

Enzymatic Saccharification of Rice Hull Cellulose

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Introduction

Plant life on the planet earth synthesizes vast quantities of carbohydrates using solar energy as shown in Fig. 1 (Biomass-I). At

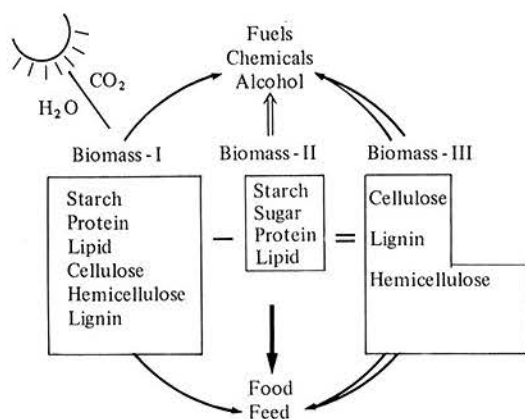


Fig. 1. Conversion of cellulosic biomass¹⁾

least, five billion tons of carbohydrates per year are provisionally estimated to be produced by land based plants alone. Of this biomass-I, about 25% is synthesized in farm fields and most of the remainder is synthesized in forests and grass lands. Furthermore, biomasses are recycled every year, and are, therefore, inexhaustible resources and are very abundant on the earth. But of these carbohydrates, only starch, accounting for a mere several percent of the total carbohydrates is presently being utilized by man directly as a food source. Cellulose, pentosan and hemicellulose (Biomass-III), which represent more than 60% of Biomass-I photosynthesized by solar energy are not being used as food at all. These still unutilized carbohydrates can

be used as food, feed and chemical material if they can be converted into glucose and xylose through hydrolysis. In other words, we do have very large amounts of carbohydrate food resources that can be stored for long periods. In the face of the worldwide food and energy crisis due to the population explosion, we must positively proceed with research and development to make food and fuels etc. from unutilized carbohydrates (Biomass-III), products of synthesis by solar energy which abundantly exists on the earth.

The past researches to convert cellulose into food have merely concerned theoretical possibility but this is likely to become of practical use as a result of the recent remarkable progress in cellulose chemistry and research on hydrolyzing enzyme.

This article analyzes the present state of and problems in research on the enzyme saccharification of cellulose and is intended for reference on cellulose saccharification technology which will soon be developed.

Cellulase enzymes and crystallinity of natural cellulosic fiber

The hydrolysis of cellulose, as a naturally occurring fiber, by cellulase is generally so slow, with a low saccharification rate, that it can not be industrialized if ordinary cellulase producing microbes are used. The difficulties in cellulose hydrolysis with enzymes are due both to strong and insoluble structure of the substrate and to the fact that several enzymes with different actions participate in the hydrolysis of cellulose. As for enzymatic saccharification, the mechanism by which crystalline cellulose is hydrolyzed into glucose remains

partially unsolved. As far as is known to date, the sequential cooperative activity of three major enzyme components in the cellulase complex are required for hydrolysis of crystalline cellulose as proposed by the scheme in Fig. 2. C_1 -enzyme (cellobiohydrolase) acts

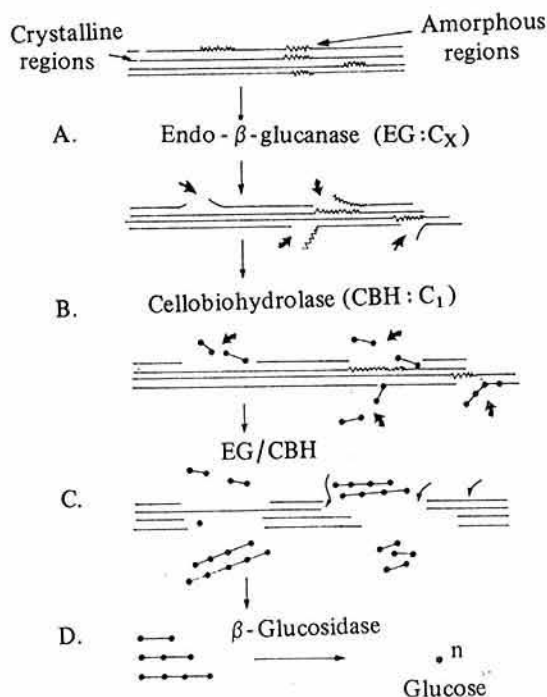


Fig. 2. Schematic representation of sequential stages in cellulolysis¹⁾

on the crystalline structure of cellulose and release cellobiose. C_X -glucanase (endo- β -glucanase) breaks down the amorphous glucose polymer to lower molecular weight compounds, and β -oligomer is converted to glucose by β -glucosidase¹⁾. Of these, C_1 -enzyme acting on the crystal structure of cellulose is particularly important and the intensity of the activity of this enzyme decidedly affects the succeeding enzyme reaction. The enzyme with the greatest activity, found on the earth to date, is *Trichoderma viride* cellulase, which produces a powerful C_1 -enzyme. But even the enzyme of this fungus takes time to decompose crystalline cellulose. Therefore, for effective enzymatic conversion of cellulose to glucose, it is exceedingly important to destroy the crystalline structure of the cellulose before exposing it to enzyme action as shown in Fig. 3. The chemical or physical pretreatment can dispense with the work of the C_1 -enzyme. Active cellulose which has had its crystalline structure already destroyed is quickly hydrolyzed by C_X -glucanase and β -glucosidase, just as the case that gelatinized starch is saccharified by amylase. Moreover, microorganisms producing C_X -glucanase occur widely and some with fairly high activity have been found.

On the other hand, the relationship between the crystallinity of natural cellulose and its

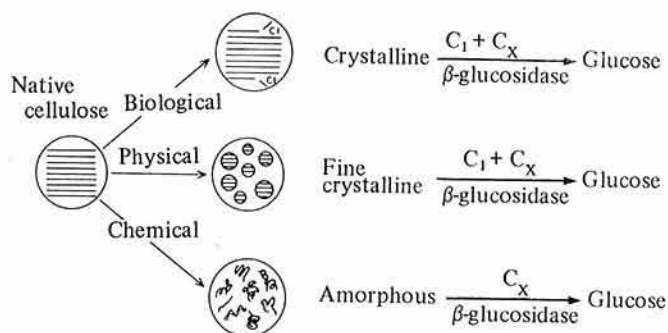








Fig. 3. Degradation of cellulose crystalline structure and action of cellulase complex. C_1 -enzyme and C_X -enzyme mean 1,4- β -glucan cellobiohydrolase and 1,4- β -glucan glucanohydrolase, respectively.³⁾

Table 1. Crystallinity of native cellulose and action of cellulase complex¹⁾

Crystallinity	X-ray diffractogram	Cellulase complex	Estimated hydrolysis
		C ₁ + C _x (<i>T.viride</i>)	Low
		C ₁ + C _x (<i>T.viride</i>)	Low
		C _x (<i>A.niger</i>)	High

enzymatic hydrolysis is shown in Table 1. Here, the three types of naturally occurring cellulose are conveniently classified according to our experimental results. The first type is composed of pure cellulose like cotton with a high degree of crystallinity and the X-ray diffractograms show a unique peak at $2\theta = 22.5^\circ$. Decomposition of highly crystalline cellulose requires C₁-enzyme to destroy the crystalline structure and the estimated decomposition rate is very low. The second type comprises not only crystallized cellulose, the main component, but also an admixture (X) containing varying quantities of lignin, hemicellulose, etc. Biomass cellulose of wood, rice hull, etc. may be classified in this type. Its X-ray diffractograms are shown according to degrees of crystallinity. Although the degree of crystallinity of the second type cellulose is usually lower than a cellulose of the first type, yet it has a crystalline structure. Therefore, the second type also requires C₁-enzyme and the following saccharification rate is low. The third type contains an admixture, but the cellulose is not of crystalline structure and shows no peak in the X-ray diffractogram. Whether plant tissues actually contain this type or not is not known. However, for bio-conversion to glucose this type cellulose does not require C₁-enzyme and can be quickly saccharified, to an extent more than 90%, by C_x-glucanase.

As can be seen from these relations, the presence or absence of crystalline cellulose greatly affects its hydrolysis rate by enzyme. The cellulose materials we are actually going to use as a resource are considered to be mainly of the second type but since these

materials have a crystalline structure, a higher enzymatic saccharification rate could be achieved if they were transformed to the state similar to the third type by destroying their crystallinity.

We shall now discuss methods to degrade the crystalline structure in an effective manner without using a C₁-enzyme.

Destruction of crystalline structure of cellulose

Prior to describing methods of destruction of the crystalline structure of cellulose, we shall examine the basic structure of crystalline cellulose.

Cellulose is a straight-chain polymer composed of cellobiose units of β -1, 4-linkage. Adjoining polymers are compactly coordinated in the lattice spaces with a displacement of 1/4 molecule. All -OH radicals in the glucose residue form intermolecular and intramolecular hydrogen bonds, thus forming a strong crystalline structure. In the case of crystal lattices, such as this, not only enzyme molecules but even water molecules cannot penetrate into cellulose molecules.

Biological, chemical and physical methods are all being tried to breakdown this strong crystalline structure of cellulose (Fig. 3). The biological method consists of destroying the crystalline structure with a C₁-enzyme produced by the above mentioned micro-organism. However, no cellulase capable of quickly destroying the crystalline structure of natural cellulose to an extent more than 90% has been found. Therefore, it is not practical to destroy a crystalline structure by this method alone, although the method can be used in combination with others.

The chemical method used to destroy the crystalline structure of cellulose consists of dissolving cellulose in a solvent and then reprecipitating it. The cellulose can be easily converted to the amorphous type by this method (Fig. 4). Cellulose thus chemically converted to an amorphous type can be quickly saccharified by C_x-glucanase, without the help of C₁-enzyme. The author used acid (sulfuric

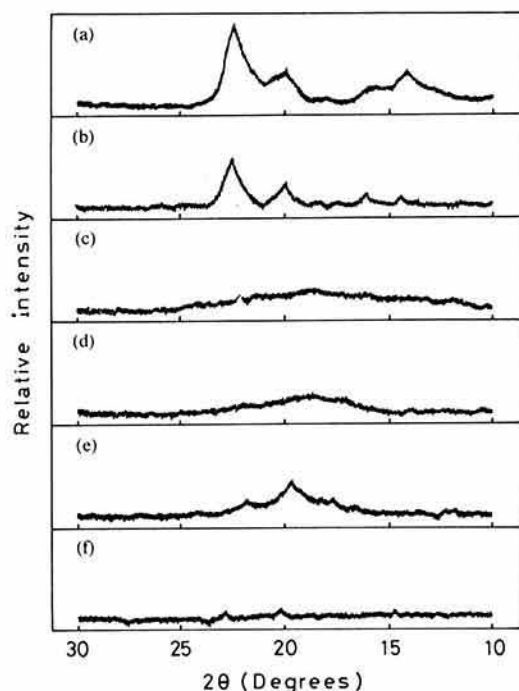


Fig. 4. X-ray diffractograms of various treated celluloses: (a), (b), (c), (d), (e) and (f): same as shown in Table 2.

acid), alkali (cadoxen) or organic (DMSO) cellulose solvents for laboratory tests and has developed a method of dissolving cellulose at low temperatures and then immediately recovering it with acetone. This method successfully converts crystalline cellulose to amorphous cellulose. Amorphous cellulose can be saccharified up to 90% by C_x -glucanase in 24 hr without C_1 -enzyme (Table 2). The use of these solvents, however, is not practical, although this method is without theoretical problems and would become practical if, in the future, one component organic solvent which is recoverable and inexpensive could be found.

The physical method consists of milling the crystalline cellulose physically—mainly by mechanical means. Natick Laboratory, an American group leading the world in this field, mills cellulose at ordinary temperature by a pot mill process. The cellulose is then decomposed by the powerful *T. viride* mutant

Table 2. Correlation between crystallinity and biological susceptibility of various treated celluloses^{a)}

Cellulose	Yield of insoluble fraction (%)	Crystallinity index (I) ^a	Saccharification (%) ^b
a) Untreated		8.8	26
b) Cryo-milled	100	4.9	36
c) Acid-treated (30 min.)	96	0.8	93
d) Acid-treated (60 min.)	93	0.7	94
e) Cadoxen-treated	98	1.0	93
f) DMSO-p-formaldehyde-treated	98	0.8	91

^a I = relative intensity of $2\theta = 22.5^\circ$ / relative intensity of $2\theta = 18^\circ$

^b Data after 48 hrs

cellulase which contains C_1 - and C_x -glucanase and is about three times as active as the naturally occurring type²⁾. This provides a practical saccharification rate. The laboratory is conducting pilot-plant research based on the results of these tests.

On the other hand, we have developed a method to breakdown cellulose structure by cryomilling. This method is ineffective to pure cotton cellulose but very effective to agricultural waste cellulose like rice hull (Fig. 5). The cryomilled agricultural waste cellulose materials show no crystallinity in analysis by X-ray diffractograms and, therefore, do not require C_1 -enzyme. They can be quickly saccharified by C_x -glucanase (e.g. commercial *Aspergillus niger* cellulase). However, the cellulose generally occurring in rice hulls, rice straws, grasses, wood scraps and other agricultural and forestry wastes contains lignin, hemicellulose, etc. as admixtures. These admixtures are strongly bonded with cellulose and impair cellulose decomposition by enzyme action. And also ligno-cellulose cannot be separated by cryomilling alone. In handling untreated cellulose materials, therefore, the removal of lignin is necessary to increase the efficiency of the succeeding step of the enzymatic hydrolysis. Lignin contained in cellulose materials can be easily removed

by heating the materials in a dilute alkali solution. Cellulose thus delignified can be quickly saccharified up to more than 90% by C.-glucanase.

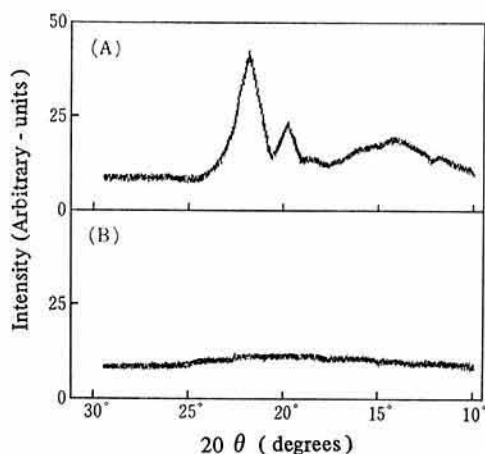


Fig. 5. X-ray diffractogram of rice hull cellulose⁹⁾

- (A) Cryomilled cotton cellulose powder
(B) Cryomilled rice hull cellulose powder

Cryomilling takes advantage of a property of solids. They become very brittle at low temperature and thus mechanical shock is most effective for breaking down the structure of cellulose. Furthermore, the efficiency of this process is high, spending only a short period. Another effect of cryomilling is the decrease in bulk. The exact time required depends on the types of materials, but with this method, cellulose materials can be pulverized in only about 10 min, as compared with 15–16 hr required to pulverize cellulose to 250 mesh by the American pot-mill process. This means a proportional decrease in the energy requirement. What is more is that liquid nitrogen used as a refrigerant in cryomilling can be supplied at a lower price (about 1/2) by using cryogenic energy from the gasification of LNG. At present, sea water is used in Japan to gasify LNG but the lower temperature energy from this process is not being utilized. Currently, interested groups are studying how this can be effectively used for the food industry. Its use

for cryomilling is one effective application.

Application to agricultural by-products

Rice hull is more voluminous than any other Japanese agricultural by-product. And its unused carbohydrate content is as much as about 14% of the total rice starch produced. Moreover, rice hull is produced in large quantities at rice mills in the suburbs of cities. It contains cellulose (38%), hemicellulose (18%), lignin (20%) and silica (18%). These components are strongly bounded as a structural support to protect the hypocotyl. So that, when saccharifying rice hull cellulose, it is necessary to pre-treat it so as to facilitate an enzyme action. The aforementioned cryomilling is effective in breaking down this physically strong structure and the liquid nitrogen used for cryomilling can be cheaply supplied by utilizing the low temperature energy of LNG. This combination of processes is a good example of the effective utilization of resources.

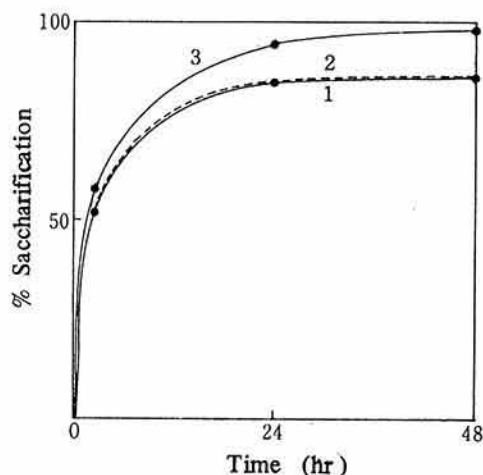


Fig. 6. Saccharification of rice hull cellulose by *T. viride* or *A. niger* cellulase⁹⁾

1. 0.25N NaOH-treated rice hull+*T. viride* cellulase
2. 0.25N NaOH-treated rice hull+*A. niger* cellulase
3. 0.25N NaOH-treated rice hull+*T. viride* cellulase+*A. niger* cellulase



Plate 1. Glucose obtained from rice hull cellulose

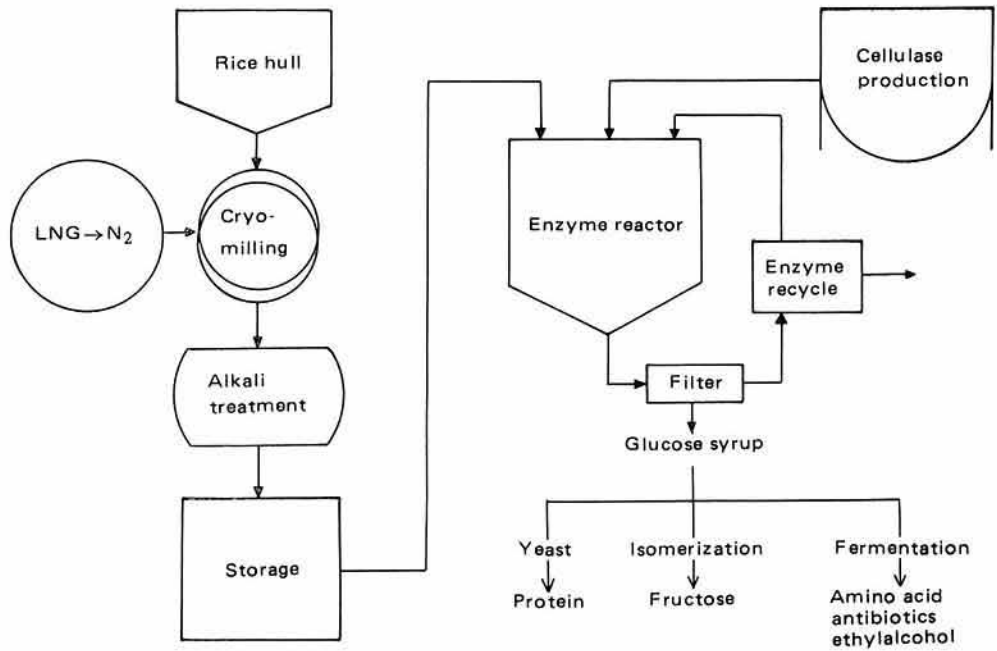


Fig. 7. Flow diagram of a pilot plant for continuous saccharification of rice hulls

The method developed by the author for the saccharification of rice hull cellulose by using the above method is as follows: In processing the rice hull, it was first cryomilled, and X-ray diffractograms of it showed no crystalline cellulose. Next, the cryomilled rice hull cellulose was delignified and then saccharified by *A. niger* cellulase (C_x-glucanase), thereby producing 5–10% glucose syrup saccharified up to more than 80% (Fig. 6). Plate 1 shows photographs of samples at different stages.

Technology to saccharify rice hull cellulose more economically is being planned at present. Fig. 7 shows the flow diagram of the continuous saccharification pilot plant used for this purpose. Rice is unhusked at a rice center neighbouring an LNG base and rice hull is obtained as the by-product. The rice hull is first cryomilled, taking advantage of the LNG, then delignified by dilute alkali treatment before charging in the reactor. Separately produced cellulase is added to the reactor to start saccharification. Sugar thus produced is recovered as glucose syrup after filtration.

Any undecomposed cellulose and enzyme are recycled. The glucose syrup obtained is used as a raw material for the production of microbial protein, for the production of fructose, and the fermentative production of ethanol, amino acids and antibiotics.

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