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On an available pretreatment for the enzymatic saccharification of lignocellulosic materials

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Summary In order to aim at a proved pretreatment for the enzymatic saccharification of lignocellulosic materials, the combined effect of the steam explosion treatment and the newly added alkali-hydrogen peroxide treatment was investigated. As a result, the following findings were obtained from comparison of the saccharification ratio (%) among the materials used: 1) Marked differences based on the lignocellulosic species characteristic of biological materials were observed. 2) Lignocellulosic samples such as rice straw, poplar, and maple chips gave enzymatic saccharification ratio (%) close to 100% by using the samples obtained after extraction with various solvents without alkali-hydrogen peroxide treatment. 3) Chip samples such as Japanese larch, loblolly pine, and Japanese cypress gave the very low enzymatic saccharification ratio (%) of 20–40% after the steam explosion and extraction with various solvents. 4) In comparison with enzymatic saccharification ratio (%) of original samples treated with steam explosion, alkali-hydrogen peroxide treatment resulted in greater effect based on the treatment of 2–2.5 times. 5) Alkali-hydrogen peroxide treatment afforded remarkable effects based on the treatment of the softwoods, such as Japanese larch and loblolly pine.

Introduction

Lignocellulosic biomass is composed of various kinds of biological resources including agricultural wastes as bagasse and crop residues, and unused forest products as waste chips (shavings) and sawdusts from a sawmill, thinning woods, barks. In order to design the effective utilization for conversion into useful substances like liquid fuels and organic chemicals by means of enzymatic saccharification of such heterogeneous lignocellulosic biomass, development of effective pretreatment is an indispensability. The necessity for pretreatment would accomplish the degradation in crystallinity of cellulosic materials, concomitant with a reduction in lignin content and an increase in the surface area of lignocellulosic substrate. Lots of various pretreatments have been so

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far attempted and the references on such research subjects are voluminous until now (Miller et al. 1975; Kelsey, Shafizadeh 1980; Fan et al. 1981; Saddler et al. 1982; Esterbauer et al. 1983; Ladisch et al. 1983; Bonn et al. 1987; Katrib et al. 1992). Various pretreatments can be classified into physical method as vibratory ball milling, physico-chemical method as steam explosion, chemical method as delignification with oxidizing agents, biological method as delignification with the fungi, and their combined method, depending on their mode of action on the lignocellulosic materials (Shimizu 1985; Moriyama, Saida 1986; Wood, Saddler 1988). The individual pretreatment has both merits and demerits, and it needs to be chosen according to the treatment method and the treatment conditions, depending on the sorts of

lignocellulosic materials. From the viewpoints of practical use, it is natural that the desirable pretreatment should be considered from the low cost and the simple treatment, and furthermore should be expected in the higher effect of pretreatment than original treatment.

Thus, this paper is concerned with the combined effect of steam explosion treatment and newly added alkali-hydrogen peroxide treatment with a view of examination of the effectiveness as a pretreatment for the enzymatic saccharification of lignocellulosic materials. Especially, the enzymatic susceptibilities for saccharification of various softwoods was focussed in this investigation.

Materials and methods

Experimental materials used

The following seven kinds of lignocellulosic materials were used: rice plant as the Gramineous plant (*Oryza sativa* L.), three species of hardwoods: namely Japanese beech (*Fagus crenata* Bl.), Japanese poplar (*Populus Maximowiczii* Henry), Japanese painted maple (Acer mono Maxim.), three species of softwoods: namely Japanese larch (Larix leptolepis Gord.), loblolly pine (Pinus taeda L.), Japanese cypress [Chamaecyparis obtusa (S.et Z.) Endl.]

The parts called as stalk or trunk in these samples were employed in this experiment.

Steam explosion treatment

Each material used was made in chip form with a chipper machine. Each chip sample prepared was subjected to a steam explosion under fixed conditions. The pressure was 28 kgf/cm^2 and the treatment temperature 232 °C was kept for 4 min in a closed vessel after the injection of steam. Then, the contents were released explosively into the atmosphere.

Successive stepwise extraction with various solvents

A part of steam explosion samples was freeze-dried and extracted stepwisely with such various solvents as water, methanol, dioxane and 1% NaOH, respectively as shown in Fig. 1.

Alkali-hydrogen peroxide treatment

A constant sample (2 g) weighed was mixed with 2% (w/v) H_2O_2 solution (100 ml) and adjusted at pH of 11.5 with 2N NaOH. The mixture was adjusted so continuously as to keep the same pH value, with stirring at room temperature during 18–24 hrs. The residue after the above treatment was recovered by filtration, and washed with 10% AcOH and water, respectively until the washings were neutral. The products were obtained as the samples for enzymatic hydrolysis by freeze-drying.

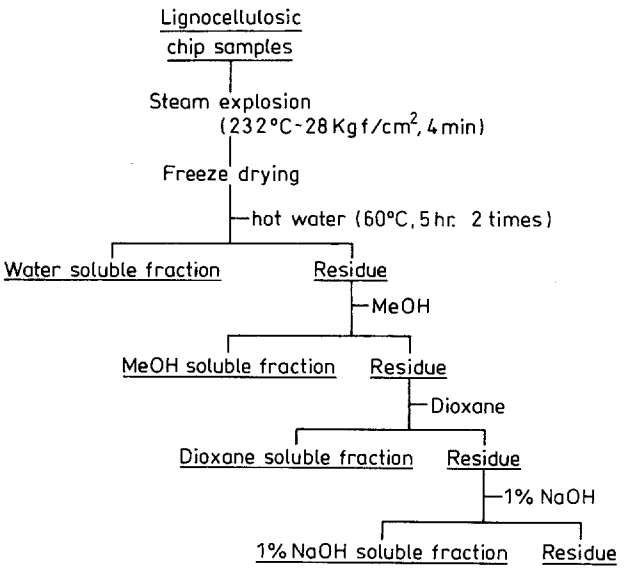

Fig. 1. A scheme of stepwise extraction with various solvents of lignocellulosic materials treated by steam explosion process

Cellulase for enzymatic hydrolysis

Commercial Meicelase CEPB-5057 originated from Trichoderma viride, which was provided by Meiji seika co., and Cellulosin AC40 originated from Aspergillus niger, which was provided by Ueda Chemical Industrial co., were employed as enzymes for hydrolysis without doing purification.

Enzymatic hydrolysis

Each of various samples weighed (1.0 g) was mixed with 0.2 M acetic acid buffer solution (pH 4.8, 50 ml). The mixture content was hydrolyzed by addition of a mixed enzyme solution (concentration: 10%, 5 ml) of commercial two kinds of enzymes (1 : 1, w/v), being kept at 40 °C under shaking (90 times/min). After fixed time (48 hrs), the saccharified residues and the filtrates were separated by filtration. The filtrate was kept in hot water of 90 °C for 10 min to inactivate the enzyme. The resulting precipitate was removed by centrifugation, and then the supernatant solution was filled up to 100 ml volume with distilled water.

Analytical methods

The reducing sugars liberated in a constant volume from the above diluted solution were determined with the dinitrosalicylic acid (DNS) method (Wood, Mahalingeshwara 1988) as the standard of D-glucose. Furthermore, the amount of D-glucose in the reducing sugars liberated was determined according to the modified enzyme method using glucose oxidase (Dahlgvist 1961; Trinder 1969). Also, the total carbohydrate contents in all samples were determined with the phenol-sulfuric acid method (Dubois et al. 1956). The lignin contents in all samples were determined as Klason lignin and acid soluble lignin according to a Tappi standard method.

Table 1. Analytical data of components extracted with various solvents from lignocellulosic materials treated by steam explosion process

Plant species	Water-soluble fraction (%)	MeOH soluble fraction (%)	Dioxane soluble fraction (%)	1% NaOH soluble fraction (%)	Residue soluble (%)
Rice	20.78	17.32	3.00	17.78	40.56
Poplar	17.24	21.34	7.36	4.64	48.48
Japanese beech	13.19	21.87	5.23	5.29	53.51
Maple	16.25	19.44	7.99	5.20	50.17
Japanese larch	11.80	21.19	0.94	4.98	60.21
Loblolly pine	10.62	12.28	3.00	5.45	67.65
Japanese cypress	16.89	8.69	3.27	3.78	66.46

All values are expressed with percent based on oven-dried samples treated by steam explosion process

Results

Table 1 shows the analytical data of components extracted with various solvents from lignocellulosic samples treated by steam explosion. Treatment by hot water extraction contributes to remove mainly water-soluble hemicellulosic components. This type of component from rice stalk is the highest among the used samples. From this experimental result, water-soluble components in hardwoods such as poplar or painted maple are expected to be relatively higher than those in softwoods such as larch or loblolly pine. Both soluble fractions obtained from MeOH extraction and dioxane extraction are supposed to correspond to the lignin degradation products caused by steam explosion treatment. These types of fractions in the Gramineae as rice stalk and hardwoods give the relatively higher values than those in softwoods. The component from 1% NaOH extraction includes alkali-soluble hemicellulosic fraction as well as lignin degradation products. This type of the Gramineae as rice stalk gives the extremely higher value, compared with that in other woods. Although this finding probably needs the detailed investigation to lead to a clear conclusion, the excess degradation of lignin and hemicellulose seems to have proceeded because the fixed treatment conditions for steam explosion were rather severe for the Gramineae as rice stalk. Consequently, lignin and hemicellulose components are supposed to be in the relatively easy extractable state after steam explosion, compared with the case of woods.

Table 2 shows the effects based on enzymatic saccharification ratio (%) of alkali-H_2O_2 treatments on various samples treated. This result leads to the conclusion as follows: 1) In samples as rice stalk, poplar, and painted maple, near 100% of enzymatic saccharification ratio (%) is expected only by various solvent extractions without alkali-H_2O_2 treatment after steam explosion. 2) In softwoods, enzymatic saccharification ratio (%) in various samples treated with no alkali-H_2O_2 treatment gives extremely low value of 20–40%. However, alkali-H_2O_2 treatment contributes to greater effect based on the treatment of 2–2.5 times, compared with enzymatic saccharification ratio (%) of original samples treated with steam explosion. 3) Alkali-H_2O_2 treatment is very effective as the combined pretreatment of the softwoods as larch and loblolly pine.

Table 3 shows the result of analytical determination of glucose contents in reducing sugars liberated by enzymatic hydrolysis. This result means that more than 80% of the

Table 2. Effect based on enzymatic saccharification ratio (%) by H_2O_2-alkali treatment

Plant species	S-I-1 (%)	S-I-2 (%)	S-II (%)	SO-I (%)	SO-II (%)	SO-I/S-I-1
Rice	31.2 (63.6)	–	100.6	72.3	99.7	2.32
Poplar	49.3 (80.7)	2.0 (3.3)	95.0 (105.3)	94.7	95.5	1.92
Japanese beech	64.7 (97.9)	5.8 (8.8)	68.6 (77.9)	79.6	71.0	1.23
Maple	54.3 (89.6)	1.9 (3.1)	99.4 (112.8)	101.0	93.7	1.86
Japanese larch	32.6 (58.9)	7.8 (14.1)	29.9 (48.9)	86.2	86.9	2.64
Loblolly pine	40.0 (75.1)	10.0 (18.9)	37.2 (55.1)	78.4	72.0	1.96
Japanese cypress	15.4 (26.7)	7.9 (13.8)	21.2 (32.8)	33.2	34.8	2.16

Note: S-I-1: Saccharification ratio (%) of lignocelluloses treated by steam explosion process
S-I-2: Saccharification ratio (%) of residual samples obtained by repeated enzymatic hydrolysis of S-I-1
S-II: Saccharification ratio (%) of final residue extracted with various solvents of lignocelluloses treated by steam explosion process
SO-I: Saccharification ratio (%) of samples obtained by H_2O_2-alkali treatment of S-I-1
SO-II: Saccharification ratio (%) of samples obtained by H_2O_2-alkali treatment of S-II

$$\text{Saccharification ratio (\%)} = \frac{B}{A} \times 100$$

A: Each oven-dried, original sample (g)
B: The amount of production of reducing sugar (g)

All values with parentheses are expressed with percent based on oven-dried lignocelluloses corrected on lignin content.
Reducing sugars were determined by using D-glucose as a standard sugar

Table 3. Analytical determination of glucose content in reducing sugars liberated by enzymatic hydrolysis

Plant species	S-I-1 (%)	S-I-2 (%)	S-II (%)	SO-I (%)	SO-II (%)
Rice	96.9	–	89.5	97.2	96.4
Poplar	86.3	109.7	84.1	89.4	91.3
Japanese beech	79.7	83.6	84.4	89.5	94.6
Maple	82.4	106.2	83.4	85.4	89.5
Japanese larch	81.6	101.9	85.1	89.2	95.3
Loblolly pine	93.1	81.4	88.2	89.6	96.2
Japanese cypress	71.5	88.1	87.5	86.3	88.4

See Note in Table 2 on explanation of S-I-1, S-I-2, S-II, SO-I and SO-II.
Glucose contents were determined with enzymatic method using glucose oxidase

reducing sugars liberated by enzymatic hydrolysis under this experimental conditions is obtained in the form of monosaccharide as glucose, and that the saccharification process could be directly connected with the following fermentation process.

Discussion

Various pretreatments so far reported for lignocellulosic materials are restricted to applicable wood species, even if the materials are treated under the most suitable

conditions because of difference among wood species characteristic of biological materials (Shimizu 1985, 1988). The steam-explosion process has a useful prospect of developing the new field for application and of managing to a routine work as one of various pretreatments. The process attracts an attention as the pretreatment in pulping and making fodder as well as that in enzymatic saccharification of lignocellulosic biomass (Dekker et al. 1983; Brownell, Saddler 1984; Iwasaki 1990). Also, the steam-explosion process makes it possible to discuss the way to utilization process of woody components separated because the process makes it easy to lead to the separation of woody components such as cellulose, hemicellulose, and lignin (Shimizu 1985).

This investigation has been performed in expectation of the combined pretreatment effect of the steam-explosion process and the newly added simple alkali-hydrogen peroxide treatment, especially in expectation of noticeable positive effect based on pretreatment toward softwoods. The combined pretreatments contribute to the disolution and removal of hemicellulosic component due to the promotion of delignification and the swelling and softening of the samples, and to further rising of enzymatic saccharification ratio due to increasing of the surface area.

Hydrogen peroxide has been originally used as a bleaching agent based on oxidation reaction in pulp and paper industry. As reviewed in the recent proceedings (Peng et al. 1989; Walsh 1991), the control of environmental public pollusion provides an important social problem, and intense interest has been shown toward alkali-hydrogen peroxide treatment as a bleaching agent for pulp instead of a bleaching with chlorine reagents. It is said that hydrogen peroxide has a property to oxidize selectively toward lignin of a herbaceous plant by formation of reactive hydroxyl radical (Gould 1984, 1985), and therefore it is supposed to contribute to effective delignification of the samples treated after the steam-explosion process.

The synergistic effect by the combined method of physical, chemical, and biological treatments has been hitherto investigated as a promising pretreatment prior to enzymatic saccharification of lignocellulosic materials (Dale 1987), as shown in various pretreatments such as wet milling plus chemical delignification, attritor milling with organosolv treatment, chemical delignification and biological treatment with the fungi. Mackie et al. (1985) and Clark et al. (1987) reported the enhancement of enzymatic saccharification caused by steam explosion treatment using wood chips impregnated with acid catalytic SO_2. Sudo et al. (1986) also described the improved enzymatic susceptibility of softwoods by the catalyzed steam explosion process in the presence of organic acids and inorganic salts. However, from a point of view in the economical and feasible application, which treatments should be combined bring forward an important choice on the above consideration. This proposing treatment method would be promising as one of the pretreatments prior to enzymatic saccharification because this alkali-hydrogen peroxide treatment or now investigating defiberation treatment with a refiner is rather simple without any special equipment after steam explosion treatment, and is further achieved in low cost.

Thus, in this study for enzymatic saccharification of lignocellulosic biomass, development of the technique for useful pretreatment pointing to energy saving and economical feasibility would be expected in the near future.

References

Bonn G.; Hörmeyer H. F.; Bobleter O. 1987: Hydrothermal and organosolv pretreatments of poplar wood and wheat straw for saccharification by a Trichoderma viride cellulase. Wood Sci. Technol. 21: 179–185

Brownell H. H.; Saddler J. N. 1984: Steam-explosion pretreatment for enzymatic hydrolysis. Biotechnol. Bioeng. Symp. 14: 55–68
Clark T. A.; Mackie K. L. 1987: Steam explosion of the softwood pinus radiata with sulphur dioxide addition. I. Process optimisation. J. Wood Chem. Technol. 7: 373–403
Dahlgvist A. 1961: Determination of maltase and isomaltase activities with a glucose oxidase reagent. Biochem. J. 80: 547–551
Dale B. E. 1987: Lignocellulose conversion and the future of fermentation biotechnology. Trends Biotechnol. 5: 287–291
Dekker R. F. H.; Wallis A. F. A. 1983: Enzymatic saccharification of sugarcane bagasse pretreated by autohydrolysis-steam explosion. Biotechnol. Bioeng. 25: 3027–3048
Dubois M.; Gilles K.; Hamilton J. K.; Rebers P. A.; Smith F. 1956: Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350–356
Esterbauer H.; Hayn M.; Jungschaffer G.; Taufratzhofer E.; Schurz J. 1983: Enzymatic conversion of lignocellulosic materials to sugars. J. Wood Chem. Technol. 3: 261–287
Fan L. T.; Gharpuray M. M.; Lee Y.-H. 1981: Evaluation of pretreatments for enzymatic conversion of agricultural residues. Biotechnol. Bioeng. Symp. No 11: 29–45
Gould J. M. 1984: Alkaline peroxide delignification of agricultural residues to enhance enzymatic saccharification. Biotechnol. Bioeng. 26: 46–52; 1985: Enhanced polysaccharide recovery from agricultural residues and perennial grasses treated with alkaline hydrogen peroxide. ibid. 27: 893–896
Iwasaki M. 1990: Explosion pulping "stake process". Japanese Tappi 44: 856–864
Katrib F. Al; Chambat G.; Joseleau J. P. 1992: Effect of pretreatment of poplar wood upon enzymatic saccharification. J. Wood Chem. Technol. 12: 355–366
Kelsey R. G.; Shafizadeh F. 1980: Enhancement of cellulose accessibility and enzymatic hydrolysis by simultaneous wet milling. Biotechnol. Bioneg. 22: 1025–1036
Ladisch M. R.; Lin K. W.; Tsao G. T. 1983: Process consideration in the enzymatic hydrolysis of biomass. Enzyme Micro. Technol. 5: 82–102
Mackie K. L.; Brownell H. H.; West K. L.; Saddler J. N. 1985: Effect of sulphur dioxide and sulphuric acid on steam explosion of aspenwood. J. Wood Chem. Technol. 5: 405–425
Millet M. A.; Baker A. J.; Satter L. D. 1975: Pretreatments to enhance chemical, enzymatic, and microbiological attack of cellulosic materials. Biotechnol. Bioeng. Symp. No. 5: 193–219
Moriyama S.; Saida T. 1986: Continuous pretreatment and enzymatic saccharification of lignocellulosics. In: Young R. A.; Rowell R. M. (Eds.): Cellulose-structure, modification and hydrolysis-. pp. 323–336. New York: Wiley-Intersci. Pub.
Peng F.; Simonson R. 1989: High-yield chemi-mechanical pulping of bagasse. I. Impregnation of bagasse with sodium hydroxide and hydrogen peroxide. Cellulose Chem. Technol. 23: 81–89
Saddler J. N.; Brownell H. H.; Clermont L. P.; Levitin N. 1982: Enzymatic hydrolysis of cellulose and various pretreated wood fractions. Biotechnol. Bioneg. 24: 1389–1402
Shimizu K. 1985: Microbial conversion of biomass. Mokuzai Gakkaishi 31: 783–792
Shimizu K. 1988: Steam explosion treatment of wood. Japanese Tappi 42: 1114–1130
Sudo K.; Shimizu K.; Ishii T.; Fujii T.; Nagasawa S. 1986: Enzymatic hydrolysis of woods Part IX. Catalyzed steam explosion of softwood. Holzforschung 40: 339–345
Tappi standard T222 om 83; Tappi useful method 250.
Walsh P. B. 1991: Hydrogen peroxide: innovation in chemical pulp bleaching. Tappi J. 74: 81–83
Wood T. M.; Saddler J. N. 1988: Increasing the availability of cellulose in biomass materials. In: Wood W. A.; Kellogg S. T. (Eds.): Methods in Enzymology, Cellulose and Hemicellulose, Vol. 160, Part A, pp. 3–11. London: Academic Press
Wood T. M.; Mahalingeshwara Bhat K. 1988: Methods for measuring cellulase activities. In: above reference pp. 87–112