THE ANAEROBIC THERMOPHILIC CELLULOLYTIC BACTERIA[1]

R. H. McBEE[2,3]

Division of Plant Nutrition, University of California, Berkeley, California

The bacteria capable of fermenting cellulose at temperatures above 50 C have been the subject of much more study than would appear to be warranted when one considers that they probably play only a minor role in the decomposition of this material under natural conditions. There have been two main reasons for this interest in a relatively unimportant group of organisms: *a*, the belief has arisen and been fostered that cellulose fermenting bacteria are different in some respect that makes their cultivation in pure culture extremely difficult when compared with other anaerobic bacteria, and *b*, the possibility of obtaining a commercially practical fermentation of cellulosic waste materials with the formation of valuable end products has been very appealing. Because rapidity of conversion in an industrial process is of prime importance and because thermophilic bacteria are often found to grow extremely rapidly, the possibility of using these bacteria in industrial cellulose fermentations has been extensively studied.

CULTURE OF THE CELLULOSE THERMOPHILES

The belief that cellulose fermenting thermophiles are difficult to isolate in pure culture started with the experiences of Macfadyen and Blaxall (28–30) who isolated with relative ease several strains of non-cellulolytic thermophilic bacteria of various sorts. They demonstrated an active fermentation of cotton and straw when it was mixed with Thames mud and incubated at 55 C but were unable to pure culture the responsible organisms, or even to obtain colonies of the cellulose fermenting bacteria.

A similar experience was recorded by Kroulik (24). He recognized that the characteristics of the cellulose thermophiles could not be adequately determined from cultures purified only by serial dilution in fluid media, the procedure used by Pringsheim (42, 43), but was compelled to use them because of his failure to obtain a pure culture. The isolation of the mesophilic *Bacillus cellulosae dissolvens* by Khouvine (20) required the use of indirect methods and again served to emphasize that cellulose fermenting bacteria were not subject to isolation by the usual techniques satisfactory for other anaerobic bacteria. The later studies by Khouvine and Soeters (21–23) on the cellulose thermophiles also failed to yield colonies of these organisms.

Langwell and Lymn (26) picked so-called "pure cultures" of cellulose fermenting bacteria from the surface of an agar plate. They grew equally well at 37 or

[1] The first part of the experimental work presented in this paper was done while employed by the Division of Industrial Research of the State College of Washington, Pullman, Washington.

[2] A. E. C. Postdoctoral Fellow in the Biological Sciences.

[3] Present address: Department of Botany and Bacteriology, Montana State College, Bozeman, Montana.

70 C, either aerobically or anaerobically and exhibited other erratic behavior from which it must be concluded that numerous species were present. The aspect of instability was added to the already confusing picture when Viljoen, Fred and Peterson (57) described the isolation of an anaerobic cellulose fermenting thermophile which they designated as *Clostridium thermocellum*. This culture irretrievably lost the ability to ferment cellulose after having been cultured on media containing glucose.

Coolhaas (2) attempted to isolate an anaerobic cellulose fermenting thermophile using all of the techniques described up to that time (1928), and, failing, doubted the purity of the cultures reported by Khouvine, Langwell, and by Viljoen *et al.*

Further study of *C. thermocellum* and of cultures obtained by enrichments failed to yield a pure culture for Tetrault (55). Microscopic observations led him to speculate on the possibility of variations and life cycles. An elaborate procedure devised by Snieszko (52) was inadequate to give a culture of demonstrable purity. He applied the technique which Cowles and Rettger (3) had used successfully with cellulose mesophiles, namely, conditioning of the medium by prior growth of a non-cellulose fermenting organism which could later be eliminated, leaving only the cellulose digester.

The study of cellulose fermenting thermophiles was undertaken systematically by Imsenecki in 1938 (14). His procedure for isolation (15) depended upon transfer of zones of medium showing no growth of microörganisms, so could not be used as a means of demonstrating culture purity. It may, however, have enabled him to obtain chance pure cultures of the desired bacteria. After extensive studies (16–19) certain instabilities, poorly defined in the published reports, were still noted. A simultaneous study by Rotmistrov (44–47) yielded a culture of a cellulose thermophile which he named *Clostridium ellipsosporogenes*. The extremely variable nature of this organism suggested that the culture was not pure, a point made and demonstrated by Imsenecki (18). Despite the lack of a pure culture, Rotmistrov recognized that a low reduction potential was necessary for satisfactory growth of these organisms. This was contradicted by Murray (33) who minimized the influence of oxygen and stressed the importance of humidity upon the growth of the cellulose fermenting bacteria.

The cellulose thermophiles were again characterized as being extremely variable by Pochon (38, 39), while the earlier work of Enebo (5–8) indicated that they could not be grown in pure culture, but required the presence of symbionts.

The isolation of two cultures of thermophilic cellulose fermenting bacteria by McBee (31, 32), through use of the technique developed by Hungate (13), gave cultures of demonstrable purity which could be used for studies on the physiology of this group of organisms. Enebo (9) subsequently was able to obtain a culture of a thermophile which formed colonies in a cellulose dextrin agar medium. Its purity was established and studies on its physiology generally confirmed the results obtained by McBee. All these cultures of undoubted purity are stable in that they retain their cellulolytic properties even after repeated subculturing on sugar media. They have not displayed any of the variability usually attributed

to these organisms, indicating that earlier beliefs were erroneous and due to observations made on cultures containing more than one species.

INDUSTRIAL CELLULOSE FERMENTATIONS

From the viewpoint of the possible commercial applications of the thermophilic cellulose fermentation the results have been uniformly disappointing, not only in failing to provide industrially practical processes, but also in failing to yield significant data which might lead to a thorough understanding of the factors involved. It is very probable that the chief difficulty in obtaining significant data has been the failure to use pure cultures.

Langwell and Lymn tried pilot plant fermentations of cellulosic materials with their purified cultures and claimed success. The data presented are sketchy, however, and suggest that there were many practical difficulties which had not been overcome. The most important of these was the recovery of the fermentation products. Enebo and Lundin (10) indicated that this might be possible by a freezing technique. It is probably quite significant that later Langwell (25) abandoned the pure culture fermentation of cellulose for one carried out by mixed cultures controlled by physical and chemical means.

Further studies on the factors governing the rate of cellulose fermentations were made by numerous investigators (8, 10, 27, 34–36, 40, 48, 49, 51, 53, 54, 56, 58–62). Their observations are so conflicting that a complete review would be of little value. For example, in one report as little as one per cent lignin had a depressing effect upon the amount of cellulose which could be fermented (34), whereas other studies (58–62) show no effect of lignin if the wood-flour is finely ground. Aeration is reported to have both a stimulating (25, 39, 56) and a depressing (45) effect upon the fermentation. In general, it can be stated that as long as the fermentation of cellulose continued, the observed effects of various physical and chemical factors were primarily due to changes in the associated microflora.

There is almost universal agreement on one point, namely, the discouragingly small amounts of cellulose on a weight per volume basis (usually less than 2 g per liter) which can be fermented in a practical period of a few days. This information, often obscured by enthusiasm, seems from the industrial viewpoint to be the most significant of all the results obtained. Reports of a higher percentage conversion (27, 35, 54) have been on fermentations of materials containing a relatively large portion of non-cellulosic compounds.

Repetition of much of this work with cultures of proven purity will be necessary before an adequate evaluation of the industrial potentialities of this fermentation can be made.

REPORTED PRODUCTS OF THE CELLULOSE FERMENTATION

The fermentation of cellulose by thermophilic bacteria has generally resulted in varying quantities of gaseous products, volatile and non-volatile acids, alcohol and sugars. There is little agreement, however, between the observations of independent workers.

The gases have almost universally consisted of carbon dioxide and hydrogen in various ratios. Methane was also produced by the cultures of Langwell and Lymn, but only at the lower temperatures. Pochon and Sarciron (39) report the formation of methane at 65 C by *Terminosporus thermocellulolyticus*. Methane production has also been observed in frankly mixed cultures (35, 59). Imsenecki (17) has stressed the point that methane is never formed by the cellulose fermenting bacteria, but instead is produced by contaminating organisms fermenting the products of the cellulose decomposition.

Most investigators report volatile acids, including always acetic acid. Formic acid was found by Pringsheim, and by Scott *et al.* Butyric acid has been found in many of the cultures, most notably by Viljoen *et al.*, Imsenecki, and Pochon. Enebo (9) observed that butyric acid was formed only by his contaminated cultures, a reversal of his previous findings. Pringsheim and McBee did not find butyric acid as a product of the thermophilic cellulose fermentation.

Non-volatile acids have not been determined in most studies. In those of Imsenecki and Enebo only lactic acid was found. McBee, in addition, found traces of succinic acid.

The main interest from the industrial aspect has been in the obtaining of ethyl alcohol. It has been found in all of the quantitatively analyzed cultures; and special attempts to increase its proportionate amount have been made by Pochon (39) and Veldhuis, Christensen and Fulmer (56).

Pringsheim, working with enrichment cultures, demonstrated the accumulation of reducing sugars in fermentations which had been stopped by the addition of antiseptics and showed the presence of both glucose and cellobiose. This accumulation of sugar was also noted by Woodman and Stewart (63), Peterson, Scott, and Thompson (36), and Tetrault (55). It was considered as a fermentation product by Pochon and Enebo. Imsenecki regarded the glucose as a normal fermentable intermediate product of the cellulose fermentation which was produced in excess to accommodate accompanying forms, and as evidence for an historically developed symbiosis which is upset by the unnatural conditions found in pure cultures. McBee found glucose to accumulate only in cultures containing more than about 0.15 per cent cellulose and demonstrated that it was a hydrolytic product formed only after fermentation had slowed down or ceased.

None of the published fermentation balances satisfactorily account for all of the cellulose fermented. There has been either a failure to recover all of the carbon initially present (17, 32) or a discrepancy in the state of oxidation of the fermentation products. A complete and satisfactory determination of all of the important products of the thermophilic cellulose fermentation has not as yet been presented.

Those products which have definitely been shown to arise from the fermentation of cellulose are hydrogen, carbon dioxide, ethyl alcohol, formic acid, acetic acid, lactic acid and succinic acid. The evidence for butyric acid is inconclusive but it has not been eliminated as a possibility. There appears to be no basis for the consideration of methane as a product of the cellulose fermentation. Glucose, by its very nature, must arise from a hydrolytic process.

SUMMARY OF CHARACTERISTICS REPORTED IN THE LITERATURE

Habitat. The cellulose fermenting thermophiles are widely distributed in nature. They were first found in soil and river mud (28, 29). Later investigations have shown them to be present in many soils and in saline bay mud.[4] Horse manure has long been recognized as an excellent source of these microörganisms (5, 14, 26, 27, 34, 35, 38, 47, 50, 55), and they have recently been cultured from the cecal contents of the porcupine (1).

The significance of the thermophilic cellulose fermenting bacteria in these habitats as well as their role in cellulose decomposition in nature is not known and has been only inadequately studied. The observations of Egorova (4) on the wide distribution of thermophilic bacteria in the Arctic regions would lead one to believe that they would be more dependent upon the presence of a suitable substrate than on what we recognize as a favorable temperature.

Morphology. The literature descriptions of the cellulose thermophiles, with but one exception, agree as to the morphology of the responsible organisms. They are described as slender, often slightly curved, gram negative, spore-forming rods. Snieszko (52) believed his culture to be weakly gram positive, and described its spores as spherical, whereas all other observations have shown ovoid spores. Since true colony formation has been reported only twice (9, 32), there is very little basis for comparison on this character. Studies based solely upon morphology have presented only a confusing picture due to the presence of many contaminants (41).

Physiology. Very little has been done on the physiology of the cellulose fermenting thermophiles in cultures of proven purity. The numerous studies on cultures of doubtful purity will not be reviewed because of their questionable value. Further discussion of the physiology of this group of organisms will be included in the section on comparative studies.

A COMPARATIVE STUDY OF THE THERMOPHILIC CELLULOSE FERMENTING BACTERIA

The isolation of the thermophilic cellulose fermenting bacteria described by McBee (32) entailed the use of procedures no more elaborate than those commonly employed in obtaining pure cultures of other anaerobic bacteria. The question then arose: Did these organisms possess special characteristics permitting their easy isolation? The answer to this could only be obtained by actually comparing all of the available cultures under identical conditions. An attempt to do this has been made. Cultures were requested as follows:

An unnamed organism described by Enebo (10) from Dr. Harry Lundin, Royal Technical University, Stockholm, Sweden.

Clostridium thermocellum (57) from Dr. P. A. Tetrault, Purdue University.

Terminosporus thermocellulolyticus (38) from Dr. J. Pochon, Pasteur Institute, Paris.

The unnamed organism of Snieszko (52) from Dr. S. Snieszko, U. S. Bureau of Fisheries, and Dr. W. H. Peterson, University of Wisconsin where Snieszko's work was done.

The unnamed organism described by Imsenecki (15) from Dr. A. A. Imsenecki, Academy of Sciences of the U. S. S. R., Moscow.

A thermophilic strain of *Bacillus cellulosae dissolvens* (21) from Mme. Khouvine, Pasteur Institute, Paris.

[4] Unpublished observation by the author.

The response to these requests was very gratifying, making available the following cultures, which for the sake of simplicity will henceforth be designated by their letter symbols: (*a*) that of Enebo, *EB*; (*b*) *C. thermocellum*, *CT;* (*c*) *T. thermocelluolyticus*, *TT;* (*d*) a previously undescribed organism recently obtained by Dr. Tetrault, *TET;* and (*e*) *B. cellulosae dissolvens* (thermophilic), *BCD*. Snieszko's culture is no longer available since it was not maintained after becoming obviously contaminated. All requests addressed to Imsenecki have been unanswered.

Each culture, as received, was opened in the manner which seemed least likely to introduce contamination and a portion was used as an inoculum for a dilution series of cellulose agar shake tubes. These were incubated at 55 C. In all instances there were formed isolated colonies of cellulose digesting bacteria from which could be obtained cultures passing a rigorous test of purity (32). In some cases colonies of contaminating bacteria were observed in the first cultures. These were not isolated, however, since the cellulose fermenters were of prime importance and there appeared to be no object in spending time on the study of contaminants of unknown origin. No efforts were made to isolate more than one type of cellulose fermenting bacterium from a single culture. The colonies in the highest dilution were all similar, so it was assumed that one selected at random would represent the organism predominant in the culture being studied.

The cellulose fermenting bacteria obtained by this procedure were used, along with the previously isolated cultures *157* and *651* (32) for a comparative study of morphological and physiological characteristics. The only culture not included in the physiological study was that received from Mme. Khouvine, *BCD*, the most recently isolated of the series.

The procedure used for routine subculturing and the preparation and analyses of quantitative cultures have already been adequately described elsewhere (13, 32). Nutritional requirements were ascertained by addition of growth factors to a mineral-base cellulose medium without agar or yeast extract. Carbohydrate fermentability was determined in the basal medium with yeast extract, substituting the test material for the cellulose. Turbidity and the production of acid and gas were used as criteria of growth and substrate utilization in these experiments.

Morphology. The vegetative cells of all of the cultures examined are very similar. They are rods, straight or slightly curved, the majority of which are 0.6 to 0.7 μ by 2.5 to 3.5 μ. They usually occur as individuals or short chains, but during the period of most active growth numerous long filamentous chains may be found in fluid media. The cells are consistently gram negative, even in cultures only a few hours old.

The spores are oval and measure about 1.2 by 1.6 μ. They form terminally, first appearing as gram positive swellings at the end of elongated gram negative cells. After the spore has become well differentiated it is soon liberated. Spores are relatively rare in cultures grown in fluid media or in cellobiose agar. Colonies in cellulose agar, however, are composed largely of spores at the time they are first visible.

Preparations from young cultures in fluid media show many cells with from 3 to 7 peritrichous flagella when stained by Gray's method. Active motility, however, has been observed in only a few instances, and not in all cultures. This failure to obtain uniform results may be due to the difficulties encountered in keeping the suspensions warm and under anaerobic conditions during the observations.

The cellular morphology of all the cultures agrees very well with the published descriptions of the cellulose thermophiles. The similarities between the cultures observed are so consistent that a differentiation cannot be made on the basis of microscopic examination.

Colonies in a 2 per cent agar medium are usually white, opaque, and lens shaped. The only culture showing any differences in this respect is strain *157* which often forms colonies in cellulose agar having irregular outlines instead of well defined borders. In cellulose agar the colonies are surrounded by clear zones from which the cellulose has been digested. If cellulose suspensions showing large variations in particle size are used, the clear zones surrounding the colonies will contain numerous bits of incompletely digested cellulose. The colonies rarely exceed 1 to 2 mm in diameter except in old cultures where they may be composed of multiple discs and over a period of months grow to a diameter of 5 mm. Surface colonies are watery, slightly convex, and translucent with a bluish fluorescence.

Growth in a fluid medium containing cellulose is restricted to the bottom of the tube. The supernatant fluid remains clear until the cellulose has been largely digested. In media containing soluble substrates the fluid is uniformly turbid from the time of inoculation. A yellowish discoloration of the cellulose occurs in fluid media. This has been observed with all of the cultures examined but is most pronounced in the cultures *EB* and *TT*. Pigmentation does not occur in colonies grown in cellulose agar or in cultures grown on substrates other than cellulose.

Growth requirements. All of the cultures grow well in a mineral-base medium containing cellulose and a trace of yeast extract (0.05 per cent). Their growth is not enhanced by larger amounts of yeast extract or by the addition of peptones, serum, plant juices, or extracts of soil or manure. Cultures carried in a fluid mineral-base cellulose medium without added growth factors failed to show growth after 2 to 3 transfers. Tests were made to ascertain the growth factor requirements. It was found that the mineral-base cellulose medium would support growth and cellulose digestion if the following materials were added in the indicated concentration per 10 ml of medium: thiamine hydrochloride, 2 γ; riboflavin, 2 γ; calcium pantothenate, 2 γ; pyridoxine, 2 γ; and biotin, 0.02 γ. The minimal concentrations required were not determined. Growth beyond a single transfer could not be obtained if any one of the listed materials was omitted from the medium. Growth and cellulose digestion after six consecutive daily transfers in the complete medium were comparable to that in a similar medium containing 0.05 per cent yeast extract, i.e., 0.1 per cent cellulose would disappear in 24 to 36 hours.

Oxygen relationships. All of the pure cultures are obligately anaerobic. A heavy

inoculum is required to give growth in media without added reducing agent. Growth in thin layers of media could be obtained only when the reduction potential was low enough to reduce added methylene blue or resazurin. This could be achieved by the addition of 0.02 per cent sodium thioglycolate or 0.01 per cent sodium sulfide.

Temperature relationships. The temperature range for the optimal rate of growth and cellulose digestion is approximately 55 to 65 C. In all cases growth at temperatures below 50 C was very slow. No growth occurred on cellulose media at temperatures above 67 to 68 C. In the case of strain *157* this was shown to be associated with the inactivation of a cellobiase. When cultures of *157* were left for long periods of time, up to 10 months, at temperatures as low as 30 C some disappearance of cellulose occurred. This reduction in the amount of cellulose may be due to a slow growth at this temperature or to continued activity of cellulase added with the inoculum, since this enzyme appears to be very stable and quite active at temperatures far below those optimal for growth of the producing organism. These long period experiments were not deemed to be of any great importance so were not carried out with the other cultures. Similar observations led Rotmistrov to conclude that these organisms were not true thermophiles. In view of the time required for the reaction, such a conclusion is hardly warranted.

Carbohydrate utilization. All of the cultures exhibited identical behavior on the carbon sources tested. Active growth and fermentation was obtained on cellulose, cellobiose, xylose, and hemicelluloses. Materials tested but not fermented were glucose, fructose, mannose, galactose, arabinose, sucrose, lactose, maltose, melibiose, trehalose, inulin, salicin, dextrin, soluble starch, inositol, sorbitol, dulcitol, mannitol, glycerol, pectin and gum arabic. Where applicable the carbon sources were sterilized by filtration of a concentrated solution. Sparingly soluble materials were autoclaved in the dry state and sterile medium added to the tube.

Effect of pH. Growth was readily initiated in cellulose media having pH values of 6.4 to 7.4. Growth was not obtained in media at pH levels above 7.6 or below 6.0. It was noted that the inclusion of calcium carbonate in the medium, a common practice, kept the pH at a level too high for the maximum rate of growth and cellulose fermentation.

Nitrogen requirements. In no instance has the inclusion of complex nitrogenous materials, other than the mentioned growth factors, been necessary for growth nor has it enhanced the rate of cellulose fermentation. Ammonium salts appear to be an adequate source of nitrogen.

Miscellaneous tests. All tests for sulfate reduction have been negative. Nitrates are not reduced to nitrites, but their inclusion in low concentration does not affect growth or cellulose fermentation. Acetylmethylcarbinol is not formed.

Products of cellulose fermentation. Cultures for the quantitative determination of the products of cellulose fermentation by the six cultures being compared were set up in all-glass flasks as previously described (32). The size of the culture, the amount of cellulose present, the amount and activity of the inoculum, and

all other conditions were kept as nearly constant as possible. The greatest variation was in the amounts of cellulose added. The time required for the cellulose to disappear in flasks inoculated with the different cultures varied from 5 to 7 days, *EB* and *TT* being the most rapid and *CT* and *TET* the slowest. Analyses of all the cultures were started on the eighth day after inoculation.

The products of the fermentation, as determined, were the same in all six cultures, varying only in amounts and percentage recovery of the cellulose (table 1). As presented in the table, the residue, varying from 9 to 15 mg of cells along with traces of unfermented cellulose, has been subtracted from the initial amount of cellulose. Analyses were performed on two aliquots of different size, yielding results which usually checked within five per cent. Succinic acid was

TABLE 1

Fermentation Balances

	CULTURES					
	651	CT	TT	157	EB	TET
Initial cellulose mg	60.0	104.4	110.5	88.5	107.5	110.5
mg atom C	2.22	3.85	4.1	3.28	3.98	4.1
Fermentation products in mM						
Hydrogen	.44	.26	.44	.51	.59	.51
Carbon dioxide	.56	.29	.43	.47	.56	.56
Ethyl alcohol	.35	.20	.21	.13	.21	.20
Formic acid	.04	.10	.04	.07	.10	.10
Acetic acid	.17	.37	.22	.27	.47	.44
Lactic acid	.21	.31	.22	.16	.08	.19
Total mg atom C	2.27	2.46	1.99	1.81	2.25	2.51
Per cent recovery	102	64	49	55	57	61
Redox Index*	1.02	1.03	1.05	1.31	1.21	1.34

* Calculated according to Erb, Wood and Werkman (11).

not determined since it had earlier been found to occur in extremely small amounts. Reducing sugars were not present.

The six cultures fall into three groups according to the per cent of the initial cellulose found in the products recovered, and the state of oxidation of the undetermined fraction.

Culture *651*, forming a class by itself, converts nearly all of the cellulose into the products listed in table 1. This has also been observed in earlier analyses (31) made on this culture.

Cultures *157*, *EB*, and *TET* form a second group in which only about 50 to 60 per cent of the cellulose can be accounted for in the fermentation products determined. The undiscovered products, representing from 40 to 50 per cent of the cellulose carbon must be in a more reduced state than cellulose because of the state of oxidation of the recovered products.

The third group, made up of cultures *CT* and *TT* shows approximately the

same discrepancy in determined carbon as does the second group. The degree of oxidation of the undetermined material, however, must be approximately the same as that of a carbohydrate to account for the good oxidation-reduction balance obtained.

Although it was not possible to separate the six cultures studied on the basis of morphology or carbon source utilization, they appear to fall into three classes according to their fermentation products. Whether or not this method can be used as a means of classifying these organisms will depend upon the nature of the unidentified compounds. These have not as yet been characterized. It is quite possible that the same materials will be found in all cultures, the varying amounts accounting for the states of oxidation shown. If, as has been observed with culture *157* (31) and *Clostridium cellobioparus* (13), the ratios of the fermentation products are quite variable and are subject to yet unrecognized factors, it may be possible to consider all of these organisms as being the same, as was indicated by the other tests.

Discussion. A comparative study of the available cultures of thermophilic cellulose fermenting bacteria has shown that it is possible to obtain pure cultures of these organisms. The technique used permits the application of the classical isolation method of picking isolated colonies from solid media. The cultures obtained have been stable in respect to their morphological and physiological characters, in some instances for periods of over two years since their isolation. Unstable organisms among the thermophilic cellulose fermenting bacteria have not been ruled out, and may exist, but they have not been encountered during the course of this study.

The nutritive requirements of the bacteria are relatively simple, permitting their cultivation on a chemically defined medium. Concomitant growth of other organisms does not increase the rate of cellulose fermentation as was reported by Imsenecki (14), but instead has an inhibitory effect.

The products of the cellulose fermentation, as far as they were determined, are the same for all of the cultures examined. It was especially noted that butyric acid was not formed, since this substance has been reported as being a fermentation product in the published descriptions of the cultures received from other workers. Preliminary observations on the culture *Bacillus cellulosae dissolvens* have also failed to disclose the presence of butyric acid. Imsenecki's report of butyric acid formation may indicate that his organisms are different from any that have been examined in this study or, as is believed probable, it may be an indication of unsuspected contamination. It is extremely unfortunate that this culture could not be obtained.

The cultures compared have shown such marked similarities in morphology and physiology, especially in their production of glucose from cellulose under certain conditions and their inability to ferment any of the hexose sugars, that it appears possible to include all of the known thermophilic cellulose fermenting bacteria in one species. It is suggested that all of the cultures of these organisms now in existence be considered as strains of *Clostridium thermocellum*, Viljoen *et al.*, 1926, unless differences greater than herein demonstrated can be shown

to exist. It will, of course, be necessary to change the description of *C. thermocellum* to fit the pure culture rather than the observations of Viljoen.

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