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AEROBIC CELLULOSE DECOMPOSITION BY BACTERIA

by

William Allen May Jr.

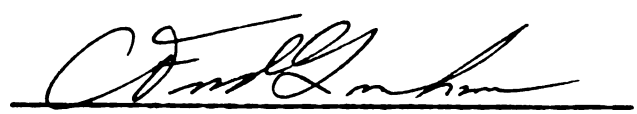
AN ABSTRACT

Submitted to the College of Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Chemical Engineering

1959

Approved 

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William A. May

ABSTRACT

Since cellulosic materials have been found to be resistant to decomposition in garbage composting processes, an investigation was made of some of the characteristics of microbiological attack on pure cellulose under conditions simulating those of a compost mixture. Filter paper was used as a substrate, and a mixed culture of bacteria obtained from garbage compost was used as inoculum. Included in the factors studied were desired nutrient composition, rate of carbon dioxide production and oxygen consumption, variation of the respiratory quotient, variation of pH, characteristics of the microbial population, products of decomposition and rate of nitrogen assimilation by the microorganisms. Work was also done to gain information concerning the limiting factors for the rate of cellulose decomposition.

An optimum concentration of $NaNO_3$ was found to be 1.4 per cent by weight of the initial mixture. Magnesium was essential for growth of the microorganisms. The optimum moisture content was from 62 to 70 per cent.

The rate of growth of microorganisms was followed by measuring the conversion of nitrate to organic nitrogen in water extracts of samples. The amount of remaining cellulose was determined by extracting the samples with water, alcohol and ether to remove the decomposition products. The rate of cellulose oxidation was followed by measuring

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oxygen uptake from the exhaust gas that had passed through the decomposing mixture. In a run which lasted eight days, 17.0 per cent of the initial cellulose was oxidized and a total of 34.3 per cent was destroyed.

It was found that the curves for the rate of cellulose destruction and for the rate of growth of microorganisms were nearly parallel. Both curves reached their maximum approximately on the fourth day of incubation. The maximum rate of nitrogen assimilation was 5.4 mg of nitrogen per gram initial cellulose per day, and the maximum rates of cellulose decomposition were 4.6 per cent of the initial cellulose oxidized per day and over 8 per cent destroyed per day.

The curves showed the activity of a decomposing mixture of cellulose to climb rapidly, then level off and decline after four to five days of incubation even though the supply of cellulose had not been exhausted. Experiments concerning the limiting factor for the rate of cellulose decomposition showed that addition of nutrient solution had no stimulating effect during the period of declining activity. An experiment using filter paper discs showed that clumping and restriction of available surface was not a limiting factor.

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SECTION I

INTRODUCTION

The ratio of carbon to nitrogen in the final product is one criteria for evaluating the quality of composted garbage and refuse. Cellulosic material, such as paper, is one of the major components of a typical garbage mixture. Since it represents a large per cent of the carbon content, an attempt to decrease the carbon to nitrogen ratio will focus on destruction of cellulose.

Finished compost often shows very little attack on the cellulosic components. Wiley and Pearce (1) reported only slight decomposition of cellulosic matter. Gotaas (2) stated that paper in composting material showed little evidence of attack by bacteria. Decomposition took place after more readily decomposable materials had been utilized, and when conditions favored growth of actinomycetes and fungi.

It was the purpose of this study to investigate some of the characteristics of microbiological attack on pure cellulose. It was hoped that some of the information gained could be applied to obtain increased decomposition of cellulosic materials in the composting process.

According to literature, the most common method of studying pure cellulose decomposition has been to suspend

the cellulose in liquid medium. In this study it was desired to simulate actual composting conditions. It was decided to use a moisture content in the range of 50 to 75 per cent and a mixed culture of microorganisms. Aerobic conditions were desired, and work was done at room temperature. A gas analysis technique was used to follow the rate of activity. Samples were dried in an oven to determine loss of weight after incubation. In one experiment an extraction technique was used to measure the actual loss of cellulose.

Factors investigated during the course of this study include desired nutrient composition, rate of CO_2 production and oxygen consumption, variation of the respiratory quotient, variation of pH, characteristics of the microbial culture, products of decomposition and rate of nitrogen assimilation by the microorganisms. Work was also done to gain information concerning the limiting factors for the rate of cellulose decomposition.

SECTION II

LITERATURE REVIEW

In the early part of this century, interest in cellulose decomposition by microorganisms was generated by its prominent role in the cycle of carbon transformation in nature. Many investigations were devoted to the type of organisms responsible and the conditions under which they attack cellulose.

A more recent stimulus to research in this field resulted from the alarming losses of cellulosic products by bacterial attack in the tropical theaters during World War II. During 1944 and 1945 teams of mycologists visited various places in the tropical belt and isolated fungi and bacteria from deteriorating cotton fabrics (3). In the last 20 years considerable interest has been directed toward the mechanism of breakdown.

A. Cellulose Attacking Microorganisms

Hutchinson and Clayton (4) reported isolation of an aerobic organism which showed growth only with cellulose as a source of carbon. Waksman and Skinner (5) studied the types of organisms found in soils capable of decomposing cellulose. Marsh, Bollenbacker, Butler and Raper (6) gave an extensive report on fungi capable of

destroying cellulose. Norman and Fuller (7) reviewed the types of microorganisms capable of decomposing cellulose. Norman and Bartholomew (8) did work with seven different mesophilic bacteria; and Viljoen, Fred and Peterson (9) studied thermophilic cellulose fermenters.

Reese (10) isolated 500 bacteria strains from decomposing fabrics. He found 8 per cent or 39 of them were capable of destroying cellulose. He divided these into two classes, an aerobic and a second type requiring little or no oxygen, but not responding to differences in oxygen concentration.

B. Favorable Conditions and Nutrient Composition

Several nutrient solutions containing no carbon have been developed for enumeration and isolation of cellulose decomposing bacteria. Dubos (11) studied effects of nutrient solution composition by immersing strips of filter paper in inoculated solutions and incubating at 28°C. He found an optimum nutrient composition to be: 0.5 grams $NaNO_3$, 1.0 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl and 0.01 g $FeSO_4 \cdot 7H_2O$ dissolved in 1 liter of distilled water. This alkaline solution (pH 7.5) favored growth of bacteria and retarded growth of fungi. He noted that decreasing concentration of $NaNO_3$ decreased the length of the incubation period for the bacteria. Using this nutrient solution, cellulose decomposition could be recorded after 36 to 72 hours.

Hutchinson and Clayton (4) used the following mixture in their work: 2 g $NaNH_4HPO_4 \cdot 4H_2O$, 1 g KH_2PO_4, 0.1 g $CaCl_2$, 0.3 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl and 0.01 g $FeCl_3$ in 1 liter of water. Walker and Warren (12) used the same mixture but found the $CaCl_2$ unnecessary.

Perlin, Michaelis and McFarlane (13) worked with an aerobic cellulose decomposing bacterium, Vibrio perimastix. They found that CO_2 was essential for growth of the bacteria, but retarded growth at concentrations over 1.2 per cent.

Many substances have been found that influence the decomposition of cellulose. For example, Fuller and Norman (14) obtained a utilization of one-third of filter paper suspended in nutrient solution over a fourteen day period. In equal time, cornstalk cellulose was far more extensively decomposed by all organisms tested. They concluded that the presence of xylan in the cellulosan component of this cellulosic material exerted a favorable influence on decomposition.

Waksman (15) reported that 80 to 95 per cent moisture favored anaerobic decomposition while 50 to 75 per cent favored aerobic cellulose decomposing bacteria. Optimum temperature range for aerobic decomposition was 20 to 28°C and 37°C was optimum for anaerobic decomposition.

Reese (10) worked with filter paper suspended in a nutrient solution. The optimum pH for Sporocytophaga

myxococcoides was from 6.5 to 7.5. After three days incubation, the optimum $NaNO_3$ concentration was from 1 to 3 grams per liter. KCl was found to be toxic at concentrations higher than about 0.05 N, but the author suggested that this might have been due to increasing total salt concentration rather than an effect of KCl. $MgSO_4$ and iron salts were found to be important to the rate of decomposition. Cu, Zn, Mo and Mn had no stimulating effect on growth.

During experimental work to determine the rate of decomposition, Reese used a medium containing 10 ml of 1 M potassium phosphate buffer solution, 1 g $NaNO_3$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.05 g $FeSO_4 \cdot 7H_2O$ and 4 g cellulose per liter. A series of 250 ml flasks were prepared and the rate of decomposition was followed by stopping each flask at a different time. He found 50 per cent decomposition of cellulose after three days. A maximum decomposition of 80 per cent was reached after six days. Reese suggested that the residue might have been bacterial substance, but no check was made.

C. By-products of Decomposition

Waksman (15) stated that as much as 30 to 40 per cent of cellulose decomposed may be converted into cell material. Heukelekian and Waksman (16) reported only CO_2 and water as waste products from decomposition of cellulose by fungi.

Nord and Vitucci (17) reviewed work reporting quantitative determination of products from decomposition of cellulose. Some work was done under aerobic conditions reporting CO_2, methane and fatty acids including acetic, butyric and valeric; but most work was done in the thermophilic range and under anaerobic conditions. Products reported include CO_2, hydrogen, methane, ethyl alcohol and higher alcohols and acetic, butyric, valeric, lactic and formic acids. Viljoen, Fred and Peterson (9) reported products from anaerobic organisms which destroyed cellulose rapidly at 65°C. The products of fermentation were acetic acid, small amounts of butyric acid, ethyl alcohol, CO_2 and hydrogen. The amount of cellulose destroyed in a 1 to 5 per cent liquid suspension varied from 70 to 95 per cent, of which 50 to 55 per cent was regained as acetic acid, 5 to 25 per cent as ethyl alcohol and the rest as small amounts of butyric acid, CO_2, hydrogen and a pigment soluble in ether.

Walker and Warren (12) found that two-thirds of cellulose decomposed under aerobic conditions was accounted for by CO_2 evolved. The remaining one-third was in the form of a mucilage substance and small amounts of pigment and other metabolic products. Perlin, Michaelis and McFarlane (13) reported products similar to those described by Walker and Warren except that a smaller amount of mucilage was found.

D. Mechanism of Attack by Microorganisms

Boswell (18) reviewed literature up to 1941 on the mechanism of enzymatic attack on cellulose. He favored evidence that decomposition was facilitated by oxidation resulting in the formation of oxycellulose. Walker and Warren (12) suggested that the mucilage substance which they isolated from decomposing cellulose was an oxycellulose and an intermediate step in breakdown. Norman and Bartholomew (8) argued that the mucilage was a product of decomposition and should be considered a bacterial gum or polyuronide gum.

In 1953, Siu and Reese (19) gave a review of previous work with emphasis on the relationship of organism to the substrate and mechanism of breakdown of the cellulose. They gave evidence for a two stage mechanism of breakdown, and suggested that as a first step natural cellulose is broken down by enzyme action to a more easily attacked form. In the second step, the cellulose molecule is converted by enzymatic reduction to cellobiose. Evidence was cited that cellulose decomposing organisms assimilated cellobiose rather than glucose.

Perlin, Michaelis and McFarlane (13) reported that the oxygen uptake rate of Vibrio perimastix was increased by additions of both glucose and cellobiose. Levinson, Mandels and Reese (20) investigated the enzymatic mechanism using paper chromatographic analysis to identify the products of hydrolysis of cellulose. Cellobiose was the

principal product of hydrolysis according to their data. They also found that in a rapidly growing culture, the cellobiose was used as rapidly as it was formed.

E. Technique of Study

The most common method of studying pure cellulose decomposition has been to suspend the cellulose in a liquid nutrient medium. Reese (10) suspended ground filter paper in inoculated nutrient solutions. The mixtures were prepared in flasks and agitated with a mechanical shaker during the incubation periods. Remaining cellulose after incubation was determined by filtering and drying. Weight of dried crucibles minus final ash weight was considered to be the weight of remaining cellulose. With this system, he investigated effects of nutrient components and concentration.

Fuller and Norman (14) suspended 3 grams of finely divided cellulosic preparations in 400 ml of nutrient solution, sterilized and inoculated it with pure cultures of bacteria and bubbled sterile, moist air through it.

Walker and Warren (12) worked on a large scale to get large quantities of metabolic products. They suspended 20 grams of chopped filter paper in 2 liters of medium and bubbled a slow stream of oxygen through it. In some of their work, the CO_2 evolved was measured by absorption in baryta bottles followed by titration. The remaining cellulose was measured by filtering the medium through a

fine linen cloth. The residue was extracted with hot water, alcohol and ether. The final residue after extraction was considered remaining cellulose.

Heukelekian and Waksman (16), working with fungi in liquid, sand and soil mediums, measured remaining cellulose by dissolving it in Schweitzer's reagent and precipitating it with alcohol. They measured CO_2 evolved by collecting it in $Ba(OH)_2$ similar to the method used by Walker and Warren (12). They also measured nitrogen assimilated by extracting the medium and converting nitrogen compounds to ammonia by distilling with MgO. The ammonia was collected in standard acid. The measured nitrogen was subtracted from the nitrogen found in an uninoculated control flask to give the nitrogen assimilated by the organisms. When nitrates were present, they were converted to ammonia using 3 grams of Devarda alloy (50% Cu, 45% Al and 5% Zn) in alkaline solution. The amount of nitrogen assimilated was shown to be directly related to the amount of cellulose decomposed.

Wiley and Pearce (1) used a gas analysis technique to follow the activity of their composting process. They forced air through the composting material and analyzed the exhaust gas for moisture and CO_2 content. After removing moisture with Drierite, they absorbed CO_2 in Ascarite. In a similar system, Moore (21) used a Beckman magnetic oxygen analyzer to measure oxygen uptake from the exhaust gas of composting garbage.

SECTION III

THEORETICAL CONSIDERATIONS

Wiley and Pearce (1) correlated their gas analysis results from composting garbage with the following relationship for organic matter destroyed:

$$C_xH_yO_z + bO_2 = xCO_2 + y/2H_2O$$

This relationship assumed that by-products other than CO_2 and water were small in comparison to the total amount of material destroyed, and they cautioned that the relationship would be expected to change with time.

For a pure cellulose substrate, this relationship for complete oxidation per monomer unit of cellulose would be:

$$C_6H_{10}O_5 + 6O_2 = 6CO_2 + 5H_2O$$

The amount of cellulose decomposed by complete oxidation could be calculated by measuring the oxygen consumed. According to this equation, 0.845 grams of cellulose will be oxidized for every gram of oxygen consumed, or for every gram of cellulose oxidized:

$$1 \text{ g cellulose} + 1.185 \text{ g } O_2 = 1.630 \text{ g } CO_2 + 0.555 \text{ g } H_2O$$

An indication of the type of microbiological attack taking place is given by the respiratory quotient (R.Q.), computed by dividing the moles of CO_2 produced by the moles of oxygen consumed. If cellulose is being decomposed by complete oxidation, the moles of oxygen consumed will equal the moles of CO_2 produced; and the R.Q. will be equal to one. If in addition to oxidation a fermentation reaction takes place, more CO_2 is produced than oxygen consumed and the R.Q. will be greater than one.

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SECTION IV

EXPERIMENTAL APPARATUS AND MATERIAL

A. Material

1. Cellulose

Whatman No. 1 filter paper was chosen as a working medium. It is almost pure alpha type or long chain cellulose. In all experiments except one, the filter paper was cut in small squares varying from 1/8 inch to 3/16 inches.

2. Nutrient Solution

A nutrient mixture was chosen on the basis of the study by Dubos (11). It was modified to the mixture listed in Table 1 so that the desired low moisture content in experimental work could be obtained. The calcium phosphate was added to the mixture to give a source of calcium.

A grey precipitate, found to be compounds of iron and magnesium phosphate, resulted on mixing the solution. When the nutrient solution was added to the filter paper, the precipitate was kept in suspension as much as possible.

TABLE 1

NUTRIENT SOLUTION

Component	Amount
Distilled water	300 ml
$NaNO_3$	6 g
$K_2HPO_4 \cdot 3H_2O$	6 g
$MgSO_4 \cdot 7H_2O$	0.337 g
KCl	0.335 g
$FeSO_4 \cdot 7H_2O$	0.018 g
$CaHPO_4$	0.252 g

3. Seed Material

A mixed culture seed was obtained from a sample of finished compost of synthetic garbage containing an initial amount of 35 per cent newspaper on a dry weight basis. The compost, prepared by Moore (21) in his study of aerobic decomposition of organic waste material, had been stored at room temperature for several weeks after completion of the run. It showed more than the usual amount of cellulose breakdown and was therefore used to seed a mixture of 5 grams of filter paper and 15 ml of nutrient solution in a 125 ml Erlenmeyer flask. The mixture was kept at room temperature in a desiccator with water in place of desiccant to give 100 per cent humidity.

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A yellow color appeared on the filter paper after three days. It lost much of its strength after a week. The paper cuts had clumped together slightly with the addition of nutrient but clumped still further due to formation of slime from the organisms. A close inspection showed the yellow discoloration to occur mainly on the surface of the clumps. This led to the addition of vermiculite to decrease clumping and preserve a greater surface for decomposition. Vermiculite is an exploded mica preparation and is bacteriologically inert.

The culture was maintained by transferring some of the decomposed cellulose into a new mixture of filter paper and nutrient solution about every twenty days. Microscopic investigation showed that a mixed culture of bacteria had developed three days after seeding. A few protozoa were present after seven days. In some samples yeasts were found after nine days which developed into a large population by the eleventh day. Protozoa were numerous in mixtures a month old. Other than yeasts, no evidence of fungi was found. No attempt was made to identify the components of the mixed bacterial culture.

B. Apparatus

1. Variation of Nutrient Composition

The equipment used to check optimum nutrient concentration consisted of a moist chamber made from a desiccator with the desiccant replaced with water.

2. Gas Analysis Apparatus

An apparatus was set up as shown in Figure 1 to pump air through a sample of cellulose in a 300 ml Erlenmeyer flask D. A Sigmamotor pump I, model T6S, with a Revco Zero-Max speed changer, model 142X, was found satisfactory for maintaining low flow rates through the system.

The incoming air was purged of CO_2 by tube A filled with Sodasorb. The air was then passed through several feet of Tygon tubing submerged in the constant temperature water bath C which was used to hold the samples between 25 and 26°C. The air was humidified in a Fisher-Milligan gas washer bottle B to prevent drying out of the samples.

Ascarite was used to collect the CO_2 produced by decomposition of the samples. Before the air reached the Ascarite filled tube J, all moisture was removed by passage through a 12 inches long, 5/8 inches inside diameter tube E filled with Drierite desiccant. In this way, the increase in weight of the tube filled with Ascarite was due to CO_2 absorbed. A second tube of Drierite F was included which could be weighed. This gave a check for saturation of the first and larger tube.

A Beckman magnetic oxygen analyzer H, model D-2, was used to measure the partial pressure of oxygen in the exhaust gas. A mercury manometer G indicated the reduced pressure in the system at the point of oxygen measurement.

The volume of the exhaust gas was measured as it left the system through a Wet-Test gas meter K.

3. Apparatus for Filter Paper Sheets

In one experiment, it was desired to decompose the filter paper as whole sheets. The Erlenmeyer sample flask in the previously described apparatus was replaced by a transparent plastic container shown in Figure 2. This container was 4 inches deep and tapered from 10 to 9 inches in width and 13 to 12 inches in length. A hole was drilled in each end of the container and fitted with rubber tubing for air circulation. Four trays were prepared as sketched in Figure 2 from 5 mm glass tubes woven in place with 1/16 inch Tygon tubing. Spacing between the glass tubes was about 1/8 inch. The trays were separated in the container by 1/2 inch diameter glass tubes running the width of the container at each end of the trays.

With this unit, discs of filter paper were spread on the trays. The cover was sealed with stopcock grease so that the container could be submerged under water for temperature control.

Figure 1. Flow Diagram of Apparatus

A. Sodasorb tube

B. Gas washer bottle

C. Water bath

D. Flask containing cellulose preparation

E. Drierite tube

F. Small Drierite tube

G. Manometer

H. Oxygen analyzer

I. Pump

J. Ascarite tube

K. Wet-Test gas meter

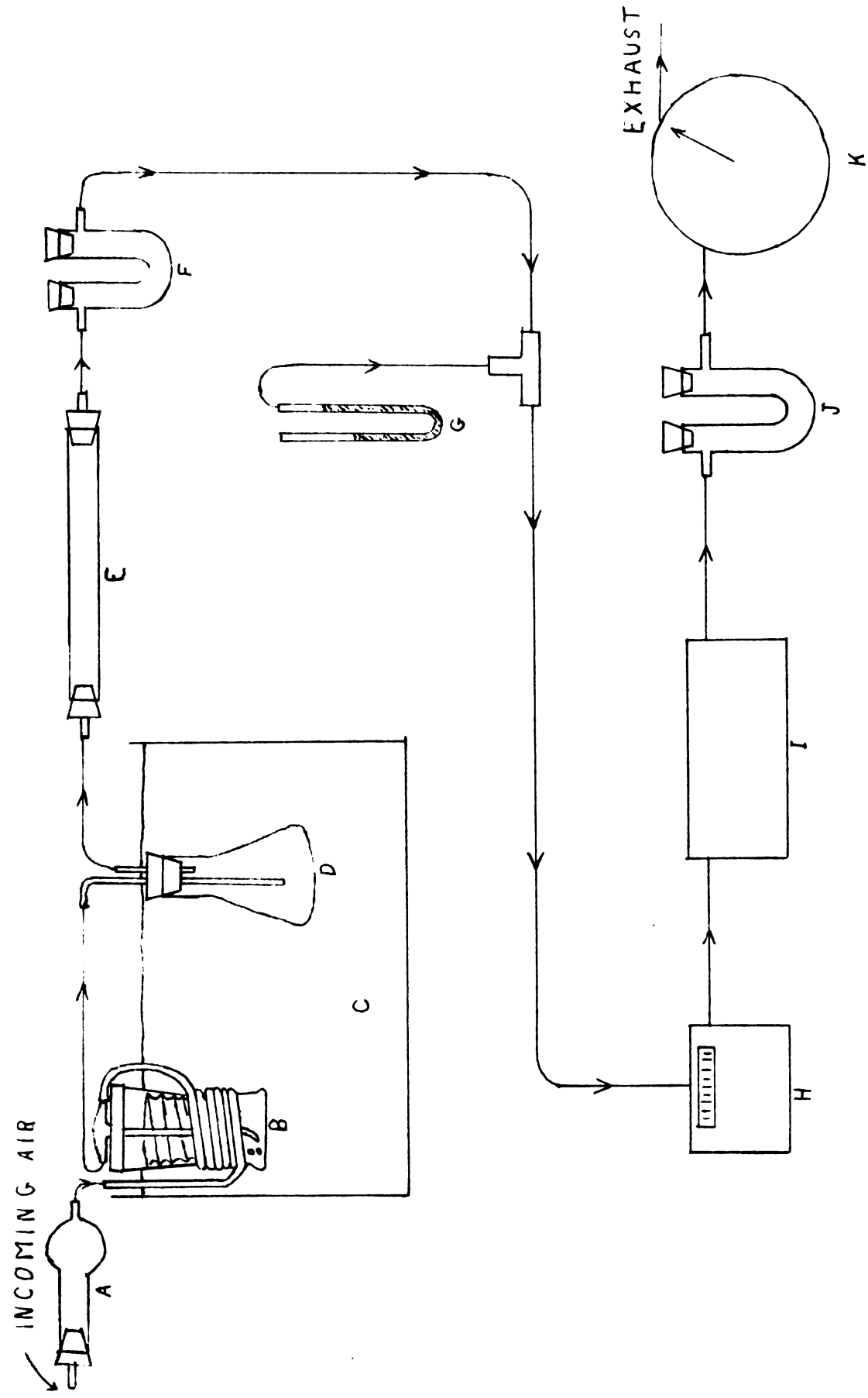
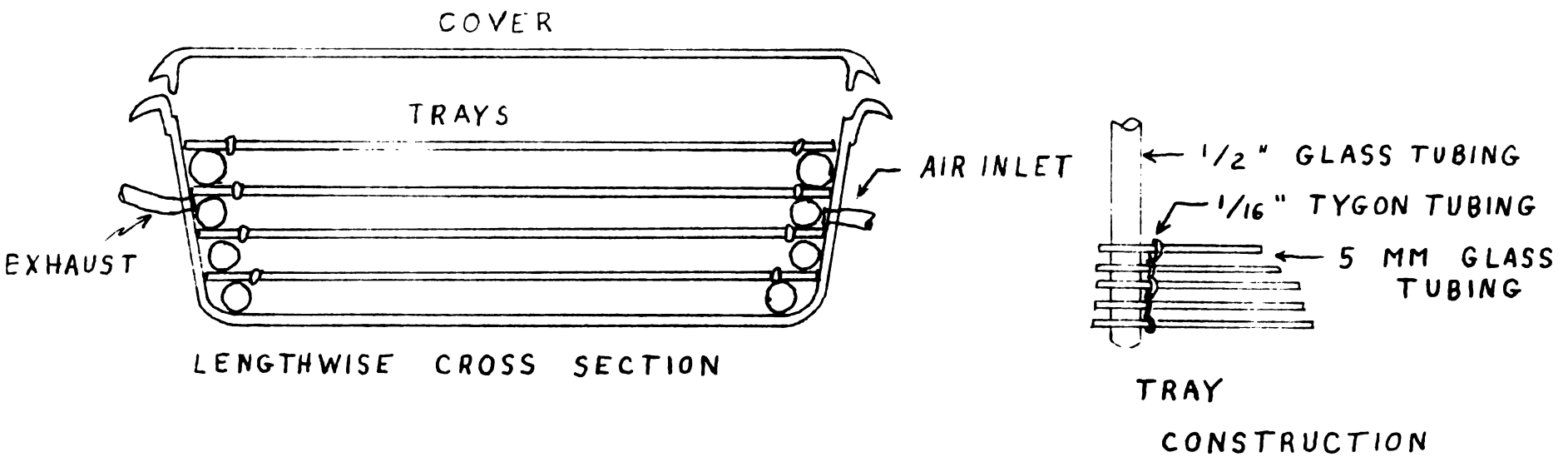


FIGURE 1. FLOW DIAGRAM OF APPARATUS

FIGURE 2. PLASTIC CONTAINER FOR PAPER DISCS



SECTION V

PROCEDURE AND RESULTS

A. Variation of Nutrient Composition

Composition of the standard mixture of filter paper, vermiculite and nutrient is given in Table 2. To determine the effect of variation of nutrient components, one component was varied in each experiment. Each time, seven samples were prepared in 125 ml flasks. Each sample contained 5 grams of filter paper, 2.5 grams of vermiculite and 15 ml of nutrient solution with a different concentration of the component to be checked.

TABLE 2

STANDARD MIXTURE

Component	Per Cent by Weight
Water	66
Cellulose	21
Vermiculite	10.9
$NaNO_3$	1.1
K_2HPO_4	0.9
KCl	0.07
$MgSO_4$	0.05
$CaHPO_4$	0.04
$FeSO_4$	0.003
Seed material	0.2

Each flask was removed from the moist chamber and shaken once a day to get mixing of the cellulose and to renew the supply of oxygen. Loss of weight of the sample at 100 per cent humidity was checked by weighing the flask containing the sample and subtracting this weight from the initial weight. Figure 3 shows the rate of weight loss at 100 per cent humidity for 3 samples with different $NaNO_3$ concentrations. This loss of weight was due to the formation of volatile products at room temperature; for example, CO_2.

At the end of twenty days, remaining dry weight of each sample was determined by drying at 105°C. This weight loss would include losses due to formation of breakdown products volatile at 105°C. Per cent weight loss for each sample was calculated after subtracting the weight of vermiculite and nutrient salts. The per cent of the component being varied in the samples was plotted against the per cent weight loss after 20 days. Figures 4 through 8 show the results for $NaNO_3$, $MgSO_4$, $CaHPO_4$ and $FeSO_4$.

The amount of weight loss after twenty days varied from 17 to 48 per cent over the range of $NaNO_3$ checked. The maximum concentration appeared to be about 1.4 per cent $NaNO_3$ in the initial mixture. It was also found that $NaNO_3$ played a role in determining the rate of decomposition as shown in Figure 3. The sample with the lower concentration had only a short lag period before activity

FIGURE 3. RATE OF WEIGHT LOSS AT 100 PER CENT HUMIDITY

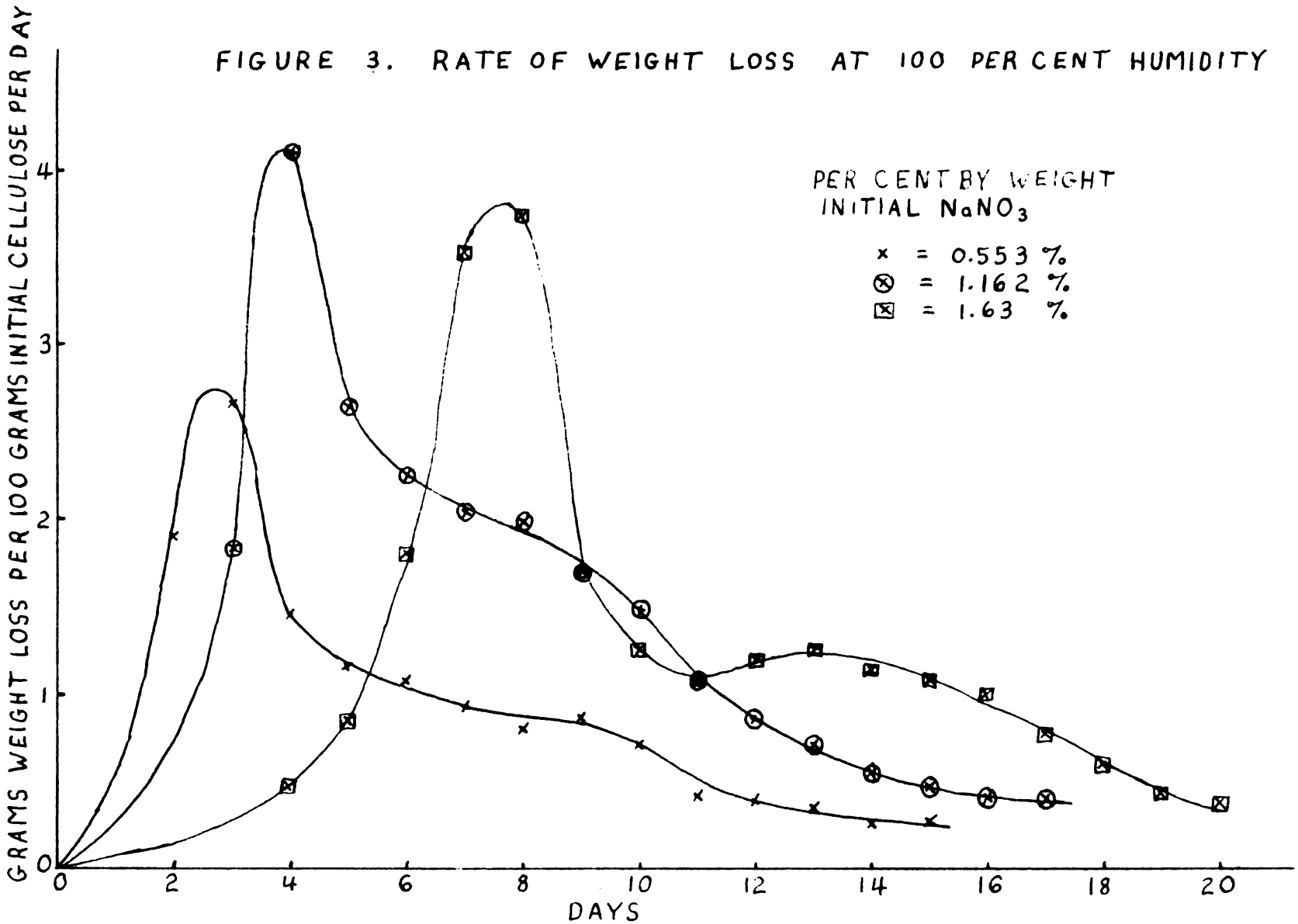


FIGURE 4. EFFECT OF $NaNO_3$ CONCENTRATION ON WEIGHT LOSS

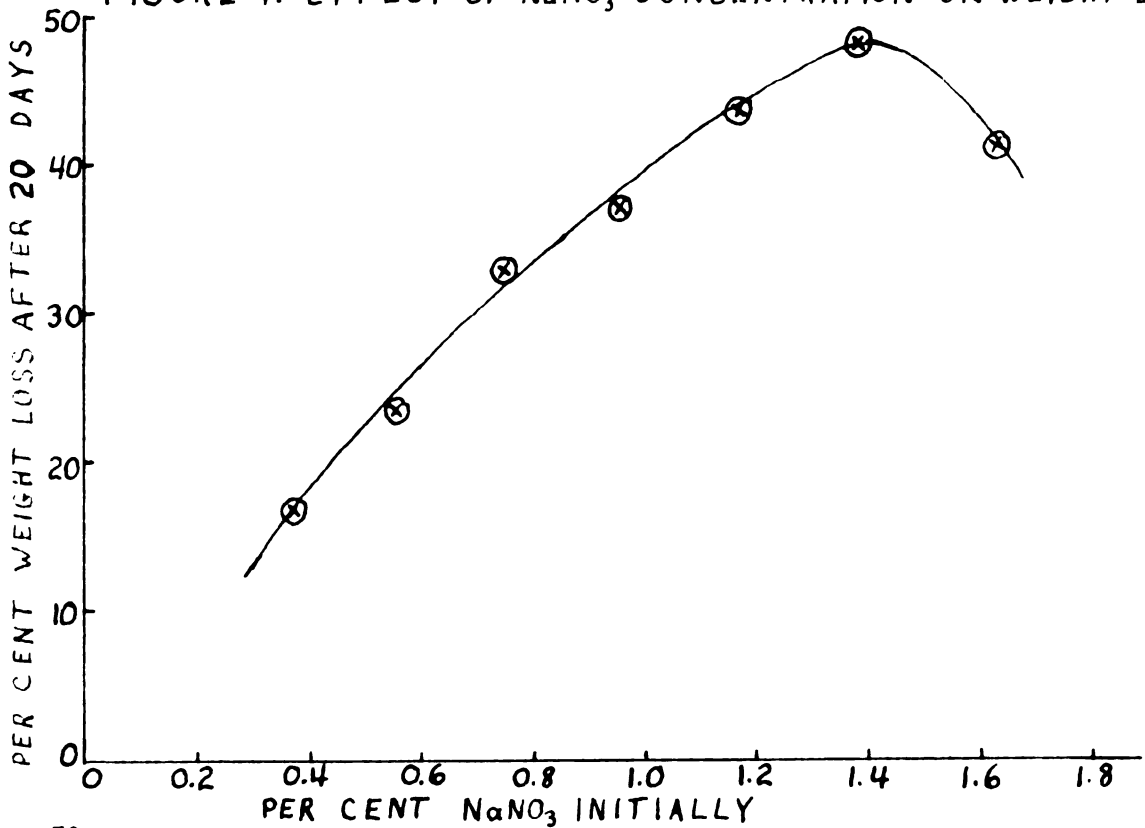


FIGURE 5. EFFECT OF $MgSO_4$ CONCENTRATION ON WEIGHT LOSS

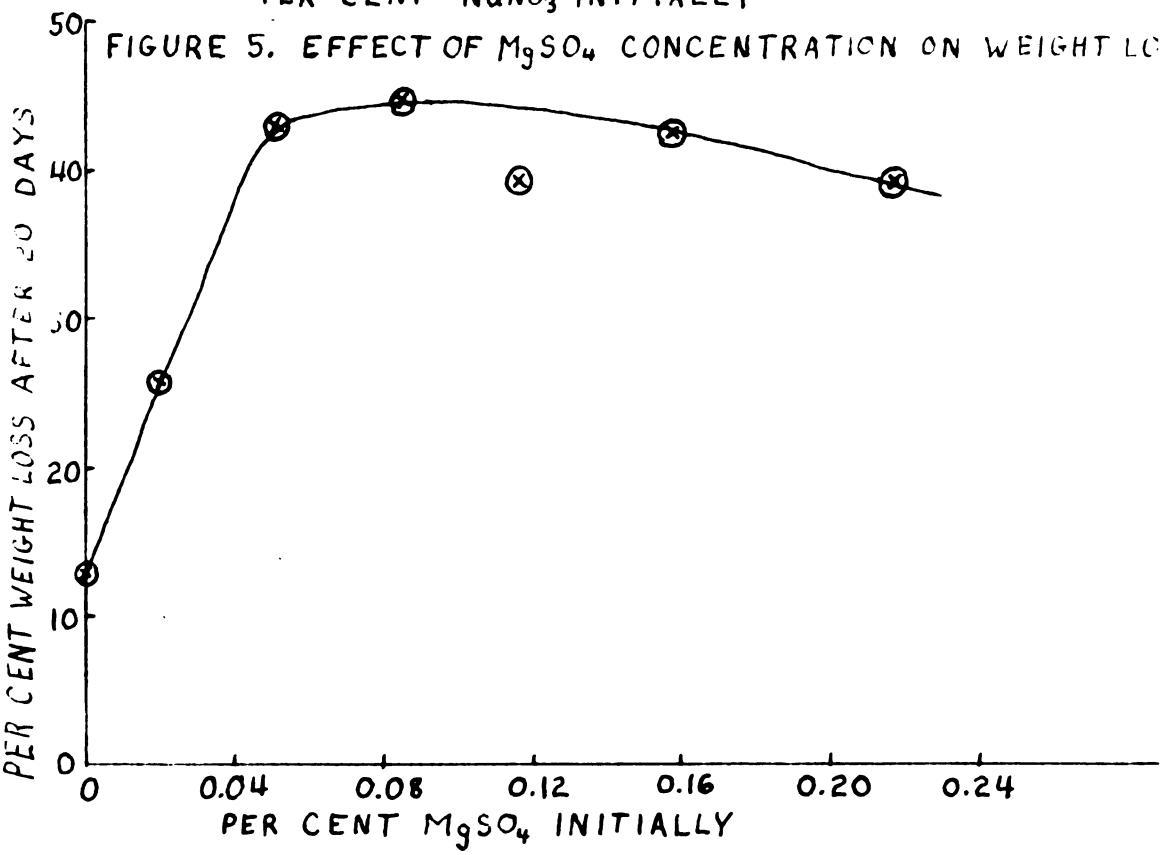


FIGURE 6. EFFECT OF $CaHPO_4$ CONCENTRATION ON WEIGHT LOSS

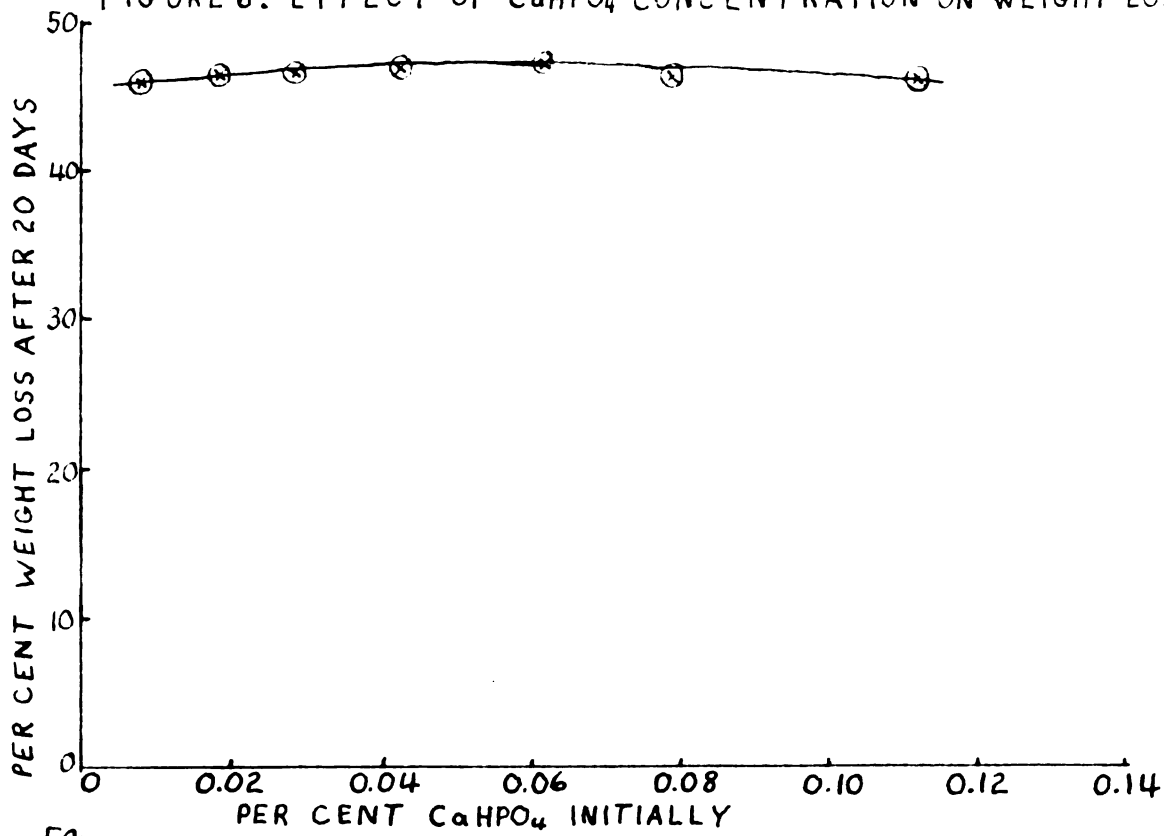


FIGURE 7. EFFECT OF K_2HPO_4 CONCENTRATION ON WEIGHT LOSS

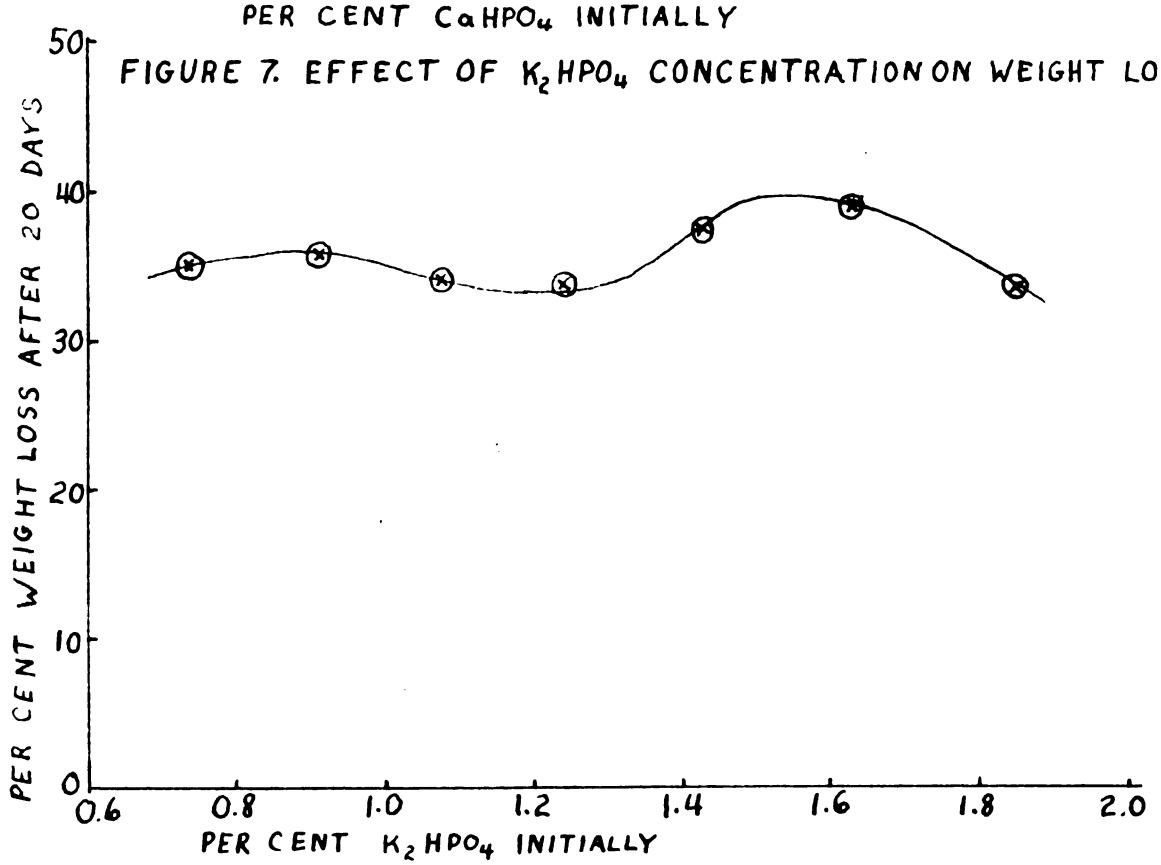


FIGURE 8. EFFECT OF $FeSO_4$ CONCENTRATION ON WEIGHT LOSS

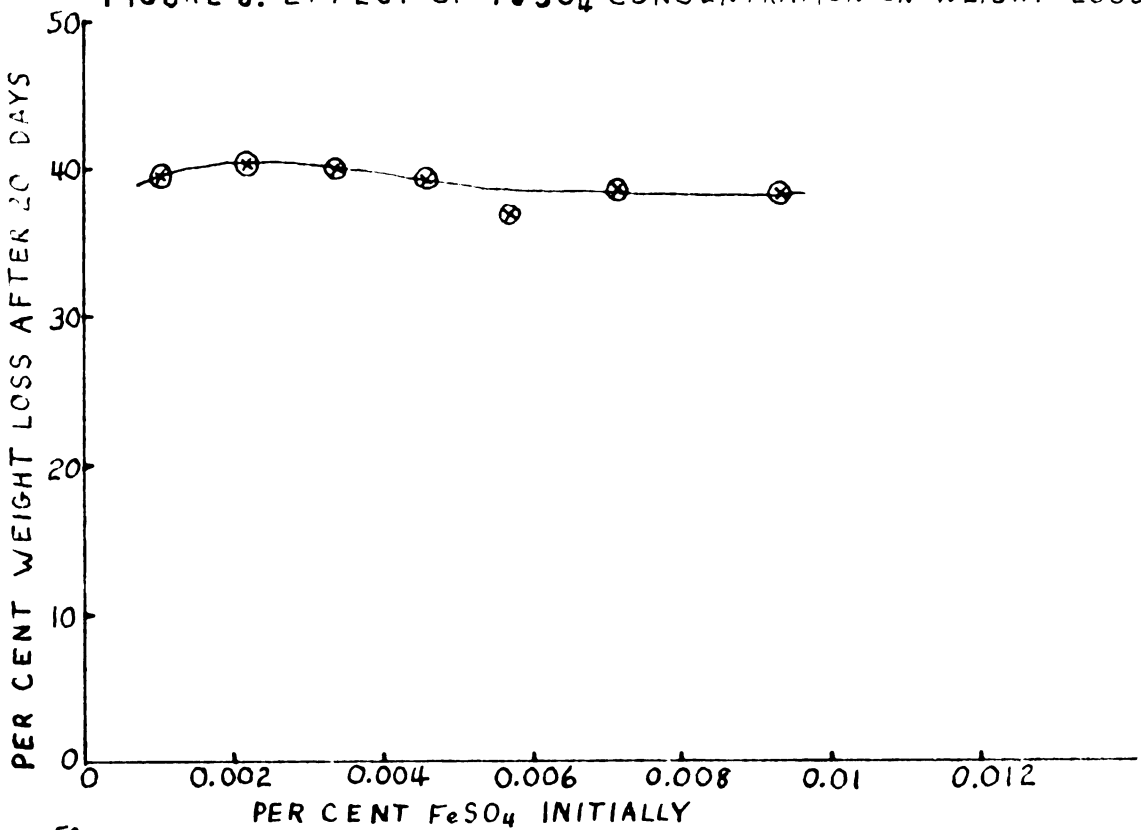
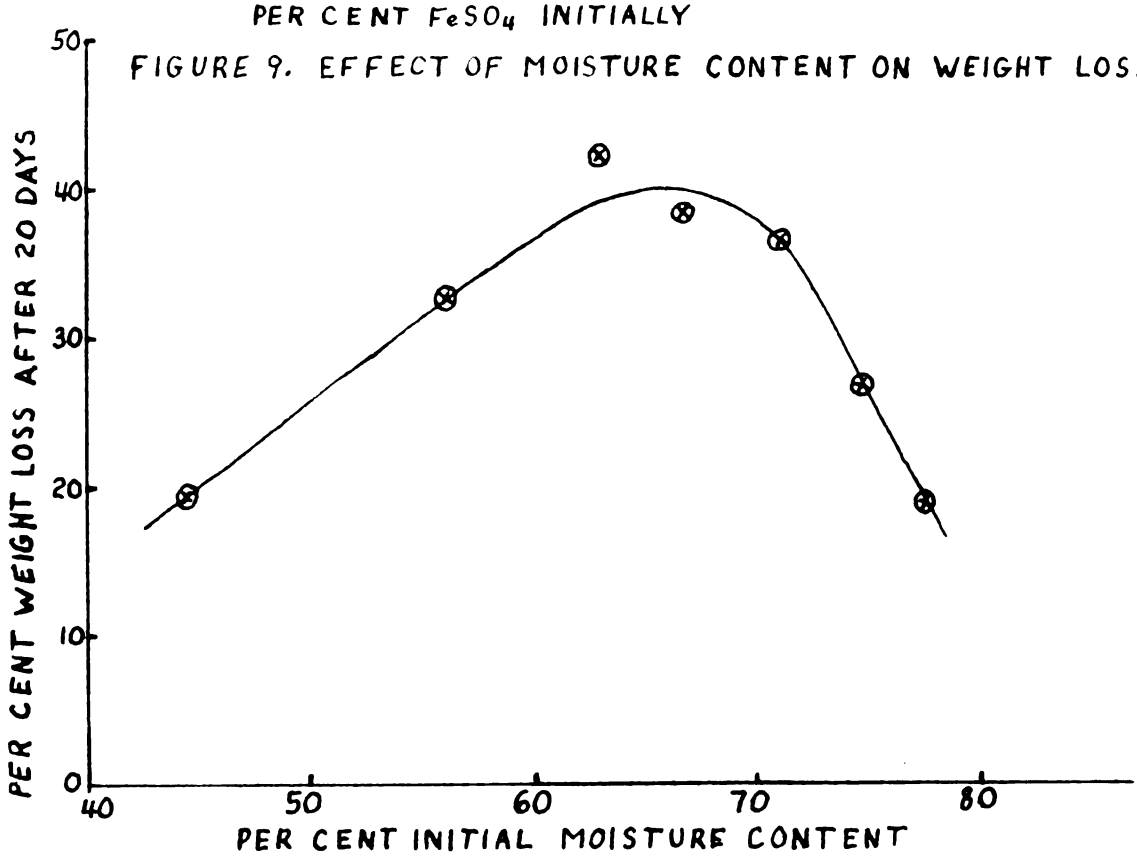


FIGURE 9. EFFECT OF MOISTURE CONTENT ON WEIGHT LOSS



became measurable. The higher concentration of $NaNO_3$ gave a longer lag period even though, up to a point, higher concentrations resulted in greater total decomposition.

The data on variation of $MgSO_4$ concentration showed clearly that a small amount of magnesium was necessary for growth of the organisms. The activity at zero concentration of this component could have been due to traces of magnesium introduced as impurity in the other components. The seed material would have supplied a small amount of $MgSO_4$ which was not considered in the calculations. Concentration of $CaHPO_4$ did not appear to be important, and it may have been that traces of calcium introduced as impurity in other components supplied the required amount of calcium. The curve for K_2HPO_4 appeared to have two maximum points. It is noted that K_2HPO_4 was the major supply of both potassium and phosphorus. The double maximum could also have been due to selectivity of different components of the mixed culture of organisms. The variation of $FeSO_4$ seemed to have little effect on the amount of decomposition over the range checked.

B. Moisture Variation

To check the effect of moisture concentration on decomposition, seven identical samples were prepared with 5 grams of filter paper, 2.5 grams of vermiculite and 15 ml of nutrient solution. Three of the mixtures were dried to a lower moisture content, and distilled water was

added to three others to increase the moisture content. This gave an initial moisture content variation as shown in Table 3. It is noted that this method of preparation did not hold nutrient concentration constant.

During the run, moisture accumulated as a product of cellulose decomposition. As a result, moisture content increased with time. Table 3 lists the final moisture content in the samples after 20 days. Figure 9 shows a plot of the per cent weight lost for the seven different moisture contents.

TABLE 3

VARIATION OF MOISTURE

Sample No.	Per Cent Initial Moisture Content	Per Cent Final Moisture Content
1	44.4	49.7
2	56.1	63.1
3	63.0	71.1
4	66.0	72.9
5	71.0	76.6
6	74.7	78.3
7	77.4	79.5

The optimum moisture content appeared to be about 62 to 70 per cent. The amount of decomposition fell rapidly for moisture contents higher than this.

With the exception of the second maximum concentration shown by the K_2HPO_4 experiment, all of the maximum points of the nutrient variation curves and the moisture

variation curve appeared to be fairly close to the concentration used in the standard mixture. This mixture was therefore used for all subsequent experiments.

C. Rate of Oxygen Consumption and CO_2 Production

Samples were prepared using one-half as much vermiculite and three times as much nutrient solution as the weight of cellulose used. For each run, the moisture content was determined for filter paper and vermiculite by drying at 105°C. These moisture contents varied between 3.5 to 5.5 per cent for cellulose and 0.5 to 1 per cent for vermiculite. Time was an important factor in these determinations since cellulose adsorbs moisture rapidly. The dry weight of cellulose and vermiculite was used for calculations in each run.

Seeding material was added in an amount of about 4 per cent of the weight of cellulose used. The seeding material, cellulose and vermiculite were mixed in a flask with the aid of a glass rod while the nutrient solution was added slowly. This insured an even distribution of the seed material.

The flask containing the sample was placed in the water bath and connected to the air flow lines. Gas analysis measurements were made every 12 hours.

A mercury manometer was used to determine the reduced pressure in the system at the point of oxygen measurement. The partial pressure of oxygen as measured

by the Beckman oxygen analyzer was divided by the atmospheric pressure less the pressure drop in the system to give the per cent oxygen remaining in the exhaust gas. The difference between this measurement and the per cent oxygen in the room was taken as per cent of oxygen used by the sample.

The volume of exhaust gas was measured after CO_2 had been removed. The weight of the CO_2 absorbed by the Ascarite, measured by weighing the tube every 12 hours, was converted to volume at 25°C and 760 mm pressure and added to the volume of exhaust gas which had been corrected to 25°C and 760 mm pressure. This gave the volume of air flowing through the oxygen analyzer. This volume, multiplied by the per cent of oxygen depleted, gave the amount of oxygen consumed by the sample. Since measurements were made every 12 hours, the average per cent of oxygen depleted was used for calculations during each period.

The final data for the six runs made in this manner are listed in Table 4. Length of run, initial dry weight of cellulose, weight loss after drying at the end of the run, per cent weight loss, total grams of CO_2 produced and total grams of oxygen consumed are tabulated.

Gas analysis data for run No. 1 are listed in Table 5. The flow rate of air was held at about 0.175 liters per hour. Remaining oxygen in the exhaust gas decreased

TABLE 4

DATA FOR RUNS NO. 1 THROUGH 6

Run No.	Length of Run, Days	Initial Cellulose, Grams	Weight Lost, Grams	Per Cent Weight Loss	CO_2 Produced, Grams	Oxygen Consumed, Grams
1	25	17.415	9.333	53.5	13.085	9.36
2	14	17.464	6.661	38.2	8.783	6.12
3	14	19.35	7.682	44.0	9.408	6.54
4	14	19.35	9.21	47.6	11.627	8.28
5	8	17.25	4.71	27.3	5.992	3.48
6	8	17.25	----*	----*	6.865	4.34

*Sample was lost before final dry weight was determined.

to as low as 7 per cent, and a total of 13.085 grams of CO_2 were absorbed by the Ascarite over a 25 day period. A total of 7.150 liters of oxygen was consumed. After being dried at 105°C, this run showed 53.5 per cent loss of weight from the initial dry weight of 17.415 grams. The initial moisture content of the mixture was 66 per cent, and the final moisture content was 75.7 per cent.

In Table 5 the oxygen consumed and CO_2 produced are given in liters at 25°C and 760 mm pressure for each 12 hour period. The cumulative amounts of gas are listed in columns 4 and 5. The oxygen uptake rates and CO_2 production rates in mg gas per day per gram initial cellulose are shown in columns 6 and 7. A plot of oxygen consumption rate versus time is shown in Figure 10. The CO_2 production rate is plotted in Figure 11.

TABLE 5. GAS ANALYSIS DATA FOR RUN NO. 1

Days	Oxygen Consumed, Liters	CO_2 Produced, Liters	Cumulative: O_2 Cons., Liters	Cumulative: CO_2 Prod., liters	O_2 Cons., Rate mg/g in.	CO_2 Prod., Rate cell./day
0.5	0.00176	0.00667	0.0018	0.0067	0.26	1.37
1.0	0.00489	0.0100	0.0067	0.0167	0.75	2.04
1.5	0.01042	0.01667	0.0172	0.0333	1.57	3.44
2.0	0.02353	0.02974	0.0407	0.0628	3.54	6.08
2.5	0.0587	0.0695	0.0994	0.1323	8.82	14.3
3.0	0.1207	0.1501	0.2201	0.2824	18.15	31.0
3.5	0.1983	0.2558	0.4184	0.5382	29.8	52.6
4.0	0.263	0.335	0.681	0.873	39.6	69.1
4.5	0.270	0.329	0.951	1.202	40.6	67.9
5.0	0.261	0.291	1.212	1.493	39.2	60.0
5.5	0.253	0.276	1.465	1.769	38.0	56.9
6.0	0.260	0.276	1.725	2.045	39.1	56.9
6.5	0.269	0.280	1.994	2.325	40.5	57.8
7.0	0.271	0.289	2.265	2.614	40.8	59.6
7.5	0.269	0.271	2.534	2.885	40.5	55.8
8.0	0.249	0.257	2.783	3.142	37.4	53.0
8.5	0.236	0.243	3.019	3.385	35.5	50.1
9.0	0.2365	0.238	3.256	3.623	35.6	49.1
9.5	0.222	0.225	3.478	3.848	33.4	46.4
10.0	0.218	0.219	3.696	4.067	32.8	45.2
10.5	0.232	0.217	3.928	4.284	34.9	44.7
11.0	0.299	0.212	4.157	4.496	34.4	43.7
11.5	0.2255	0.206	4.383	4.702	33.9	42.5
12.0	0.215	0.196	4.598	4.898	32.3	40.4
12.5	0.199	0.183	4.797	5.081	29.9	37.3
13.0	0.1885	0.174	4.985	5.255	28.3	35.5
13.5	0.178	0.163	5.163	5.418	26.8	33.2
14.0	0.169	0.151	5.332	5.569	25.4	30.8
14.5	0.157	0.145	5.489	5.714	23.6	29.6
15.0	0.149	0.136	5.638	5.850	22.4	27.8
15.5	0.142	0.125	5.781	5.975	21.4	25.5
16.0	0.128	0.117	5.909	6.092	19.25	23.9
16.5	0.1166	0.108	6.026	6.200	17.45	22.0
17.0	0.1076	0.099	6.133	6.299	16.2	20.2
17.5	0.0987	0.0917	6.232	6.394	14.8	18.7
18.0	0.0897	0.084	6.322	6.478	13.5	17.1
18.5	0.0816	0.0778	6.404	6.556	12.25	15.9
19.0	0.0757	0.0723	6.480	6.628	11.4	14.75
19.5	0.0700	0.0684	6.550	6.696	10.5	13.9
20.0	0.0666	0.0645	6.617	6.760	10.0	13.15
20.5	0.0626	0.0606	6.680	6.821	9.42	12.35
21.0	0.0600	0.0589	6.740	6.880	9.03	12.00
21.5	0.0584	0.0562	6.798	6.936	8.78	11.45
22.0	0.0555	0.0534	6.853	6.989	8.35	10.8
22.5	0.0530	0.0517	6.906	7.041	7.96	10.55
23.0	0.0527	0.0506	6.959	7.092	7.92	10.3
23.5	0.0492	0.0489	7.008	7.141	7.40	9.95
24.0	0.0477	0.0467	7.056	7.188	7.18	9.52
24.5	0.04705	0.0456	7.103	7.233	7.08	9.30
25.0	0.0466	0.0450	7.150	7.278	7.01	9.20

FIGURE 10. RATE OF OXYGEN CONSUMPTION FOR RUN NO. 1

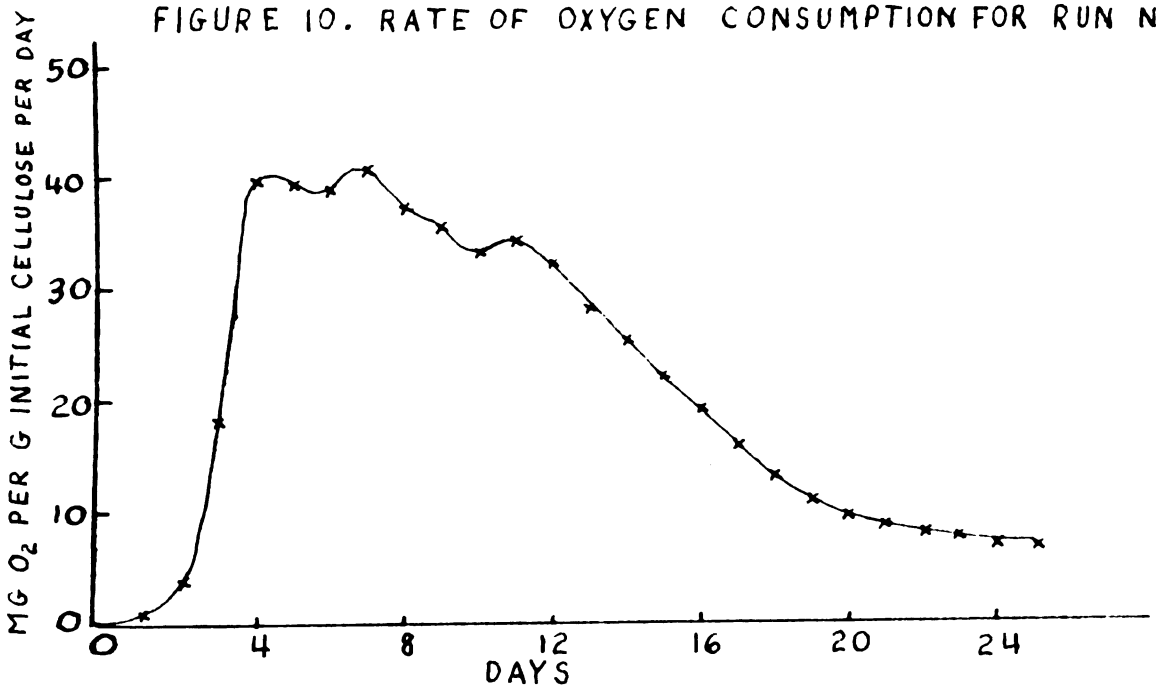
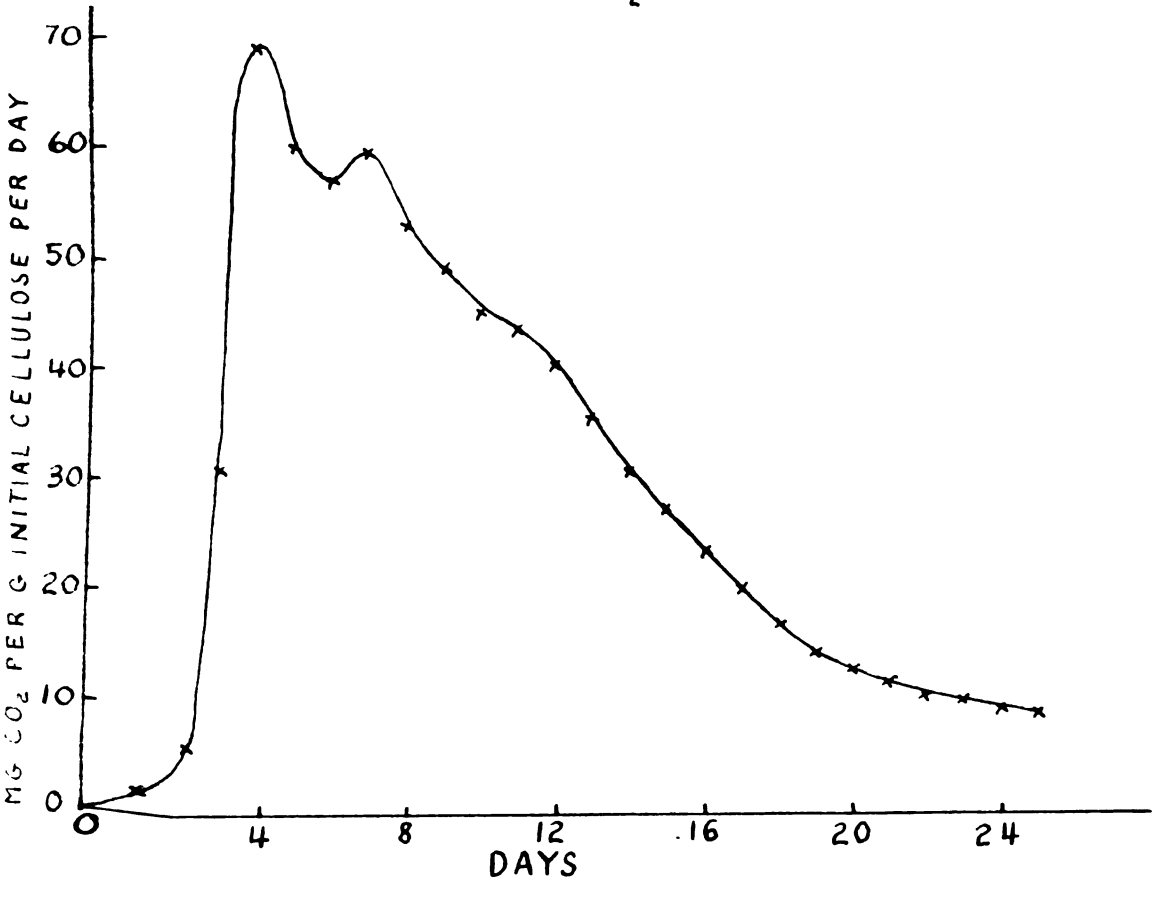


FIGURE 11. RATE OF CO_2 PRODUCTION FOR RUN NO. 1



One to two days of incubation were necessary before activity became measurable. Oxygen consumption and CO_2 production indicated rapid growth of microorganisms from the second until the fourth day. This was followed by a leveling off period and a rapid decline of activity after 7 to 10 days. The maximum oxygen consumption rate was 40.8 mg per day per gram initial cellulose, and the maximum CO_2 production rate was 69.1 mg per day per gram initial cellulose.

D. Addition of Nutrient Components at the Point of Maximum Oxygen Consumption

To gain information concerning the reason for the leveling off of activity after four days of incubation, the effect of addition of nutrient components on the rate of oxygen consumption was checked. These additions were made after the leveling off point in the oxygen uptake curves had been reached.

Runs No. 2 and 3 were run parallel at a flow rate of 0.4 liters per hour. Remaining oxygen in the exhaust gas decreased to as low as 12 per cent. To check the possibility that activity slowed because of depletion of nitrogen supply, 1.1 grams of $NaNO_3$ in 11.3 ml of water were added to sample No. 3 on the seventh day. It is noted that this addition increased the initial moisture content of the mixture from 66 to 70 per cent.

The gas analysis data for these runs are listed in Table 6. The oxygen consumption rates are plotted in Figure 12. The maximum oxygen consumption rate was 49.7 mg oxygen per gram initial cellulose per day for run No. 2 and 53.0 mg oxygen per gram initial cellulose per day for run No. 3.

Results showed no stimulating effect. Production of CO_2 and consumption of oxygen in sample No. 3 had been running slightly higher than in sample No. 2. After the addition of $NaNO_3$, CO_2 production and oxygen consumption in sample No. 3 fell below that of sample No. 2.

Runs No. 5 and 6 were conducted in parallel at a flow rate of about 0.5 liters per hour. Remaining oxygen in the exhaust gas dropped as low as 14 per cent. On the fifth day, 20 ml of complete nutrient solution were added to sample No. 6 to see if any component supplied in the original nutrient solution acted as a limiting factor. Table 7 lists the gas analysis data for these runs, and the oxygen consumption rates are plotted in Figure 13. The maximum oxygen consumption rate was 46.1 mg oxygen per day per gram initial cellulose for run No. 5 and 57.6 mg oxygen per day per gram initial cellulose for run No. 6.

Only a slight increase in oxygen consumption rate was observed after the addition of nutrient solution. Sample No. 5 appeared to lag behind sample No. 6 during the course of the run and did not reach as great a maximum

TABLE 6

GAS ANALYSIS DATA FOR RUNS NO. 2 AND 3

Days	Oxygen Consumed, Liters		CO_2 Produced, Liters		Oxygen Cons. Rate mg/g in.cell./day	
	No. 2	No. 3	No. 2	No. 3	No. 2	No. 3
0.5	0	0.0015	0.0150	0.0100	0	0.2
1.0	0	0.0045	0.0142	0.0144	0	0.7
1.5	0.0048	0.0076	0.0172	0.0217	0.7	1.1
2.0	0.0212	0.0347	0.0317	0.055	3.2	5.2
2.5	0.054	0.112	0.070	0.143	8.4	16.8
3.0	0.135	0.237	0.166	0.295	24.8	35.6
3.5	0.248	0.338	0.293	0.408	37.2	50.6
4.0	0.312	0.354	0.375	0.418	46.8	53.0
4.5	0.331	0.320	0.376	0.337	49.7	48.0
5.0	0.303	0.297	0.310	0.286	46.5	44.5
5.5	0.257	0.282	0.259	0.273	38.6	42.3
6.0	0.234	0.264	0.238	0.261	35.0	39.6
6.5	0.234	0.260	0.232	0.258	35.0	38.9
7.0	0.229	0.247	0.226	0.247	34.5	37.0*
7.5	0.222	0.239	0.217	0.224	33.4	35.5
8.0	0.217	0.232	0.213	0.226	32.6	34.8
8.5	0.208	0.211	0.200	0.206	31.2	31.7
9.0	0.205	0.191	0.192	0.196	30.7	28.6
9.5	0.190	0.1815	0.180	0.181	28.6	27.1
10.0	0.175	0.1755	0.170	0.175	26.2	26.2
10.5	0.1655	0.1615	0.160	0.162	24.8	24.2
11.0	0.1585	0.150	0.154	0.151	23.8	22.4
11.5	0.149	0.140	0.143	0.139	22.1	21.0
12.0	0.140	0.132	0.138	0.128	21.0	19.8
12.5	0.137	0.119	0.130	0.116	20.5	17.9
13.0	0.131	0.105	0.126	0.108	19.7	15.7
13.5	0.122	0.1005	0.121	0.099	18.2	15.1
14.0	0.119	0.093	0.117	0.093	17.9	13.9

*$NaNO_3$ added.

TABLE 7

GAS ANALYSIS DATA FOR RUNS NO. 5 AND 6

Days	Oxygen Consumed, Liters		CO_2 Produced, Liters		Oxygen Cons. Rate mg/g in.cell./day	
	No. 5	No. 6	No. 5	No. 6	No. 5	No. 6
0.5	0.0013	0.0014	0.041	0.0128	0.19	0.22
1.0	0.00815	0.00577	0.0506	0.0156	1.2	0.94
1.5	0.0176	0.0174	0.0566	0.0278	2.65	2.6
2.0	0.0453	0.0638	0.0857	0.0762	6.8	9.6
2.5	0.0986	0.166	0.149	0.190	14.9	25.0
3.0	0.162	0.316	0.239	0.393	24.5	47.6
3.5	0.226	0.382	0.303	0.456	34.1	57.6
4.0	0.294	0.336	0.371	0.379	44.4	50.6
4.5	0.306	0.290	0.379	0.316	46.1	43.7
5.0	0.264	0.270	0.297	0.293	40.1	40.6*
5.5	0.216	0.275	0.243	0.319	32.6	41.5
6.0	0.201	0.271	0.221	0.312	30.2	40.8
6.5	0.193	0.232	0.213	0.268	29.0	35.0
7.0	0.195	0.217	0.214	0.247	29.3	32.7
7.5	0.199	0.209	0.220	0.244	30.0	31.4
8.0	0.192	0.206	0.209	0.227	28.9	31.0
---	0.04**		0.04**			

*Nutrient solution added.

**Correction for content of air in the system at the close of the run.

FIGURE 12. EFFECT OF $NaNO_3$ ADDITION ON OXYGEN CONSUMPTION

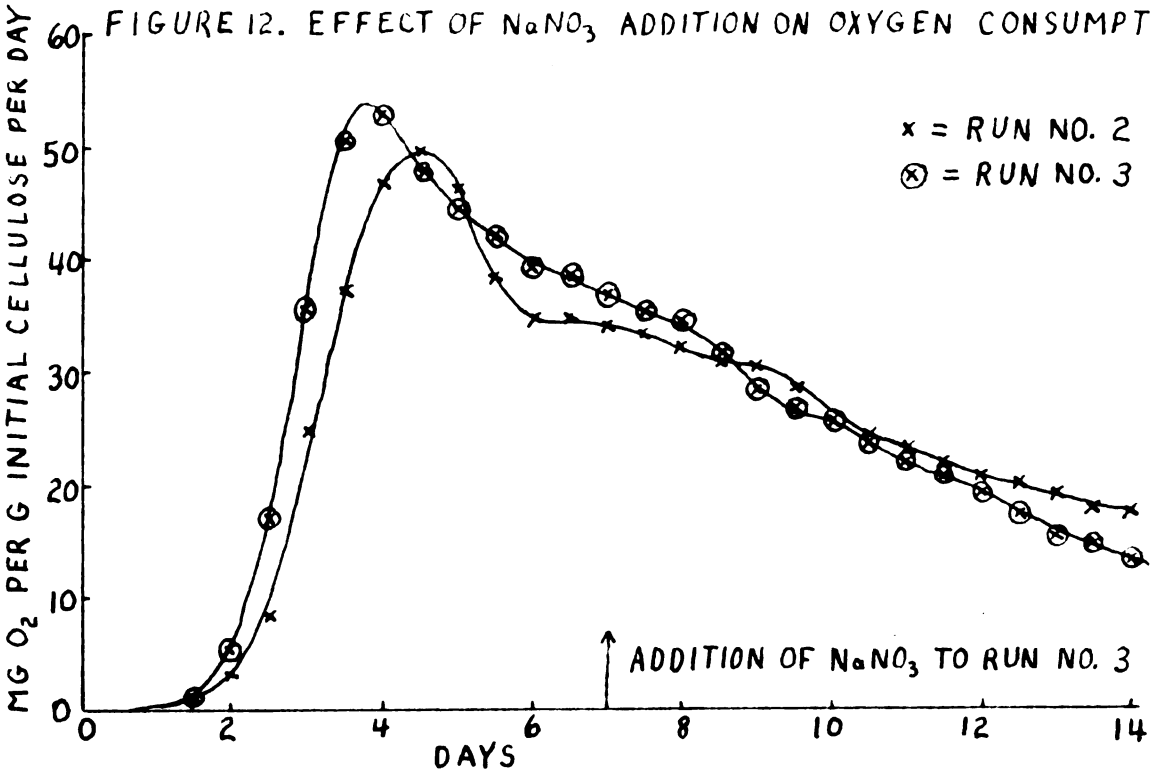
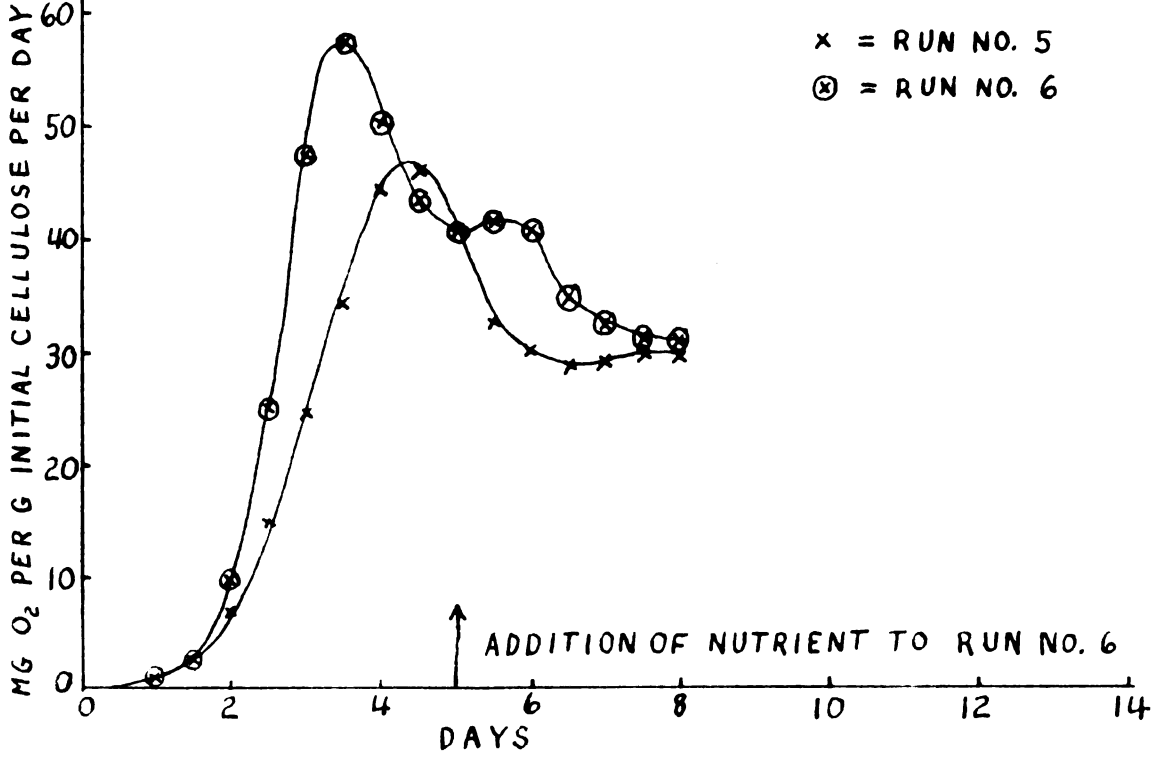


FIGURE 13. EFFECT OF NUTRIENT ADDITION ON OXYGEN CONSUMPTION



rate. It is noted that sample No. 5 also had a secondary maximum at a corresponding point in its development even though no addition of nutrient solution was made to this sample.

E. Effect of Increased Surface

It was thought that the clumping of the filter paper might be a limiting factor for rate of decomposition. To check this, run No. 4 was made in the plastic container. Since each paper disc was spread individually on the glass grids, practically all of the paper's surface was available for attack by microorganisms and open to the supply of oxygen.

The initial dry weight of the 36 discs of filter paper which were spread on the trays was 19.345 grams. The seed material was suspended in the nutrient solution, and 1.9 ml of this solution were added to each paper disc. A flow rate of 0.3 liters of air per hour was maintained, and remaining oxygen in the exhaust gas decreased to as low as 7 per cent.

Table 8 lists the gas analysis data for this run. The maximum oxygen consumption rate was 55.0 mg per day per gram initial cellulose, and the maximum rate of CO_2 production was 80.3 mg per day per gram initial cellulose. The oxygen consumption rate is plotted in Figure 14, and the CO_2 production rate is plotted in Figure 15. The flow rate was increased to 0.4 liters per hour on the eleventh

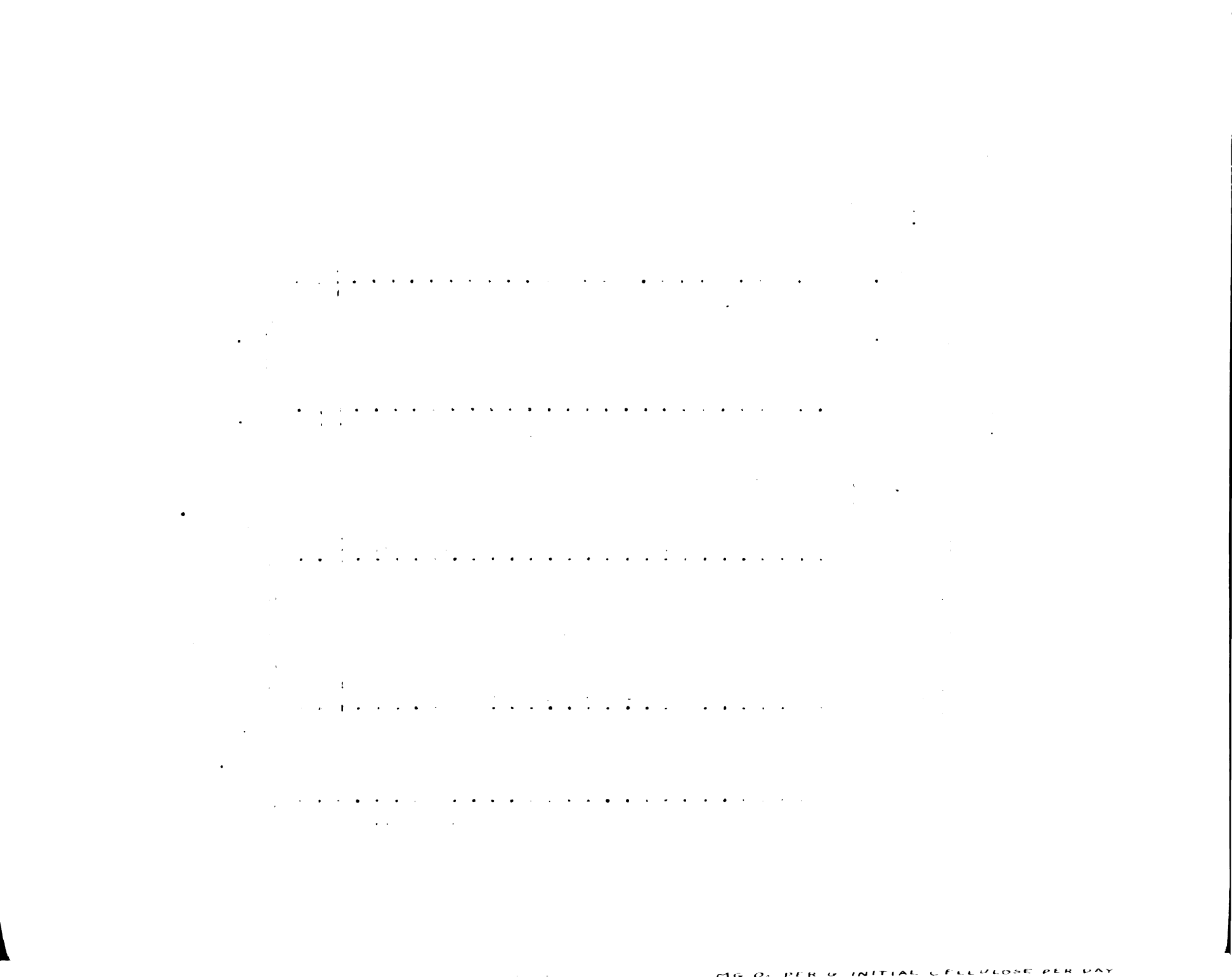
TABLE 8

GAS ANALYSIS DATA FOR RUN NO. 4

Days	Oxygen Consumed, Liters	CO_2 Produced, Liters	O_2 Cons., Rate mg/g in.cell./day	CO_2 Prod., Rate
0.5	0.0041	0.0106	0.5	2.0
1.0	0.0088	0.0111	1.2	2.1
1.5	0.0121	0.0139	1.7	2.6
2.0	0.0266	0.0294	3.6	5.5
2.5	0.0813	0.0885	11.0	16.5
3.0	0.1905	0.219	25.8	40.8
3.5	0.308	0.360	41.7	67.0
4.0	0.381	0.432	51.6	80.3
4.5	0.394	0.422	53.5	78.5
5.0	0.392	0.406	53.3	75.5
5.5	0.405	0.405	55.0	75.4
6.0	0.401	0.396	54.5	73.6
6.5	0.397	0.376	54.0	70.0
7.0	0.358	0.340	48.8	63.3
7.5	0.328	0.307	44.6	57.1
8.0	0.297	0.279	40.3	51.9
8.5	0.264	0.249	35.8	46.4
9.0	0.229	0.220	31.0	41.0
9.5	0.202	0.192	27.4	35.7
10.0	0.182	0.171	24.7	31.8
10.5	0.164	0.154	22.3	28.6
11.0	0.1485	0.141	20.2	26.2
11.5*	0.153	0.148	20.8	27.5
12.0	0.161	0.159	21.8	29.6
12.5	0.157	0.156	21.4	29.0
13.0	---	---	---	---
13.5**	0.297	0.296	---	---
14.0	0.145	0.144	19.7	26.8

*Air flow had been increased from 0.3 to 0.4 liters per hour.

**Data for a 24 hour period.



MG. O. PER G. INITIAL CELLULOSE PER DAY

FIGURE 14. OXYGEN CONSUMPTION RATE FOR RUN NO. 4

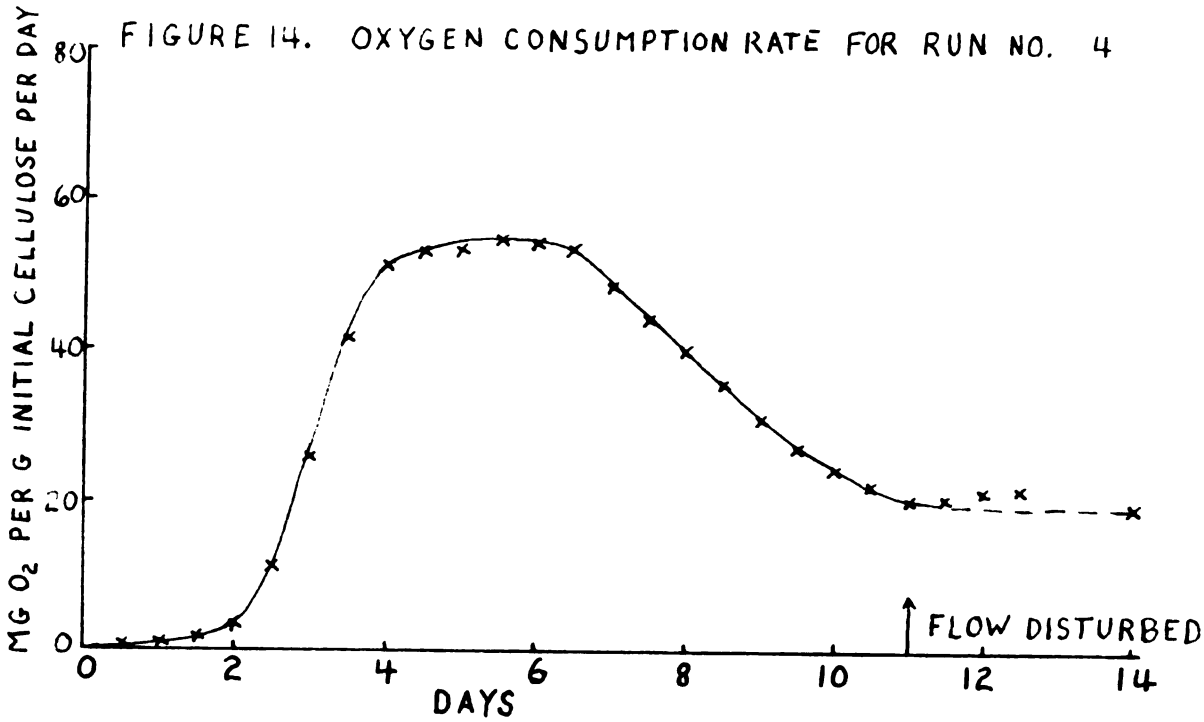
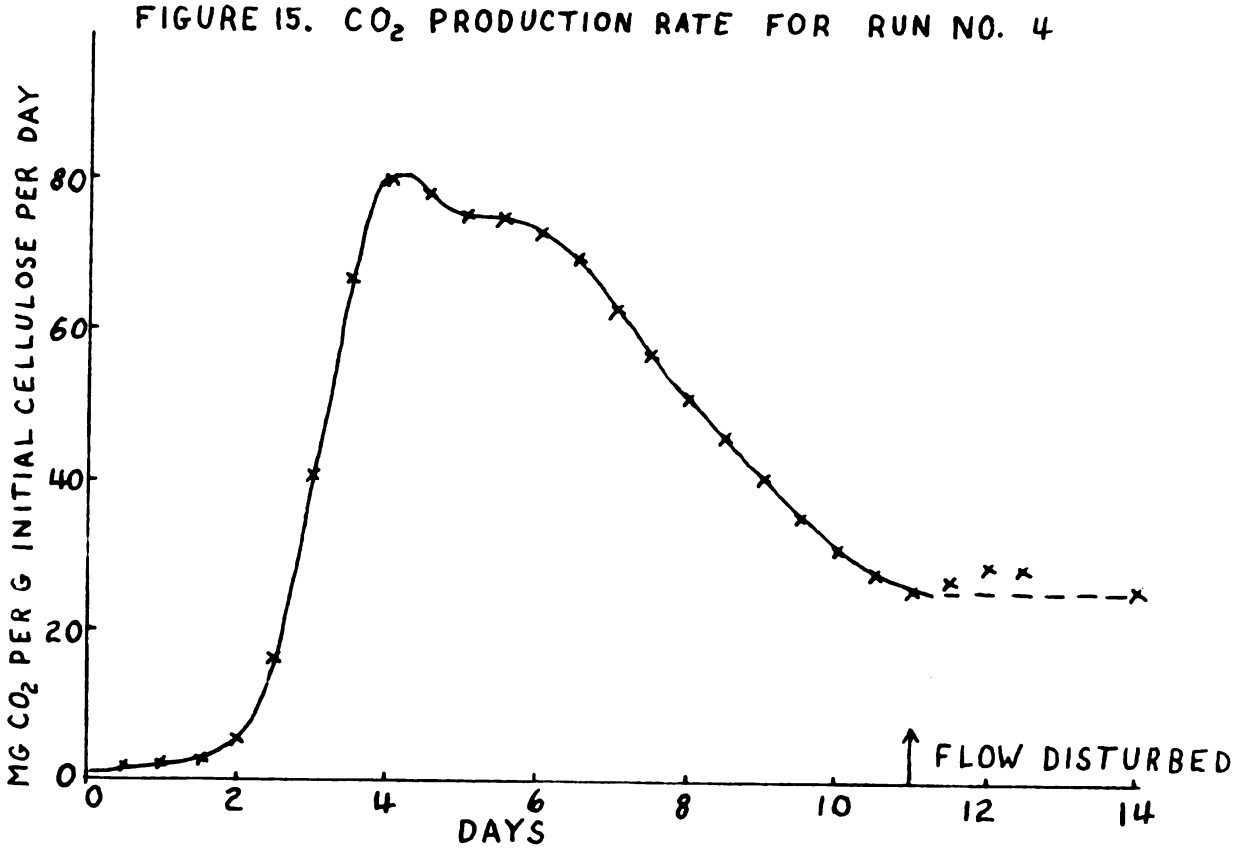


FIGURE 15. CO_2 PRODUCTION RATE FOR RUN NO. 4



day. This change resulted in a disturbance of the measured oxygen uptake and CO_2 production due to the large volume of the container.

For comparison with other data, run No. 4 is plotted along with runs No. 1 and 3 in Figure 16. Run No. 4 appeared to maintain its maximum oxygen uptake rate longer than run No. 3, and both runs reached a higher rate than run No. 1. Runs No. 3 and 4 reached a maximum rate of 53 and 55 compared to a maximum rate of 40.8 mg oxygen per day per gram initial cellulose for run No. 1. Run No. 4 showed a very rapid decline of activity after leveling off. Results indicated that clumping of filter paper was not a limiting factor.

F. Nitrogen Assimilation and Total Cellulose Destruction

In preliminary work, attempts were made to follow the conversion of $NaNO_3$ to organic nitrogen using the Kjeldahl method of organic nitrogen determination. Curves were obtained for nitrate conversion using this method, but work was hampered by what appeared to be a conversion of the nitrate ion to ammonia in the presence of cellulose during the Kjeldahl procedure.

A method was worked out to measure the remaining nitrate rather than organic nitrogen. This method was similar to the procedure described by Heukelekian and Waksman (16). A mixture of Al, Cu and Zn in the presence of NaOH was used to convert the nitrate to ammonia. The

FIGURE 16. COMPARISON OF RUNS NO. 1, 3 AND 4

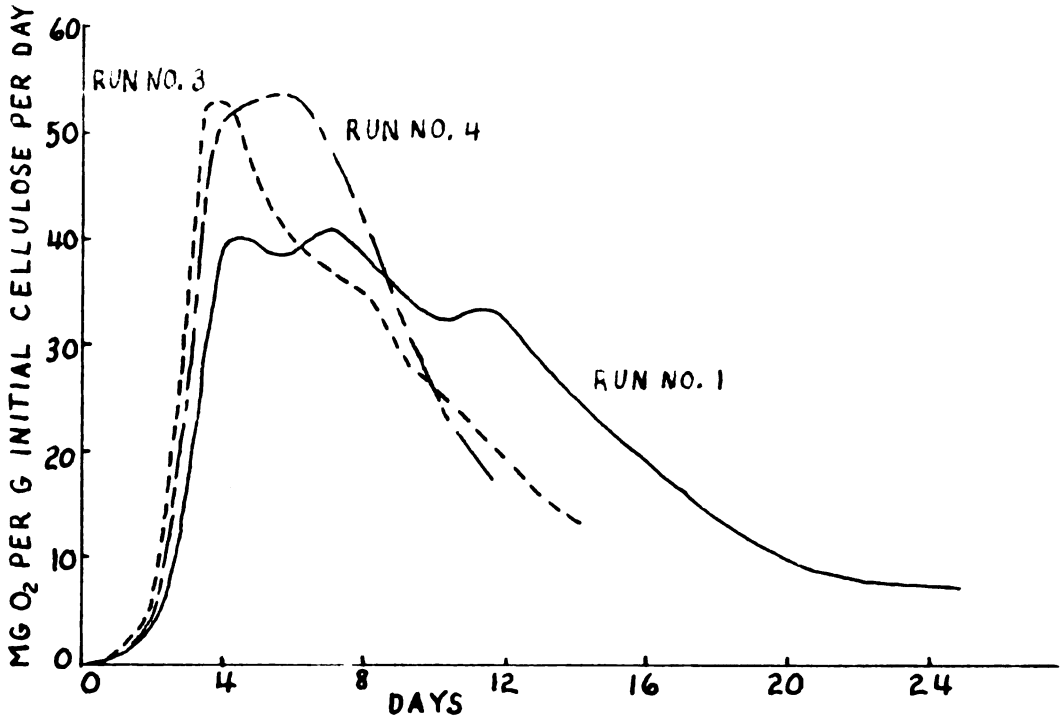
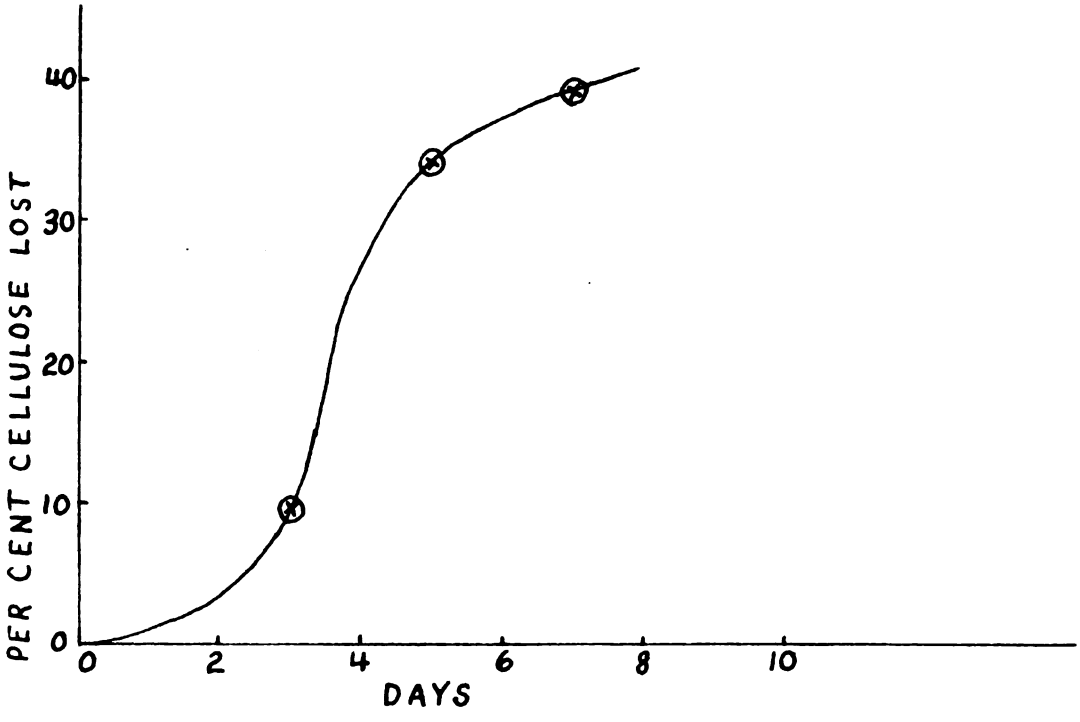


FIGURE 17. TOTAL CELLULOSE DESTROYED IN RUN NO. 7



ammonia was collected in standard acid and titrated to determine the amount of nitrate which had been present. The procedure is given in the Appendix.

A mixture was prepared using 60 grams of cellulose, 30 grams of vermiculite, 180 ml of nutrient solution and 1.8 grams of seed material. Samples were taken initially and on the third, fifth and seventh day. These samples were extracted with water, and the extract was analyzed for nitrate. To determine the amount of cellulose destroyed, the residue was further extracted with alcohol and ether before drying at 105°C. The dry weight was taken as vermiculite and remaining cellulose. The samples were ashed at 800°C for three hours to determine the amount of vermiculite. Checks of vermiculite alone showed it to be 97.8 per cent ash. Ash from filter paper in the samples was considered negligible and salts from the nutrient had been removed with the water extract.

Four samples were taken for each day. Data from these samples are given in Table 9. Since ash content should remain constant, calculations were made using it as a basis. Initially the mixture contained 0.576 grams of nitrogen in the form of nitrate. The initial ash content was 29.3 grams or 10.7 per cent of the initial moist weight of the mixture. This gives 19.7 mg of nitrate per gram ash or 0.21 per cent of the initial moist weight. Initially there should have been 1.97 grams of cellulose per gram of ash.

TABLE 9

NITROGEN DETERMINATION DATA

Day	Sample No.	Total Moist Weight, Grams	Dried Wt. After Extraction, Grams	Ash, Grams	Nitrogen as Nitrate, Grams
0	1	7.426	2.418	0.579	0.01425
	2	6.187	2.013	0.468	0.0129
	3	11.109	3.525	0.806	0.02165
	4	7.357	2.387	0.645	0.0131
3	1	3.544	1.197	0.313	0.00464
	2	3.261	1.117	0.272	0.00476
	3	3.783	1.301	0.314	0.00484
	4	5.045	1.652	0.512	0.00725
5	1	4.437	1.342	0.416	0.00138
	2	3.374	1.043	0.312	0.00116
	3	5.401	1.632	0.527	0.00134
	4	5.453	1.609	0.612	0.00231
7	1	4.812	1.360	0.477	0.00071
	2	4.180	1.169	0.363	0.00035
	3	3.199	0.905	0.294	0.00056
	4	3.149	0.834	0.333	0.00064

The actual measurements are listed in Table 10. The per cent nitrogen as nitrate measured initially was slightly low. The initial ash per cent deviated almost 3 per cent from the expected value. This deviation resulted from some of the vermiculite settling to the bottom. Likewise, the calculations tied to the ash have a large deviation from their expected values. The average measured values were taken as the correct initial condition.

The per cent of cellulose lost was calculated using

TABLE 10

NITROGEN DETERMINATION CALCULATIONS

Day	Sample No.	Per Cent Nitrate Nitrogen	Per Cent Ash	G Nitrate Nitrogen Per G Ash	G Cellulose Per G Ash
Calc. initial value		0.21	10.7	0.0197	1.97
0	1	0.192	7.8	0.0246	3.16
	2	0.209	7.6	0.0276	3.29
	3	0.195	7.3	0.0269	3.36
	4	0.178	8.8	0.0203	2.69
	Average	0.194	7.9	0.0248	3.12
3	1	0.131	8.8	0.0148	2.81
	2	0.146	8.3	0.0175	3.09
	3	0.128	8.3	0.0154	3.13
	4	0.144	10.2	0.0142	2.21
	Average	0.137	8.9	0.0155	2.81
5	1	0.031	9.4	0.0033	2.21
	2	0.034	9.3	0.0037	2.33
	3	0.025	9.8	0.0025	2.08
	4	0.042	11.2	0.0038	1.61
	Average	0.033	9.9	0.0033	2.06
7	1	0.015	9.9	0.0015	1.84
	2	0.008	8.7	0.0010	2.21
	3	0.018	9.2	0.0019	2.07
	4	0.020	10.6	0.0019	1.49
	Average	0.015	9.6	0.0016	1.90

the data in Table 10. The fraction of cellulose remaining at any time would be the measured cellulose per gram ash divided by the initial cellulose per gram ash. Thus, 9.9 per cent of the initial cellulose had been destroyed by the third day. Likewise, 34.0 and 39.1 per cent of the cellulose had been destroyed by the fifth and seventh day. These three values are plotted in Figure 17.

Nitrogen converted to an organic form was calculated by subtracting the value of remaining nitrate nitrogen per gram ash from the initial nitrate nitrogen per gram ash. This value was then expressed as mg nitrogen converted per gram initial cellulose. These calculations are tabulated in Table 11. The cumulative nitrogen converted per gram initial cellulose is plotted in Figure 18. Figures 17 and 18 are very similar showing that nitrogen conversion and cellulose destruction were nearly parallel. The maximum possible conversion was 15.2 mg nitrogen per gram initial cellulose as calculated from the initial nitrogen content.

To get a rate curve of nitrogen conversion, the cumulative nitrogen conversion curve given in Figure 18 was taken to be correct. Points were taken from it as listed in Table 12 and changed into rates as mg nitrogen converted per gram initial cellulose per day. These rates are plotted in Figure 19.

Gas analysis measurements were also made for this run. Results are given in Table 13. Since the weight of the mixture was always changing due to removal of samples,

TABLE 11

CUMULATIVE NITROGEN CONVERSION

Days	G Nitrate Nitrogen Per G Ash	G Nitrogen Converted Per G Ash	MG Nitrogen Converted Per G Initial Cellulose
0	0.0248	0	0
3	0.0155	0.0093	5.7
5	0.0033	0.0215	13.2
7	0.0016	0.0232	14.2

TABLE 12

RATE OF NITROGEN CONVERSION

(Data taken from Figure 18)

Day	MG Nitrogen Converted Per G Initial Cellulose	MG Nitrogen Converted Per G Initial Cellulose Per Day
0.5	0.1	---
1.0	0.4	0.6
1.5	1.0	1.2
2.0	2.0	2.0
2.5	3.5	3.0
3.0	5.7*	4.4
3.5	8.4	5.4
4.0	10.8	4.8
4.5	12.3	2.8
5.0	13.2*	1.8
5.5	13.6	0.8
6.0	13.9	0.6
6.5	14.1	0.4
7.0	14.2*	0.2

*Actual measurement

TABLE 13

GAS ANALYSIS DATA FOR RUN NO. 7

Days	CO_2 Produced Per G Initial Cellulose, Liters	O_2 Consumed Per G Initial Cellulose, Liters	Oxygen Consumption Rate, mg/g in.cell./day
0.5	0.0002	0	0
1.0	0.0003	0.0001	0.12
1.5	0.0008	0.0005	1.68
2.0	0.0017	0.0012	3.6
2.5	0.0034	0.0026	8.4
3.0	0.0073	0.0060	18.95
3.5	0.0141	0.0115	36.4
4.0	0.0199	0.0165	52.0
4.5	0.0212	0.0175	54.4
5.0	0.0173	0.0162	51.1
5.5	0.0164	0.0147	46.3
6.0	0.0147	0.0137	42.9
6.5	0.0141	0.0130	41.0
7.0	0.0137	0.0128	40.3
7.5	0.0136	0.0128	40.3*
8.0	0.0143	0.0135	42.5
8.5	0.0139	0.0132	41.5
9.0	0.0132	0.0119	37.4*
9.5	0.0113	0.0108	34,3
10.0	0.0101	0.0091	28.6**
10.5	0.0083	0.0075	18.0
11.0	0.0075	0.0064	15.4
11.5	0.0066	0.0060	14.4
12.0	0.0062	0.0057	13.7
12.5	0.0060	0.0052	12.5
13.0	0.0057	0.0050	12.0
13.5	0.0054	0.0045	10.8

*Addition of $NaNO_3$

**Addition of fresh filter paper

FIGURE 18. CUMULATIVE NITROGEN CONVERSION

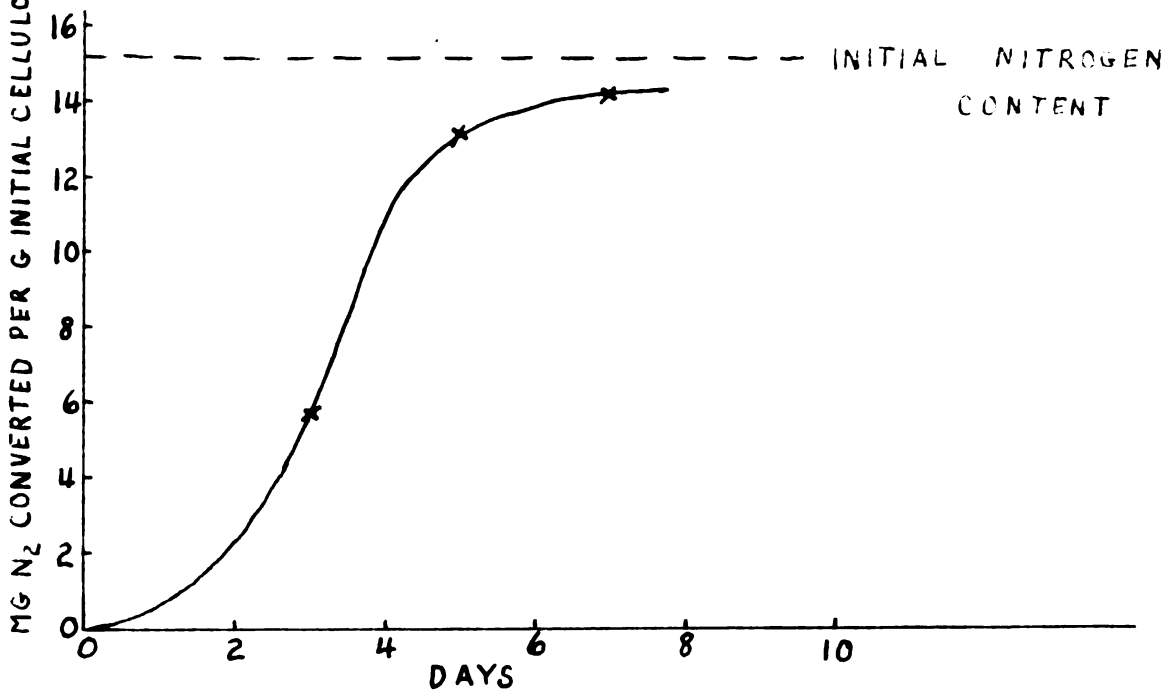
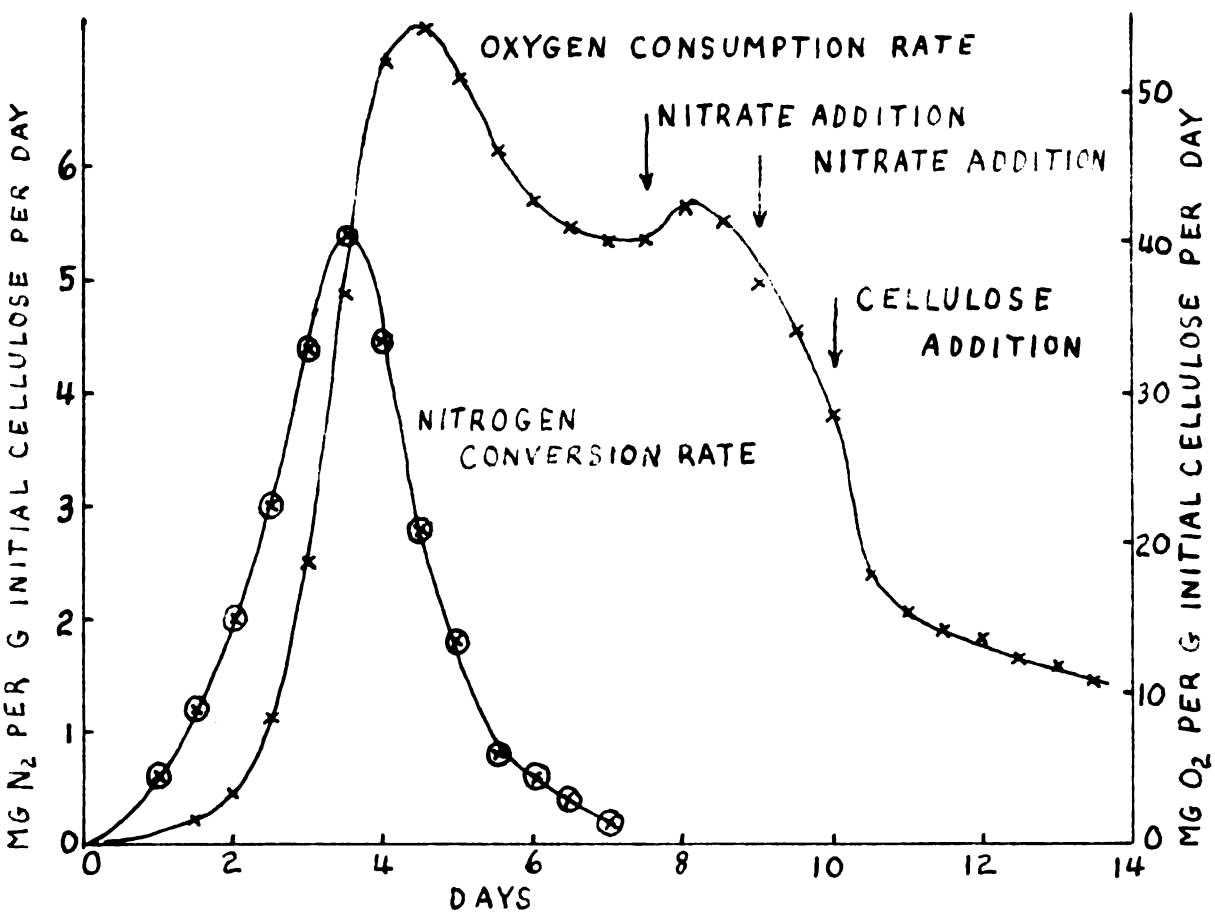


FIGURE 19. OXYGEN AND NITROGEN CONSUMPTION RATES



these measurements were made as grams of CO_2 produced and liters of oxygen consumed per gram ash and converted to liters per gram initial cellulose. The rate of oxygen consumption was calculated in mg oxygen per gram initial cellulose per day and is plotted in Figure 19. The maximum rate of oxygen consumption was 54.4 mg per day per gram initial cellulose and occurred about 4.5 days after the start of the run. The maximum rate of nitrogen conversion was approximately 5.4 mg nitrogen per gram initial cellulose per day and appeared to precede the maximum rate of oxygen uptake by about one day.

Since measurements indicated that nearly all the $NaNO_3$ had been used, it was thought that nitrate addition might have some effect. After 7.5 days, 0.139 grams of $NaNO_3$ in 14 ml of water were added to the mixture. A slight rise in oxygen uptake followed, but this rise corresponded to the normal pattern of other oxygen consumption curves. To check this, a second addition of 0.256 grams of $NaNO_3$ in 23 ml of water were added on the ninth day. This had no stimulating effect on the curve. It is noted that the first addition raised the moisture content of the mixture by 2 per cent, and the second addition raised it an additional 4 per cent. On the tenth day, 6 grams of fresh filter paper were added. The oxygen consumption rate, which had been declining rapidly, leveled off but did not climb. The points of addition are marked on Figure 19.

The nitrogen conversion data was taken as a measure of the growth of bacteria in the mixture. The cumulative per cent of the total nitrogen available which had been converted is plotted on a semilogarithmic graph against time in Figure 20. The curve shows a nearly logarithmical rate of conversion followed by a leveling off period. This corresponds to the general behavior of a bacterial growth curve, indicating that nitrogen conversion can be considered a measure of the growth of bacteria in the cellulose mixture. According to this curve the maximum growth of the microbial population was reached on the fifth day. This is in agreement with all other data on the activity of the culture including oxygen consumption rates and CO_2 production rates.

If points are taken from the curve for total cellulose destruction in Figure 17, a plot of the rate of cellulose destruction can be made. This was done to obtain the curve in Figure 21. The maximum rate of cellulose destruction of 8 per cent of the initial cellulose per day appeared to occur about 3.5 to 4 days after the start of the run. This corresponds with the maximum rate of nitrogen conversion by the bacteria as shown in Figure 19, and precedes the maximum rate of oxygen consumption.

The total weight of nitrogen converted and weight lost after extraction during run No. 7 are listed in Table 14 and plotted in Figure 22. From these data, grams of cellulose destroyed per gram of nitrogen assimilated

FIGURE 20. NITROGEN CONVERSION PLOTTED AS A GROWTH CURVE

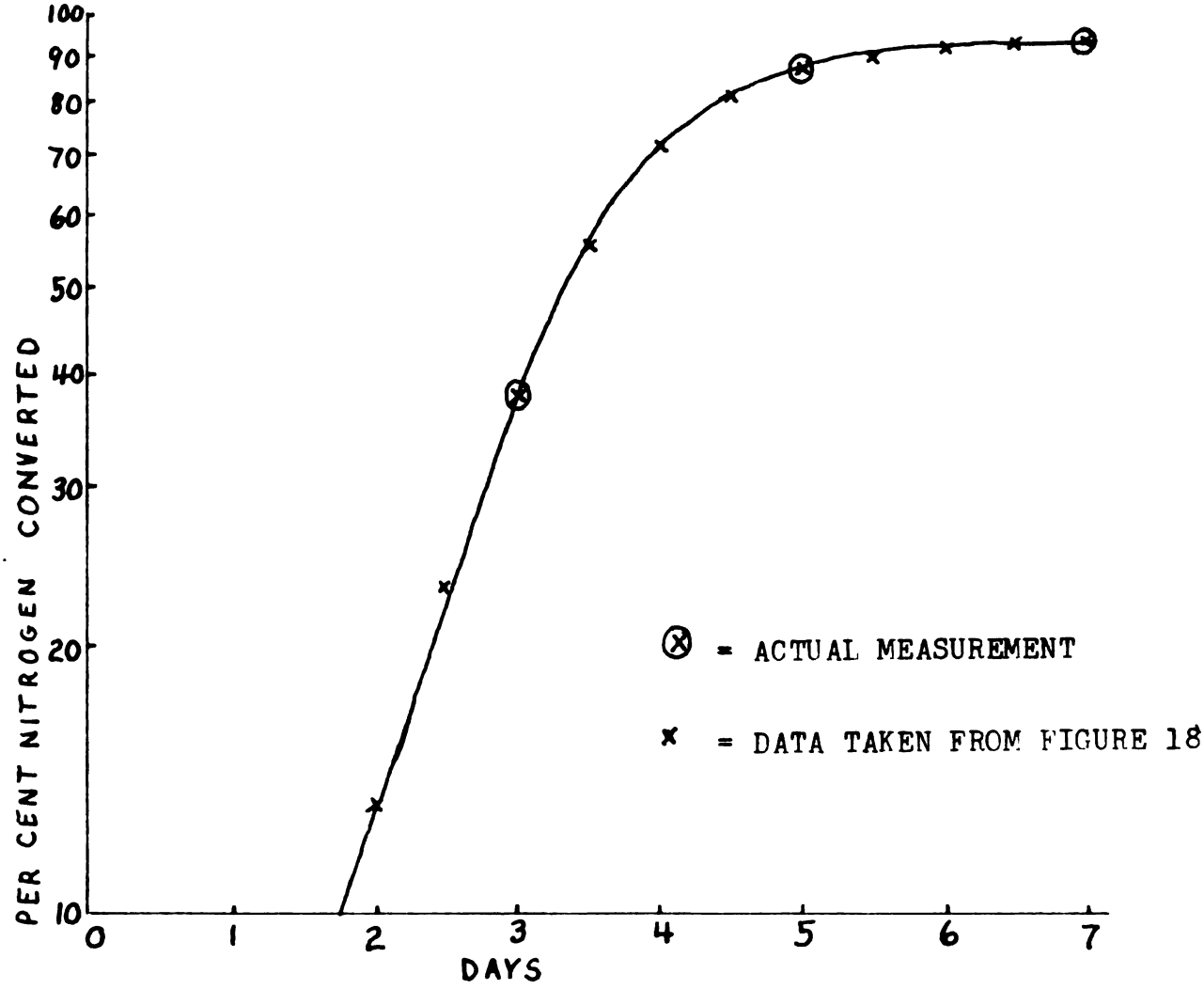


FIGURE 21. RATE OF WEIGHT LOSS AFTER EXTRACTION

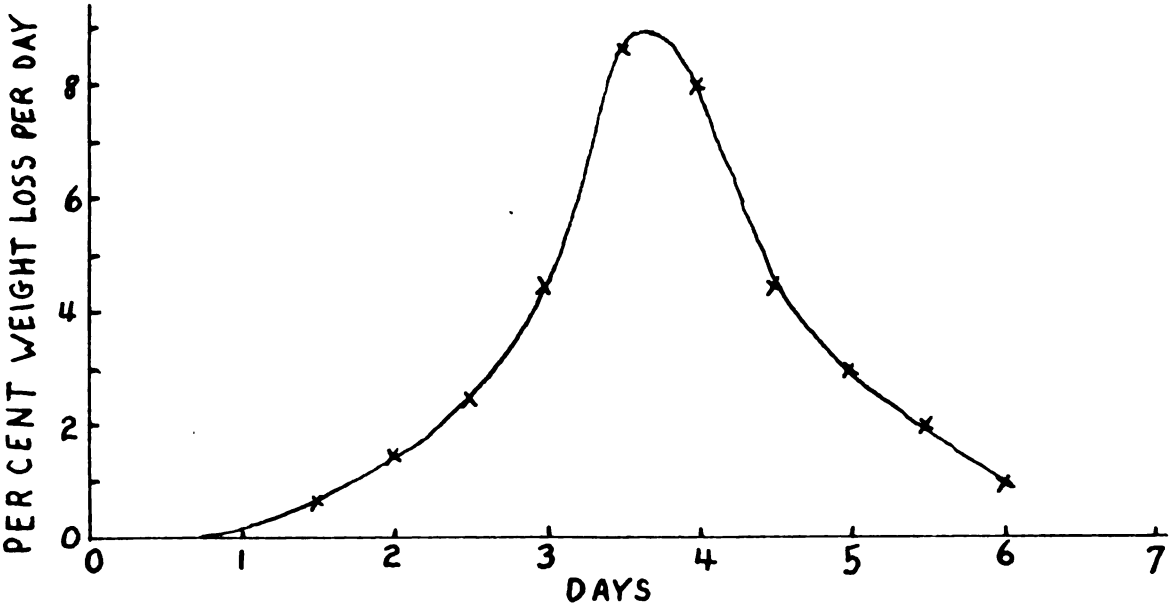
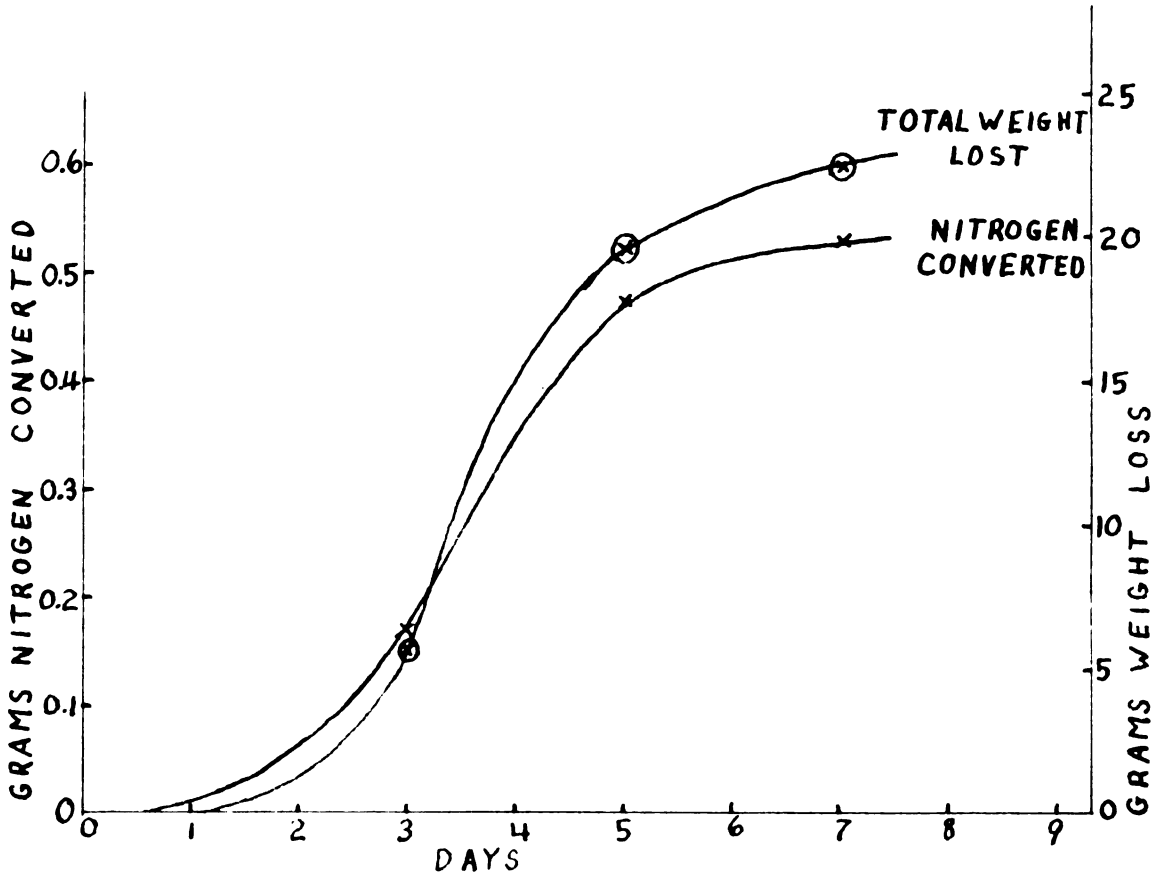


FIGURE 22. TOTAL NITROGEN CONVERSION AND CELLULOSE BREAKDOWN



are calculated. After a week of incubation, 42 grams of cellulose had been destroyed per gram of nitrogen assimilated.

TABLE 14

NITROGEN CONVERSION AND CELLULOSE LOSS

Days	Nitrogen Converted, Grams	Total Weight Loss, Grams	G Weight Loss Per G N_2 Converted
3	0.169	5.7	33.8
5	0.478	19.6	41.0
7	0.531	22.5	42.4

G. Variation of pH

To check pH it was necessary to have mixtures which could be sampled. Large mixtures were prepared and analyzed in parallel with gas analysis samples. The pH of samples taken from run No. 7 was also measured. Results are tabulated in Table 15. The pH of the nutrient solution used in each mixture is listed.

The pH of these mixtures started at about 8.2, which was slightly higher than the pH of the nutrient solution. The pH tended to increase during the run. This would explain the absence of fungi in the mixed culture seed, since a high pH favors aerobic bacteria over fungi. The dip in pH of run No. 5A on the third day is the only indication of a pH change at the point of leveling off of

activity. The other runs showed no change in pH from the third to the fifth day.

TABLE 15

VARIATION OF pH

Sample:	No. 2A*	No. 5A*	No. 7
Nutrient solution:	7.8	8.2	7.9
Days			
0	8.2	8.3	7.9
3	8.6	7.6	8.2
5	8.6	8.0	8.2
7	8.4	8.5	8.8
9	8.6	---	---
11	8.7	---	---
14	9.0	---	---

* "A" indicates a large mixture used for sampling and analyzed in parallel with the designated gas analysis run.

H. Variation of Respiratory Quotient

An indication of the type of microbiological attack taking place is given by the respiratory quotient which is equal to the moles of CO_2 produced divided by the moles of oxygen consumed. Table 16 lists the average R. Q. for each day during runs No. 1 through 5. The variation is plotted in Figure 23 for runs No. 1 and 2.

The R. Q. started out very high and decreased rapidly. Normally it was nearly one by the fifth or sixth day and remained near one for the rest of the run. It dropped

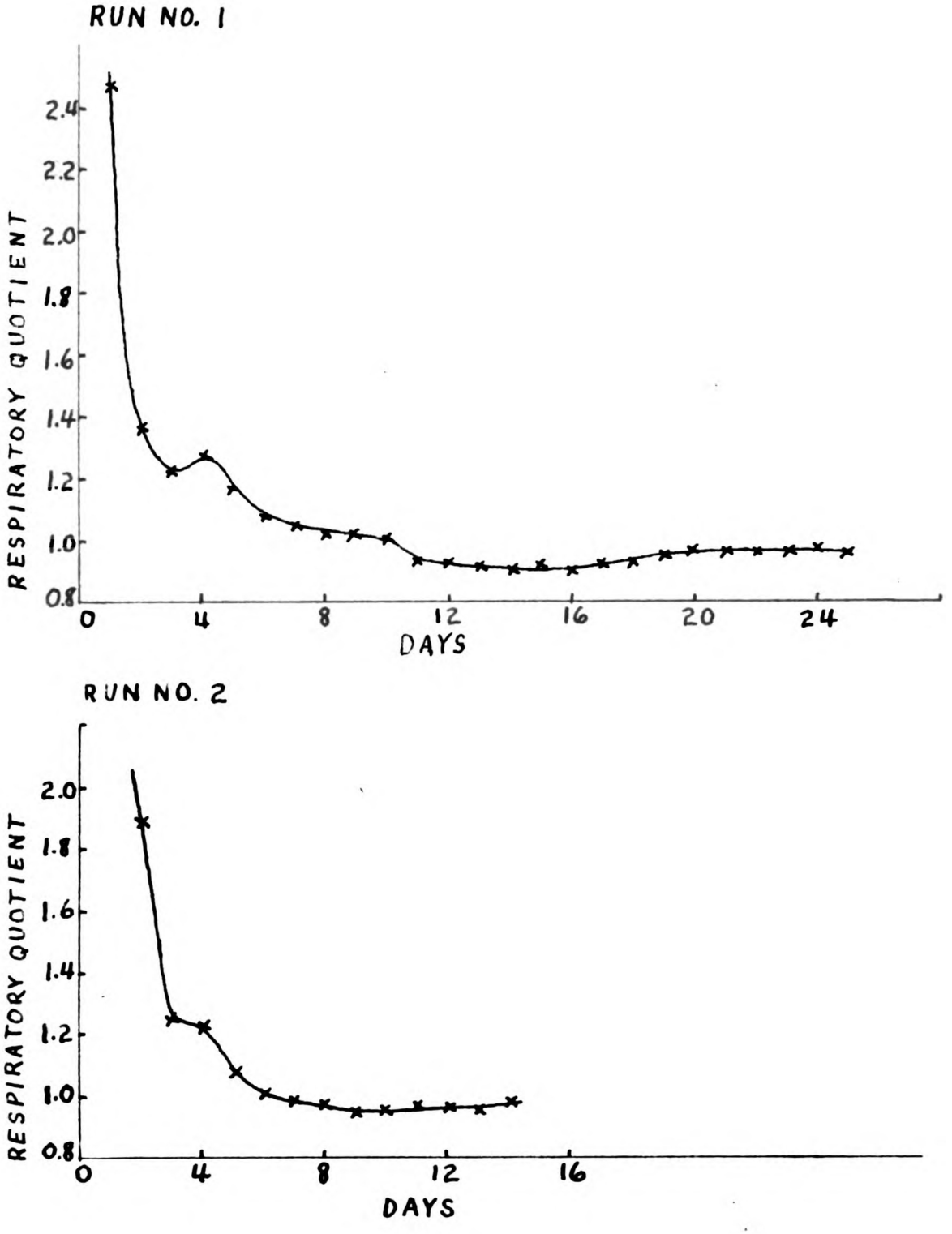
slightly below one for the latter part of the run. The high R. Q. value during the first of the run indicated fermentation occurring.

TABLE 16

VARIATION OF RESPIRATORY QUOTIENTS

Sample: Day	No. 1	No. 2	No. 3	No. 4	No. 5
1	2.47	---	2.46	1.68	9.7
2	1.36	1.89	1.81	1.12	2.27
3	1.22	1.25	1.26	1.13	1.49
4	1.28	1.23	1.19	1.15	1.29
5	1.17	1.08	1.01	1.05	1.19
6	1.08	1.01	0.98	0.99	1.11
7	1.05	0.99	0.99	0.95	1.10
8	1.02	0.98	0.96	0.94	1.09
9	1.02	0.95	1.00	0.95	
10	1.01	0.96	1.00	0.95	
11	0.93	0.97	1.00	0.94	
12	0.935	0.97	0.98	0.98	
13	0.92	0.96	1.00	---	
14	0.905	0.99	0.99	---	
15	0.92				
16	0.90				
17	0.925				
18	0.93				
19	0.955				
20	0.975				
21	0.975				
22	0.965				
23	0.97				
24	0.99				
25	0.97				

FIGURE 23. DAILY RESPIRATORY QUOTIENTS



I. By-products from Decomposition

The amount of cellulose broken down completely into water and CO_2 can be calculated from the measured oxygen consumption. For every gram of oxygen consumed, 0.845 grams of cellulose will be oxidized. Likewise, the rate of cellulose oxidation can be calculated from the rate of oxygen consumption. The weight of cellulose decomposed by oxidation each day during run No. 1 is tabulated in Table 17. The cumulative per cent of cellulose oxidized is also listed. These quantities are plotted in Figures 24 and 25. Figure 24 is essentially the same curve as shown in Figure 10 for oxygen uptake rate since cellulose decomposed by oxidation is a direct conversion from oxygen consumption. These curves show that the rate of cellulose destruction by complete oxidation reached a maximum of 3.4 per cent of initial cellulose per day on the fourth day, then leveled off and declined rapidly after the eleventh day. Oxygen consumption rates for the other runs indicated a higher maximum rate of cellulose oxidation followed by a more rapid decline of activity. A maximum rate of 4.6 per cent of the initial cellulose per day was reached in run No. 4.

According to oxygen consumption measurements, a total of 43.5 per cent or 7.90 grams of the initial cellulose in run No. 1 was decomposed by complete oxidation. The actual weight loss of the residue after drying at 105°C was 9.333 grams or 53.5 per cent as plotted in Figure 25. This leaves 1.43 grams or 8.2 per cent of the

TABLE 17

CELLULOSE OXIDIZED IN RUN NO. 1

Days	O_2 Consumed Per Day, Liters	Cellulose Oxidized, Grams	Per Cent Per Day	Cumulative Per Cent
1	0.00674	0.00743	0.043	0.043
2	0.03395	0.0375	0.215	0.258
3	0.1794	0.198	1.13	1.39
4	0.461	0.509	2.92	4.32
5	0.531	0.586	3.36	7.68
6	0.513	0.565	3.24	10.92
7	0.540	0.596	3.42	14.34
8	0.518	0.572	3.28	17.62
9	0.4725	0.522	3.00	20.62
10	0.440	0.486	2.79	23.41
11	0.461	0.509	2.92	26.33
12	0.4405	0.486	2.79	29.12
13	0.3875	0.428	2.46	31.58
14	0.347	0.383	2.20	33.78
15	0.306	0.338	1.94	35.72
16	0.270	0.298	1.71	37.43
17	0.224	0.248	1.42	38.85
18	0.1884	0.208	1.19	40.04
19	0.1573	0.174	1.00	41.04
20	0.1366	0.151	0.867	41.91
21	0.1226	0.1355	0.778	42.69
22	0.1139	0.1255	0.720	43.41
23	0.1057	0.1165	0.670	44.08
24	0.0969	0.1070	0.615	44.69
25	0.09365	0.1033	0.594	45.28

FIGURE 24. RATE OF CELLULOSE DECOMPOSITION BY COMPLETE OXIDATION

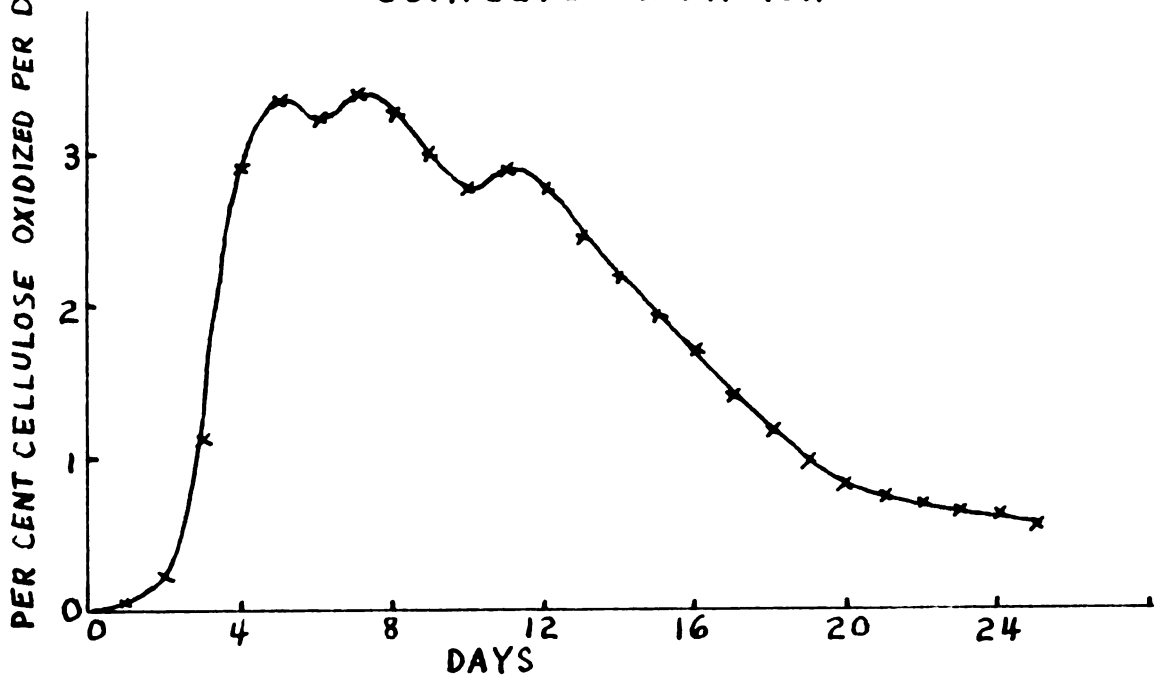
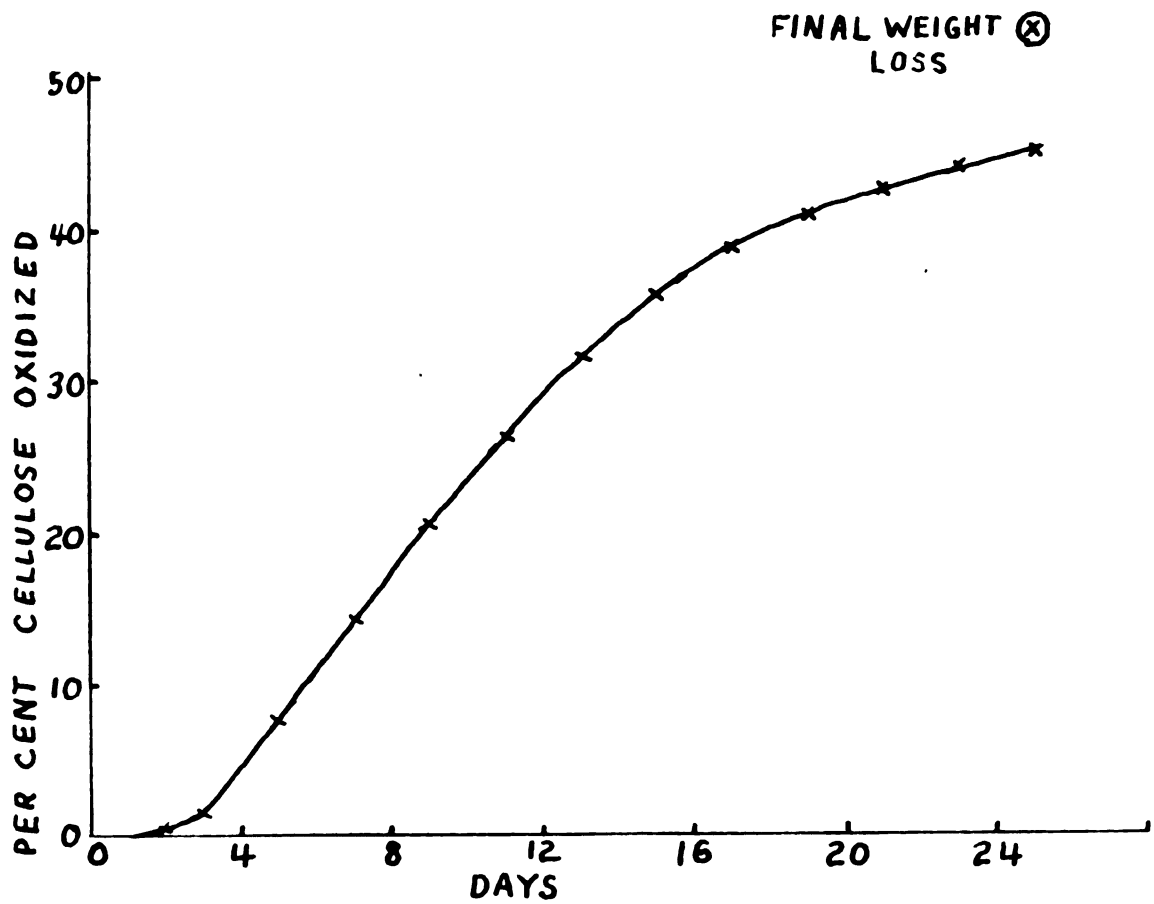


FIGURE 25. CUMULATIVE CELLULOSE OXIDATION



initial weight not accounted for by oxidation of cellulose or remaining residue.

Table 18 lists per cent of initial cellulose which was oxidized, per cent loss in weight after drying at 105°C and difference between loss of weight from cellulose oxidation and actual loss after drying for all runs made. Sample No. 6 was lost before the final dry weight was determined.

TABLE 18

CELLULOSE OXIDIZED IN RUNS NO. 1 THROUGH 6

Run No.	Length, Days	Cellulose Oxidized, Per Cent	Weight Loss After Drying, Per Cent	Difference Between Oxidation and Loss of Weight, Per Cent
1	25	45.3	53.5	8.2
2	14	29.8	38.2	8.4
3	14	31.6	44.0	12.4
4	14	36.1	47.6	11.5
5	8	17.0	27.3	10.3
6	8	21.2	---	---

Evidence was found from variation of the R. Q. that fermentation occurred during the early part of each run. The relative amount of fermentation going on should be indicated by the amount of excess CO_2 being produced over that expected from complete oxidation. Figure 26 shows the excess CO_2 per gram initial cellulose per day produced during run No. 1. The data were computed from Table 5.

The rate of excess CO_2 production reached a maximum of 15 mg per day per gram initial cellulose on the fourth day. After the tenth day, less moles of CO_2 were being produced than moles of oxygen consumed. This was probably due to the fact that during this period not only carbohydrates were oxidized, but also other components such as proteins and fats, which may have been synthesized by the bacterial culture.

Figure 27 shows the relative amount of cumulative excess CO_2 compared to the total CO_2 produced during run No. 1. After seven or eight days, the amount of excess CO_2 had leveled off and became less important to the total amount of decomposition.

Data are listed in Table 19 showing the excess CO_2 at the end of the run for runs No. 1 through 6. The per cent of the initial cellulose weight represented by the amount of excess CO_2 is also listed. The full weight of the CO_2 molecule was taken to have come from the cellulose because all oxygen uptake from the air had already been considered in the CO_2 from oxidation; thus, the excess CO_2 must have taken its oxygen content from the cellulose. The ratio of excess CO_2 to the grams of unaccounted weight loss after drying was calculated and listed in Table 19. The unaccounted weight loss was obtained by subtracting the weight of cellulose oxidized from the actual loss of weight after drying at 105°C.

FIGURE 26. RATE OF EXCESS CO_2 PRODUCTION IN RUN NO. 1

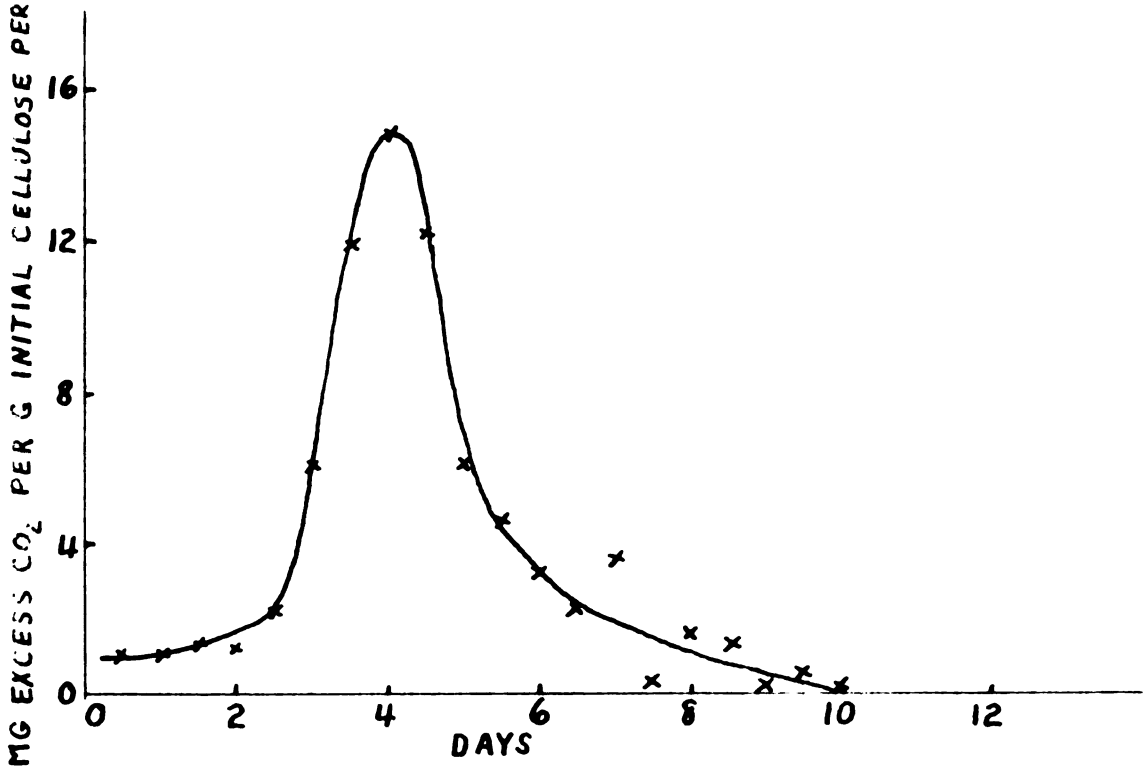


FIGURE 27. CUMULATIVE CO_2 PRODUCTION FOR RUN NO. 1

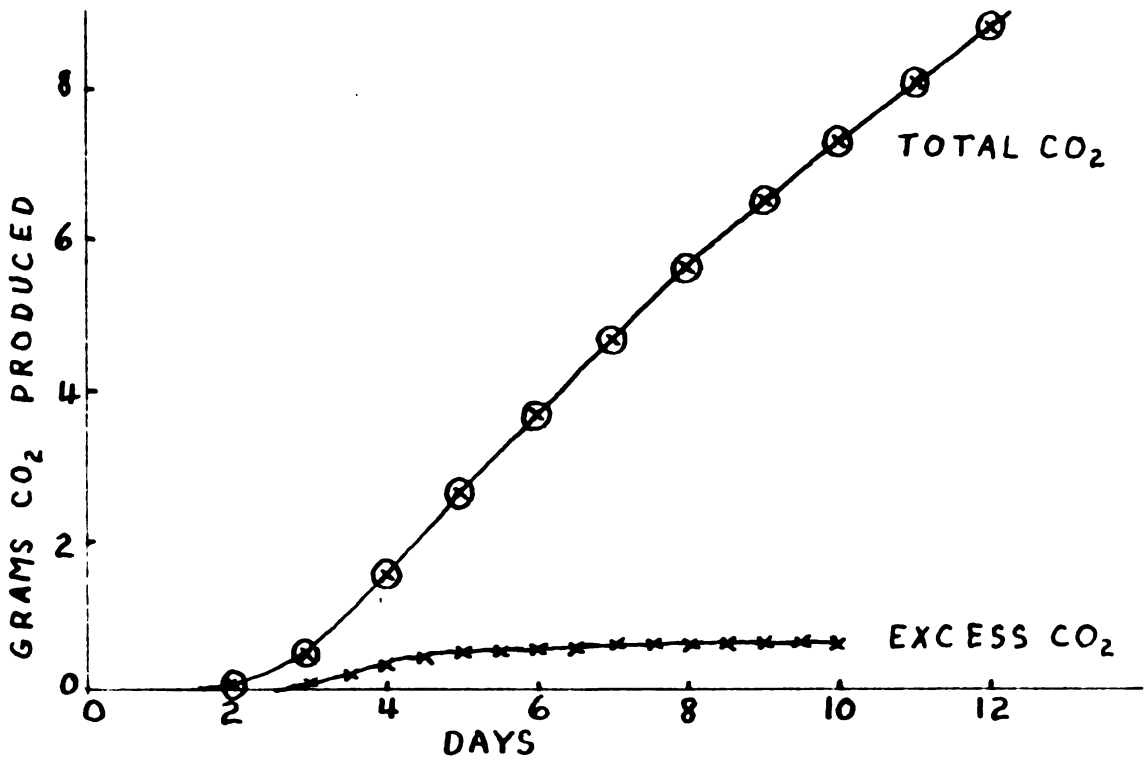


TABLE 19

EXCESS CC_2 IN RUNS NO. 1 THROUGH 6

Run No.	Length, Days	Excess CO_2, Grams	Per Cent Initial Weight	Excess CO_2 Per Gram Unaccounted Weight Loss, Grams
1	25	0.215	1.2	0.15
2	14	0.313	1.8	0.21
3	14	0.415	2.4	0.19
4	14	0.490	2.5	0.21
5	8	1.205	7.0	0.68
6	8	0.905	5.3	----

The results of Table 19 show that the excess CO_2 is quite significant for runs that lasted only 8 days but is rather small for the runs which lasted longer.

If fermentation occurred, it would be expected that products other than CO_2 would be formed that might be volatile at 105°C. To check for these products, the volatile matter from run No. 5 was condensed and analyzed for carbon. The flask containing the sample was held at 105°C in a small oven, and all volatile matter evolved was condensed in a three neck flask outside of the oven. The noncondensable matter was exhausted through Drierite and Ascarite to collect CO_2.

During the first 24 hours of drying, 0.339 grams of CO_2 were absorbed in the Ascarite. About 40 ml of colorless liquid had been condensed. A mixture of 25 ml of 0.25 N $K_2Cr_2O_7$ solution and 75 ml of concentrated H_2SO_4

were added to the condensed material. During the next 12 hours of drying an additional 20 ml of condensate were collected. The Ascarite tube absorbed 0.157 grams of CO_2 during this period which could have come directly from the sample or from oxidation of carbonaceous material in the condensate. After all volatile matter had been collected, the condensate and acid-dichromate mixture was refluxed for three hours. An additional 0.015 grams of CO_2 were collected while refluxing.

A total of 0.511 grams of CO_2 was collected as volatile products at 105°C, the majority of which evolved directly from the sample as CO_2. The total weight lost in run No. 5 was 27.3 per cent of the initial weight of cellulose. The cellulose lost by oxidation was 17.0 per cent. The weight of CO_2 from volatile products during drying represented about 3.0 per cent of the initial weight of cellulose, and therefore only a fraction of the 10.3 per cent difference between weight lost and cellulose oxidized.

Gas analysis data for this run showed a total of 1.20 grams of CO_2 evolved over the amount expected from the complete oxidation of cellulose as calculated from oxygen consumption. This represents 7.0 per cent of the initial weight of cellulose. As shown in Table 20, cellulose oxidized, excess CO_2 evolved and CO_2 collected during drying gave a rough account of the dried weight loss in run No. 5.

To determine the nature of the final dried residue of run No. 5, it was extracted with water, alcohol and ether. Extracts with water were done with hot water which cooled in the time necessary for filtration. Aliquot

TABLE 20

MATERIAL BALANCE OF WEIGHT LOSS AFTER DRYING

	Weight, Grams	Per Cent Initial Weight of Cellulose
Cellulose oxidized	2.94	17.0
Excess CO_2	1.20	7.0
CO_2 as volatile products	0.51	3.0
	4.65	27.0
Weight loss after drying	4.71	27.3
Difference	0.06	0.3

portions of the extracts were dried on a water bath to determine the weight of suspended and dissolved solids. Four 250 ml water extracts contained a total of 0.683 grams of solid material after subtracting the weight of nutrient salts. An alcohol extract of 300 ml contained 0.096 grams of solid material. An ether extract of 300 ml contained 0.024 grams of solid material. A final cold water extract of 1500 ml contained 0.360 grams of solid material. The complete material balance for run No. 5 is given in Table 21.

The residue after extractions was ashed at 800°C for three hours. The initial weight of vermiculite in the sample was 9 grams. The final ash weight was 8.8 grams. This checked exactly with the experimentally determined ash content of 97.8 per cent for vermiculite.

TABLE 21

MATERIAL BALANCE FOR RUN NO. 5 AFTER EXTRACTION

	Weight, Grams	Per Cent of Initial Cellulose Weight
Initial cellulose	17.25	100.00
Cellulose oxidized	2.94	17.04
Excess CO_2	1.205	6.99
CO_2 from drying	0.511	2.96
Solids in water extracts	1.043	6.05
Solids in alcohol extract	0.096	0.56
Solids in ether extract	0.024	0.14
	5.819	33.74
Remaining cellulose	11.33	65.68
Total	17.15	99.42
Unaccounted weight	0.10	0.6

Figure 28 shows a plot of cellulose oxidized during run No. 5. The final weight losses before and after extraction are shown. The remaining material after extraction was 11.33 grams giving a weight loss of 34.3 per cent.

The per cent cellulose lost was calculated for run No. 7 using data in Table 10. The results are listed in Table 22 together with the cellulose oxidized as calculated from the oxygen consumption. These two quantities are plotted in Figure 29.

Figure 29 shows that the total cellulose destruction curve began to level off before the cellulose oxidation curve. The curve also demonstrates that the fraction of cellulose oxidized became increasingly larger in time.

FIGURE 28. CUMULATIVE CELLULOSE OXIDIZED IN RUN NO. 5

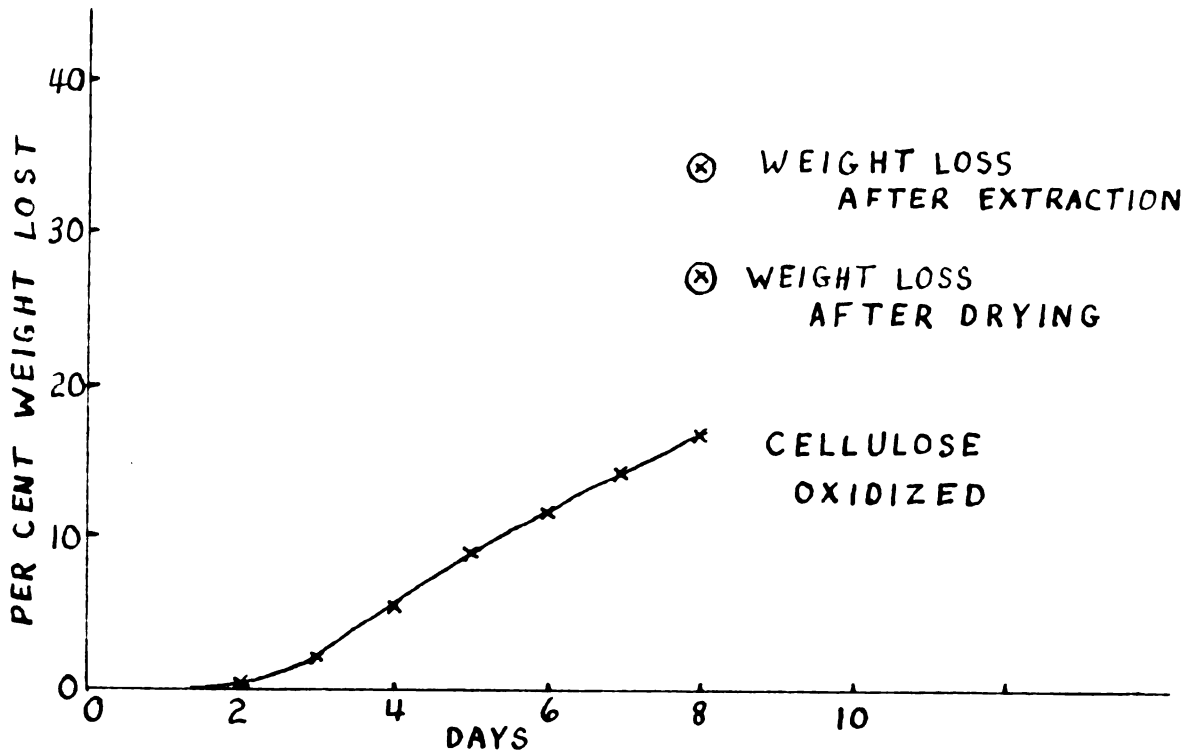


FIGURE 29. CUMULATIVE CELLULOSE LOST IN RUN NO. 7

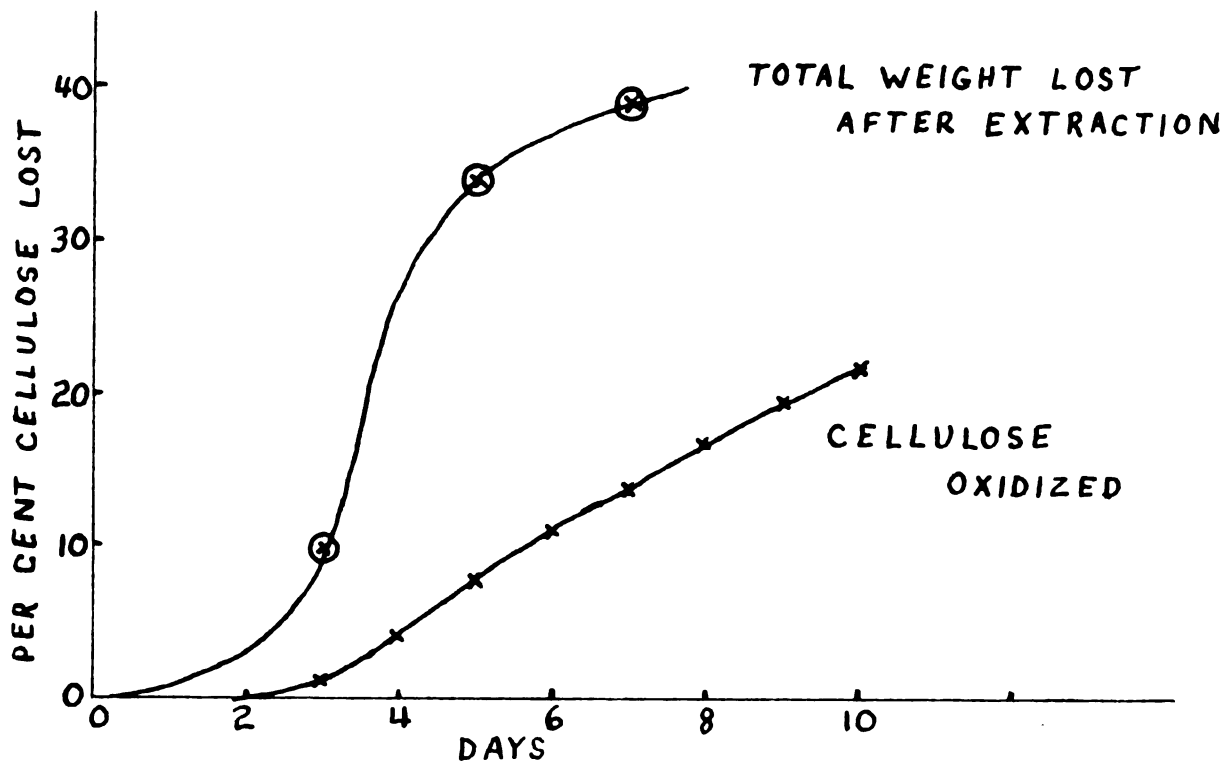


TABLE 22

CELLULOSE LOST IN RUN NO. 7

Days	Per Cent Cellulose Lost	Per Cent Cellulose Oxidized
3	9.9	1.15
4	---	4.25
5	34.0	8.0
6	---	11.1
7	39.1	14.0
8	---	16.9
9	---	19.8
10	---	21.8

An obvious by-product from cellulose decomposition is the cell material of the microorganisms. The amount of this material can be estimated from data on nitrogen conversion and approximate nitrogen content of dried bacteria. The "Handbook of Biological Data" (22) lists a range of from 8 to 14 per cent nitrogen content in the dry weight of bacteria. The average is about 10 per cent.

In run No. 7, 0.538 grams of nitrate nitrogen had been converted to organic nitrogen by the seventh day. At 10 per cent nitrogen, this represents 5.38 grams of dried bacteria. To estimate the per cent of this weight which came from the nutrient components, the average ash content and nitrogen content of the bacteria were considered. The "Handbook of Biological Data" listed a range of from 4 to 14 per cent ash with an average of about 8 per cent.

Combining these two figures gives about 18 per cent of the dry weight of the bacteria due to nutrient salts. This leaves approximately 4.4 grams of weight due to assimilation of cellulose, which represents nearly 7 per cent of the initial cellulose. This is a significant amount and shows that the rapid climb of the total cellulose destruction curve could be due largely to the weight assimilated by the organisms. The growth of these microorganisms would be expected to follow the nitrogen assimilation curve shown in Figure 22.

The amount of material extracted with water after eight days for run No. 5 appears to be low, since it is only 6 per cent of the initial weight of cellulose and should account for both the weight of bacteria and the weight of by-products formed, such as mucilage, which would not be volatile at 105°C. A microscopic examination of a large mixture ran parallel with run No. 5 showed growth of yeast and protozoa on the seventh day. It might be that formation of these microorganisms had started in sample No. 5, and these would not pass through filter paper as easily as bacteria. Also, this sample had been dried before extraction which might have affected it.

Of the four samples taken on the seventh day from run No. 7, the water extracts of two were centrifuged for 30 minutes at 3500 revolutions per minute. The substance which settled out was considered mucilage, some of the

bacteria and other high molecular weight by-products which had been extracted with water. The supernatant still had a cloudy white color, and a microscopic check showed it to be a suspension of bacteria. The first sample gave 0.046 grams of settled material from 0.875 grams of remaining cellulose, and the second gave 0.041 grams from 0.796 grams of cellulose. This is 5.26 and 5.15 per cent or an average of 5.2 per cent of the remaining cellulose. This would amount to 1.8 grams based on the remaining weight on the seventh day.

On the seventh day an excess of 2.9 grams of CO_2 had been evolved, and 8.1 grams of cellulose had been oxidized in run No. 7. From nitrogen conversion data, it was estimated that the bacteria had assimilated 4.4 grams of cellulose. If it is assumed that the 1.8 grams of centrifuged material contained a negligible amount of the bacteria, the combined weight of these four quantities is 17.2 grams and accounts for about 30 per cent of the initial cellulose. This is 9 per cent less than the measured loss of weight after extractions. Other losses of weight not considered are solids extracted by alcohol and ether, volatile products present and products which did not settle with centrifuging.

SECTION VI

DISCUSSION

As much as 50 per cent of the initial weight of cellulose has been lost over a twenty day period of attack by the cellulose decomposing microorganisms used in this study. By extracting the residue, it was shown that the actual breakdown of cellulose was greater than the weight loss after drying. At the maximum activity during run No. 1, 3.5 per cent of the initial weight of cellulose was being oxidized per day. In run No. 4, a maximum rate of 4.6 per cent per day was reached and maintained for two days. Extraction data from run No. 7 indicated a maximum rate of over 8 per cent of the initial cellulose being destroyed per day. Thus, cellulose can be extensively decomposed under conditions simulating the compost process.

The variation of $NaNO_3$ concentration from 0.4 to 1.6 weight per cent resulted in a variation in amount of weight loss after drying from 17 to 48 per cent with the maximum occuring at 1.4 per cent $NaNO_3$ in the initial mixture. Reese (10) reported an optimum concentration of $NaNO_3$ from 1 to 3 grams per liter in work with cellulose suspended in a liquid medium. The figure reported by Reese is about 0.2 per cent by weight and is not comparable with results of this study.

Nitrogen concentration was found to play a role in determining the rate of decomposition as shown in Figure 3. This is in agreement with observations made by Dubos (11); namely, that increasing concentration of $NaNO_3$ increased the lag period before rapid growth of the bacteria.

The presence of magnesium was found to be essential for growth of the organisms in this study. Reese (10) reported $MgSO_4$ to be important to the rate of decomposition in his work. Results for $CaHPC_4$, $FeSO_4$ and K_2HPO_4 showed only a small variation of amount of decomposed cellulose over the range of concentrations checked.

Since nutrient components were varied from a standard mixture, the curves of Figures 4 through 8 all have a point of identical composition. At these points it would be expected that the final weight loss of material in each experiment would be comparable. The actual amounts are listed in Table 23.

TABLE 23

WEIGHT LOSS AT STANDARD COMPOSITION

Component Varied	Per Cent Weight Loss
$NaNO_3$	42.5
$MgSO_4$	43
$CaHPO_4$	47
K_2HPO_4	36
$FeSO_4$	40

The results cover a range from 36 to 47 per cent or an 11 per cent spread. This is not interpreted as the magnitude of experimental error for each curve since the data in most cases followed a relatively smooth curve. However, the data were collected over a period of four months, during which time the room temperature showed 5 to 10 degree variations; and the mixed culture seed may have changed to some extent.

From gas analysis data it was shown that the weight loss of material after drying could be only partially explained by cellulose oxidation as calculated from oxygen consumption. Evidence was found from variation of the R.Q. that fermentation occurred during the early part of each run. It was expected that products volatile at 105°C would have been formed as a result of fermentation, but analysis of volatile matter during drying of run No. 5 showed only about 3 per cent of the initial weight of cellulose represented by the CO_2 obtained from products evolved during drying. The majority of this CO_2 was collected directly from the sample rather than by oxidation of volatile products. Thus, it would appear that if many products of fermentation are formed, they must be used up during the course of further activity.

The nitrogen conversion data was taken as a measure of the growth of bacteria in the mixture. From the amount of nitrogen converted, it was found that the weight of the bacteria cells formed during the decomposition process was

significant. On the seventh day the bacteria represented about 7 per cent of the initial weight of cellulose in run No. 7. This weight was not removed in samples that were dried in an oven.

From considerations of fermentation and synthesis of bacterial cell material, it would be expected that the total cellulose destroyed would be greater than the cellulose oxidized in the earlier part of the run. During the phase of rapid growth of bacteria in the first 4 to 5 days, a relatively large portion of the cellulose broken down would be converted into cell material and into products of fermentation or of enzymatic degradation. Evidence that this was actually the case is given by the data plotted in Figure 29. The curve for cumulative cellulose destroyed climbed rapidly and leveled off sooner than the cumulative curve of cellulose oxidation.

In run No. 7, the maximum rate of cellulose oxidation of 4.6 per cent per day was reached about 4.5 days after the start of the run as shown by the oxygen uptake rate plotted in Figure 19. The maximum rate of total cellulose loss appeared to be about 8 per cent per day and occurred about 3.5 to 4 days after the start of the run as shown in Figure 21. It is noted that the maximum growth of bacteria according to the nitrogen conversion data shown in Figure 19 nearly corresponds with the maximum rate of total cellulose destruction. The delayed maximum oxygen uptake rate is interpreted to be a result of its dependence on the

total number of bacteria present.

The amount of cellulose destroyed per gram of nitrogen assimilated was listed in Table 14. After a week, 42 grams of cellulose had been destroyed per gram of nitrogen assimilated. Heukelekian and Waksman (16) reported about 30 grams of cellulose decomposed in a liquid medium for every gram of nitrogen assimilated after 24 to 38 days of incubation. Shorter incubation periods gave less cellulose decomposition per gram of nitrogen assimilated. This ratio was higher for experiments done in sand and soil mediums. They reported as much as 43 grams of cellulose decomposed per gram of nitrogen assimilated after 2 weeks incubation in a soil medium. The ratio of total weight lost to grams of nitrogen assimilated found in this study are comparable to the soil medium as reported by Heukelekian and Waksman. The tendency for the ratio to become greater with increasing length of incubation was also observed.

The maximum rate of cellulose destruction found under the conditions of this study was 8 per cent of the initial cellulose per day. Only limited data have been found in literature of the daily rate of cellulose destruction in other experimental work. Reese (10) gave a curve showing the cumulative amount of cellulose destruction which climbed rapidly and leveled off after five days similar to the curve shown in Figure 17 of this study. The curve shown by Reese reached a maximum of 80 per cent decomposition before leveling off. He found 75 per cent of

the cellulose remaining after 2 days and 50 per cent after 3 days. This would give a rate of 25 per cent of the initial cellulose per day and is three times the rate of maximum decomposition found in this study. Reese worked with bacteria and filter paper in liquid suspension.

Attempts were made to determine the reason for leveling off of activity after three to four days of incubation. It was found that addition of $NaNO_3$ and of the complete nutrient solution after reaching the point of maximum activity had no stimulating effect. When clumping of the filter paper was prevented by spreading the paper discs on glass grids, the oxygen uptake rate still leveled off after four days. Addition of fresh filter paper was not found to be stimulating. The pH showed no significant change at the point of maximum activity.

Perlin, Michaelis and McFarlane (13) reported concentrations of 1.2 per cent CO_2 to retard growth of the organism with which they worked. Although this point was not specifically checked, no evidence was found in the data of this study that the rate of cellulose decomposition was related to flow rate or concentration of oxygen or CO_2. However, the range of flow rates used in this study varied only from 0.175 to 0.5 liters per hour. None of these rates kept concentration of CO_2 below 6 per cent at the point of maximum activity. Low flow rates were necessary to get accurate oxygen consumption measurements. No definite conclusion could be drawn as to the limiting factor for the rate of cellulose decomposition in this study.

SECTION VII

CONCLUSIONS

1. Cellulose was extensively attacked under the conditions described in this study.

2. Maximum cellulose destruction occurred from three to five days after mixing the cellulose (filter paper) with nutrient solution and bacterial seed.

3. The maximum rate of nitrogen assimilation by the bacteria occurred at nearly the same time as maximum cellulose destruction and slightly preceded the maximum rate of oxygen consumption.

4. Neither nutrient supply nor available surface appeared to be the limiting factor for the rate of cellulose decomposition.

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APPENDIX

PROCEDURE USED FOR NITRATE DETERMINATION

1. The weighed sample (from 3 to 10 grams wet weight) was dissolved in 150 ml of distilled water.

2. The suspended matter was filtered out and rinsed with 250 ml of distilled water. (The residue was further extracted with alcohol and ether, then dried and finally ashed at 800°C for three hours.)

3. The water extract containing the nitrate was placed in a Kjeldahl flask. To this was added 75 ml of saturated NaOH solution and glass beads to prevent bumping. Paraffin wax and paraffin oil were added to prevent frothing.

4. The solution was boiled until 300 ml had been distilled over. (A final portion of this was usually checked to see if ammonia was still being evolved.)

5. The solution was cooled and 1 g Al, 0.5 g Zn, 1 g Cu and 400 ml of distilled water were added. A blank was always ran and subtracted from final results as a correction for ammonia in the distilled water.

6. The solution was distilled, allowing several hours for reaction, until 300-400 ml of distillate had been collected in a measured amount of standard H_2SO_4 solution.

7. The acid solution was titrated with standard NaOH to find the amount of acid used by the ammonia.

8. The amount of nitrogen as nitrate was calculated as follows:

$$g\ N_2 = 1.4 \times \underline{N}\ \text{Acid} \times \text{ml acid used up by the } NH_3$$