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Studies on the Utilization of Straw for Production of Glucose Isomerase

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볏짚을 이용한 Glucose Isomerase 생산에 관한 연구

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Abstract

The hemicellulose fraction of ryegrass straw was extracted with NaOH and used for production of glucose isomerase by *Streptomyces flavogriseus*. Up to 25% crude hemicellulose (20% as pentosan) could be obtained by treating straw with 1 to 24% NaOH. The yield of hemicellulose was increased proportionately with increasing NaOH concentration up to 4%, but the rate of increase was slowed thereafter. The optimum condition for hemicellulose extraction from ryegrass straw was to treat straw with 4% NaOH for 3hrs at 90°C or 24hrs at 30°C. Highest level of glucose isomerase activity (3.04 units/m*l* culture) was obtained when the organism was grown for 2 days at 30°C on 2% straw hemicellulose. The organism also produced a good quantity of glucose isomerase on xylan, xylose or H_2SO_4-hydrolysate of straw. The hemicellulose-extracted straw residue could be used as animal feed, because the residue had 75% higher digestibility and 20% better feed efficiency for weanling meadow voles than the untreated straw.

Introduction

Each year vast amount of crop residues are produced (about 60% of the total crop production), which have not been effectively utilized because they are bulky, difficult to digest and have little fuel energy per unit volume. Agricultural crop residues consist mainly of cellulose. hemicellulose, lignin, pectin, and other plant carbohydrates. Because of the nature, crop residues can be best utilized for fuel, fiber, fertilizer, feed and food, and production of chemicals.

Ryegrass straw contains about 25% hemicellulose and about 65% of it is xylose, which has been used as an inducer-substrate for glucose isomerase production by many microorganisms. Purified xylose is expensive, so, production cost of glucose isomerase from xylose is high. Grass straw could be used as a substrate for production of glucose isomerase of an organism capable of producing the enzyme from straw was found. Chen et al[2] (1978)

reported the isolation of an organism which they identified as a strain of *Streptomyces flavogriseus*. The organism produced a good quantity of glucose isomerase when grown on straw hemicellulose.

This paper reports the production of straw hemicellulose and glucose isomerase that was obtained by growing *S. flavogriseus* ons traw hemicellulose. Utilization of straw residue obtained after extraction of hemicellulose is also disscussed.

Material and Methods

1. Microorganism

A strain of *Streptomyces flavogriseus* was isolated from soil and used for production of glucose isomerase. The cultural characteristics are reported elsewhere (Chen et al, 1978).[2] The organism was grown on a medium containing 2.5% corn steep liquor, 0.1% $MgSO_4$ and different level of straw hemicellulose. PH of the medium was adiusted to 7.0.

2. Substrate

Straw hemicellulose was prepared as follow: Ten g of sun dried annual ryegrass straw was mixed with 100m*l* of 1~24% NaOH and kept at 30~120°C for 1~48 hr. Treated straw was then expressed through 8 layers of cheese cloth and the residual straw was washed twice with distilled water. The filtrates were combined to make the final volume of 100 m*l* and the pH adjusted to 5.0 with HCl. To the filtrate 150 m*l* of 95% ethanol was added and the mixture was set at room temperatture for 24 hr. The precipitate was collected and washed with 70% ethanol to remove lignin and other solubles. The precipitate was then dehydrated with 95% ethanol and the ethanol was removed by filtration and vaccum drying at 50°C. The dry product was used as straw hemicellulose.

3. Analytical procedures

Pentosan was determined according to the modified method of AOAC (1970).[1] Aabout 0.2g of crude hemicellulose was placed in a 200 m*l* distillation flask containing 100 m*l* of 12% HCl and several pieces of boiling chips. The flask was connected to a condensor and heated to distill 30 m*l* in 10 min. During the distillation 12% HCl was constantly replaced and the total volume of 360 m*l* of distillate was collected.

Forty m*l* of phloroglucinol solution (7.3 g of phloroglucinol in one liter of 12% HCl) was added to the distillate. After standing overnight, the precipitate was collected on a Gooch crucible and washed with 150 m*l* of distilled water.

Glucose isomerase was determined according to the cystein-carbazole method of Dishe and Borenfreund (1951).[3] Cellulose, hemicellulose, lignin, and ash were deptermined by the method of Goering and Van Soest (1970).[4] *In vitro* rumen digestibility (IVRD) was determined by the modified method of Mellenberger et al. (1970).[9] Crude protein was determined by multiplying a factor of 6.25 to the difference between total N and NH_3-N. Total N was determined by micro-Kieldahl method of Perrin (1953)[10] and NH_3-N was determined by the method of Jackson (1960).[7]

4. Animal feeding trial

Weanling meadow voles (*Microtus canicadus*) were Prpvoded by L. G. Forslund of Oregon State University. About 3 week old, uniform size, male voles were randomly selected from 14 litters. Age and average weight of voles at the begining of each experiments were 21 day and 18.5 g, respectively. A group of 10 animals were subiected to each diet, The voles were fed from an aluminum cup (10 cm diameter and 5 cm deep). Average daily weight gain and feed consumption were measured during 8 day feeding period after 2 days adjustment period to the diet. The diet consited of soybean meal 120 g, corn oil 50 g, mineral mix (Jones and Foster, 1942)[8] 20 g, ground corn 510 g, and 300 g of hemicellulose-extracted straw. Control diet contained 810 g of ground corn and had no straw.

Results and Discussion

Hemicellulose in grass straw was easily extracted by soaking the straw in 4 to 24% NaOH at 100C for 3 hr or at room temperature for 24 hr. As much as 25% hemicellulose (20% as pentosan) could be obtained by treating the straw with 15% NaOH for 3 hr at 100°C (Fig. 1). The level of hemicellulose extracted was gradually increased as the concentration of NaOH increased upto about 10%, thereafter the rate of increase wal slowed. The rate of decrease in residual straw was inversly proportional to that of hemicellulose removal. Almost 85% of initial straw was solubilized by treating straw with 10% NaOH at 100°C for 3 hr.

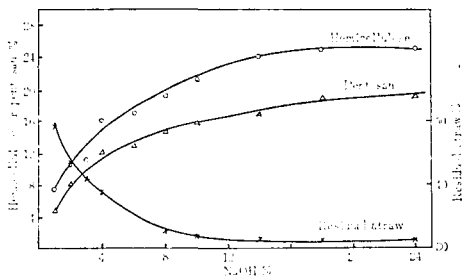

Fig. 1. Effect of NaOH Concentration on the Extraction of Hemicellulose from Ryegrass Straw. One part of ryegrass straw was extracted with 10 parts of NaOH at 100 C for 3 hrs.

Extraction of hemicellulose from grass straw was facilitated by combined application of heat and NaOH. When treated with 4% NaOH for 3 hr, the highest yield of hemicellulose was obtained at 90°C (Fig. 2). The yield was declined as the temperature was further increated, probably due to decomposition of hemicellulose. When straw was treated with 4% NaOH at 30°C for 24 hr, it produced 15.5% hemicellulose and 12.4% pentosan. This level was equivalent to that obating at 90C for 3 hr. Thus, choice of optimum condition depended on the economic and convenience of using high tmeperatureshort time or low temperature-long time. For our study, 30°C for 24 hr was chosen as a standad conditions for extraction of hemicellulose.

With the standard condition, hemicellulose was rapidly extracted during the first 12 hr and then the rate of extraction slowed thereafter. (Fig. 3).

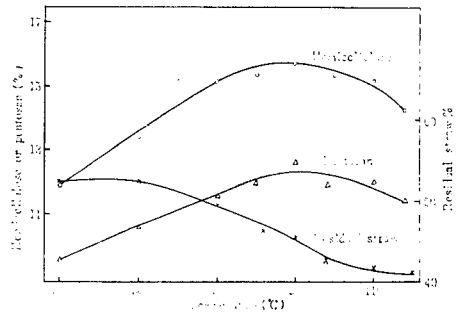

Fig. 2. Effect of Temperture on the Extraction of Hemicellulose from Ryegass Straw. One part of ryegrass straw was extracted with 10 parts of 4% NaOH at different temperature for 3 hrs.

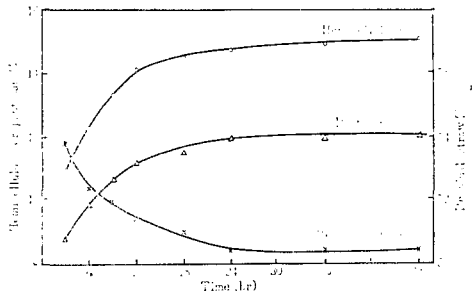

Fig. 3. Time Course of Hemicellulose Extraction from Ryegrass Straw.
One part of ryegrass straw was extracted with 10 parts of 4% NaOH at 30C.

After 24 hr, there was no appreciable degree of hemicellulose extraction nor disappearance of residual straw.

The organism produced a high level of glucose isomerase when grown on straw hemicellulose, xylan or xylose (Table 1). Optimum substrate concentration for the enzyme production was 2% hemicellulose, from which 3.04 units/ml of enzyme was produced. Specific activity of the enzyme was also highest at this conentration. Eventhough microbial cell growth attained maximum at 1%

hemicellulose, the level of enzyme production was significantly lower at this concentration. Optimum concentration for xylan and xylose was 3.0% and 1.25%, respectively.

Table 1. Effect of Substrate Concentration on Cell Growth and Production of Glucose Isomerase by *S. flavogriseus.* [a]

Substrate (%)	cell Growth (mg protein/ml culture)	Enzyme production[b] (units/ml culture)	Relative activity (%)	Specific activity (Units/mg protein)
Hemicellulose				
0	···	0	0	0
0.5	1.51	0.67	22	0.44
1.0	2.02	2.17	71	0.98
1.5	2.02	2.28	75	1.13
2.0	2.02	3.04	100	1.50
3.0	2.02	2.48	82	1.23
Xylan				
0.5	1.51	0.93	31	0.62
1.0	1.55	2.20	72	1.41
1.5	1.68	2.35	77	1.40
2.0	1.51	2.67	88	1.77
3.0	1.82	2.89	95	1.59
Xylose				
0.5	1.74	1.85	61	1.06
1.0	1.76	2.35	77	1.26
1.25	2.24	2.89	95	1.29
1.5	2.24	2.81	92	1.25

(a): The organism was grown at 30°C for 48 hr on a medium containing 2.5% corn steep liquor, 0.1% $MgSO_4$ and different level of straw hemicellulose.

Table 2. Chemical Composition and *in vitro* Digestibity of untreated Straw and Straw Residues after Hemicellulose Extraction. (% dry matte)

Composition and in vitro digestibility	Untreated straw	Straw residue[a]	Washed straw[b] residue
Cellulose	41.9	48.6	66.6
Hemicellulose	26.3	14.2	11.0
Cell soluble matter	28.2	23.4	15.9
Lignin	6.9	5.4	7.1
Ash	1.0	0.7	0.6
Protein	3.8	2.0	1.4
In vitro digestibility	44.9	78.6	56.2

(a) Straw residue after hemicellulose was extracted with 4% NaOH. PH of the substrate was 12.0.

(b) The hemicellulose extracted straw residue was washed with water. until free of extractable hemicellulose. PH of the substrate was 7.0.

NaOH is often used to improve the digestibility of cellulosic crop residues (Han, 1978).[5] By treating cellulosics with NaOH, the fiber structure is believed to be swollen and water holding capa-

Table 3. Response of Weanling Meadow Voles for Diets Containing Straw Residues after Hemicellulose Extraction.

Diet	Weight gain[a] (g)	Feed intake[a] (g)	Feed efficiency (g feed/g wt. gain)
Control	0.241	3.56	14.8
Untreated straw	0.222	4.15	18.7
Straw residue[b]	0.241	3.78	15.7
Washed straw[c] residue	0.194	3.69	0.91

(a): Average per day of 10 animals for 8 day feeding period.
(b): Straw residue after hemicellulose was extracted with 4% NaOH. PH of the substrate was 12.0.
(c): The straw residue was washed with water until free of extractable hemicellulose. PH of the substrate was 7.0.

city and penetability of microorganisms and enzymes are increased. The straw residue obtained after the hemicelluose is extracted also increased the digestibility from 45% to 78.6% (Table 2). This level of increase was almost equivalent to that obtained by NaOH treatment (without extraction of hemicellulose) of ryegrass straw (Han et al, 1978).[6] Thus, removal of hemicellulose does not appear to adversely affect the digestibility of straw. Thus, the hemicellulose-extracted straw residue could be beneficially used for animal feed, because the low diqestibility was cited as the major obstracle in using straw as animal feed (Han, 1978).[5]

The increased digestibilty of straw residue was diminished when the residue was thoroughly washed with water to remove all the soluble hemicellulose. Therefore, the extend of washing the alkali treated straw should be adjusted to balance the maximum recovery of hemicellulose and maximum increase in digestibility of treated residue.

An animal response was studied with a diet containg the alkali treated straw residue (Table 3). Weight gain, feed intake and feed efficiency were significantly increased by alkali treatment. This effect, however, was diminished when the straw residue was thoroughly washed. The pH of the alkali treated straw was 12.0, but it did not adversely affect the palatablity of the diet for weanling meadow voles. The weight gain, feed intake, and feed efficiency value of the straw residue diet were almost equivalent to that of control (corn) diet.

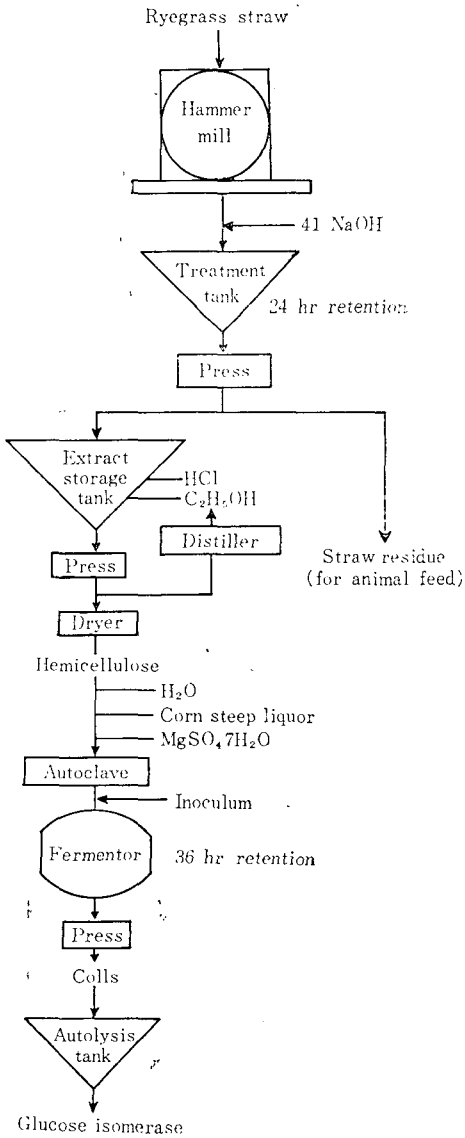

Fig. 4. Flow Diagram of Straw Utilization Process.

isomerase was produced by growing *Streptomyces flavogriseus* on straw hemicellulose. The hemicellulose was extracted from straw with NaOH and the residual straw was used as animal feed. The schematic diagram of the process is shown in Fig. 4. The process is simple and inexpensive compared to the process where pure xylose is used as an inducer-substrae. The process utilizes straw in its entirety for production of enzyme and animal feed.

요 약

독보리짚을 NaOH로 처리하여 헤미셀로오즈 부분을 추출하여 이것을 *Streptomyces flavogriseus*에 의한 글루코오스 이성질화효소를 생성하는데 사용하였다. 짚을 1%~24%의 NaOH를 처리함으로써 천연 헤미셀룰로오즈를 농도 25%까지 얻을 수 있었고 헤미셀룰로오즈 수율은 NaOH 농도를 4%까지 증가시킬 때까지는 비례적으로 증가하였다. 그러나 증가율은 NaOH 농도가 더 증가하면 낮았다. 독보리짚에서 헤미셀룰로오즈를 추출하는 최적조건은 짚을 4% NaOH로 90°C에서 3시간 처리하거나 30°C에서 24시간 처리하여 균체를 2% 짚—헤미셀룰로즈에서 30°C, 2일간 키웠을 때, 글루코오스 이성질화 효소활성도(3.04 units/ml 배양액)가 최고값에 달하였다. 또한 균체를 xylan, xylose 또는 짚의 황산가수분해물에 키웠을 때 글루코오스 이성질화 효소를 상당한 량을 생성되었다. 헤미셀룰로오즈를 추출해내고 남은 짚 찌꺼기는 동물 사료로 사용할 수 있는데, 이는 젖을 갖 뗀 초원 들쥐에 그 찌꺼기를 사료로 주었을 때, 처리하지 않은 짚을 준 경우보다 75%나 더 높은 소화가능도를 나타내며 사료로서의 효율은 20%가 더 높은 것으로 나타났음으로 알 수 있었다.

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