobe, utilised cellulose and cellobiose, but not glucose and like the rumen bacteria above [56] showed reduced growth on cellulose if accompanied by agitation. Cellulose powder (Avicel) was reported to be completely degraded. Culture filtrates were examined for cellulase (on Avicel), CM-cellulase and cellobiase (β-glucosidase). The assay for cellobiohydrolase or exo-glucanase (apparently misprinted as enzyme EC.3.2.1.74, exo-1,4-β-glucosidase [63]) employed Avicel with crude culture filtrates containing CM-cellulase and as such is better regarded as an estimate of cellulase rather than of cellobiohydrolase activity. Table 3 [62] suggests about 14% solubilisation of Avicel was achieved in 1 h. In 4 h the same crude filtrates provided equal amounts of glucose and cellobiose from the cellodextrin substrates, cellotriose and cellotetraose whilst hydrolysis of cellulose powder (MN 300 in 24 h) by ten-fold concentrated culture filtrates gave twice as much glucose as cellobiose. Although cellobiase activity was not detectable in 2 h its presence in the concentrated preparation over 24 h could account for the observed pattern of hydrolysis which is similar to that expected from the known properties of purified enzymic components of cellulolytic fungi where CM-cellulase and β-glucosidase (cellobiase) cooperate in endo- and exo-actions, particularly on the larger dextrins (see below under "The Enzymes"). A similar thermophilic anaerobic Clostridium sp. isolated from manure degraded filter paper in two days at 60°C but showed less than 1% solubilisation of fibrous cellulose (cotton) in 1 h [64].

As is evident above, possession of thermophilic characteristics does not in itself ensure greater amounts of cellulase or more effective activity; nor is Avicel a particularly good choice of cellulose to assess cellulolytic activity (see later in this section under Cellvibrio sp. [90] and Cellulomonas sp. [92]). Thermophilic fungi such as Chaetomium thermophile, isolated from refuse compost, have shown more promise in their