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The Hydrothermal Degradation of Cellulosic Matter to Sugars and their Fermentative Conversion to Protein

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Synopsis

For the hydrothermal degradation of cellulosic matter, an apparatus was developed in which water is used as extraction medium. Samples, 0.15 g each, of pure cellulose (filter paper), natural straw, and ^{14}C-labeled straw were treated at temperatures of between 200° and 275°C. Of the inserted cellulose, 65.7% was recovered at the optimum temperature as sugars and hydroxymethylfurfural. It was possible to degrade the straw selectively: at lower temperatures, the hemicellulose part of the plant matter was converted to xylose and arabinose; and then at higher temperatures, the cellulose was converted to glucose and cellobiose. At the same time, a certain amount of the sugars was transformed to furfural compounds. The growth behavior of the yeast *Candida utilis* (strain Weissenbach) was analyzed, using cellobiose, xylose, and glucose (standard) as carbon sources. The growth curves applying cellobiose were nearly identical to those of glucose. Xylose showed lower productivity than the hexoses. The main products of the hydrothermal degradation can, therefore, be used favorably as nutritive substances for this protein-producing yeast.

INTRODUCTION

Organic materials are derived mainly from three natural sources: fossil fuels, plants, and animals. Food for man has been supplied practically only by the latter two. Nowadays, single-cell protein production from paraffins is gaining in importance; and after its introduction as animal feed, there are now certain expectations that this protein may also be added to the list of human foods.

The great shortage of food—especially of protein—in many parts of the world has led to investigations as to how far other resources can be utilized. The large amount of uneconomically employed and totally unused plant material has attracted much thought and research work.

The annual above-ground world production of plant matter[1] is 45×10^{12} kg dry weight in woodland and 47×10^{12} kg dry weight in nonwoodland areas. This gives a total of 92×10^{12} kg dry weight of plant matter, which is only 20% of the maximum potential in the global land ecosystem but nevertheless over 35 times the weight of the petroleum production in the year 1972.

The utilization of the plant matter is not very efficient. Nearly 30% of the

cut wood in an industrial country (U.S.A.) is lost as logging, veneer, or sawlog residue.[2] The straw of the major cereals is often unused or constitutes a pollution hazard by open field burning. The weight of the straw may amount to as much as 100% to 400% of the grain harvest, depending on the type of crop. The world cereal crop[3] was 1.2×10^{12} kg in the year 1972 (wheat, barley, oats, rye, rice, and maize). Lesser crops, such as sorghum, millet, etc., would increase this amount somewhat (by approximately 7%).

Several other cellulosic wastes are burned or buried; for example, the municipal residues in the U.S.A. contain nearly 0.1×10^{12} kg used paper.[2]

The first extensive process where waste wood could be used, with the aim of obtaining glucose and corresponding fermentation products, was acid hydrolysis. In Germany, the Bergius process (concentrated acids at normal temperature) and the Scholler process (dilute acids at 160° to 180°C) were in use, but were discontinued after World War II as uneconomical. In the U.S.S.R., large factories using these processes are still in operation, and an output of 0.9×10^9 kg yeast obtained by fermentation of the sugar solutions is envisaged.[4]

Another method for the utilization of plant matter is the application of cellulase, an enzyme which can degrade cellulose directly to glucose. Some microorganisms (fungi and bacteria) possess cellulase activity to transform the native cellulose into reactive cellulose. In a second step, this reactive cellulose can then be transformed to monomere sugars by the cellulase of a larger number of fungal and bacterial species. Very fine or hot grinding of wood enables the cellulase, which normally degrades only reactive cellulose, to convert this material directly to glucose. The energy required for the grinding, however, makes its economical applicability questionable.[4]

We have concentrated our efforts on the purely thermal degradation of cellulosic and hemicellulosic matter, with water as extraction medium ("hydrothermal process"). In earlier, mainly static experiments,[5] it was shown that a significant amount of the degradation products was recovered as sugars. At the same time, the further degradation of glucose at the relevant temperatures was determined.[6] In this way, several important parameters for the process were obtained, so that an apparatus could be constructed where the extraction medium, water, was continuously passed through the reaction vessel, as described in the following point 2.

Due to the small size of the reaction vessel (0.5 ml), the amount of reaction products was not enough for experiments to obtain growth curves for the cultivation process with the original hydrothermal solutions. The analysis of the fermentation parameters (point 3), using the yeast *Candida utilis,* was therefore carried out with larger quantities of the three main sugars which occur in the reaction solutions.

APPARATUS FOR HYDROTHERMAL DEGRADATION

A stainless steel apparatus was developed[7] able to withstand the equilibrium pressure of water up to 300°C. In Figure 1, the scheme of this device is reproduced. A piston pump (1) presses water from a container (2), by-passing a safety valve (3), through a pressure compensation vessel (4) to the preheating system (5). With a thermocouple in (6), the preheating controller (7)

controls the temperature in (5) at a level somewhat below the desired reaction temperature.

The reaction temperature is reached at the entrance of the pressure vessel (8). The main heat controller (9) adjusts the temperature to the selected value through a signal from a thermocouple which is mounted in the pressure vessel (10). In this vessel, the waterflow is split into two streams. The outer stream ascertains that the walls of the reaction vessel (11) are at the proper temperature and then leaves the pressure vessel through the heat exchanger (12) and the valves (13) and (14). The other part of the stream flows through reaction vessel (11), a heat exchanger (15), and two valves (16) and (17) into a fraction collector (18).

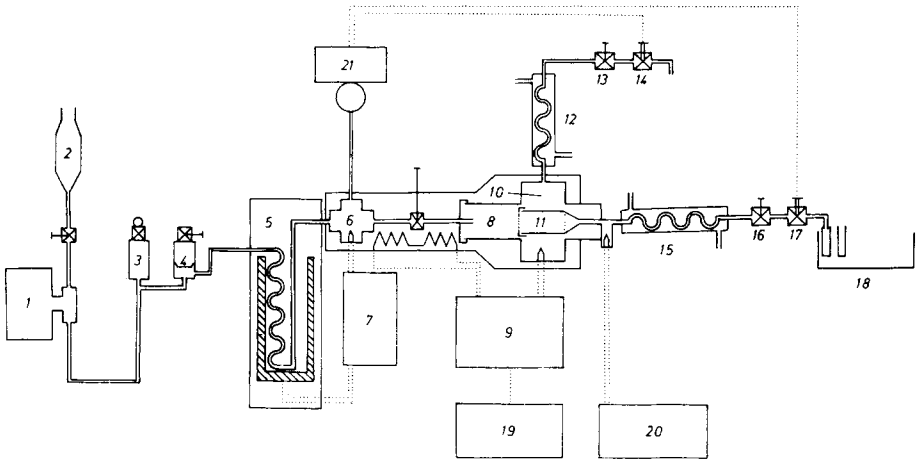


Fig. 1. Apparatus for hydrothermal degradation: Piston pump (1), water container (2), safety valve (3), pressure compensation vessel (4), preheating system (5), temperature and pressure control point (6), preheating controller (7), pressure vessel entrance (8), main heating controller (9), pressure vessel (10), reaction vessel (11), heat exchanger (12), manually controlled valve (13), automatically controlled valve (14), heat exchanger (15), manually controlled valve (16), automatically controlled valve (17), fraction collector (18), temperature recorders (19) and (20), and automatic pressure control (21).

The temperatures in the pressure vessel and in the main stream immediately after the reaction vessel are recorded (19, 20). The pressure at the exits of both water streams can be controlled manually with the valves (13) and (16), or automatically (21) between an upper and lower pressure level by operating the valves (14) and (17).

The reaction vessel (11) has a volume of approximately 0.5 ml. Therefore, only up to 0.15 g straw or cellulose can be introduced for hydrothermal treatment.

HYDROTHERMAL DEGRADATION OF CELLULOSIC MATTER[7]

Three different materials were analyzed: pure cellulose (filter paper), natural straw, and ^{14}C-labeled straw. Characteristic results of the different series of experiments are given, as follows.

TABLE I
Hydrothermal Degradation of Pure Cellulose at 264°C for 22 Minutes

Sample weight, mg	Residue, %	Glucose, %	Cello-biose, %	Fructose, %	Xylose, %	Hydroxy-methyl-furfural, %	Sum of anal. subst., %
149	12.8	42.6	4.8	5.2	1.0	12.1	78.5

Degradation and Yields of Pure Cellulose

Filter paper (Schleicher u. Schüll Nr. 589[2]) was introduced into the reaction vessel and water passed through at temperatures between 203° and 275°C at a flow rate of approximately 1 ml/min. The reaction time was between 22 and 45 min. The glucose content of the effluent solution was determined enzymatically.[8]

Figure 2 gives the degradation of cellulose and the yield of glucose, both as percentage of the total cellulose. It is obvious that the optimum conditions are at 264°C, where over 40% of the degraded cellulose are recovered as glucose.

In Table I, the reaction products analyzed for the experiment at 264°C are given. In addition to the 42.6% glucose, 11% are obtained as cellobiose, fructose, and xylose. Including the hydroxymethylfurfural and the residue, the sum of the analyzed substances amounts to 78.5% of the original cellulose. The missing 21.5% was at first a problem, but was then explained as described in a later section.

Degradation and Yields of Natural Straw

After a series of experiments, it was found that the degradation can be selectively carried out, so that first the hemicellulose and then the cellulose is

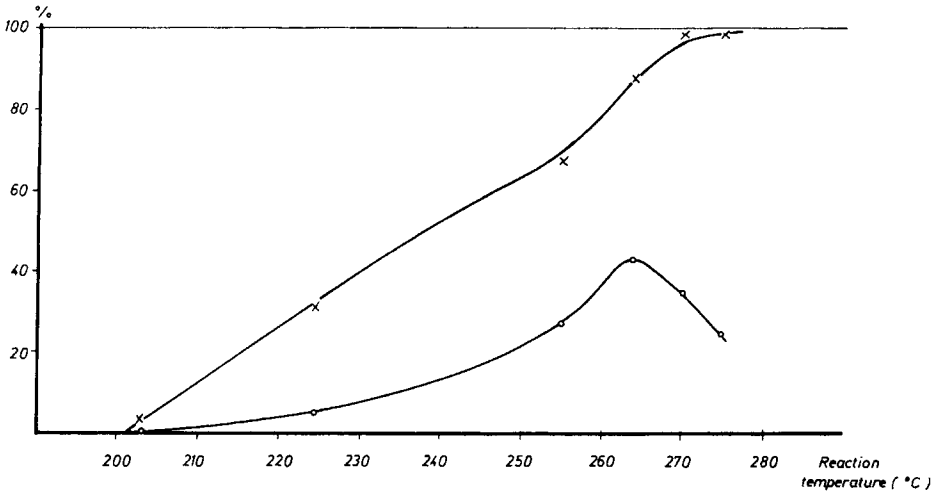


Fig. 2. Degradation of cellulose (×) and yield of glucose (○). Reaction period was 22 to 45 min at indicated temperatures.

converted. This can be achieved by conducting the hydrothermal process at two different temperatures. Figure 3 shows an example of such an experiment. At first, the temperature was set at 218°C and then raised to 260°C. The reaction solution at the lower temperature contained only xylose and arabinose (from hemicellulose) and at the higher temperature, glucose and cellobiose (from cellulose). The flow rate was 1.23 ml/min.

Although the separation of the cellulose components was successful, there still remained a problem, because only 15% of the converted straw was recovered as sugars (glucose, cellobiose, xylose, and arabinose). The undetermined part of the reaction solution was considerable. The following experiments with ^{14}C-labeled straw answered this question to a large extent.

Degradation and Yield of ^{14}C-Labeled Straw

^{14}C-Labeled wheat straw with a specific activity of 185.5 μCi/g carbon and a carbon content of 40.45% was hydrothermally treated in the same way as described above: Up to fraction 6, the temperature was 219°C and later, 259°C. The flow rate was first 0.91 and later 1.11 ml/min; 4.6% of the original sample was found as residue after the reaction.

From each fraction, 5 ml was carefully evaporated to dryness (in a vacuum desiccator at 40°C) and the total dry matter of the reaction determined. The sum amounted to slightly over 100% of the original sample weight. At the same time, the activity of 1 ml of each fraction was measured by the liquid scintillation method (Packard-Tri-Carb 3380).

In Figure 4, the results of these experiments are shown. The dry weight and activity percentages of each fraction in relation to the sum of all ten fractions are given in dependence of the fraction number. The fraction numbers are marked in the middle of each fraction volume.

Certain features were characteristic for the experiments with ^{14}C-labeled straw. The total weight and activity of the fractions were in good agreement

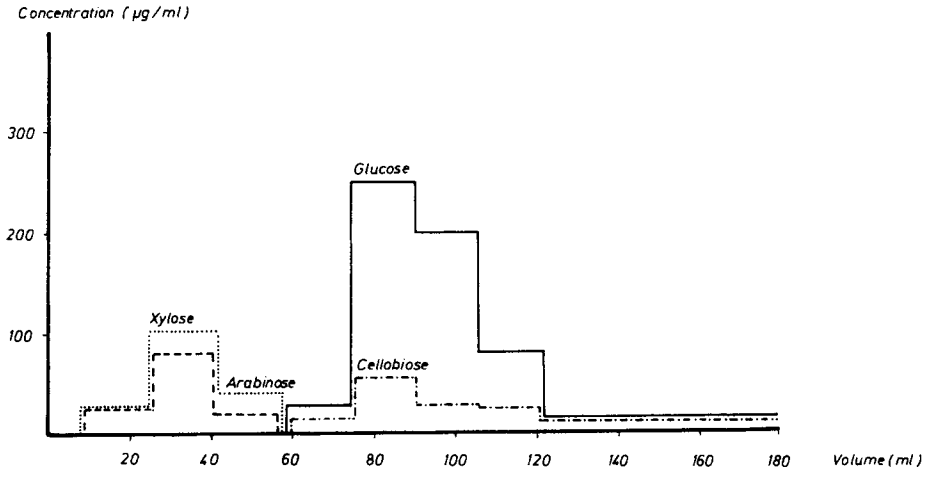


Fig. 3. Degradation products of natural straw in the reaction solution. At 218°C, the hemicellulose is degraded (xylose and arabinose), and at 260°C, the conversion products of cellulose (glucose and cellobiose) are determined in the effluent solution.

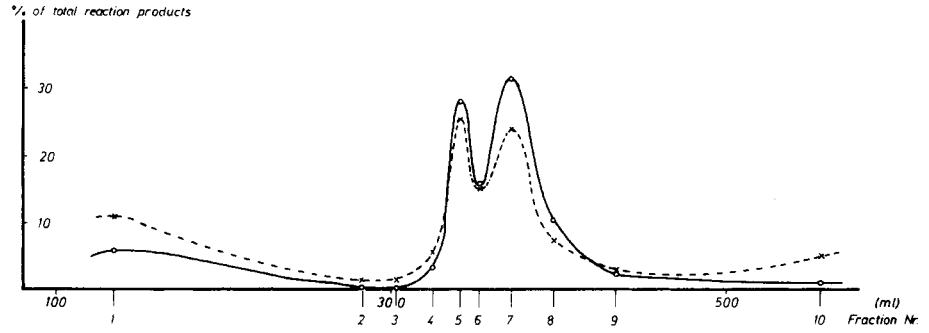


Fig. 4. Distribution of the substance weights (dry substance) (×) and activity (○) after hydrothermal degradation of ^{14}C-labeled straw. Weight and activity of each fraction are given as percentage of the sum of all ten fractions. Temperature rise between both peaks from 219° to 259°C.

with the corresponding values of the original sample. In the individual fractions, however, there were distinct differences: the weight percentages at the beginning and end of the experiments were higher than the percentage of the activity. As compensation, the activity percentage was higher in the region of the peaks where the main sugar content occurred.

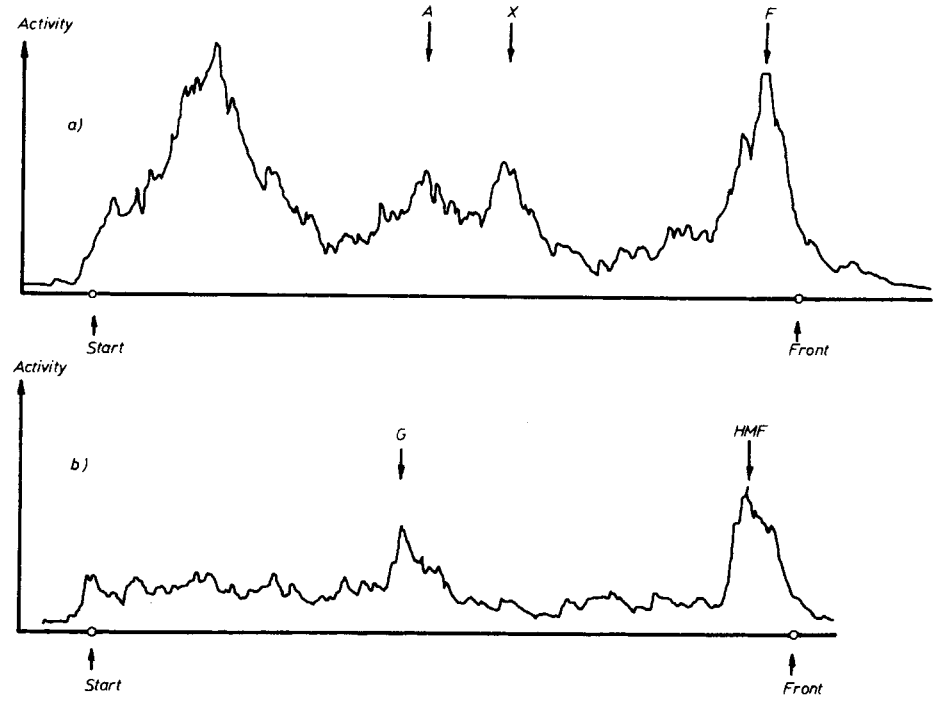


Fig. 5. Scanning of thin-layer chromatograms (hydrothermal degradation of ^{14}C-labeled straw). 100 μl of the reaction solution was applied to DC Plastikfolien Kieselgel (Merck) and a mixture of isopropanol, ethylacetate, and water (1.7:3.3:1) was used to develop the chromatogram.[14] (a) Fraction from the hemicellulose part; first peak unknown substance, A = arabinose, X = xylose, and F = furfural. (b) Fraction from the cellulose part; G = glucose, HMF = hydroxymethylfurfural.

Two points can mainly be made responsible for this discrepancy. First, a certain amount of inorganic salts and certain low molecular products (for instance amino acids) will appear at the beginning of the degradation process, and with their lower specific activity reduce the relation of activity to dry weight. Second, part of the sugars formed is further degraded to furfural compounds, which have a higher specific activity (loss of water) and so tend to increase the above relation in the fraction with the main sugar content.

In Figure 5, two scanning diagrams of thin-layer chromatograms are reproduced. In Figure 5a, the hemicellulose fraction is analyzed. The first peak after the start is not yet definitely determined but consists most probably of uronic acids. This relatively large peak accounts for a substantial part of the nonanalyzed substances in the natural straw experiments. The other three peaks are xylose (X), arabinose (A), and furfural (F). In addition, it was found that even with especially careful application of the reaction solution on the thin-layer chromatograms, considerable losses of furfural compounds occurred.

The scanning diagram of a cellulose fraction thin-layer chromatogram is reproduced in Figure 5b. Here, the main peaks of glucose (G) and hydroxymethylfurfural (HMF) appear in the middle and at the end of the chromatogram. The higher molecular substances, as, for instance, cellobiose, are here not yet resolved as single peaks. Using other solvents, good separation of these compounds was obtained, as will be shown in a further publication.

The unknown substance of the hemicellulose fraction (Fig. 5a) and the losses of the furfural compounds on the thin-layer chromatograms accounted to a large extent for the difference between the original sample weight and the sum of the determined products in the experiments with the inactive material (pure cellulose and natural straw).

The experiments showed certain differences between natural and ^{14}C-labeled straw. The high yield of furfural compounds indicated that the active straw can be more easily degraded by the hydrothermal process.

The results demonstrate at the same time that the apparatus developed can be used successfully for the analytical and structural studies of natural and synthetic high-polymer substances.

GROWTH BEHAVIOR OF YEAST *(Candida utilis)* ON THE SELECTED DEGRADATION COMPOUNDS

As already mentioned, the amount of the reaction products was too small to obtain growth curves of the microorganism. It was, however, of special interest to compare the growth behavior of the chosen microorganism, a typical fodder yeast, *Candida utilis* (strain Weissenbach), using the four main sugars of the hydrothermal process (glucose, cellobiose, xylose, and arabinose) as carbon source.

In a preliminary series of tests,[9] it was demonstrated that, after a 30-hr incubation period, all four sugars were completely consumed, so that practically no traces could be detected on the thin-layer chromatograms.

Growth curve measurements had to be made in order to determine the difference in the growth behavior of the yeast on the various nutrition media. For this purpose, a fermentation apparatus with a special automatic analysis system, as described in the next point, was used.

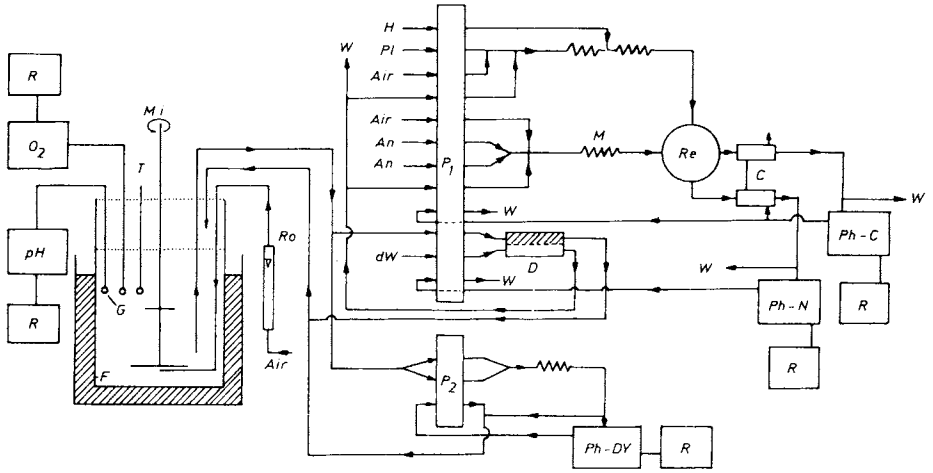


Fig. 6. Fermentation apparatus and automatic analysis system. Fermentation vessel (F), mixer (Mi), thermometer (T), glass electrode (G), pH meter (pH), recorder (R), oxygen monitor (O_2), air supply (Air), rotameter (Ro), pumps (P_1) and (P_2), hypochlorite (H), phenol (Pl), anthrone (An), distilled water (dW), dialyzer (D), mixing coil (M), reactor (Re), cooling coils (C), photometer for total carbohydrate content (Ph-C), photometer for ammonium nitrogen content (Ph-N), photometer for dry yeast content (Ph-DY), and waste (W).

Fermentation Apparatus and Automatic Analysis System

In Figure 6, the scheme of the fermentation apparatus and the adjoining analytical system is shown. A modified laboratory fermentation vessel (New Brunswick Scientific Co.) with 5-liter content was employed (F). The lower flat blade turbine (Mi) was replaced by a miniaturized aeration device of the Vogelbusch system. The rotation speed of the impeller was adjusted to 715 rpm, which could be measured with a photoelectric rotation meter.

The air flow was kept at 280 liters/hr. Foaming in the fermentation vessel was controlled by adding Glanapon 2000 F. The fermentation vessel was placed in a water container where a thermostat and cooling coils kept the water at 29°C. The temperature in the fermentation vessel was maintained precisely at 30°C (T).

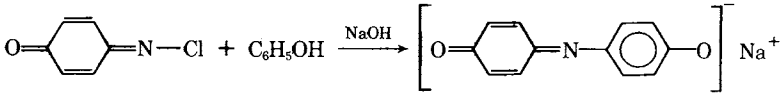
Five reaction parameters were continuously determined and recorded (R) by an automatic analysis system, as shown in Figure 6:

pH Value. The pH value was measured with a glass electrode (G), using the instrument PHM 256, Radiometer Copenhagen.

Dissolved Oxygen (O_2). The dissolved oxygen concentration in the fermentation fluid was determined polarographically (Yellow Springs Instruments Co., Mod. 53, Biological oxygen monitor).

Ammonia Nitrogen. The determination was carried out according to Hoffman and Teicher[10] by applying the Berthelot reaction

$$NH_3 + C_6H_5OH + NaOCl \xrightarrow[\text{(catalyst)}]{Na_4[Fe(CN)_5NO_2]}$$



indophenolblue

The hypochlorite (H) and phenol (Pl) reagents were introduced by the peristaltic pump (P_1). The dye developed in the reactor (Re) at 90°C was measured in the photometer (Ph-N) at 600 nm.

Total Carbohydrates. The hexoses and pentoses were determined continuously after their transformation into the corresponding furfural compounds (with sulfuric acid) and reaction with the anthrone (An) reagent[11,12] at 635 nm in a filter photometer (Ph-C).

Dry Yeast (DY). Yeast dry matter was determined[13] continuously, using a filter photometer Ph-DY (Carlo Erba Mod FF 27).

Growth Curves of *Candida utilis*

Candida utilis (strain Weissenbach) was precultured in shake flasks in two stages. These cultures were used to inoculate the 5-liter fermentation vessel in order to obtain larger amounts of yeast. When portions of 60–85 g were available, growth curve experiments were carried out, using 2.5 liters of culture medium of the following composition: D(+)-glucose or D(+)-cellobiose or D(+)-xylose, 75 g; KH_2PO_4, 2.5 g; KCl, 2 g; $MgSO_4 \cdot 7H_2O$, 1 g; $ZnSO_4 \cdot 7H_2O$, 0.5 g. A pH of 4.9 was set with H_3PO_4 (20%).

For the 2.5 liters of the medium described above, an initial volume of 3 ml $(NH_4)_2SO_4$ solution and the precultivated yeast were added. The further nitrogen requirement was covered by the same $(NH_4)_2SO_4$ or an NH_4OH solution, containing in both cases 1 g N/10 ml. The experiment was conducted over a 7.5-hr fermentation period.

The growth curves of cellobiose were surprisingly similar to those of glucose. Only xylose had a clearly lower productivity than the hexoses examined (see Table II). The yields were in all cases close to 50%.

In Figure 7 the growth behavior of *Candida utilis*, using cellobiose as carbon source, is given. Because cellobiose is one of the main components of the degradation products and at the same time characteristic for another class of sugars (higher molecular dissolvable carbohydrates), the following results have special relevance.

In Figure 7a, the reaction rates and the oxygen concentrations are given. After a relatively slow start, yeast extract (2.5 g indicated by arrows) was added. This caused a sharp drop in the oxygen concentration and an increase in the consumption of cellobiose and ammonia, as well as an acceleration of the dry yeast (DY) production. Figure 7b gives the volumetric rates of the ammonia and cellobiose consumption as well as that of the dry yeast production per liter and minute.

TABLE II
Yields and Productivity of *Candida utilis* Cultured on Different Carbon Sources

Carbon source	Yeast production, g DY	Productivity, g DY/l. hr
Glucose	36.5	2.0
Cellobiose	39.8	2.16
Xylose	34.8	1.57

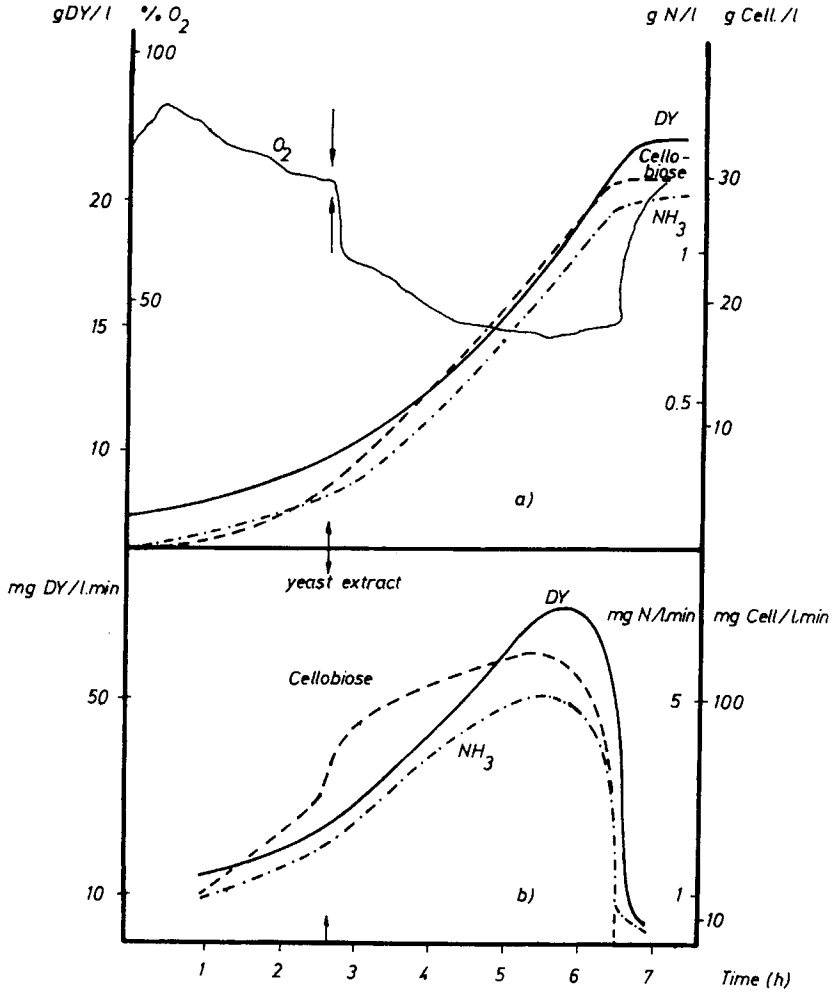


Fig. 7. Growth behavior of *Candida utilis* on cellobiose as carbon source. (a) O_2 = oxygen content (%), consumption of ammonia and cellobiose (in g N/l. and g cellobiose/l.) and the formation of dry yeast (g DY/l.). (b) Growth curves (volumetric rates) in milligrams per liter and minute (mg/l. min).

The results show that the main compounds obtained by the hydrothermal degradation process are efficiently used as carbon source by the yeast *Candida utilis*.

DISCUSSION

In the case of pure cellulose, it was shown that 65.7% of the introduced matter could be recovered as sugars and hydroxymethylfurfural when the optimum reaction temperature was chosen. In addition to the 12.8% residue, the remaining part of 21.5% were not analyzed substances. A portion of the latter percentage will be higher molecular products as, for instance, cellotriose. The recoverable amount in a larger scale apparatus should, therefore, be higher than 65.7%.

As regards straw, it was demonstrated by the experiments with ^{14}C-labeled straw that, within experimental error, the degradation products added up to 100% of the original sample weight. It can be assumed that the larger part of these products can be used either as chemical raw material, direct food reserves, or carbon sources for microorganisms.

The fermentation experiments proved that the applied hexoses and pentoses (glucose, cellobiose, and xylose) are valuable carbon sources for *Candida utilis*, with growth yields of approximately 50%. Even if wood substances are included, it should be possible to convert by the hydrothermal process 60% of the plant matter into usable sugars and furfural compounds. Taking only 50% of the reaction compounds as fermentable products, *Candida utilis* would yield 25% of the introduced plant matter as yeast.

Assuming that the world annual cereal crop produces twice the weight of straw as of grain, a 2.4×10^{12} kg straw harvest would be available. The 25% transformation by the hydrothermal process and microorganism *Candida utilis* would amount to 0.6×10^{12} kg yeast per year. This yeast would contain approximately 0.3×10^{12} kg protein, which is three times the protein value of the whole world cereal crop.

Ten per cent of the annual above-ground plant matter production could yield 4.6×10^{12} kg fermentable sugars. When transformed to yeast, this would constitute ten times the protein content of the world cereal crop.

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