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ENZYMATIC SACCHARIFICATION OF VARIOUS CELLULOSIC MATERIALS

The possibilities of saccharification of cellulosic wastes in the presence of cellulolytic enzymes of *Aspergillus wentii* and *Trichoderma viride* have been discussed. Corn-cobs, cellulosic masses and fine-particles wastes from the pulp and paper industry are the most susceptible materials to enzymatic action. The alkaline treatment of cellulosic materials 3–4 times increases their susceptibility to *T. viride* enzymes. Since the percent of pulp and paper industry wastes saccharification conducted for 24 h at 50°C, pH 5:2 without pretreatment is as high as 47.4–52.5, hence those materials after enzymatic hydrolysis can be used in the production of single cell microbial protein (SCP).

1. INTRODUCTION

In view of rapid increase in world population and the deepening protein deficiency, the so far underestimated cellulosic waste materials are becoming a potential source of food.

For the time being there is no lack of cellulose. Within one year 1.46×10^9 tons of biomass are renewed by photosynthesis, at the same time, however, the resources of cellulose decrease systematically [1]. More and more amounts of cellulose are utilized by the chemical industry (pulp and paper industry included).

Hence, e.g. the world consumption of paper and cardboard amounting to 128×10^6 tons in 1970 will increase to 218×10^6 tons in 1980 [10] followed by increasing amounts of waste products. Annual production of waste in U.S.A. is 1010×10^6 tons, comprising 250×10^6 tons of municipal refuse (of which cellulose waste is 40–50%) and 200×10^6 tons of agricultural waste [25]. Their reuse is rather low, amounting to 18–20% and 1%, respectively. The increasing amounts of wastes entail the cost of their storage and degradation which in U.S.A. amounted to \$ 1.5×10^9 tons in 1968 [27].

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These facts have inspired the studies on the possible enzymatic saccharification of wastes from wood processing industry (sawdust, bark and branches), pulp and paper industry (waste paper, pulp, solid components of wastewater) and from cellulosic fractions of municipal waste.

In Poland the utilization of straw whose annual production is estimated as being equal to 30×10^6 tons [23] seems also to be an essential problem. However, the economic and organizational reasons, i.e. large volume of this raw material, its availability depending on the season, transportation and storage difficulties, cause an increasing of straw price. In Great Britain the prices of straw (in £/t) in successive years were the following [3]:

	1971/1972	1973/1974	1974/1975
min	2.6	5.5	14.0
max.	5.2	7.0	40.0

Considering the above fact and not resigning from the attempts of straw saccharification, the chief attention of scientists is focussed on the possible utilization of municipal and industrial wastes [4, 8, 12, 13, 17, 27].

Under ideal conditions processes of cellulose degradation should be included in a closed cycle (fig. 1) [2].

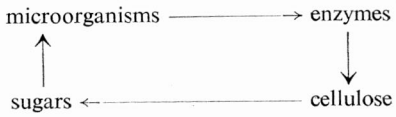

Fig. 1. Microbiological degradation of cellulose
Rys. 1. Mikrobiologiczna degradacja celulozy

Products from particular phases can be separated and used in other processes. Thus, for instance, enzymes produced by microorganisms growing on substrates with cellulose, after being separated from biomass, are used in controlled processes of cellulose degradation to monosaccharides. Those monosaccharides in turn are used in fermentation processes and are a potential source for a number of products (fig. 2).

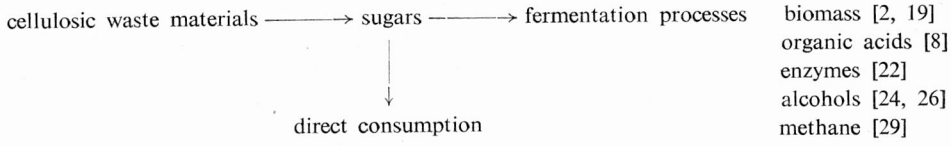

Fig. 2. Potential products which can be obtained from waste cellulose
Rys. 2. Potencjalne produkty możliwe do uzyskania z odpadów celulozowych

Biomass formed in fermentation processes, being the growth effect of yeast [6], bacteria [8], or a mixed culture of those microorganisms [9], is an excellent source of full-value protein, vitamins and antibiotics [12].

2. CHARACTERISTICS OF CELLULOSIC WASTE MATERIALS

In the optimization of the saccharification processes the insolubility of cellulose is the most essential problem, hence the extent of reaction degree closely depends upon the area available for enzymes. Many cellulosic waste materials contain lignins that do not inhibit cellulolytic enzymes, but being combined e. g. with silicates they restrict effectively the access of celluloses to the substrate [8]. The chemical composition of various vegetable raw materials in great part depends not only on the kind and age of the plant but also on the climatic zone of its habitat, site of sampling, methods used for determining and many other factors. Hence, quantitative composition of the separate components in cellulosic raw materials varies within a wide range. The data referring to chemical analysis of some cellulosic waste materials are given in table 1.

Table 1

Chemical composition of various raw cellulose materials (in % of dry matter)
Skład chemiczny różnych surowców celulozowych (% s. m.)

Kind of raw material	Holocellulose	Lignins	Protein	Free nitrogen	Ash	Extraction components	References
Linters	90	0.84	3.27	3.69	0.42	1.69	[18]
Rice straw	34-35	4-4.5	4.5-5.0	36.7-42.0	17.36	1.5-2.4	[8, 18]
Straw (wheat, oat)	37-46	7.4-8.0	2.7-7.7	35.5	6.3-10.4	1.2-2.0	[18]
Corn-cobs	37	10.4	2.61	46.89	2.06	0.87	[18]
Pine sawdust	63-69	26-32	—	—	0.2-0.4	4.4-5.9	[23]
Pine sawdust	75	8.45	0.42	13.16	0.21	2.89	[18]

Many factors (such as cellulose delignification, the swelling effect of acids and bases, radiation, preliminary oxidation in $Fe^{+2} - H_2O_2$ system, heating in the reduction medium or mechanical crushing) decrease the degree of cellulose crystallinity and at the same time increase its susceptibility to cellulolytic enzymes.

Each of the factors mentioned, apart from the considerable increase in saccharification costs, creates additional difficulties. In the process of cellulose heating, besides a reactive cellulose an exceptionally hydrolysis-resistant oxidized form is produced; the hydrolysis products are characterized by the dark colours [12]. Due to the cellulose swelling, the substrate becomes fluffy and not easily applied in the desired concentrations in enzymatic reactors; it is accompanied by large amounts of wastewater.

It seems that the optimal variant of a preliminary preparation of cellulosic substrates is to desintegrate them at room temperature and conduct the saccharification process in the presence of enzymatic preparations containing the whole complex of enzymes indispensable in the hydrolysis of cellulose.

3. MATERIAL AND METHODS

3.1. ENZYMATIC PREPARATIONS

Enzymes used in the saccharification process came from the filtrates of culture media of *Aspergillus wentii*, *A. niger* and *Trichoderma viride*. Enzymatic preparations were applied both in liquid form and isopropanol precipitate. A commercial preparation of cellulose produced by Novo firm was also used in the investigations. The saccharification degree of cellulosic material was defined by quantitative determining of the reducing sugars produced in the hydrolysis and weight loss of the sample analysed.

3.2. REDUCING SUGARS

They were determined quantitatively by the colorimetric method of SOMOGYI-NELSON [14, 21]. Results were read from a curve made for the solution of glucose.

3.3. PROTEIN

The protein content in preparations was determined by Lowry's method at the wavelength of 565 nm [11] and using an analytical curve made for albumins.

3.4. PRETREATMENT OF CELLULOSIC MATERIALS

In order to increase the susceptibility of cellulosic material to enzymatic activity, the following procedures have been applied:

1. The disintegrating of cellulosic products. This process was conducted in the mill of the Bąk type (produced by factory of farming implement "Jawor"). The product obtained was sifted on sieves aiming to obtain a possibly homogenous fraction, size of particle being $\varphi = 0.3\text{–}0.5 \times 10^{-3}$ m.

2. Heating in alkaline medium:

a) crushed straw; it was at first treated with 0–8.0% of NaOH at an elevated pressure (1 atm, 9.81×10^4 Pa) for 2 hours; then it was filtered, thoroughly washed with water and dried at 80°C;

b) cellulosic substrates; they were treated with 1.2 and 3% amonia at room temperature for 24 hours.

3. Heating in a reducing medium (saturated sodium sulphite) at 200°C for 1 hour.

3.5. CELLULOSIC SUBSTRATES

The following cellulosic materials were saccharified: brich sawdust, sunflower husks and stems, straw (rye, wheat, oat), corn-cobs, cellulosic masses (birch and fir-spruce), sulphite cellulose, linters and fine-particle waste from the waste paper grinding. The latter seems to be particularly interesting since besides cellulose this waste material contains large amounts of kaolin and glues used as filling material in the production of paper is still poorly utilized (in building). Huge amounts of those substances being discharged into rivers constitute hazards to their biocenosis, and, moreover, many tons of valuable saccharides are lost.

The data on the saccharification degree of CM-cellulose, filter paper and xylan are given for comparative reasons.

3.6. DETERMINATION OF THE SACCHARIFICATION DEGREE

The calculations based on the amount of the reducing sugars (converted in glucose), produced in a given time (0–48 h) in the reaction mixture, were performed according to the formula:

$$\frac{162 \times \text{mg of reducing sugars/ml} \times 100}{180 \times \text{mg of substrate/ml}} = \% \text{ of cellulose saccharification,}$$

The reaction was conducted at 45–50°C in the 100 ml flasks. They were placed in the laboratory shaker, and the samples for determination were periodically taken.

The degree of cellulose degradation was also determined from the weight loss of analysed sample. To this end a definite amount of aerial dry substrate was carefully washed with water and subject to the enzymatic hydrolysis. Thereupon the samples were quantitatively transferred on the filters, and after having washed with distilled water they were dried up again to constant weights. Analogical procedure was used to the control sample, containing buffer in place of enzyme. The degree of saccharification was calculated from the proportion:

$$\frac{m_0 - m_t}{m_0} \times 100 = \% \text{ of cellulose saccharification,}$$

where

m_0 — mass of control sample in time t_x,
m_t — mass of sample examined in time t_x,
x — time of enzymatic hydrolysis 0–48 h.

3.7. DETERMINATION OF CELLULOSE CONTENT IN WASTE FROM THE PAPER AND PULP INDUSTRY

To determine exactly the saccharification degree of cellulose in waste paper, its content was determined quantitatively according to the acid hydrolysis method [15].

It has been found that of 1.9% of dry matter stated in industrial waste 24.75% falls to cellulose, the remaining 75.25% falls to glues and kaolin which do not disturb the process of enzymatic hydrolysis of cellulose.

3.8. DETERMINATION OF THE ACTIVITY OF ENZYMATIC PREPARATIONS

There exist many controversies concerning the mechanism of hydrolytic activity of enzymes of cellulolytic complex and terminology of the separate enzymes [16, 28]. In the present paper the following terms have been assumed:

xylanase, β-1.4 xylan xylanohydrolase,

cellulase C_x, β-1.4 glucan endoglucanase (it hydrolyzes CM-cellulose),

cellulase C_1, exocellulase β-1.4 glucane cellobiohydrolase (it saccharificates filter paper).

Activities of enzymes have been expressed in mg of reducing sugars produced from xylane, filter paper and CM-cellulose by 1 ml of filtrate from the culture medium of molds (unit/ml) or by 1 mg of a dry enzymatic preparation (unit/mg).

The quantity of enzyme which under optimal reaction conditions induced the production of 1 mg of reducing sugars (converted in glucose) during 1 h has been assumed as the unit of enzyme activity.

4. RESULTS

Efficiency of the cellulosic materials hydrolysis depends on a number of factors of which the most important are: type of substrate, its concentration and kind of pretreatment, character of enzymatic preparations, temperature, pH and the process duration. Many of these factors are mutually dependent, hence the whole saccharification process is extremely complicated.

The disintegrated cellulosic raw materials, depending on their origin (annual or perennial plants) and the way of their processing in the production process (wood pulp, cellulosic industry wastes, filter paper) undergo enzymatic hydrolysis in various degrees (table 2).

The most susceptible to cellulolytic enzymes of both the moulds are modified cellulosic substrates (spruce pulp, sulphite cellulose, paper industry wastes) and some farming wastes (corn-cobs, rye straw). Their saccharification degree ranges within 14–50%. Big differences in the degradation of substrates are observed, while comparing the enzymatic effects of *A. wentii* and *T. viride*. The enzymes of *A. wentii* hydrolyze xylan in 43% and CM-cellulose merely in 10%, while the saccharification of CM-cellulose due to cellulase C_x in *T. viride* is as high as 33%. This fact finds its confirmation in the saccharification degree stated for the separate cellulosic substrates. *T. viride* hydrolyzes the substrates with high contents of cellulose (cotton, linters, filter paper, sulphite cellulose) much quicker and more efficiently than *A. wentii*.

Both thermal and alkaline treatments increase the cellulose susceptibility to hydrolases (table 3); in native substrates this effect was higher than in the case of the newsprint paper.

Cellulosic materials are most often modified with soda lye. This treatment is connected with relatively low material cost and easy neutralization of alkaline wastewater. Saccharification efficiency of the soda lye treated substrate (table 4) is 3–4 times higher compared with that of a native substrate reaching 40–50%. The loss in mass of the samples analysed is equally high.

Table 2

Hydrolysis of cellulose wastes by a cellulolytic complex of *A. wentii* and *T. viride*
Hydroliza odpadów celulozowych przez kompleks celulolityczny *A. wentii* i *T. viride*

1% substrate	*A. wentii*			*T. virde* 9414		
	Saccharification percent in time (hours)					
	4	24	48	4	24	48
Birch sawdust	0.1	2.0	2.5	0.6	2.5	5.5
Sunflower stems	1.0	3.3	4.7	3.6	7.3	9.5
Rye straw	2.5	4.3	6.1	4.5	7.2	13.6
Wheat straw	1.6	2.3	5.4	3.3	6.5	9.9
Oat straw	2.5	5.2	6.3	2.4	3.1	6.3
Corn-cobs	3.7	10.1	12.4	11.9	20.8	24.6
Birch cellulosic pulp	0.4	3.2	5.1	2.7	7.8	18.0
Spruce cellulosic pulp	5.7	11.8	13.4	4.1	12.3	20.7
Sulphite cellulose	2.2	6.3	15.8	8.9	29.8	49.7
Wastes from cellulose industry	4.9	13.9	22.2	10.4	16.4	—
Cotton (fibres)	0.4	1.0	2.7	2.1	7.2	11.6
Cotton (linters)	0.6	1.3	2.8	2.7	9.0	22.5
Filter paper	0.6	1.6	6.8	3.9	20.4	31.4
Solko-floc cellulose	1.9	5.8	7.4	—	—	—
CM-cellulose	5.1	9.1	9.9	20.4	27.0	33.0
Xylan	13.7	34.9	42.8	12.1	21.0	28.3

— was not determined

Experimental conditions: 0.5 g of ground substrate + 30 ml of 0.05 M acetic buffer (pH 4.6) + 20 ml of filtrate from culture medium (*A. wentii* or *T. viride*) of the activities of xylanase equal to 40 units/ml and 27 units/ml and of cellulase C_x being 9.8 units/ml and 16 units/ml were incubated in flasks on shaker at 45°C.

Table 3

Change in the susceptibility of cellulosic substrates to *A. wentii* enzymes due to some modifying factors
Zmiana podatności substratów celulozowych na działanie enzymów *A. wentii* pod wpływem niektórych czynników modyfikujących

Kind of pretreatment	5% substrate			
	Saccharification percent of substrate with respect to native substrate			
	Rye straw	Newsprint paper	Birh sawdust	Sunflower stems
Native substrate	100	100	100	100
1% ammonia	141	135	204	125
2% ammonia	147	132	228	127
3% ammonia	153	126	240	143
Sodium sulphite, 200°C	194	135	161	107

Experimental conditions as in table 1.

Table 4

Effect of alcaline treatment of rye straw on the degree of saccharification by *T. viride* 9414 enzymes
Wpływ alkalicznej obróbki słomy żytniej na stopień scukrzenia enzymami *T. viride* 9414

Substrate pretreatment	Saccharification percent of the substrate of the concentration in incubated mixture		Mass loss percent of the substrate of the concentration in incubated mixture	
	5%	10%	5%	10%
Control	13.4	12.0	12.1	11.8
0.0% NaOH	19.0	15.2	23.0	21.8
2.4% NaOH	26.0	21.5	37.0	36.0
4.4% NaOH	37.1	32.2	45.0	43.1
5.5% NaOH	41.0	32.5	47.3	43.8
6.6% NaOH	43.5	32.9	52.8	49.3
8.0% NaOH	49.0	37.5	62.0	55.2

Experimental conditions: 5 or 10 gr of substrate (native and after alkaline treatment under previously described conditions) was poured with 20 ml of filtrate from culture media of *T. viride* and 70 or 75 ml of 0.05 M acetic buffer (pH 4.6) and incubated at 50°C for 24 h. Xylanase and cellulase C_X activities amounted to 43 units/ml and 24.5 units/ml of the filtrate, respectively.

Table 5

The effect of pH on the hydrolysis degree of the pulp and paper industry wastes
Wpływ pH na stopień hydrolizy odpadów przemysłu papierniczego

Enzyme source	pH	Percent of substrate saccharification in time (hours)			
		2	4	24	48
A. wentii	4.0	4.7	4.8	11.6	14.8
	4.6	3.6	4.6	11.0	19.1
	5.2	4.9	5.4	13.9	22.2
A. niger	4.0	11.1	12.7	24.6	31.8
	4.6	8.3	11.2	32.4	34.8
	5.2	11.8	12.2	28.8	41.3

Experimental conditions: 25 ml of aqueous suspension of pulp and paper industry wastes (dry weight 1.9%, cellulose content — 24.75% of dry weight) + 5 ml of 0.05 M acetic buffer of different pH values + 5 ml of filtrate from culture media + 0.005% of methiolate were incubated at 45°C for 48 h. The activities of moulds in unit/ml of preparations were

	A. wentii	*A. niger*
xylanase	64.0	114.0
C_X	10.8	12.5
C_1	0.4	1.8

Considering the fact that during alkaline treatment at high temperatures most hemicelluloses which are also valuable carbohydrates are washed out, further investigations aimed to find optimal conditions for saccharification of non-pretreated cellulosic wastes.

Results from the investigations on the effect of pH on the saccharification degree of pulp and paper industry wastes due to *A. wentii* and *A. niger* are presented in table 5.

In the first stage of enzymatic hydrolysis (up to 4h) the optimum activities of both the enzymatic preparations were stated at pH 4.0 and 5.2 which proves the presence of at least two enzymes: cellulase (optimum activity at pH 4–4.2 [7]) and xylanase (optimum activity at pH 5.2 [7]). With the increasing reaction time the optimum pH value has been established as ranging within 4.6–5.2. Such a close correlation between reaction time and optimum pH for the activity of enzymatic preparation is related to the change in the medium activity during cellulose saccharification which is followed with activation or inhibition of various enzyme groups of the cellulolytic complex.

The composition of the complex of glycosides degrading vegetable polysaccharides depends to a great extent on the origin of the enzymes [5, 20, 28]. It is known, for instance, that *T. viride* moulds produce a cellulolytic complex including exo- and endo-β-1.4 glucanases (cellulases C_1 and C_x) in a quantitative ratio guaranteeing an optimal hydrolysis of the native cellulose. *A. wentii*, in turn, does not hardly produce β-1.4 glukane cellobiohydrolase (C_1), but large amounts of endoglucanase (C_x) and xylanase. Different compositions of enzymatic preparations influence the hydrolysis degree of different vegetable materials (table 2). Hence, it seemed interesting to combine the enzymatic preparations of *A. wentii* and *T. viride* with the commercial preparation — cellulase of the firm Novo for the application to the saccharification process. Since the pulp and paper industry wastes contain cellulose almost exclusively, thus the investigations were performed on corn wastes which because of their more differentiated chemical composition could be assumed as a more raliable substrate. Results are shown in table 6.

From the data obtained, it follows there exist no strict proportionality between the amount of the enzyme used and the degree of substrate saccharification. A relatively lower saccharification percent (at higher enzyme concentration) is caused not only by catabolic repression but also by a small amount of amorphous regions in a cellulosic substrate, available for enzymatic activity.

While analysing the data connected with the application of the combined enzymatic preparations (table 6) it seems that under the experimental conditions presented above, there is an interaction of enzymes produced by the investigated strains which would justify further investigations on the application of the combined enzymatic preparations to the saccharification of cellulose.

β-1.4 gluconases and probably β-1.4 xylanases belong to the induced enzymes [16] produced by moulds in substrates containing an appropriate carbon source. That is why the straw used formerly in the culture media of *A. wentii* was replaced by waste products from the pulp and paper industry. Enzymes obtained from the filtrates of the above substrates were used in saccharification of those waste products. Results are presented in table 7.

Table 6

The effect of the amount of enzymes and their origin on the saccharification degree of corn wastes

Wpływ ilości i pochodzenia enzymów na stopień scukrzania odpadów kukurydzy

Enzyme source	Amount of enzyme (mg)	Degree of saccharification in time (hours)			
		2	4	24	48
A. wentii	5	1.7	2.1	6.1	8.0
	10	2.9	3.7	10.3	12.8
T. viride 9414	5	3.9	7.6	14.5	16.2
	10	4.0	11.9	20.9	24.6
Cellulase „novo"	10	4.1	12.5	24.0	—
A. wentii + *T. viride*	5+5	3.9	11.8	21.8	25.3
A. wentii + cellullase "novo"	5+5	6.3	17.5	28.0	—

Experimental conditions: 5 (10) mg of dry enzymatic preparation + 0.3 g (1%) of substrate + 30 ml of 0.025 M acetic buffer (pH 5.2) + 0.005% merthiolate were incubated at 50°C for 48 h. The activities in unit/ml preparations of were:

	A. wentii	*T. viride*	cellulase "novo"
xylanase	10.5	5.6	2.1
C_x	1.5	2.4	4.3

Table 7

Saccharification of pulp and paper industry wastes by induced enzymes of *A. wentii* and combined enzymatic preparations of *A. wentii*, *T. viride* 1.03 and cellulase "novo"

Scukrzanie odpadów przemysłu papierniczego przez indukowane enzymy *A. wentii* i połączone preparaty enzymatyczne *A. wentii*, *T. viride* 1.03 i celulazę „novo"

Kind of enzymatic preparation	Percent of substrate saccharification in time (hours)			
	1	3	6	24
A. wentii	6.2	11.4	15.6	23.5
T. viride 1.03	1.4	3.4	3.8	3.8
Cellulase „novo"	12.0	16.9	20.5	34.9
A. wentii + *T. viride*	4.8	10.0	12.5	19.8
A. wentii + *cellulase* "novo"	13.6	29.3	35.4	52.5
T. viride + *cellulase* "novo"	11.4	19.1	28.0	47.4

Experimental conditions: 10 ml of cellulose waste suspension + 2.5 ml of enzyme solution + 2.5 ml of 0.05 M acetic buffer (pH 5.2) + 0.005% methiolate were incubated on a laboratory shaker at 50°C. The activities of xylanase and cellulase in separate preparations in unit/ml were:

	A. wentii	*T. viride*	cellulase "novo"
xylanase	60.7	2.8	8.4
C_x	13.0	1.0	17.4
C_1	0.4	0.2	0.9

Combined enzymes contained 50% of the separate preparations.

The same saccharification effect (ca 23%) which was achieved within 48 h in the case of enzymes from standard substrates (table 5) was obtained within 24 h by applying a cellulolytic complex produced on the inducing substrate. The application of combined enzymatic preparations also substantially increases the saccharification degree of wastes, amounting to 20–52.5% within 24 h. The highest degree of cellulosic wastes saccharification can be obtained by applying the mixture of *A. wentii* or *T. viride* enzymes with commercial preparation of cellulose produced by the firm Novo.

Preliminary attempts of yeast (*Candida utilis*) culture on enzymatic hydrolysates of cellulosic wastes gave positive results. Results of investigations are to be published in a separate paper.

5. CONCLUSIONS

The so far applied methods for the control of cellulosic wastes are the permanent and continuously increasing hazard of the environment, in particular of water and air. At the same time, the problem of future deficiency of nutritive protein is more and more often taken into consideration. In such a situation it seems logically justified to undertake the studies on the possible saccharification of cellulose wastes and utilization of the hydrolysis products as substrates in production of SCP.

Of the cellulosic substrates investigated the highest degree of saccharification has been found for corn-cobs, cellulosic masses and fine-particle wastes from the pulp and paper industry. The increase in saccharification degree is closely connected with the increased number of amorphous regions, thereby with the better hydratation of cellulose molecules.

Thermal and alkaline treatment, depending on the kind of substrate, increases 1.5–4 times its susceptibility to cellulases. In view of the fact that pretreatment increases the saccharification cost, requires an additional energy and apparatus it seems plausible to find the procedures which would eliminate the above shortcomings.

The rate and efficiency of saccharification process is conditioned to a large extent by the pH of reaction mixture, it is also essential that the optimum pH depends on the reaction time. For mould of *Aspergillus* genus the optimum pH of enzyme activity amounts to 4.6–5.2 when saccharification lasts for 24–48 h. The lack of the proportionality between the hydrolysis time and the saccharification degree is due to inactivation of enzyme during reaction and to inhibitory effect of the glucose which is being produced.

From the results obtained, it also follows that the amount of the enzyme added and the saccharification degree are not simply proportional. An increase of the enzyme concentration at a constant initial concentration of the substrate results in inhibition of the saccharification rate at each stage of hydrolysis. A synergic effect of the separate enzymes of the cellulolytic complex is visible with respect to the fine-particle fraction of pulp and paper industry wastes. Their saccharification is due to the combined preparations of *A. wentii* or *T. viride* with cellulose "novo" amounts to about 50% within 24 h. It is of interest that such a high saccharification percent of waste cellulosic material was achieved

without the pretreatment of the substrate; hence this waste can be utilized as a potential source of carbohydrates for the production of SCP (single cell protein). Summing up, it can be stated that:

for every cellulosic material optimal saccharification conditions should be determined by establishing for the given time (24–48 h) and temperature (45–50°C) of the process an optimum pH of reaction as well as the amount and composition of the added enzymatic preparation;

selection of the substrate should depend on the local conditions, taking account of the fact that in order for the saccharification process to be justified economically, it should be inexpensive (including the costs of its accumulation, transport and labour) and its pretreatment limited to the minimum.

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ENZYMATYCZNE SCUKRZANIE RÓŻNYCH MATERIAŁÓW CELULOZOWYCH

W pracy omówiono możliwości scukrzania odpadów celulozowych przy udziale enzymów celulolitycznych pleśni *Aspergillus wentii* i *Trichoderma viride*.

Najbardziej podatne na działanie enzymów są kolby kukurydziane, masy celulozowe i drobnocząsteczkowe odpady przemysłu papierniczego. Obróbka alkaliczna materiałów celulozowych zwiększa 3–4 krotnie ich podatność na działanie enzymów *T. viride*. Frakcja odpadów przemysłu papierniczego, bez wstępnej obróbki, w temperaturze 50°C, w czasie 24 godzin i przy pH 5,2, ulega scukrzeniu w 47,4–52,5%, co wskazuje na możliwości wykorzystania jej po uprzedniej hydrolizie enzymatycznej do produkcji białka mikrobiologicznego (SCP).

ENZYMATISCHE VERZUCKERUNG VERSCHIEDENER ZELLULOSEDERIVATE

Im vorliegenden Beitrag werden die Möglichkeiten der Verzuckerung verschiedener Zelluloseabfallstoffen mit Hilfe der zellulolytischen Enzyme des *Aspergillus wentii* und *Trichoderma viride* beschrieben.

Der Enzymwirkung unterliegen sehr leicht Maiskolben, Zellstoff und feindispergierte Abfallstoffe der Papierindustrie. Eine Voralkalysierung aktiviert 3–4 mal die Zellulase von *T. viride*. Abfallstoffe der Papierindustrie werden bei 50°C, einer Reaktion pH = 5,2 und einer Reaktionszeit von 24 Stunden — d.h. ohne irgend einer Vorbehandlung — in einer Menge von 47,4 bis 52,5% verzuckert. Wird eine enzymatische Hydrolyse vorgeschaltet, dann besteht die Möglichkeit der Herstellung von mikrobiellen Eiweiß (SCP).

ФЕРМЕНТАТИВНОЕ ОСАХАРИВАНИЕ РАЗЛИЧНЫХ ЦЕЛЛЮЛОЗНЫХ МАТЕРИАЛОВ

В работе обсуждены возможности осахаривания целлюлозных отходов при участии целлюлолитических ферментов плесени *Aspergillus wentii* и *Trichoderma viride*.

Наболее податливыми на действие ферментов являются кукурузные початки, целлюлозные массы и мелкочастичные отходы бужамной промышленности. Щёлочная обработка целлюлозных материалов увеличивает в 3-4 раза их податливость на действие ферментов *T. viride*. Фракция отходов бумажной промышленности, без предварительной обрайботки, при температуре 50°C, в течение 24 часов и при pH 5,2, подвергается осахарению в 47,4-52,5%, что указывает на возможности использования её после предварительного ферментативного гидролиза для производства микробиологического белка (SCP).