ISOLATION AND STUDY OF AN APPARENTLY WIDE-SPREAD CELLULOSE-FERMENTING ANAEROBE, CL. CELLULOSOLVENS (N. SP.?)[1]

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The decomposition of cellulose by microbiological agents has for many years attracted considerable attention. This is not at all surprising, for cellulose occurs in enormous quantities in nature and is of great economic importance. Furthermore, its extreme resistance to most chemical and biological influences makes it all the more interesting.

The earlier investigators who attacked the problems of cellulose decomposition by bacteria dealt with crude cultures only, and were inclined to the belief that the process was essentially an anaerobic one. However, in 1904 Van Iterson observed that cellulose could be destroyed under aerobic conditions, and described the organisms concerned. As a result, research has, for the most part, been concentrated on this phase of the problem, and there have been isolated many aerobic bacteria, facultative anaerobes and fungi, which are active in the process. Among the workers in this field are Kellerman and McBeth (1912) and Bradley and Rettger (1927). Thaysen and Bunker have reviewed the subject from many aspects in their "Microbiology of Celluloses, Hemicelluloses, Pectin and Gums" (1927).

The anaerobic bacteria, on the other hand, have received relatively little attention, at least in so far as the isolation of pure cultures is concerned. This is probably due to the fact that, while easy to demonstrate in mixed cultures, they are extremely exacting in their growth requirements and are very difficult to obtain

[1] This paper is based upon the senior author's Doctorate Thesis deposited in the Yale University Library.

by themselves. As far as the writers are aware, only three investigators have made claims of isolation of pure strains. First among these was Omeliansky (1902), who published several papers in which were described two cellulose fermenting anaerobes, *B. fossicularum* and *B. methanicus*, obtained from river mud and horse feces by growth in an inorganic salt medium containing cellulose. Both were thin rods which formed spherical terminal spores. Cellulose was essential to these organisms and was, indeed, the only carbohydrate which they could attack. Carbon dioxide and various organic acids were formed while, in addition, *B. fossicularum* produced hydrogen, and *B. methanicus*, methane. Omeliansky was unable to secure colony formation on any solid medium and had to resort to repeated heatings and dilutions to secure his isolations. Kellerman and McBeth believed that these two organisms were not in reality pure cultures, since they were able to obtain several contaminating forms from them, including a cellulose-destroying facultative anaerobe.

In 1923 Khouvine described another anaerobe, *B. cellulosae-dissolvans*, which was strictly specific for cellulose and dependent upon it for growth. This organism was similar to Omeliansky's in its inability to attack sugars and to grow on solid media, but it could be distinguished from the other by the formation of oval spores. Isolation was secured by repeated heating, diluting and growing in a special fecal extract medium containing cellulose. By her technique Khouvine was able to demonstrate the presence of the organism in the excreta of man and of the herbivora, and in numerous soils. The products of fermentation were found to be carbon dioxide, hydrogen, organic acids and ethyl alcohol.

The third report deals with a description of *B. cellulosam-fermentans* isolated by Werner in 1926 from the intestines of rose beetle larvae. Morphologically it is similar to Khouvine's organism, but the oval spore is slightly smaller. In Werner's hands the isolation methods used by the preceding workers were unsuccessful; he adopted the following technique. An enriched culture of the organism was developed in the usual way in Omeliansky's medium. A bit of the decomposing paper was next streaked thoroughly over the surface of a nutrient agar plate which was

then incubated for twenty-four hours. At the end of this time all areas of visible growth were cut out with a sterile knife and the same plates incubated further. If no new growth became apparent, the surface of the agar was wiped off with a bit of sterile cotton in order to gather up the dormant spores of the desired organism, and the cotton planted in a tube of medium. Disintegration of the filter paper followed in from four to ten days. The cultures were assumed to be pure when no growth occurred on ordinary nutrient agar, incubated both aerobically and anaerobically for several days, and when microscopic observation showed no contaminating forms. Werner could obtain growth only in the presence of cellulose, as apparently no other carbohydrates were utilized. The only solid medium on which growth took place was the cellulose agar of Kellerman and McBeth, and on this the organism developed only occasionally, and in contact with fibers. Gas formation was observed, but the fermentation products were not investigated.

From the foregoing brief summary it is apparent that the isolation of cellulose-fermenting anaerobes is fraught with difficulties. Not the least of these is the uncertainty of knowing whether or not the cultures are pure, for failing colony formation or single cell isolation, the present-day methods of obtaining pure strains are open to criticisms.

This paper deals with the isolation, growth upon solid medium, and study of an anaerobic organism which is morphologically similar to those described by Omeliansky.

Media

1. Omeliansky's medium

Ammonium sulphate or peptone	1.0 gram
Di-potassium phosphate	1.0 gram
Magnesium sulphate	0.5 gram
Sodium chloride	trace
Calcium carbonate	excess
Water	1000cc.

2. Fecal extract medium

Peptone	1.0 gram
Di-potassium phosphate	1.0 gram
Sodium chloride	1.0 gram

Calcium carbonate	excess
Fecal extract	250 cc.
Water	750 cc.

The fecal extract was prepared by extracting horse feces with ten parts of water, filtering through paper, autoclaving, and passing through a Berkefeld candle.

3. Beef infusion broth

Beef infusion	1000 cc.
Peptone	5.0 grams
Di-potassium phosphate	1.0 gram
pH	7.0

In all of the above media cellulose was added in the form of a strip of filter paper placed in each tube.

ANAEROBIC METHODS

All culture media were autoclaved and cooled immediately before inoculation. Anaerobic conditions were obtained, as a rule, by placing the cultures in jars, exhausting with a vacuum pump until the liquid boiled (or, in the case of agar media, to a constant pressure), admitting carbon dioxide and re-exhausting several times, and finally sealing off the jar after the last exhaustion. Incubation was always at 37°C.

THE PREVALENCE OF OBLIGATE ANAEROBIC CELLULOSE-FERMENTING BACTERIA

The presence of spore-bearing cellulose-fermenting bacteria in soils, muds, feces, and in fact wherever cellulose is undergoing decomposition, is easily demonstrable. Dügelli (1921) found the following numbers per gram of soil: garden, vineyard, and meadow, 367; field, 350; and marsh, 1.1. In the present investigation samples from numerous sources were collected in sterile tubes. In the case of the soils, the surface was first scraped away and then material taken to a depth of three inches. The fecal specimens were always from fresh excreta.

Ten-gram portions of a well-mixed sample were placed in dilution bottles containing enough sterile water to bring the final volume to 100 cc. and thoroughly shaken. From these bottles, 10 cc. transfers were made to bottles containing 90 cc. of water,

and so on through several dilutions. One cubic centimeter amounts of the different dilutions were then added to tubes containing about 15 cc. of Omeliansky's medium with peptone. These tubes were heated at 80°C. for ten minutes, cooled, and placed under anaerobic conditions. The presence of spore-forming cellulose fermenters was shown by disintegration of the filter paper, accompanied by gas formation, in from five to ten days.

Numerous samples of soils and feces, besides those listed, were examined, and positive results almost invariably obtained. Mud from the bottom of a salt water pond was particularly active. Feces from four dogs seemed to be devoid of cellulose fermenting

TABLE 1

Anaerobic spore-forming cellulose-fermenting organisms from various sources

SOURCE	SMALLEST AMOUNTS OF NORMAL SAMPLE TO ATTACK CELLULOSE	EQUIVALENT OF DRY SAMPLE	ORGANISMS PER GRAM DRY SAMPLE
	grams	*grams*	
1. Lawn	0.01	0.009	110
2. Marsh	0.01	0.004	250
3. Potato field	0.01	0.009	110
4. Pine woods	No attack		
5. Rich garden soil	0.001	0.0009	1,100
6. Cow feces	0.01	0.002	500
7. Human feces	0.01	0.003	333
8. Rabbit feces	0.05	0.03	33
9. Dog feces	No attack		

anaerobes, a fact rather to be expected, since dogs are essentially carnivorous.

This method, at best, is very inaccurate and gives only a rough idea of the numbers of organisms present. There were, undoubtedly, many more than the determinations showed. The limited number of samples taken does not make it permissible to generalize too broadly, but one may conclude from these findings that cellulose-fermenting anaerobic spore-formers are widely distributed in nature, and that they probably play a more important rôle in nature's economy than is usually attributed to them. A further study of this rôle should reveal some interesting facts,

particularly in so far as the digestion of cellulose in the intestine is concerned.

ISOLATION

In all of the cultures in which active decomposition of cellulose was observed an organism was present which morphologically resembled those described by Omeliansky. The rods appeared slender and often slightly curved; they were about 0.5 micron in diameter and from 2 to 6 micra long. A spherical terminal spore varying from 1.0 to 1.5 micra in diameter was often seen either attached to the rod or free. It is this organism or one morphologically similar to it which has been described by different workers in the field of cellulose fermentation.

The present attempts to effect its isolation were made from horse feces by the preliminary enrichment culture method. The medium described by Khouvine was employed, except that horse, instead of human, fecal extract was added. After heating in this medium for fifteen minutes at 80°C. and incubating in a vacuum the forms that persisted were few in number, with the typical cellulose-fermenting type predominating.

Both Omeliansky and Khouvine seemed able to eliminate the contaminating forms by repeated transfers and heatings. This method, however, was not successful in Werner's work, nor was it effective in the present investigation. In some instances, where all contaminants had apparently been eliminated as far as microscopical and cultural studies showed, no further growth of the cellulose organism could be demonstrated.

The dilution method was tried, but the results were equally unsatisfactory. The last tube which revealed cellulose decomposition always contained a contaminant.

Attempts were next made to produce colonies on various solid media. Meat extract and meat infusion agar with and without glucose, maltose and soluble starch, and tomato agar, casein digest agar and silica gel were used. The addition of precipitated cellulose prepared by the method described by Kellerman and McBeth was tried in several of the above media. In no case was colony formation observed.

Numerous methods of obtaining anaerobic conditions were also tried. The exhaustion and replacement system already described, Marino's cupped plates (1907), growth with non-spore forming bacteria as recommended by Sturges and Rettger (1919), and deep agar shake tubes were alike unsuccessful.

In a few instances colonies of organisms resembling the one desired were noticed, but from these no growth or cellulose decomposition could be obtained. This observation tallies with that of Choukevitch (1911) who, in studying the intestinal flora of the horse, was able to isolate an organism which was morphologically similar to Omeliansky's. He, also, failed to make it attack cellulose in pure culture.

At this point a device used originally by Winogradsky (1890) and later by Werner was tried in a modified form. Nutrient agar plates were streaked with a small bit of washed paper from an almost pure fermenting culture of the cellulose organism. The plates were incubated aerobically for two days at 37°C. Colonies of contaminants developed along the first parts of the streak, but the more thinly streaked areas showed no growth. Small portions of agar were cut out from these clear parts with a sterile spatula and transferred to tubes of fecal extract medium and to Omeliansky's solution. In some of these tubes decomposition of the paper was apparent in from four to ten days, but on examination there was always found an oval spore-producing contaminant.

Since the cellulose-attacking type was microscopically the most abundant form in the culture with which the plate had been streaked, it seemed highly probable that its spores predominated along the parts of the streaked agar which showed no growth, and that, therefore, some of these spores must have been transferred to tubes of medium where they failed to develop. In these tubes the spores remained dormant unless an occasional contaminant served to make conditions favorable for their germination. Such associative influence could easily explain the results obtained in this experiment as well in many of the previous unsuccessful attempts to secure growth.

Acting on this suggestion, another plate was treated as described above, but in this instance the small portions of agar were

transferred to tubes which were inoculated also with a non-spore former, *Bact. aerogenes*. In most of these tubes decomposition of the paper was observed after suitable incubation. Some of them contained contaminating spore forms, but others seemed to have only the cellulose fermenting organism plus *Bact. aerogenes*. The last-mentioned tubes were heated at 80°C. for fifteen minutes to kill all vegetative cells, and purity tests were made by inoculating various media and incubating both aerobically and anaerobically. No evidences of contamination could be seen. The cultures were considered pure, therefore, and from them inoculations were made into tubes of fecal extract medium, some of which also received *Bact. aerogenes*. The tubes containing the

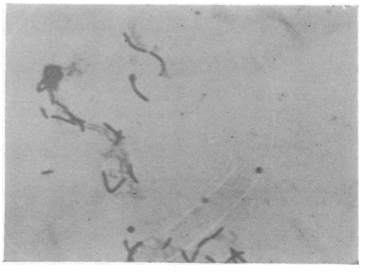

FIG. 1. PHOTOMICROGRAPH SHOWING VEGETATIVE CELLS, A SPORANGIUM AND SPHERICAL SPORES. 1000×

associated organism consistently showed cellulose decomposition; the others did not.

By using the same principle (association with *Bact. aerogenes*) and the dilution method a second isolation was effected.

The failure to obtain growth in pure culture without the influence of some associated organism (*Bact. coli*, *Proteus vulgaris*, and an unidentified spore-forming anaerobe were likewise effective) seemed to indicate either that the food requirements of the cellulose organism were not satisfied by any of the media used or that bacterial association is absolutely essential in its development. The former hypothesis seemed to be the plausible one.

In further efforts to find a suitable medium, cellulose meat infusion was tried and found to be very satisfactory. It is quite useless so long as any contaminating forms are present, since these as

a rule develop so luxuriantly as to overgrow the cellulose organism. In this meat infusion medium, pure culture decomposition of the filter paper was observed in from three to five days. The body of the liquid became only slightly turbid, indicating that the organism grows abundantly only in contact with the cellulose fibers. Gas bubbles were evolved slowly, often forming blisters in the paper. In the course of several days after the fermentation set in the paper disintegrated and fell to the bottom of the tube, and the reaction subsided, probably interrupted by the increased H-ion concentration. The pH at this time was about 5.6.

MORPHOLOGY

Vegetative cells in liquid media are about 0.5 micron in diameter and from 2 to 6 micra in length, sometimes slightly curved and often occurring in pairs. Chains of more than two cells are not seen.

The spores form as dark-staining granules in the end of the rod, usually when it has reached a length of 2.5 to 3.5 micra. This granule swells to a diameter of 1.0 to 1.5 micra. The free spores are almost perfect spheres.

STAINING REACTIONS

The organism stains readily with all of the common dyes. The Gram stain is rather uncertain. As a rule the cells seem to be Gram-negative.

MOTILITY

The organism is non-motile when viewed by the usual hanging drop method.

FERMENTATION REACTIONS

It has been observed by all workers in the field of anaerobic cellulose fermentation that their organisms have been highly specific in their carbohydrate requirements. Indeed, there appears to be no mention in the literature of attack on any substance except cellulose.

In the present investigation, such common carbohydrates as glucose, maltose, and soluble starch were occasionally used in efforts to find some soluble energy source to replace the filter paper, as this in its natural form or as precipitated cellulose is very inconvenient for incorporation in agar media. In none of these attempts could growth be uniformly obtained, although at times and after prolonged periods of incubation there were some evidences of development. With a pure culture available, and a medium which favored development, the study of the fermentation characteristics of the organism was begun.

Valley (1929) has shown that ordinary Dunham inverted vial fermentation tubes are quite adequate for the study of fermentation by anaerobes, provided 0.1 per cent of cysteine hydrochloride is added to the medium. The writers have found that the organism under investigation develops very nicely in such tubes, even when the cysteine is omitted. In fact, when inoculations were made into these tubes and into ordinary culture tubes incubated *in vacuo*, attack on the cellulose occurred in both after the same period of incubation. These simple fermentation tubes were employed, therefore, in the following study.

The basic medium used was the standard meat infusion broth to which one per cent of the various carbohydrates was added. The reaction was adjusted to pH 7.0 to 7.2, and after autoclaving at 15 pounds for ten minutes was tested again to make certain that no change had occurred. With the pentoses, arabinose and xylose, a change in the color of the medium to a deeper yellow accompanied by a drop in reaction to pH 6.4 was observed, indicating some degree of decomposition. Alkali was added to bring the reaction back to the original level.

All of the carbohydrates were tubed in quadruplicate. Two tubes of the sugar medium, with and two without strips of cellulose, constituted a set. Controls of plain meat infusion, with and without cellulose, were used.

The inoculum was 0.1 cc. of a seven-day culture in cellulose meat infusion medium. Care was taken to transfer no cellulose fibers in the process of inoculation.

The following substances were employed:

Pentoses
Arabinose
Xylose

Hexoses
Glucose
Levulose
Mannose

Trisaccharides
Melizitose
Raffinose

Polysaccharides
Soluble starch
Dextrin
Inulin
Cellulose

Alcohols
Adonitol
Dulcitol
Erithrytol
Glycerol
Inositol
Mannitol
Sorbitol

Disaccharides
Lactose
Maltose

Glucosides
Amygdalin
Salicin

Gums
Gum Arabic

The data presented in table 2 show that this organism is highly restricted in its ability to utilize carbohydrates. At times, an occasional tube containing a test substance alone showed some evidences of fermentation. This was infrequent, however, and was probably the result of a partial hydrolysis during autoclaving. The slight but consistent fermentation of soluble starch may also be attributed to this cause.

Furthermore, these results confirm the reports of other workers that glucose is not utilized by the anaerobic cellulose fermenting organisms. This is all the more interesting inasmuch as it is generally believed that, if bacteria are able to decompose any sugar, they can attack glucose.

It is also significant that the cellulose is readily attacked in the presence of all of the substances used except arabinose and xylose. Glycerol seems to exert a complete inhibition upon the development of the organism, and lactose a partial restraint. Even dextrin, which is readily available as an energy source, does not prevent an attack upon the more complex cellulose.

Of more practical value from the point of view of a study of the anaerobe is its ability to ferment dextrin. Cysteine meat infusion agar containing this substance served as an excellent medium on which to secure colony formation. After three days' incubation

TABLE 2

Showing the action of the isolated organism on carbohydrates, alcohols and glucosides

CARBOHYDRATES, ETC.	CARBOHYDRATES, ETC., ALONE			CARBOHYDRATES, ETC., AND CELLULOSE		
	Gas observed	Final per cent gas	Final pH	Gas observed	Final per cent gas	Cellulose decomposition
	days			*days*		
Control	—	—	7.2	3	65	+
Arabinose	5	30	6.2	4	45	−
Xylose	6	20	5.8	4	40	−
Glucose	—	—	7.2	3	60	+
Levulose	—	—	7.2	5	60	+
Mannose	—	—	7.2	3	55	+
Lactose	—	—	7.2	—	—	−
Maltose	—	—	7.2	5	55	+
Sucrose	—	—	7.2	3	60	+
Melizitose	—	—	7.2	5	50	+
Raffinose	—	—	7.2	4	60	+
Soluble starch	4	2	6.9	3	55	+
Dextrin	4	25	6.5	3	65	+
Inulin	—	—	7.2	4	60	+
Cellulose	3	65	5.6	4	65	+
Salicin	—	—	7.2	4	40	+
Amygdalin	—	—	7.2	4	40	+
Adonotol	—	—	7.2	4	50	+
Dulcitol	—	—	7.2	5	55	+
Erythritol	—	—	7.2	5	50	+
Glycerol	—	—	7.2	—	—	−
Inositol	—	—	7.2	4	50	+
Mannitol	—	—	7.2	3	60	+
Sorbitol	—	—	7.2	4	55	+
Gum arabic	—	—	7.2	4	60	+

in vacuo or under hydrogen, small, discrete colonies appeared. These seldom grew to more than 0.5 mm. in diameter, even after prolonged incubation. They were round and had a transparent, dew-drop appearance. The body of the colony was finely granular in structure, with a smooth edge. Inoculations from these

colonies always gave growth in cellulose meat infusion in from two to four days, and caused decomposition of the filter paper. As a rule, relatively few spores were seen in pure cultures, even after continued holding under aerobic or anaerobic conditions.

THE INFLUENCE OF VARIOUS CARBOHYDRATES UPON THE AMOUNT OF CELLULOSE DESTROYED

In the study of the fermentation characteristics of the anaerobe it was noticed that, whenever cellulose and other carbohydrates were present, the cellulose was attacked in preference to the latter, except in the cases of arabinose and xylose. In view of the fact

TABLE 3

Showing the influence of glucose, starch and dextrin upon the amount of cellulose decomposition

CARBOHYDRATES	ORIGINAL WEIGHT PAPER	AMOUNT OF PAPER DESTROYED	PER CENT OF GAS	FINAL pH
	mgm.	*mgm.*		
1. Cellulose only	85	17	45	6.4
2. Cellulose only	84	*	0	7.4
3. Cellulose + glucose	87	16	45	6.4
4. Cellulose + glucose	85	14	50	6.4
5. Cellulose + soluble starch	86	16	55	6.4
6. Cellulose + soluble starch	88	15	55	6.4
7. Cellulose + dextrin	86	11	50	6.4
8. Cellulose + dextrin	82	10	55	6.4
9. Cellulose (uninoculated)	84	2	0	7.2

* No growth.

that both dextrin and, to a slight extent, soluble starch could be used without preventing the fermentation of the cellulose, it seemed of interest to determine their influence upon the amount of cellulose that is decomposed. Glucose was also included in the experiment, as its failure to be utilized is so unusual.

Weighed strips of paper were placed in fermentation tubes containing 15 cc. of meat infusion, pH 7.2. These were inoculated with 0.1 cc. of an actively fermenting cellulose meat infusion culture, care being taken to transfer no cellulose fibers. The cultures were observed daily, and at the end of eight days were taken

from the incubator and final determinations made. The filter paper used in the experiment had been dried at 105°C. to constant weight. After the fermentation in the culture tubes had subsided, the contents of each tube were filtered through a Gooch crucible similarly dried to constant weight, and washed several times alternately with one per cent hydrochloric acid, one per cent potassium hydroxide and distilled water. The crucibles with their contents were then thoroughly dried and the weight determined.

It will be seen from the figures in table 3 that the presence of dextrin inhibits to some extent the decomposition of the cellulose, probably because the dextrin is partly responsible for the lowering of the pH. On the other hand, neither soluble starch nor glucose have any effect upon the cellulose fermentation.

GAS PRODUCTION

The observation has been made by all workers in the field of anaerobic cellulose fermentation that gas is consistently produced in their cultures. Omeliansky believed that he could produce a methane as well as a hydrogen fermentation by his two supposedly pure cultures. Khouvine, however, found only hydrogen and carbon dioxide in the evolved gas, and Werner, although he failed to make analysis of the gas from his pure culture fermentation, found no methane in the product of crude cultures.

In the present experiment the apparatus described by Werner (1926) was used. This consists of two bottles connected with an inverted U tube which passes through a cotton plug in one (the overflow bottle), and through a two-hole rubber stopper in the other (the culture bottle). This tube should reach to the bottom of each bottle. Through the second hole of the rubber stopper a short glass tube passes. This is connected by a piece of soft rubber tubing about an inch long to another short glass tube plugged with cotton. The soft rubber tube is supplied with a pinchcock with which to control the flow of gas.

The cellulose is placed in the culture bottle, each bottle is filled over half full with the medium (in this case the standard meat infusion), the stopper and cotton plug are tightly fitted, and the

whole system is autoclaved. Care must be taken not to have the pinch-cock close the rubber tube, as the air must have a means of exit in the autoclave. It is advisable to cover the rubber stopper with cotton and then with paper, in order that no contaminating organisms may enter the bottle through its junction with the glass.

After autoclaving, the medium is immediately sucked up to fill the culture bottle completely so that no air can enter. When the whole system has cooled, the cotton plug in the short tube at the top of the culture bottle is withdrawn, using appropriate precautions, and the inoculum is admitted by means of a pipette.

In this experiment 1.0 cc. of an actively fermenting pure culture of the cellulose decomposing anaerobe was used as inoculum.

Active gas evolution began after three days' incubation and lasted for five days. At the end of this period slight action was still evident, but the culture was removed and analyzed.

Data on the fermentation are listed below.

Volume of culture bottle	240 cc.
Weight of cellulose used	1.178 gram
Weight of cellulose recovered	0.746 gram
Weight of cellulose destroyed	0.432 gram
Gas evolved 1st day of fermentation	15 cc.
Gas evolved 2nd day of fermentation	40 cc.
Total gas evolved in five days	130 cc.
Amount of normal acid as titrated with N/10 NaOH	6.4 cc.

The data on the gas volumes are not at all accurate for several reasons. Pressure and temperature were ignored, as was the amount of gas which was dissolved in the culture medium. Two analyses of the gas made at different stages of its evolution, in a modified Orsat apparatus described in the Bureau of Mines Bulletin No. 197, showed that of the gas about 75 per cent was hydrogen, and the remainder carbon dioxide.

SUMMARY

1. There has been isolated from horse feces a cellulose fermenting anaerobe (for which the name *Clostridium cellulosolvens* is proposed) morphologically similar to the organisms described by Omeliansky. The rods are thin, often slightly curved, and form spherical terminal spores.

2. Colony formation can be secured on a peptone beef infusion agar medium containing dextrin and cysteine. This medium is not selective and cannot be used for direct isolation, but is of value in obtaining the desired organism from enriched cultures. The best liquid medium devised for cellulose decomposition by this organism consists of a peptone beef infusion phosphate broth containing a strip of filter paper.

3. Cellulose, dextrin, arabinose and xylose alone of the materials tried are attacked. Glucose is not utilized, a fact in contradiction of the so-called theory of carbohydrate gradients. The presence of dextrin or glucose in the medium seems to have little or no effect upon the amount of cellulose decomposed.

4. As far as they have been determined, the products of cellulose decomposition by this organism are carbon dioxide, hydrogen and organic acids.

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